Proceedings of the 8th International Conference on Controlled Atmosphere and Fumigation in Stored Products

Edited by:

Guo Daolin, Shlomo Navarro, Yang Jian, Tao Cheng,
Jin Zuxun, Li Yue, Liu Yang and Wang Haipeng

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EDITED BY

Guo Daolin, Shlomo Navarro, Yang Jian, Tao Cheng,
Jin Zuxun, Li Yue, Liu Yang and Wang Haipeng

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Preface

International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF) is one of the two foremost international conferences in protection of stored products. It is held in different countries every four years after the first conference in 1980. The first conference had been held in Rome of Italy in 1980, and then the conferences had been held separately in Perth of Australia, in Singapore, in Winnipeg of Canada, in Nicosia of Cyprus, in Fresno of America and in Brisbane of Australia. This is the 8th CAF conference (CAF 2008) held in Chengdu, P. R. China. It's the first time to hold CAF in China.

The theme of CAF 2008 is Controlled Atmosphere and Fumigation——Green, Safe, Harmony and Development. Green is an eternal topic in protection of stored products. Safe is the safeguard of life and property. Harmony is a common wish of people. Development is a common goal of human beings. Surrounding this theme, this conference includes ten session topics such as 1) Advances in the basic studies on the application of Controlled Atmospheres (CA) and Fumigation, 2) Substitutes for Methyl Bromide and replacement technologies, 3) Safety of products under CA and Fumigation (effects on quality of stored products), protection of the environment, human health issues, 4) Application technologies and safe practices of CA and Fumigation treatments, 5) Sealing techniques and CA engineering, 6) Effects on insect control and Economic Thresholds (ET), 7) Operation, regulation, and technique standards for CA and Fumigation, 8) CA and Fumigation——Insect resistance and management strategies, 9) Achievements of CA and Fumigation, and development trends, 10) Technology transfer and international cooperation in CA and Fumigation. A platform will be offered to communicate the innovative fruits in research and development of controlled atmosphere and fumigation in stored products, to discuss the development of this field, to strengthen international communication and cooperation, and to promote the common development to contribute more for human beings.

During the preparatory work of CAF 2008, the Organizing Committee had call for papers in global range and received 181 papers. After review by experts, session chairs, organizing committee and CAF Permanent Committee (CAF PC), confirmed 137 papers for conference presentation in conference. According to the suggestion of CAF Permanent Committee, the conference proceedings will be published before conference with title Proceedings of the 8th International Conference on Controlled Atmosphere and Fumigation in Stored Products.

In preparation and organization of this conference, we had obtained great supports from State Administration of Grain of P. R. China (SAG), Chinese Cereals and Oils Association (CCOA), China Grain Reserves Corporation and China National Cereals, Oils and Foodstuff Co. (COFCO). We had also got the unselfish supervise from Chair and Secretary of CAF PC, Dr. J. Banks and Dr. S. Navarro, got support and help from members of CAF PC, such as Dr. Yonglin Ren etc, session chairs. On behalf of Organizing Committee of CAF 2008, I sincerely appreciate them all. Here, I also want to give special appreciation to Prof. Wang Jinjun, Dr. Shlomo Navarro, Dr. Jim Leesch, Mr. Wang Yuejin, Dr. Jonathan Banks, Dr. Tom Batchelor, Dr. An Yulin, Prof. Dagmar Klementz, Prof. Zhang Hongyu, Dr. Yonglin Ren, Dr. Ronald Noyes, Mr. Wang Yanan, Prof. Digvir Jayas, Prof. Wang Dianxuan, Prof. Greg Daglish, Dr. Zeng Ling, Dr. Dirk Maier, Prof. Cao Yang, Dr. Pat Collins, Prof. Deng Yongxue, Dr. James Throne, Dr. Bhadriraju Subramanyam, Dr. Niu Xinghe and Mr. David Fienberg for their huge contribution to paper review and editing.

Even though we try our best to guarantee the quality in review and editing, time is very limited for the publication of the papers before conference, and English is not the mother language in China, some oversights or flaws are hard to avoid. We look forward to your kind understanding.

Liu Xinjiang, Conference chair

Obituary

An appreciation of Jonathan Ezra Donahaye
(1935 – 2008)

Jonathan Donahaye was born in Toulouse, France in 1935. He obtained his B. Sc. at the University of London, Imperial College before emigrating to Israel in 1960 where he was one of the founders of the Stored Products Research Laboratory, Ministry of Agriculture in Jaffa. In 1986, he submitted his dissertation to the Hebrew University of Jerusalem where he was duly awarded his doctorate. In 1989 he spent a sabbatical year with the World Hunger Program, at Brown University, Rhode Island, USA. In 1992, he was promoted to Grade A Research Scientist, equivalent to University Professor, at the Israel Agricultural Research Organization (ARO) and 2 years later Jonathan was elected as Head of the Department of Stored Products, at the ARO.

He served as lecturer and consultant for the Israel Foreign Assistance Service and FAO on the conservation of stored products in the Far East, Africa and South America. Searching for viable, innovative and appropriate technologies that can be easily implemented where they are needed, Jonathan became a leading researcher in developing non-chemical postharvest technologies; he worked on chilling systems, on modified atmospheres, and on the hermetic storage of products that need protection from insects, mites and mold damage in semi-permanent plastic storage structures.

Jonathan was known for his many talents. He loved woodwork, and he built guitars and lutes. Among other activities he also established a website on fountain pens (http://www. FTIC. info/Donahaye/ConwayStewart/) and another on the insects that he loved, Insects of the Land of Israel (http://www. FTIC. info/Donahaye/insects/israeliinsects. html).

Jonathan retired in 2000, but continued with his professional interests and served as consultant on various missions, and in publications within a consultancy company he established together with his colleagues. He edited the book of Proceedings of the International Conference on Controlled Atmosphere and Fumigation in Stored Products, which was held in Australia in 2004, and established a website for this book (http://www. ftic. info/CAFSITE/CAF. html).

Jonathan was active in publications of post-harvest technologies and was on the Editorial Board of the Journal of Stored Products, from 1999 until his death.

He passed away on January 23, 2008. He leaves his wife Marcel, a daughter and a son, and 6 grand children. We all will miss Jonathan; he will remain in our memories as a talented entomologist, an excellent scientist, a very good companion, and as a person with goodwill to help others.

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Abstract Session

Current Situation and Development Tendency of Controlled Atmosphere and Fumigation Technology in Chinese Grain Storage

—Council Director of Chinese Cereals & Oils Association (CCOA)
Zhu Changguo

Dear Honorific Chairman Banks, Ladies & Gentlemen,

First of all, please allow me to welcome all of you on behalf of Chinese Cereals & Oils Association (CCOA).

Here, I would like to avail myself of this opportunity to briefly introduce Chinese grain storage technologies, especially developments in low temperature grain storage, controlled atmosphere and fumigation technology.

Ⅰ. Chinese grain storage technology has a long history. Research shows that it is at least 7 000 years since our ancestors began to consciously store grain and other seeds. 7 000 years ago in southern China, people used fence-style depots to aerate and store grain; 5 000 yeas ago in central China, people adopted underground vaults to store grain by using matting materials to decrease oxygen content. Records show ancient people began to utilize plant-sources medicines to kill insects in stored grain about 2 000 years ago. From this perspective, China is one of the oldest cradles of low temperature, controlled atmosphere and fumigation technology.

Ⅱ. I will broadly outline the scientific and technological developments on Chinese modern grain storage scientific technologies, and then particularly illuminate the technological developments in low temperature, controlled atmosphere and fumigation.

A. History of Chinese Grain Storage Technology

1. The Chinese government attaches great importance to the development of grain storage science and technology. In the 1950s, China established academic research institutions and began the research in grain storage; in the 60s and 70s, a few middle and high grain schools were established successively which not only cultivated grain intellects but also carried out fruitful researches.

2. China's grain storage facilities were constructed by stages whose capacity is over a few hundred million tons. In the 1990s, China constructed some modern grain storage depots with the loans from the World Bank and China's national debt. These depots are large warehouse, squat silos and vertical silos), which feature large-scale gain bulks, high-level grain layers' (Single depot capacity is 5 000t – 10 000t). In recent years, enterprises' yearly average grain storage capacity totals 1.5 – 2.0 hundred million tons. Farmers' yearly average capacity totals 2.5 – 3.0 hundred million tons. For this reason, China has particularly popularized such technologies as 'grain inspection, machinery aeration, grain cooling and recirculative fumigation' among grain storage enterprises. Not only have China's storage equipment and technologies greatly improved, but also the storage technologies have occupied forefront places in the world.

3. Applied basic theories and applied technologies are the two main aspects of Chinese grain storage research. Depending on our own development experiences and research fruits and using the results of foreign grain storage ecological research, we have summarized a Chinese character grain storage 'ecosystem theory system' to guide grain storage and depot construction in the future. The system includes: reasonable partition of Chinese grain storage ecological zones; reasonable depot types selection and design in different zones; grain depot equipment in different depot types and zones, rational configuration of machinery and special safety-ensuring grain storage equipment for different depot types and zones; the best storage techniques and economic running modes of different kinds of grain in varied depot types and different zones; management and economics evaluation of different storage scales, modes and methods; evaluation indexes and system on safe grain storage technology. Chinese Safe Grain Storage Study researches the tripartite relations among the subject (grains, oil seeds and their products), object (ecological factors including biologic and abiologic) and sociology (management, cost and benefit), which is the development and extension of grain storage study and grain storage ecology.

4. CCOA is a national first-class association whose Storage Branch is an academic organization in which domestic and foreign experts, scholars and a few hundred thousand scientific staffs communicate and cooperate with each other. This branch trains party members and individual member every year, meanwhile, expands cooperation and communication with international academic organizations.

B. Developments in some key fields in Chinese grain storage science & technology. To make this question easy, I will firstly introduce 'ecological grain storage and green grain storage'. 'ecological grain storage' means fully using and controlling the ecological conditions which are favorable to stored grain quality, such as low temperature, low oxygen, therefore ensuring safe grain storage. 'Green grain storage' means trying to adopt technologies that have no contamination on stored grain. The two concepts are consistent to each other. In China, ecological & green grain storage means taking 'low temperature' as the major technology complemented with controlled atmosphere technology in the suitable zones.

1. Low temperature and quasi-low temperature grain storage. In China, the average temperature of grain bulk in the depot below 15℃ with the highest temperature not over 20℃ is called 'low temperature grain storage'; grain bulk's average temperature below 20℃ with the highest temperature not over 25℃ is called 'quasi-low temperature grain storage'. In 2005, China built a large, low-temperature experimental grain depot directly subordinate to the National Grain Reserves in Chengde, Hebei Province. It has a storage capacity of 15 000 tons and its grain storage temperature is controlled below 20℃, which has successfully achieved the designed standards after a few years' operation. Major technological approaches to low temperature and quasi-low temperature are natural low temperature (including overground and underground natural low temperature), machinery aeration, machinery cooling (grain cooling, special cooling machines). From experiment results of nearly 20 national grain depots all over China, machinery aeration in squat silo can effectively decrease stored grain's temperature. In large warehouse, if grain bulk's height is not over 6m, using centrifugal fan, axial-shaft fan and mixed airflow fan could well decrease the temperature and preventing dewing.

Low temperature grain storage technology has brought about corresponding technology research, for example, spray-cooling the exterior of grain depot to reduce temperature, using anti-radiation dope to lower temperature, heat insulation on the ceiling of grain depot to control temperature, different press-top technologies in the grain bulk to insulate heat and keep cool, solar technology to insulate heat and keep cool etc. Based on the previous research of different materials' heat conductibility properties, researchers have proposed the technologies of 'static heat insulation' and 'dynamic heat insulation' for different grain depots of different ecological regions. The grain inspection technology, machinery aeration technology and grain cooling technology have their respective technical regulations.

2. Controlled atmosphere grain storage. In china, controlled atmosphere grain storage research falls into two major phases: the first phase is in the 1960s and 70s, which focused on the study of using different materials for N_2 & CO_2 – filled, or vacuum, small-package storage of grain, edible oil and their processed products; the second phase is from the 1970s when Chengdu Grain Storage Research Institute (CSR) of State Administration of Grain led the development of *Research on Modern Grain Fresh-Keeping Technology*, started comprehensive research on CO_2 controlled atmosphere grain storage technology, and performed small-scale depot experiments. The research explored not only the airtight materials, methods and effects but also the causes of quick deterioration of stored grain under controlled atmosphere after it was exposed to normal air. In 1990s, CSR and Henan Industrial University led the research on the construction of CO_2 controlled atmosphere grain depot. The project was the first time to adopt large-scale system of CO_2 supply, realized centralized CO_2 supply, and developed an automatic depot CO_2 concentration monitoring & analysis system. The longest time of pressure half life of Mianyang depot in Sichuan (air tightness decreases from 500Pa to 250Pa) reaches 12min.

In 2000, after the completion of Sichuan Mianyang CO_2 controlled atmosphere grain depot, some other CO_2 controlled atmosphere grain depots were also constructed in other regions, whose total depot capacity reaches 215 thousand tons.

Since 2005, with the rising of CO_2 price, emphasis is switched to N_2 grain storage. Based on N_2

grain storage demo-application in Jiangsu Nanjing Grain Depot and Guangxi Fangchenggang Grain Depot directly under the National Grain Reserves, the National Grain Reserves invested to build 16 controlled atmosphere depots in a bid to further optimize and expand the demo application. Presently a storage capacity of 320 thousand tons has been put to use, and it is expected to reach 850 thousand tons by the end of 2008.

Besides, the *CO_2 Controlled Atmosphere Grain Storage Technology Regulations* has already been promulgated and brought into effect.

3. Recirculative fumigation technology. From the middle 1960s up to now, the main fumigant in China is phosphine. For this reason, China has deepened the correlated researches which include medical effects (dosage, obturation time and insecticidal effects), factors affecting medical effect (different insect species, different insect states, different temperature, different grain type's adsorption intensity, different obturation conditions and performances, influence of grain bulk's airflow on medicinal effect), different operating methods of fumigation technologies (general fumigation, recirculative fumigation, intermittent fumigation and slow-releasing fumigation) which include the effect-enhancing experiment of phosphine fumigation to decrease O_2 concentration and to increase CO_2 concentration. At the end of 90s, nearly 20 National Grain Depots practical phosphine recirculative fumigation experiments proved that in squat silo and large warehouse recirculative fumigation could force phosphine gas to evenly distribute in the grain bulk and prevent stored grain insects effectively, that the three medicine application methods (using phosphine steel-bottle, recirculative fumigation outside of depot and under-film recirculative fumigation in the depot) have the same effects, and that effects of both one-time and several-time medicine application of under-film recirculative fumigation are affirmed. In practice, technologies of medicine application on grain surface and whole-depot recirculative fumigation, dynamic deliquescence of medicine application at the wind path entrance are also put to use. The total phosphine dosage in China has decreased by 60% after the popularization of recirculative fumigation technology. In addition, the *Phosphine Recirculative fumigation Technology Regulation* has already been promulgated and put into effect.

4. Comprehensively phasing out Methyl Bromide (MB) in grain storage industry.

Supported by U. N. Industry Development Organization (UNIDO), and organized and led by State Administation of Grain and Ministry of Environmental Protection, we have accomplished the MB substitute technology research, realized the goal of phasing out MB in country-wide grain storage industry, and therefore we have fulfilled the Chinese government's magnificent commitment to the international community and have made important contribution to protecting ozonosphere and human's living environment. The main steps are as follows.

Firslty we have confirmed the substitute technology of the industry which has laid foundation for realizing MB phasing outin the industry. Through comprehensive investigation, we have grasped the situation of MB application in grain industry and confirmed that phosphine under-film recirculative fumigation and phosphine and CO_2 mixing fumigation are the main alternative technologies to phase out MB.

We have compiled the training and publicity materials, providing teaching materials for training subprograms and substitute technological expansion and application. We have also compiled *Training Material of Phasing Out MB in Grain Storage Industry*, *Phasing Out MB in Grain Storage Industry Handbook* and *Phasing Out MB in Grain Storage Industry Multimedia Coursebook*.

The third is carrying out technology and management training, enhancing managing and technological personnel's knowledge and skill. Through 6 terms of training, we have completed MB substitute technology training of 387 grain storage managing and technological personnel from 128 units that used MB. We have also organized 2 terms of overseas training program for a total of 20 experts and managing personnel.

The fourth is to equipment configuration and inspection to ensure phasing out achievements. We choose 34 grain depots nationwide as demonstration depots. Through public bidding, we have designated 2 equipment providers in China which provide and install phosphine under-film recirculative fumigation equipment. Meanwhile, we executed 2 phases (4 times) of of on-site inspection of 'equipment providers' supply, installation and commissioning.

The fifth is to promulgate a joint proclamation of forbidding MB, fulfilling our commitment to the international community. Proclamation of *Comprehensively Forbidding MB in Grain Storage In-*

dustry (*No.* 4) jointly promulgated by State Administration of Grain and Ministry of Environmental Protection on 26th of Sep. ,2006 definitely stipulated that 'From 31st of Dec. ,2006, any grain depot of grain storage industry is not allowed to use Methyl Bromide(MB) as fumigant.' The mainstream media hugely publicized and reported the MB phasing out campaign in Chinese grain storage industry In 2007, the total consumption of MB in Chinese grain storage industry decreased to zero, realizing our commitment to the international commitment. The UNIDO, State Administration of Grain and Ministry of Environmental Protection convened a summarizing and commending conference in Beijing in May of 2008.

The sixth is to establish a long-term mechanism of phasing out MB. In order to consolidate the existing achievements, preventing MB grain storage enterprises to reuse MB, we established a long-term, two-layered mechanism: technical assistance and supervision to establish MB phase-out guarantee capability. The long-term mechanism includes the tracking, supervision and evaluation system for MB substitute technology application effects and the supervision and management system.

Ⅲ. Development Tendency in the Future

In the coming 5 – 10 years, with a view to the green, ecological and harmonious development tactics, China will upgrade the traditional grain storage technology, further popularize low temperature and quasi-low temperature grain storage technology in Northern China, promote N_2 and CO_2 controlled atmosphere technology in hot, humid southern China, trying to ensure 60% application of low temperature, quasi-low temperature and temperature controlling technology in grain storage . As for the grain depots that are not suitable to be equipped with the low temperature and controlled atmosphere storage equipment, we will continuously revamp the air-tightness of these depots, in a bid to comprehensively promote phosphine recirculativ fumigation, vigorously carry out research on plant-resources pesticide and biological prevention technology, develop phosphine substitute products, and to minimize dosage of medical agent. We will achieve the goal of 'high quality, high nutrition, high benefit' and 'low waste, low pollution, low cost' in grain storage. At the same time, we shall increase capital investment to improve two hundred million farmers' grain storage conditions step by step and to comprehensively realize scientific grain storage.

Looking to the future, we believe that Chinese grain storage technology will also achieve relatively rapid progress along with modern bio-technology, material technology and information technology.

Thank you very much!

SESSION 1

ADVANCES IN THE BASIC STUDIES ON THE APPLICATION OF CONTROLLED ATMOSPHERE (CA) AND FUMIGATION

Chairpersons:
Shlomo Navarro, Israel
Wang Jinjun, China
Jim Leesch, USA

0101

Advances in Postharvest Pest Control on Perishable Commodities Using Ultralow Oxygen Treatment and Low Temperature Phosphine Fumigation

Yong – Biao Liu

Abstract: Recent research in postharvest pest control on fresh fruits and vegetables for export markets have resulted in promising ultralow oxygen (ULO) treatments and low temperature phosphine fumigation treatments for a variety of pests on different commodities. Lettuce aphid (*Nasonovia ribisnigri*), western flower thrips (*Frankliniella occidentalis*), and black widow spiders (*Latrodectus hesperus*) were successfully controlled on head lettuce, broccoli, and table grapes respectively, without negative impact on product quality. Tolerance of lettuce to ULO treatment varied among cultivars and was also affected by pre-treatment storage. One week storage under normal or CA conditions prevented injury to lettuce by the subsequent ULO treatment for control of western flower thrips. In general, shorter treatment at higher temperature had less impact on lettuce quality as compared with longer treatment at lower temperature for control of western flower thrips. Low temperature fumigation with diluted pure phosphine gas was effective for control of western flower thrips on lettuce, broccoli, asparagus, and strawberries without negative effects on product quality. A successful commercial trial in a refrigerated reefer container also demonstrated the efficacy of low temperature phosphine fumigation for control of the thrips and its safety for the postharvest quality of all products. Complete control of lettuce aphid and leafminer (*Liriomyza langei* Frick) were also achieved with low temperature phosphine fumigation without negative effects on lettuce quality. These advances provided promising controlled atmosphere and fumigation treatments for commercial development. The ULO treatment also has potential to be used for postharvest pest control on organic products and facilitates international trade of organically produced agricultural commodities.

Introduction

Fresh fruits and vegetables present a unique challenge for postharvest pest control because of their requirements for cold storage to preserve quality and their sensitivity to fumigants. Typically, chemical fumigations for pest control of fresh fruits and vegetables requiring cold storage are conducted at ambient temperatures. The warm up of products for fumigation treatment compromises product quality and shelf life. Most vegetables are fragile and delicate and susceptible to injury by fumigants. For lettuce, fumigation with methyl bromide for pest control causes injury and quality reduction. The lack of safe and effective treatment for postharvest pest control hinders export of U. S. lettuce and other fresh products to overseas markets. The global phase out of methyl bromide production also heightens the need to develop alternative treatments. Although its use for pest quarantine treatment is exempt from phasing out at present, this status may change in the future. On the other hand, organic products have gained popularity around the world in the recent years. Postharvest pest quarantine treatments which are compatible with organic products would be needed for international trade of organic products.

There are a wide range of alternatives being studied for postharvest pests control on perishables, including controlled atmosphere (CA), irradiation, temperature treatment, and alternative fumigants (Mangan and Hallman 1997, Mitcham 2001, Fields and White 2002). Most CA studies focus on combinations of reduced oxygen and elevated CO_2 for pest control. But the efforts over the last 20 years have yielded little progresses due to adverse effects on product quality (Mitcham et al. 2001, 2003). Some products such as iceberg lettuce are very sensitive to CO_2 (Lipton et al. 1972). Recent studies on CA treatment with ultralow oxygen (ULO treatment hereafter) have resulted in successful control of lettuce aphid, western flower thrips, and black widow spider on different fresh products (Liu 2005, 2007, 2008a, 2008b; Liu et al. 2008).

Among alternative fumigants, pure phosphine fumigation at low temperature has been used successfully to control quarantined pests

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on fresh fruits and vegetables in Chile (Horn and Horn 2004, Horn et al 2005) and is being studied for pest quarantine treatments on a variety of fresh commodities in several countries (Klementz et al 2005, Liu 2008c). Our research also shows that fumigation with bottle pure phosphine gas at low temperature is promising for insect control on vegetables. In this paper, recent and current research on ULO treatment and low temperature phosphine fumigation are presented and discussed.

Ultralow Oxygen Treatments

ULO treatment for insect control on lettuce. ULO treatment was studied for control of lettuce aphid, *Nasonovia ribisnigri* (Mosley) and leafminer, *Liriomyza langei* Frick, quarantined pests in Japan, on iceberg lettuce and for control of western flower thrips, *Frankliniella occidentalis*, a quarantined pest in Taiwan, China, on lettuce and broccoli. ULO treatments with different durations, temperatures, and oxygen levels were tested to determine responses of the insects and effects on product quality. Selected ULO treatments were tested on lettuce or broccoli to verify their efficacy in insect control and preservation of product quality.

Lettuce aphid was subjected to ULO treatments with 0.015% – 0.025% O_2 at 1, 5, and 10℃ for 1 to 3 days. Complete control of lettuce aphid was achieved in a 3 day treatment at 1℃, 2 day treatment at 5℃, and 1 day treatment at 10℃ (Table 1). In large-scale tests in 562 liter box chambers with commercial iceberg lettuce, a 2 day treatment at 6℃ and a 3 day treatment at 3℃ with 0.015% – 0.025% O_2 achieved complete control of lettuce aphid. No negative effect on lettuce quality was detected after two weeks of post-treatment storage. Therefore, the selected treatments have potential in postharvest control of lettuce aphid on iceberg lettuce.

Western flower thrips was found to be more tolerant to ULO treatment than lettuce aphid. ULO treatments with different durations, temperatures, and much lower oxygen levels were studied to determine effective treatments for the control of the thrips. Thrip mortality increased with reduced oxygen level and increased treatment time and temperature. At 0.003% O_2, over 99.6% mortality rates of thrips was achieved in three ULO treatments of 2, 3, and 4 days at 10, 5, and 1℃ respectively (Table 1). Although there were no injuries to lettuce surface leaves and there was no reduction in visual quality for treated lettuce, about 9 to 33% of lettuce heads sustained injury to heartleaves. The 2 day ULO treatment with 0.003% O_2 produced the lowest injury rate to heartleaves and the injury increased with increased treatment duration. The amount of injured leaves was small (<2 g per head). There were also some variations among the lettuce cultivars in susceptibility to heartleaf injury by ULO treatments.

Pre-treatment storage of lettuce was also studied to determine whether it affected lettuce tolerance to ULO treatment. For control of western flower thrips lettuce was stored under normal atmosphere and under controlled atmosphere (CA) with about 3% O_2 at low temperature for one week and was then compared with fresh lettuce for their response to 2 day ULO treatment with 0.003% O_2 at 10℃.. Lettuce which had been stored for one week under normal or CA condition tolerated the ULO treatment while over 30% of fresh lettuce sustained minor injury to heartleaves. Therefore, pre-treatment storage at low temperature enhanced tolerance of lettuce to the subsequent insecticidal ULO treatment. A sequential combination of CA storage and the ULO treatment was demonstrated to be effective against western flower thrips and lettuce aphid and safe for all seven lettuce cultivars tested. The study indicated that ULO treatment can be made safer for lettuce through pre-treatment storage to increase lettuce tolerance.

Leafminer flies were more tolerant to ULO treatment than western flower thrips and no successful ULO treatment was developed for leafminer flies (Table 1). Previous CA treatments which resulted in over 95% mortality of leafminer flies caused only 14% – 44% mortality of leafminer pupae (Liu 2003). Therefore, ULO does not seem to be suitable for leafminer control.

ULO treatment for thrips control on broccoli. ULO treatments at a low temperature of 1℃ were studied for controlling western flower thrips on ice-packed broccoli. Complete control of thrips was achieved by a 5 day ULO treatment with 0.003% O_2. Oxygen level affected efficacy of ULO treatment. At a higher oxygen level of 0.03%, a 6 day treatment killed approximately 85% of thrips while a 10 day treatment killed all thrips. The 5 day ULO treatment with 0.003% O_2 was successfully tested on iced commercial broccoli of several cultivars without any noticeable negative effects on shelf-life and postharvest quality. The production of

off-odor mainly methanethiol was a major concern for ULO treatment. The 5 day ULO treatment for thrips control did not result in a detectable off-odor. A 10 day ULO treatment with a higher oxygen level did result in the production of off-odor at the end of the treatment. The 5 day ULO treatment provided a safe and effective alternative to methyl bromide fumigation for postharvest control of western flower thrips on broccoli.

ULO treatment for black widow spider control on table grapes. Western black widow spider, *Latrodectus hesperus* Chamberlin & Ivie, was subjected to ULO treatments at different temperatures. Complete control of the spiders was achieved in 1 day ULO treatments with 0.5% O_2 or lower at 1℃ and in 1 day low oxygen (2%) treatment at 15℃. Oxygen level and temperature greatly affected spider mortality. At 1℃, as oxygen level was decreased from 2% to 0.5%, spider mortality increased from 0 to 100%. At 2% O_2, as temperature was increased from 1 to 15℃, spider mortality increased from 0% to 100%. The 1 day ULO treatment with 0.5% O_2 at 1℃ was tested on harvested table grapes of the 'Thompson Seedless' and Flame Seedless' varieties. The treatment had no negative effects on grape quality. Because of the relatively short treatment time, effectiveness at low storage temperature and the easily attained oxygen level, we conclude that the ULO treatment has good potential to be implemented commercially for control of black widow spiders on harvested table grapes.

Low Temperature Phosphine Fumigation

Phosphine fumigation for control of western flower thrips. Fumigation with diluted pure phosphine at a low temperature of 2℃ was studied to control western flower thrips and to determine its effects on the quality of treated lettuce, broccoli, asparagus, and strawberry. Complete control of thrips was achieved in ≥18 hour fumigation treatments with ≥ 250 ppm phosphine (Table 2). One day fumigation treatment with 1 000 ppm phosphine was tested on lettuce and broccoli. One day fumigation treatments with 500 ppm and 1 000 ppm phosphine were tested on asparagus and strawberry. Visual quality of lettuce, broccoli, and asparagus was evaluated after 2 weeks of post-treatment storage. Strawberry quality was evaluated immediately after fumigation and after 1 week of post – treatment storage. For all the above mentioned products, there were no significant differences in postharvest quality between the treatments and the controls and there were no injuries caused by the fumigation treatments. Therefore, phosphine fumigation at low temperature is promising for postharvest control of western flower thrips on lettuce, broccoli, asparagus, and strawberry.

A commercial scale fumigation trial was conducted in a 40 ft (12.19 m) reefer container with half-full load of iceberg and romaine lettuce and small loads of broccoli, asparagus, and strawberries. Gaseous phosphine was injected into the container through the fresh air vent using the Horn Dyluphos System (Horn et al. 2005). The treatment lasted 18 hours with an average phosphine concentration of about 600 ppm at 2℃. The treatment was terminated by 4 repeated 30 min aerations separated by a 30 min interval with the door closed to maintain a stable temperature in the container. Phosphine level in the head space at the end of the aeration was < 0.3 ppm. The treated products were sealed in the container for additional 3 days to simulate shipping and phosphine level in the container was monitored. One day after treatment termination, phosphine level was below the detection limit of 0.01 ppm of ultralow phosphine detector tubes. In the trial complete control of thrips was achieved. The quality of lettuce, broccoli, and asparagus was evaluated after two weeks of post-treatment storage. Strawberry quality was evaluated immediately after fumigation and after one week of post-treatment storage. There were no negative effects on the quality of any of the treated products. The results from the trials indicate that it is feasible to conduct commercial fumigation with pure phosphine at a low temperature in reefer containers for pest quarantine control.

Phosphine fumigation for control of lettuce aphid and leafminer on iceberg lettuce. Lettuce aphid and leafminer flies and pupae were subjected to low temperature phosphine fumigation treatments. Leafminer flies were much more susceptible to phosphine fumigation than leafminer pupae. At 500 ppm of phosphine, complete control of leafminer flies was achieved in the 24 h treatment and complete control of leafminer pupae was achieved in the 72 h treatment. At 1 000 ppm of phosphine, all treatments ranging from 12 h to 72 h achieved complete control of leafminer flies and 48 h or longer treatments resulted in complete control of leafminer pupae. At 2 000 ppm of phosphine, all treatments ran-

ging from 24 h to 72 h resulted in complete control of leafminer pupae and the 24 h treatment also resulted in complete control of leafminer flies (Table 2).

Lettuce aphid was more tolerant to phosphine fumigation than the leafminer. The 48 h treatment with 2500 ppm phosphine and 72 h treatment with 2000 ppm phosphine did not completely control the lettuce aphid. The 72 h treatment with 2500 ppm phosphine and the 48 h treatment with 3400 ppm phosphine, did achieve complete control of lettuce aphid (Table 2). Both treatments were safe for iceberg lettuce. Yet, given the very high phosphine concentrations and long treatment durations used, there could be problems in implementing such treatment practically due to the cost and the feasibility of maintaining such a high phosphine level. More research is needed to improve the phosphine fumigation treatment for control of lettuce aphid.

Discussion

The large variations in susceptibility to ULO treatments among pest species tested indicate limitations as well as opportunities of ULO treatment for postharvest pest control. Aphids and thrips can be controlled effectively and safely by ULO treatment on lettuce and broccoli respectively. However, pre-treatment storage is needed to prevent injury to iceberg lettuce for control of western flower thrips using ULO treatment. Low temperature is not effective (Table 1). with ULO treatment for the leafminer, The most remarkable finding is that black widow spiders can be easily controlled with a short ULO treatment. The treatment has good potential of being adopted commercially because the oxygen level used in the treatment is not very far from the low oxygen used in CA storage of table grapes. Like most other postharvest treatments, ULO is not suitable for all pests. However, effective treatments can be developed for pests within certain pest groups that have been demonstrated to be susceptible to ULO treatment. Many other pest groups have yet to be explored for susceptibility to ULO treatment. The present examples of success seem to suggest that ULO treatment is a promising alternative for postharvest pest control. More research efforts are warranted to explore ULO treatment for a wide variety of pests on various fresh commodities and to develop successful ULO treatments for commercial applications.

The enhanced tolerance of lettuce through pre-treatment storage suggests that lettuce susceptibility to ULO treatment can be modified significantly. This is possibly due to acclimation to postharvest storage. Other fresh commodities may exhibit similar characteristics of increased tolerance to extreme CA conditions including ULO after a certain length of acclimation to postharvest storage. Therefore, future research should consider modifying postharvest physiology of the fresh products in developing CA-based postharvest pest control technology rather than focusing solely on efficacy in controlling target pests.

The results of low temperature phosphine fumigation research are very encouraging. The successful control of western flower thrips, leafminer flies and pupae, and lettuce aphid with low temperature phosphine fumigation indicates that phosphine is a promising as an alternative to methyl bromide for postharvest pest control on perishables. In comparison with methyl bromide fumigation, pure phosphine fumigation under low temperature has the advantage of keeping products at storage temperature, thereby avoiding negative effects on product quality associated with warming up of the products as in methyl bromide fumigation. However, for pests like lettuce aphid, long treatment time and high phosphine level is nec essary to achieve successful control. This makes low temperature phosphine fumigation less practical. In comparison, lettuce aphid can be controlled more easily with ULO treatment.

Longer treatment at lower phosphine concentrations was more effective in controlling insects than low temperature phosphine fumigation as demonstrated with western flower thrips. Similarly, low dose phosphine fumigation with longer treatment time was suggested for controlling aphids on cut flower because of its higher efficacy as compared with shorter fumigation with higher phosphine concentrations (Karunatne et al. 1997).

Phosphine is very toxic to humans and health problems related to exposure to low levels of phosphine were also reported (Bond 1984, Chaudhry 1997, Brautbar and Howard 2002). However, studies on phosphine residue in perishable products are very limited. Phosphine fumigated fruits were reported to have mild off-taste and the off-taste disappeared after 5 – 6 days of storage (Horn and Horn 2004). For phosphine fumigated table grapes, no phosphine could be detected after they were processed to release phosphine residue 9 days after

fumigation by chromatography with detection limit of 0.003 ppm (Klementz et al. 2005). In the U. S., the maximum residue limit (MRL) for phosphine is 0.1 ppm for stored products and 0.01 ppm for fresh and processed food stuff. Phosphine has low solubility in water (Bond 1984). Even though phosphine has low solubility in water, phosphine residue may still be a health concern (Chaudhry 1997). For low temperature phosphine fumigation of fresh commodities, because of longer treatment time and high water content of the products, there is a need for more research on phosphine residue in fresh products. Research efforts are needed to quantify the amount of phosphine absorbed by the products and how fast and how much of the absorbed phosphine will dissipate to ensure that phosphine fumigated fresh commodities do not impose a risk to human health.

In summary, both ULO treatment and low temperature phosphine fumigation showed promise in postharvest pest control on fresh commodities. For ULO treatment, its effectiveness against most pest species and effects on the quality of most perishable commodities have not been explored. Recent studies also showed limitations of ULO treatment in term of ineffectiveness against some groups of pests and its negative impact on product quality. There are also needs for efforts from industry to develop the ULO treatment for commercial use. However, given that the ULO treatment is compatible with organic products and there is a heightened awareness of health risks related to chemical pesticides, ULO treatment is an important technique to be studied and developed for postharvest pest control on perishable commodities. For low temperature phosphine fumigation, successful use of the technology in Chile and our studies all indicate that it is very promising in postharvest pest control on fresh commodities. However, more research is needed to determine phosphine residue and its fate in treated products to ensure their safety to human health Currently, there are also a lack of low temperature fumigation chambers or suitable commercial coolers for low temperature fumigation treatments. Fumigation in refrigerated shipping containers seems to offer a viable alternative for commercial use of low temperature phosphine fumigation.

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Table 1. Comparison of insect mortality in response to ultralow oxygen treatments

Temp(℃)	Time(day)	O_2(%)	Mortality (%)		
			Lettuce aphid	Western flower thrips	Leafminer flies
1	2	0.015	95		
	3	0.015	100		
		0.003		85	
	5	0.05	99	51	43
		0.003		100	
5	1	0.015	90		
	2	0.015	100		
	3	0.003		100	90
10	1	0.015	100	65	
	2	0.003		100	86

Table 2. Comparison of insect mortality in response to fumigation treatment with pure phosphine at 2℃

Time(h)	PH_3(ppm)	Mortality (%)			
		Western flower thrips	Leafminer flies	Leafminer pupae	Lettuce aphid
12	500	89.2	90.4		
	1000	98.5	100.0		
18	250	100.0			
	500	100.0			
24	500	100.0	100.0	91.3	
	1000	100.0	100.0	98.2	
	2000		100.0	100.0	
48	500			99.7	86.1
	1000			100.0	89.3
	2000				95.8
	3400				100.0
72	500				98.1
	1000				99.2
	2000				99.5
	2500				100.0

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0102

The Mortality Response of *Sitophilus oryzae* (L.) Eggs to Diurnal Interrupted Doses of Phosphine (PH_3)

Stephen Beckett

Abstract: Fumigant distribution in grain is inherently variable, with movement occurring by gas expansion and contraction, convection and diffusion. Diurnal changes in temperature and wind velocity, and weather changes more broadly, drive movement which is further influenced by the type of storage facility and the degree of gas tightness. Particular near boundaries, , this can prevent occurrence of lethal concentrations. Such conditions may have implications for resistance selection in stored grain insects. However, there is limited information on insect mortality responses to variable concentrations similar to those often confronting industry. This paper reports on initial results from a study that investigates the impact of repeated sub-lethal doses of phosphine on the mortality of eggs from three strains of *Sitophilus oryzae* that are susceptible, slightly resistant and moderately resistant to the fumigant. The treatments used were designed to represent fumigant environments during simple diurnal fluctuations. Results are compared to those obtained from equivalent continuous treatments and are discussed in reference to phosphine toxicology with regard to the practical implications for successful fumigation.

Key words: phosphine, diurnal interrupted doses, *Sitophilus oryzae*, eggs, mortality

Introduction

Considerable variations in phosphine dosage occur during industrial fumigations carried out in a large range of storage structures. Reasons include gas dispersal, the level of gas-tightness, grain sorption and weather conditions. The effects of gas expansion and contraction, convection and diffusion can lead to uneven concentrations which are particularly noticeable at the storage boundaries when sealing is poor. This can prevent lethal concentrations being maintained and may have implications for resistance selection in stored grain insects.

Diurnal patterns of gas expansion and contraction occur due to the daily temperature cycle. The amount of fumigant at certain locations can follow these oscillating conditions causing repeated treatment interruptions, particularly near the periphery. This phenomenon has been reported by several authors[3;13]. Certain weather conditions[2] that also have a diurnal pattern[11] can further affect distribution and leakage. Uniform cyclical[2] environmental events such as lower temperatures and wind velocities that have similar impacts on fumigant movement, may coincide,. This can continually cause, a period of several hours each morning of gas expansion, that results in gas loss through the leaks.

Most data on insect mortality due to phosphine have been determined using continuous treatments at constant concentrations. However, there have been some investigations of mortality under variable concentrations reflecting those occurring during industrial fumigations. These studies were reviewed by Daglish et al[6]. Attempts to determine mortality in terms of the Concentration × time (Ct) product have not been very fruitful. However, Daglish et al.[6] were able to relate the mortality of *Rhyzopertha dominica* to $C^n t = k$. Nevertheless, predicting insect mortality under variable or interrupted fumigant concentrations, remains inadequate.

Specific information is sparse on the mortality response of stored grain insects to repeated sub-lethal doses or interrupted treatments of phosphine. Bond and Upitis[4] found that two sub-lethal 5h doses of phosphine within 24 h caused greater mortality of *Tribolium confusum* and *Sitophilus granarius* adults than one 10h treatment at the same concentration, and Hobbs and Bond[9] reported a similar response in *T. castaneum*.

The present paper reports initial results of an investigation exploring the response of insects to the effects of diurnally interrupted doses of phosphine. Data are presented on the mortality response of eggs of *S. oryzae* whichrepresent a development stage tolerant to phosphine[12] and allow for a specific definition of the mortality response. The treatments were de-

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signed to reflect simple fumigant environments that might occur under conditions described above. The results give insight into overall treatment times required under such circumstances and demonstrate the particular toxicological effects of phosphine on insect physiology.

Materials and Methods

Bioassays

Three strains of *S. oryzae* were compared. These included a long standing phosphine susceptible strain (LS2), a weakly resistant strain (QQSO1111) and a moderately resistant strain (QSO335). Levels of resistance were determined by the FAO method for resistance measurement[7] with moderate resistance considered commercially significant[7]. Between 24 and 48 h old eggs were obtained for bioassay by allowing adult insects to lay for 24h on soft wheat at 30℃/12% grain moisture content (mc) 48h before treatment. The adults were then discarded and replicates were setup for treatment by placing 25g of infested grain in 50 ml jars.

Fumigation

Fumigations were conducted in closed desiccators (2.5 6.4L) at 30℃/60% relative humidity (r. h.). Humidity was generated with 100mL of aqueous glycerol solution (specific gravity 1.185 – 1.190) held in a dish at the bottom of each desiccator. Phosphine was produced by adding aluminium phosphide tablets to 10% (v/v) aqueous sulfuric acid[1]. The concentration of this source gas was established using a Gow Mac (Model 11-625) gas density balance in a Tracor (MT-150) gas chromatograph fitted with a HayeSep Q 80/100 mesh column. The volume of gas required for fumigation was determined with respect to desiccator volume, gas purity and atmospheric pressure. The appropriate dose was then injected with a gastight syringe into each desiccator through a septum in the lid. The concentration of each dose was then confirmed from peak areas calibrated with 4 standards and integrated automatically using Varian Star software. The standards were established using a Tracor (MT-150) gas chromatograph fitted with a flame photometric detector and a GSQ column (30 m, 0.53 mm i.d.) run at 110℃, or on a Varian CP3800 pulse flame photometric detector AT/Q column (30 m, 0.53 mm i.d) run at 200℃.

Treatments

Diurnally interrupted treatments covered a range of phosphine doses from 36h at 0.67mg/L to 6h at 4mg/L are presented in Table 1. In this manner, all treatments had the same concentration X time (Ct) product. Each treatment was run for three days. Each treatment was divided into into three equal parts with each part administered at the same time each day (e.g. $12 \times 3 = 36$h) (Fig. 1). Fifteen replicates were setup at the start of each diurnal treatment. At the end of each day's treatment, 5 replicates were removed to be assessed for mortality. Thus, one set of replicates was treated on day 1, one set was treated on day 1 and 2, and one set was treated on day 1, 2 and 3. Reduction in emergence was measured against controls as a surrogate for mortality. After each day as dose accumulated, lethal time (LT) predictions could be made as to the ultimate number of diurnal doses needed for a given % mortality.

An equivalent continuous treatment was conducted for each interrupted treatment in order to determine the degree to which interruption affected efficacy (Fig. 1). Continuous treatments were comparable not only in Ct product but also in terms of time at the points of assessment during the treatment (Table 1). For example, if the series of doses under an interrupted treatment regime were 1mg/L for 8h, or 8h on two successive days or 8h on 3 successive days; under the continuous treatment, doses were 1mg/L for 8h, or 16h, or 24h. The same number of replicates was used for both interrupted and continuous treatments.

Table 1. Phosphate doses for diurnal interrupted 3 days treatments duration and equivalent continuous treatments

mg/L	ppm	Diurnal interrupted treatments		Continuous treatments	Ct(mg · h/L)
		Days/treat.	Duration time/day (h)	Duration times/treatment (h)	
0.67	480	3	12	12,24,36	24
1.00	720	3	8	8,16,24	24
1.33	960	3	6	6,12,18	24
2.00	1440	3	4	4,8,12	24
4.00	2880	3	2	2,4,6	24

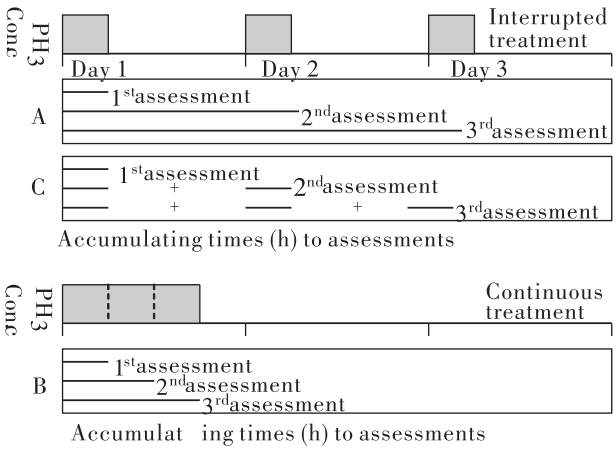

Fig. 1 Schematic description treatment conditions and assessment times

For the first 5 days of development at 30℃/60% r. h., *S. oryzae*appears to have roughly the same level of phosphine tolerance (Beckett and Seneviratna, unpublished data). Thus, the same degree of tolerance was assumed for eggs exposed to both interrupted and continuous treatments. After fumigation, the desiccators were aired for at least 6 h under a fumehood. The insects were then either held at 30℃ 60% r. h. before receiving another treatment the following day or incubated at 26℃/60% r. h. for about 5 weeks to be assessed for emerging adults relative to controls. Mortality data were analyzed using probit transformations[8] with an Excel program (Annis, unpublished)

Results

Table 1 shows predicted $LT_{99.9}$ values for eggs of the three strains of *S. oryzae* exposed to the range of phosphine concentrations, which were administered either as diurnal interrupted treatments (Fig. 1 A) or as continuous treatments (Fig. 1 B) and figure 2a, b and c. Figure 1℃. shows results for diurnal interrupted treatments with the time where no fumigation occurred.

$LT_{99.9}$ values for each strain subjected to diurnal interrupted treatments tend to be greater at higher concentrations for shorter treatment times. $LT_{99.9}$ values for the moderately resistant strain (QSO335) were much greater than those for the slightly resistant strain (QQSO1111) and the susceptible strain (LS2) (147.2h cf. 84.4h cf. 76.5h at 1mg/L respectively and 212.5h cf. 101.1h cf. 84.7h at 4mg/L respectively). $LT_{99.9}$ values of each strain subjected to continuous treatments were lesser at higher concentrations for shorter treatment times. $LT_{99.9}$ values for all treatments were greatest for the moderately resistant strain and least for the susceptible strain (69.7h cf. 42.8h at 1mg/L and 19.4h cf. 13.2h at 4mg/L). $LT_{99.9}$ values for each strain subjected to diurnal interrupted treatments where the times without fumigation were excluded were also lesser at higher concentrations for shorter treatment times. Again, $LT_{99.9}$ values for all treatments were greatest for the moderately resistant strain and least for the susceptible strain (54.2h cf. 32.1h at 1mg/L and 20.2h cf. 9.3h at 4mg/L). $LT_{99.9}$ values determined for diurnal interrupted treatments in this way were usually less than those for the equivalent continuous treatment.

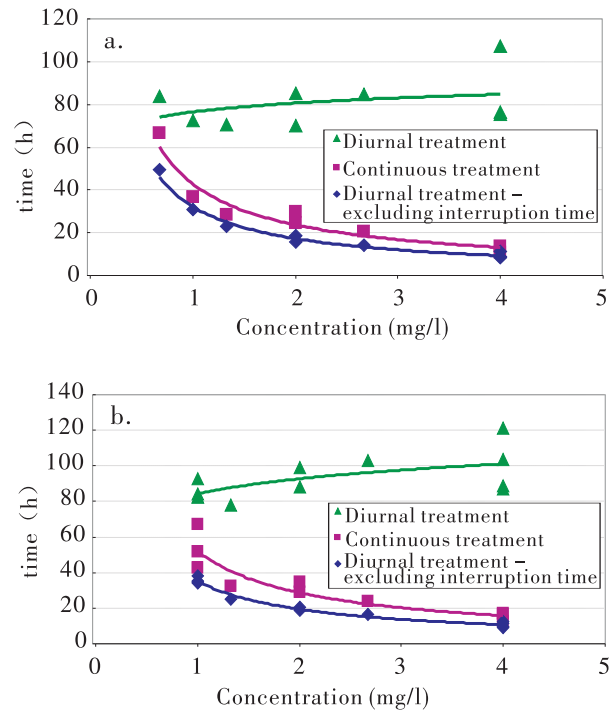

Fig. 2 $LT_{99.9}$ values for eggs of a) susceptible strain, b) mildly resistant strain and c) moderately resistant strain of *S. oryzae* exposed to a range of phosphine concentrations administered as either diurnal interrupted treatments or as continuous treatments. Results for diurnal interrupted treatments are also shown, with the periods of interruption excluded from analysis.

Discussion

Results presented in this paper are preliminary. However, they show that a phosphine treatment administered as a simple interrupted dose over several days greatly extends the time required for 99.9% mortality of *S. oryzae* eggs relative to an equivalent continuous dose (Fig. 2a, b and c). Over the range of concentration X time combinations that were used, all giving a

CT product of 24mg h/L, the predicted time required to obtain $LT_{99.9}$ appeared to increase for all three insect strains at treatments that have higher phosphine concentrations and shorter treatment times. The relative difference in predicted treatment time between diurnal interrupted treatments and continuous treatments for a given concentration is therefore greater at higher concentrations, since under continuous treatment, the time needed to obtain $LT_{99.9}$ is less at higher concentrations. The greatest relative difference in $LT_{99.9}$ values between the two treatments regimes was exhibited by QSO335 (147.2h cf. 69.7h at 1mg/L and 212.5h cf. 19.4h at 4mg/L) followed by QQSO1111 (84.4h cf. 52.0h at 1mg/L and 101.1h cf. 15.8h at 4mg/L) and LS2 (76.5h cf. 42.8h at 1mg/L and 84.7h cf. 13.2h at 4mg/L).

If $LT_{99.9}$ values were determined for diurnal interrupted treatments where only the time when eggs were under fumigation was used for the y axis, the correlation of $LT_{99.9}$ to concentration and time is similar to that occurring during continuous treatments with treatment time decreasing at increased concentrations and $C^n t = k$ where $n < 1$. However, the values of $LT_{99.9}$ determined for interrupted treatments are generally lower for the three strains relative to those for the equivalent continuous treatment. This not only indicates that the toxic affects of phosphine do not decline in the period between fumigations in the diurnal cycle (which in this study can be up to 22h/d) but also implies that efficacy may be increased by the event. A possible explanation is that breaks in dosage may bring more fumigant to the cell over time since the rate of absorption decreases over time as saturation approaches[5]. Phosphine toxicity involves the build up of hydrogen peroxide in the mitochondria due to the inhibition of enzymes such as catalase[9] and there is an evidence that respiration is greater after fumigation than during the fumigation[10]. Therefore, diurnal interrupted treatments increase mitochondrial hydrogen peroxide due to the presence of greater amounts of oxygen and hydrogen phosphide.

While interrupting fumigation may have some toxicological benefits at the doses tested in this study, in reality, the extended time required for sufficient insect mortality under diurnally fluctuating conditions means serious control problems may arise if major variations in dosage are not sufficiently minimized. However, at the lower phosphine concentrations, diurnally fluctuating doses do not extend the necessary treatment time by much. This is evident by the apparent convergence of the two $LT_{99.9}$ data sets as concentration decreases. Further work is required at lower phosphine concentrations where treatment times are extensive and efficacy relies on tolerant insect development stages progressing to susceptible stages. It is important to clarify the impact of diurnally fluctuating doses on the effectiveness of phosphine fumigation because any reduction in efficacy will have implications for the development of insect resistance.

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0103

Respiration of *Tribolium castaneum* (Herbst) at Different Oxygen Concentrations

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Abstract: The main content of this research is the respiration rate of the egg, larvae of 7 – 10d instars (young larvae), larvae of 18 – 22d instars (old larvae), pupae and adults of Tribolium castaneum (Herbst) exposed to atmospheres containing 21%, 15%, 10%, 5%, 3%, 2% and 1% oxygen in nitrogen at 30℃. The respiration rate (RR) of them was determined with Warburg respiration instrument. The results indicated that respiration rate of the five stages was different at different oxygen atmospheres. At 21% and 15% oxygen atmospheres, RR of egg was the least, followed by young larvae, adult, pupae and old larvae. At 10% and 5% oxygen atmospheres, RR of young larvae was the least followed by egg, adults, pupae and old larvae. At 3%, 2% and 1% oxygen atmospheres, RR of young larvae was similar to egg, followed by adults, pupae and old larvae. Obviously, the downtrend of RR of the five stages was different, RR of old larvae was the highest, RR of egg and young larvae was the least, and the value of them was at the same level. With the reduction of, the model of RR at the different stages there was a decrease of the logarithm, which is $y(\text{egg}) = 0.44\ln(x) + 0.12$, $y(\text{young larvae}) = 0.52\ln(x) + 0.01$, $y(\text{old larvae}) = 0.90\ln(x) + 1.22$, $y(\text{pupae}) = 0.63\ln(x) + 0.57$ and $y(\text{adult}) = 0.36\ln(x) + 1.08$. The decreasing rate of the RR of the five stages was different, the reduction in the rate of old larvae was the greatest, followed by pupae, young larvae and egg and the last was the adult. At reduced oxygen levels, the decrease of respiration rates of all stages was proportional to the oxygen levels. Under 10% oxygen atmospheres, RR of two stages of larvae were significantly different ($a = 0.01$). Under 5% oxygen atmospheres, RR of egg and adult were significantly different ($a = 0.01$). And under 3% oxygen atmospheres, RR of pupae was markedly restrained and differed significantly ($a = 0.01$). Hence, the reduction of RR and the reduction in degree of RR were related to the death of the insects in low oxygen atmosphere.

Key words: respiration rate, *Tribolium castaneum*, low oxygen, stages

Preface

At present, although chemical fumigants are still extensively used as major pesticides for stored products in various fields, the substantial use of chemical fumigants can result in certain problems such as residues on food, environmental pollution and etc., Countries have already begun to limit the use of chemical pesticides and and qre developing registration of new chemical pesticides gradually. In consideration that the status of methyl bromide will be eliminated gradually in the whole world, the high PH_3 resistance of pests and some new-type fumigants can be used for only on special products and situations, and the developments of the new-type fumigants which are green, environmental-friendly and effective are necessary. Low oxygen used as a gas pest killing technology has common advantages over fumigants. It is a grain storage technology which can kill the pests or inhibit the growths and developments of pests through reducing the oxygen content in the environment by natural or artificial methods. One can also can raise the temperature and increase the CO_2 content in the environment to achieve a more effective and more rapid controlling effect. It is obvious that low oxygen grain storage technology has advantages such as safety, green and has no environmental pollution. It conforms to the green grain storage idea which is promoted strongly in China.

At present, CO_2 rich and low oxygen grain storage technology is popularized in China. We need to understand the respiration characteristics of stored grain pests under the low oxygen condition to explain the mechanism of pest control at low oxygen for the improvement of modified atmospheres technology. We need to provide the theoretical basis for application of the low oxygen pest controlling technology. The purposes of this experiment were 1) to research the

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respiration changes of *Tribolium castaneum* (Herbst) under the low oxygen condition through determination of its respiration rates at various stages under different oxygen concentrations, 2) provide a basis for research on mechanism of pest killing by low oxygen and 3) recommendation of the effective concentration for controlling of stored grain pests by low oxygen. At the same time, the research on respiration rates of insects under low oxygen condition has significant meaning for grain temperature rises caused by heat production of the insect's metabolism and physiological reactions of insects to different environments[1].

Materials and Method

Materials

Instruments and equipments

SKW-3 type micro-respiration apparatus, Shanghai Gongda Industry and Trade Corporation;

Electric thermostatic incubator, Shanghai Precision Instruments Co., Ltd.

Orsat gas analyzer, Shangdong Leling Xinghua Glass Instruments Factory

Reagents

Industrial oxygen, purity ≥ 99.7%; high purity nitrogen, purity ≥ 99.9996%. All produced by Beijing Bei Temperature Gas Factory.

Test insect

The test insect was *T. castaneum* which had been cultured in the Academy of State Administration of Grain for dozens of generations, and was taken from the Yiyang depot, State Grain Reserves.

Test Method

Cultivation of the test insects

Clean the wheat and place it into an oven of 80℃. adjust the water content, then store in refrigerator for use. Prepare the feed with whole wheat flour and yeast at the ratio of 95:5 for culture of *T. castaneum*. Add *T. castaneum* into the prepared feed and culture it under the condition of temperature being 30 ± 1℃ and relative humidity (RH) being 75% ± 5%. Record the kind, species and culture date of the insect. After 3 days, screen the adults out and put them into another prepared culture bottle to culture them, and put the screen underflow which contain the eggs back into the original bottle and culture them, and perform tests for the test insects at various stages and ages [2].

Selection of the test insects

Confirmed days of the test insects by stages and ages and the necessary quantities of the test insects for each treated group are shown in table 1.

Table 1. Stages and quantities of the test insects

Stages	Quantity(/group)	Age(d)
egg	400	0 – 1
Young larva	50	7 – 10
Old larva	10	18 – 22
Pupa	10	1 – 2
Adult	10	10 – 14

Note: the age of larva is calculated from egg, the age of pupa is calculated from pupation and the age of adult is calculated from eclosion.

Test method

Use SKW-3 type micro-respiration apparatus, according the standard pressure reduction procedure[3], at 30℃, perform test of respiration rates for the test insects under the conditions of which oxygen concentrations are 21%, 15%, 10%, 5%, 3%, 2% and 1% separately.

Test method of respiration rate under the normal oxygen concentration

Perform test of oxygen consumption (per hour) for test insects under the condition of temperature being 30℃ and oxygen concentration being 21%. Perform parallel test for 4 groups.

Test method of respiration rate under low oxygen condition

According to the characteristic of piezometric pipe of SKW-3 type micro-respiration apparatus, set the upper mouth of the piezometric pipe as the air inlet, and the mouth at the side of the reaction bottle as the air outlet. Charge the mixed gas of nitrogen and oxygen at a certain ratio from the upper mouth of the piezometric pipe into the reaction bottle and open the side mouth of the reaction bottle at the same time to discharge the original gas in the reaction bottle, and thus create the needed low oxygen environment through this gas discharging method. Perform the test of gas concentration with an Orsat gas analyzer. When the gas concentration in the reaction bottle reaches the requirement of the test, stop venting and test the airtightness after sealing. Determine if the airtightness is good or bad through observation and see if there is difference in height between two liquid levels in the U-tube of the piezometric pipe.

At 30℃, perform the test of oxygen consumption (per hour) for the test insects under the conditions of oxygen concentrations being 15%, 10%, 5%, 3%, 2% and 1%. Separately,

perform parallel test for 4 groups.

Weighing

Take out the test insects after above test finished and dry them at 80℃ to constant weight, and record the data.

Data processing method

According to calculation formula of respiration rate: respiration rate = oxygen consumption/(weight of test insect × time), perform calculation on collected original data to obtain the oxygen consumption each hour and per mg of test insect, i. e. respiration rate.

Analyze the change trend of oxygen consumption in unit time for the test insect under different oxygen concentrations with EXCEL software; perform analysis of variance on oxygen consumption in unit time for *T. castaneum* at various stages under seven different oxygen concentrations described above with SAS (Statistics Analysis System) data processing software and compare significance of effects of different low oxygen concentrations on respiration rate.

Results and Analysis

Respiration Rates of the Egg of *T. castaneum* under Different Oxygen Concentrations

From Fig 1, we can see that under the conditions of oxygen concentrations being 21%, 15%, 10%, 5%, 3%, 2% and 1%, the corresponding respiration rates of the egg of *T. castaneum* are 1.50 ± 0.15, 1.43 ± 0.13, 1.11 ± 0.19, 0.78 ± 0.06, 0.36 ± 0.04, 0.38 ± 0.04 and 0.32 ± 0.04 (μl/mg · h). Analytically, there is a logarithmic relationship between respiration rate of the egg of *T. castaneum* and oxygen concentration, and the mathematical model is $y = 0.44\ln(x) + 0.12$ ($R^2 = 0.92$). From the analysis of variance of the data, we see that during the process of oxygen concentration reduction from 21% to 1%, the respiration rate of egg reduces gradually. When the oxygen concentration is reduced to 5%, the respiration rate of the egg is strongly reduced. ($a = 0.01$). This indicates that when the oxygen concentration is below 5%, the respiration rate of egg is inhibited strongly by the low oxygen environment.

Respiration Rates of the Young Larva of *T. castaneum* under Different Oxygen Concentrations

From Fig 2, we can see that under seven oxygen concentrations from 21% to 1%, the corresponding respiration rates of the young larva of *T castaneum* are 1.75 ± 0.07, 1.64 ± 0.13, 0.96 ± 0.06, 0.55 ± 0.01, 0.45 ± 0.01, 0.38 ± 0.05 and 0.25 ± 0.03 (μL/mg · h.)

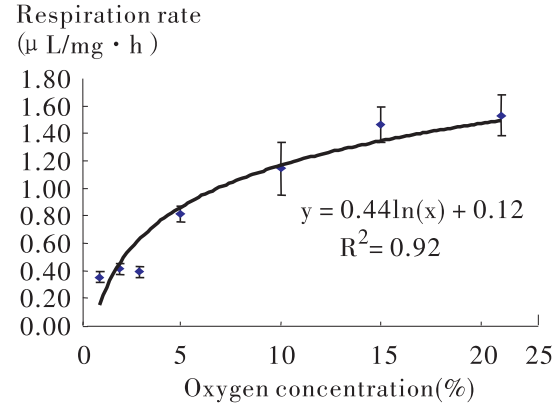

Fig. 1 Respiration rate of the egg of *T. casta* neumunder different oxygen concentrations at 30℃

Analytically, there is a logarithmic relationship between respiration rate of the young larva of *T. casta*neum and oxygen concentration, and the mathematical model is $y = 0.52\ln(x) + 0.01$ ($R^2 = 0.87$). The model type is the same as that of the egg, except that of the coefficient. From analysis of variance for the data, we can see that during the process of oxygen concentration reduction from 21% to 1%, the respiration rate of the young larva of *T. casta*neum reduces gradually. When the oxygen concentration is reduced to 10%, the respiration rate of the young larva reduces greatly ($a = 0.01$). This indicates that when the oxygen concentration is below 10%, the respiration rate of the young larva is inhibited strongly by the low oxygen environment

Comparison of Respiration Rates of the old Larva of *T. castaneum* under Different Oxygen Concentrations

From Fig 3, we can see that at 30℃ and the same oxygen concentration series, the corresponding respiration rates of the old larva of *T. castaneum* are 3.99 ± 0.19, 3.60 ± 0.06, 3.22 ± 0.06, 2.74 ± 0.06, 2.11 ± 0.13, 2.02 ± 0.05 and 1.13 ± 0.03 μL/mg · h. Analytically, there is a logarithmic relationship between respiration rate of the old larva of *T. castaneum* and oxygen concentration, and the mathematical model is $y = 0.90\ln(x) + 1.22$ ($R^2 = 0.99$). The model type is the same as that of the egg and young larva, except for the coefficient. From the analysis of variance data, we see that with the reduction of oxygen concentration, the respiration rate of the old larva is reduced gradually; when the oxygen concentration reduced to 10%, the respiration rate of the old larva is greatly reduced ($a = 0.01$) This indicates that when the oxygen concentration is below 10%, the respiration rate of the old larva is strongly

inhibited by the low oxygen environment.

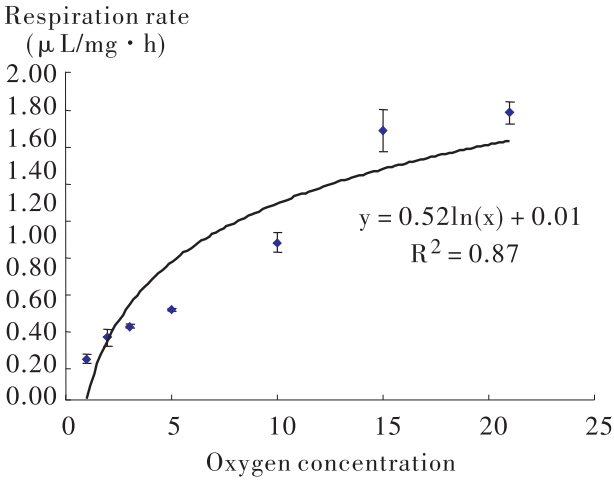

Fig. 2 Respiration rate of the larva (7 – 10 days) of *T. castaneum under* different oxygen concentrations at 30℃

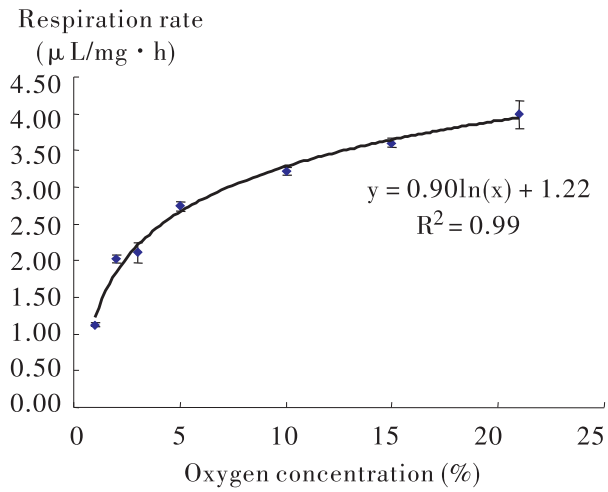

Fig. 3 Respiration rate of the larva (18 – 22 days) of *T. castaneum* (Herbst) under different oxygen concentration

Comparison of Respiration Rates of the Pupa of *T. castaneum* under Different Oxygen Concentrations

From Fig 4, we can see that under the same test condition described above, the corresponding respiration rates of the pupa of *T. castaneum* are 2. 34 0. 04, 2. 17 ± 0. 15, 2. 08 ± 0. 17, 1. 98 ± 0. 01, 1. 53 ± 0. 03, 0. 69 ± 0. 02 and 0. 47 ± 0. 01 μL/mg · h. Analytically, at 30℃, there is a logarithmic relationship between respiration rate of the pupa of *T. castaneum* and oxygen concentration, and the mathematical model is $y = 0.63\ln(x) + 0.57$ ($R^2 = 0.89$). The model type is the same as those of those three test insects described above. From analysis of variance of the data, we see that with the reduction of oxygen concentration, the respiration rate of the pupa is gradually reduced; but the difference with the above three test insects is that when the oxygen concentration is reduced to 3%, the respiration rate of the pupa is greatly reduced ($a = 0.01$). This indicates that the respiration rate of the pupa is strongly inhibited by the low oxygen environment.

Comparison of Respiration Rates of the Adult of *T. castaneum* under Different Oxygen Concentrations

From Fig 5, we can see that under the same test condition described above, the respiration rates of the adult of *T. castaneum* are correspondingly 2. 05 ± 0. 03, 1. 97 ± 0. 04, 1. 95 ± 0. 08, 1. 80 ± 0. 04, 1. 61 ± 0. 03, 1. 49 ± 0. 05 and 0. 79 ± 0. 02 μL/mg · h. Analytically, at 30℃, there is a logarithmic relationship between respiration rate of the adult of *T. castaneum* and oxygen concentration, and the mathematical model is $y = 0.36\ln(x) + 1.08$ ($R^2 = 0.85$). From analysis of variance of the data, we see that during the process of oxygen concentration reduction from 21% to 1%, the respiration rate of the adult reduces gradually. When the oxygen concentration reduces to 5%, the respiration rate of the adult reduces greatly ($a = 0.01$). This indicates the respiration rate of the adult is inhibited strongly by the low oxygen environment and this result is the same as that of the egg.

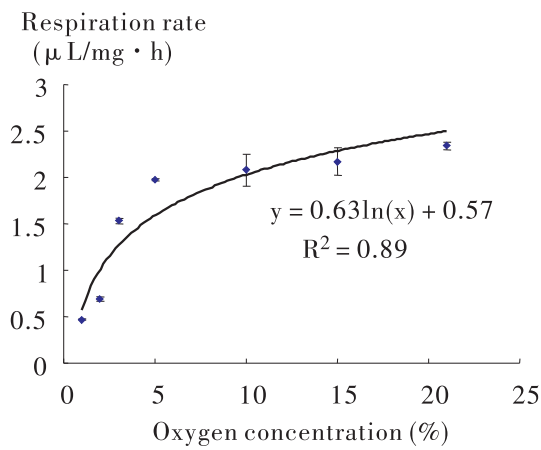

Fig. 4 Respiration rate of the pupa of *T. casta – neum* under different oxygen concentrations at 30℃

Discussion

Shijun performed research on respiration rates of the pupa of *Cacoecimorpha pronubana* under oxygen concentrations of 21%, 10%, 8%, 6%, 4%, 2% and1%[4]. Emekci reported on respiration rates of eggs, young larvae, old larvae, pupae and adults of *T. castaneum* and *Rhizopertha dominica* (Fabricius) under oxygen concentrations of 21%, 15%, 10%, 5%, 3%,

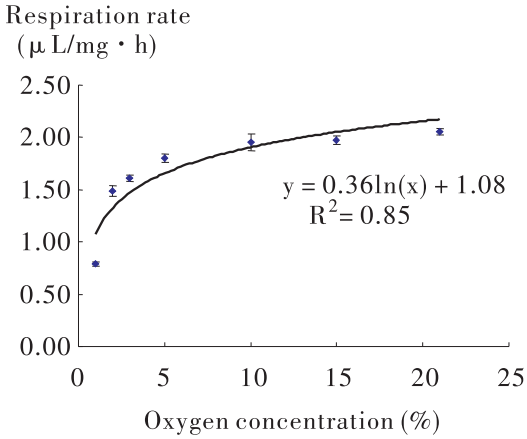

Fig. 5 Respiration rate of the adult of *T. castaneum* under different oxygen concentrations at 30℃

2% and 1%[5,6]. They found that with reduction of the oxygen concentration, the corresponding respiration rates of the test insects were gradually reduced, which is in agreement with the findings of the present article.

Navarro found that when the oxygen concentration was reduced to 3%, the respiration rate of *Ephestia cautella* (Walker) was strongly inhibited [6]. White (1981) and Campbell (1990) reported on respiration rates of *Oryzaephilus surinamensis* Linne and *Cryptolestes ferrugineus* (Stephens) and obtained the same results[7,8]. In the present research it was also found that, when the oxygen concentration was reduced to 3%, the respiration rates of *T. castaneum* at various stages were all strongly inhibited.

For most insects, gas exchange is performed through a tracheal system. According to the difference in the pressure gradient of oxygen, oxygen will enter the tissue of insect from a step-wised branched trachea, through the spiracles. When oxygen exists, energy producing substances will be oxidized and decomposed to CO_2 and H_2O and will produce considerable ATP. However, under a low oxygen environment, the partial pressure of oxygen in air is relatively small, which inhibits the input of oxygen to some extent and results in decreasing of respiration rate of insect and reduction of energy supply. Since the energy supply is reduced the insect will increase ATP production through anaerobic respiration glycolysis. But it still can not supply the normal energy for the insect survival[9]. A product of anaerobic respiration-lactic acid has a harmful effect on insect[10]. It is obvious that under low oxygen condition, the reduction of the respiration rate of insect can result in a deficiency of APT production and accumulation of toxic metabolites which finally leads to the death of insect. The findings of the present work show that the effects of the low oxygen on respiration rates of *T. castaneum* are different at various stages of the development of the insect. When the oxygen concentration is ≤ 10%, the reductions of respiration rates of the young larva and old larva of *T. castaneum* are significant. When the oxygen concentration is ≤5%, the reduction of respiration rate of the egg and the adult of *T. castaneum* is significant. When the oxygen concentration is ≤3%, the reduction of respiration rate of the pupa of *T. castaneum* is significant. However, when the environmental oxygen concentration is below 3%, the respiration rates of the egg, larva, pupa and adult of *T. castaneum* are significantly inhibited. Current researche shows that when the oxygen concentration is below 3%, each stage of the store grain pest can be controlled effectively[11]. Annis reported that the low oxygen concentrations of 1% – 5% has lethal effect on the adults of stored grain pests[12]. The present article shows that when the oxygen concentration is 1% – 5%, the respiration rate of the adult of *T. castaneum* is obviously inhibited. The reduction of the respiration rates of stored grain pests under the low oxygen condition can represent differences in sensitivities of pests to the low oxygen environment, and the mathematical models developed on respiration rates of stored grain pests under the low oxygen condition help us establish more economic and more effective stored grain pests controlling strategy.

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0104

Toxicity Effects of High Carbon Dioxide Modified Atmospheres in Combination with Sulphur Dioxide on the Rice Weevil *Sitophilus oryzae*

Jordi Riudavets[1*], Rosa Gabarra[1], Mª José Pons[1], Cristina Castañé[1], Oscar Alomar[1], Sonia Guri[2,3]

Abstract: Control of insects in processed food by means of modified atmospheres (MA) is sometimes a relatively long treatment to accommodate the requirements for marketing in the food industry. Therefore, it is necessary to find alternative methods or additives that permit the reduction the length of the treatments and to achieve a high control efficacy. The objective of this laboratory study was to evaluate the feasibility of combining high carbon dioxide (CO_2) MA and low concentrations of a food additive, sulphur dioxide (E-220) (SO_2), to control adults and eggs of the rice weevil *Sitophilus oryzae*. The combination of two different CO_2 concentrations (70% and 95%) and four SO_2 concentrations (35 ppm, 50 ppm, 150 ppm and 3%) were tested for a range of exposures times. The addition of SO_2 to a MA containing 70% CO_2 significantly increased the mortality of both eggs and adults of *S. oryzae*. In comparison, when increasing the CO_2 concentration form 70% to 95%, adult mortality and emergence reduction rates of *S. oryzae* were much less influenced by the addition of SO_2. Different SO_2 sorption and desorption curves were observed in flour wheat, rice and almonds. Residual contents of SO_2 after the treatment were low except for the 3% SO_2 concentration treatment. Seven days after the treatment, residual levels in all treatments were very low or undetectable. Therefore, SO_2 could be effective in reducing the exposure times needed to control this pest species with high CO_2 MA.

Key words: modified atmospheres, food additives, carbon dioxide, sulphur dioxide, *Sitophilus oryzae*, pest control, stored products.

Introduction

Modified atmospheres (MA) with a high carbon dioxide (CO_2) content are safe and environmentally friendly pest control methods for raw and manufactured food products[1,2]. The main advantages of using high CO_2 MA are that they are effective for the control of a wide range of pest species, and they can be used for the treatment of different food products without an accumulation of toxic residues after the treatment. However, control of insects in processed food by means of MA is sometimes too long for the marketing requirements of the food industry. Data on the effects of different CO_2 types of treatments and dosages are available for many species and stages of stored product pests under many particular sets of conditions[3,4]. The recommended exposure time to achieve complete pest control at the most appropriate gas concentration may take from several days to weeks, according to CO_2 concentration, developmental stage and species. For example, to control one of the most tolerant species to MA, the rice weevil *Sitophilus oryzae*, the estimated LT_{95} values using 40% to 100% CO_2 MA range between 1 and 4 days for adults and between 3 and more than 5 days for eggs[5]. However, 12 days at 90% CO_2 and more than 12 days at 50% CO_2 are needed to achieve full mortality for eggs and pupae of this species[6].

Since MA with high concentrations of CO_2 causes permanent opening of the spiracles of insects, a synergistic toxic effect of CO_2 when combined with other compounds has been sought[7]. For example, adding CO_2 to certain fumigants such as acrylonitrile, methyl bromide, phosphine, and hydrogen cyanide increases their toxicity and permits the reduction of exposure times[8].

Sulphur dioxide (SO_2) is a gas accepted as a food additive (E-220). Although it has a large history as a food preservative due to its antimicrobial properties in a range of food products and beverages, it has also been used for the control of some insect pest of grapes during storage[9,10,11]. Official dosages approved for the treatment of food products range from 50ppm in

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cereal by-products, to 500ppm in nuts and 2000ppm in dried fruits.

The objective of this paper is to present preliminary results of on-going research on the evaluation of the effectiveness of combining high CO_2 MA with low concentrations of SO_2, for the control of the rice weevil *S. oryzae*. A second objective was to determine the residual SO_2 levels in samples of rice, flour and almonds treated with this gas mixture.

Materials and Method

Insect mortality

Sitophilus oryzae used for the experiments came from colonies maintained on brown rice at IRTA (Cabrils, Barcelona). Experiments were conducted with adults and eggs. All laboratory studies were conducted in a climatic chamber at 25 ± 1℃ and 70% ±10% RH.

The experimental arenas were ventilated plastic cages (7.5 cm diameter and 3 cm high) containing 50g of brown rice. The sides of the arenas were painted with *Fluon* in order to avoid the escape of insects during the treatment.

Fifty adults were added to arenas. For obtaining eggs, adult weevils (1280 adults/kg of brown rice) were added and allowed to oviposit for 7 days. Afterwards, adults were removed with a 2.0 mm mesh sieve and rice with eggs was used for the experiments.

Two different experiments were carried out:

1. To evaluate the effectiveness of two high CO_2 modified atmospheres, 70% CO_2 and 95% CO_2, combined with SO_2 (0%, 35 ppm, 50 ppm, 150ppm and 3%) against adults and eggs of *S. oryzae*.

2. To evaluate the mortality responses of *S. oryzae* adults exposed to a modified atmosphere of 70% CO_2 with and without the addition of 35 ppm of SO_2 for a range of exposure times.

Insects were exposed to each gas mixture in sealed glass desiccators (200 mm diameter). Three cages of each stage were exposed in the same desiccators and five replications were made for each gas mixture and exposure time according to each experiment.

Gas mixtures were previously prepared in cylinders and gas concentration verified by the company that made up the gases mixtures (Carburos Met licos S. A.). Modified atmospheres were balanced with N_2 and 5% O_2. Gas mixtures from the cylinders were continuously flushed into the desiccators at a pressure of 2 bars until the desired gas concentrations were reached. A gas analyzer (Abiss model TOM 12) was used to check the concentrations of CO_2 and O_2 inside the desiccators during the experiment.

After the treatment, desiccators were opened and cages were kept in a climatic chamber at 25 ± 2℃ of temperature and 70 10% r. h. To evaluate mortality on adults, the number of living adults in each cage was counted after 24h. The percentage of mortality was calculated using the initial number of individuals placed in each cage. Sets of control cages were used to determine the percentage of natural mortality. For eggs, emerging weevil progeny was counted after 9 weeks. The number of living adults in each arena was counted and the percentage of mortality was calculated using the number of individuals emerged in the control cages. Results were subjected to one-way analysis of variance (ANOVA) (SAS System for Windows 8.02. SAS Institute, Cary, NC. 2001). For the second experiment, time-mortality data for each treatment were subjected to Probit analysis by the POLO-PC computer program (LeOra Software Polo Plus 1.0 2002 – 2007) to determine LT_{50}, and their respective 95% confident intervals.

SO_2 sorption and desorption in samples of rice, wheat flour and almonds

The experimental arenas were glass Petri dishes (9 cm diameter and 2 cm high) containing 25 g of three different food products: polished rice, wheat flour, and almonds. Three different gas mixtures were used: 70% CO_2 with 50ppm SO_2, 90% CO_2 with 150ppm SO_2, and 70% CO_2 with 3% SO_2. Gas mixtures were balanced with N_2 with 5% O_2.

Products were exposed to the modified atmosphere in sealed crystal desiccators (200 mm diameter). Three Petri dishes were exposed in the same desiccator to each gas mixture, except in the case of the mixtures containing 3% SO_2 where only one Petri dish was exposed in each desiccator. Four different exposure times were tested: 1, 2, 3 and 7 days. Three desiccators were used for each product and exposure time. Gas mixtures were previously prepared in cylinders and gas concentration verified as in the previous experiments. For the treatment, gas mixtures were continuously flushed through the desiccators for 2 minutes at a pressure of 2 bars. Desiccators where then kept in a climatic chamber at 25 ± 1℃. After the treatment, a gas

analyzer (Abiss model TOM 12) was used to check CO_2 and O_2 levels inside desiccators. SO_2 content in the food product was determined by the method of *Monier-Williams*[12]. Analysis were conducted immediately after opening desiccators, and 3 h, 24 h and 7 days after the end of the treatment. Treated samples were held in the laboratory at room temperature. SO_2 levels in untreated control samples were also analyzed in three samples of rice and wheat flour, and six samples of almonds.

Results and Discussion

Insect mortality

When exposed to a MA of 70% CO_2, the mortality rates of *S. oryzae* adults were strongly influenced by the addition of SO_2 (Table 1). Adding SO_2 significantly increased the percentage mortality from 7.8%, without SO_2, to 28% with 35ppm, to 62.7% with 50ppm and to total mortality when combined with 3% SO_2.

When increasing the CO_2 concentration from 70% to 95% and the exposure time from 20 h to 24 h, adult mortality in the treatments without SO_2 increased from 7.8% to 54.4%. However, the addition of 50ppm SO_2 to the 95% CO_2 MA did not increase the mortality when compared to the treatment without SO_2, as had been found with 70% CO_2 (Table 1). Only the addition of 150ppm of SO_2 resulted in a significant increase in mortality compared to the treatment without SO_2.

The mortality of adults exposed to a MA of 70% CO_2 with and without the 35ppm of SO_2 for a range of exposure times are given in Fig. 1. Time-to-death curves show that mortality of adults was higher for the range of 20 h to 48 – h in presence of SO_2, although variability observed was high, mainly after 20 hours exposure. At the LT_{50} level, adults exposed to the combination of SO_2 and CO_2 were more susceptible by approximately 6 h, than adults exposed to CO_2 alone (Table 2).

The reduction of adult emergence was significantly higher when exposed eggs to 70% CO_2 in combination with 35ppm of SO_2 than without SO_2 (Table 3). After 120 hours treatment, the number of emerging adults in each control cage averaged 237. When treated with 70% CO_2 the number of emerging adults was reduced to an average of 6.6 per cage, ranging from 6 to 10 in all cages. Only one adult in one cage emerged when treated with the same CO_2 modified atmosphere in combination with 35ppm of SO_2. No adult emergence was recorded at 70% CO_2 and 3% SO_2.

With the emergence of adults from egg exposure, the same trend was observed as with adult mortality, when increasing the CO_2 concentration form 70% to 95%, the addition of SO_2 did not reduce the emergence of *S. oryzae* in comparison to the non-SO_2 treatment (Table 3). Although nearly a reduction of 10% in emergence was observed in average between the highest SO_2 content (150ppm) and the treatment without SO_2, no significant differences were found among treatments according to the ANOVA.

Analysis of sulfur dioxide residues

The initial residues of SO_2 detected in almonds, wheat flour and rice before the treatments were 3.6 ± 1.85ppm, 23.6 ± 12.80ppm, and 0ppm, respectively.

When treated with a MA of 3% SO_2, different sorption curves were observed for the three food products during the study. Wheat flour reached the highest values (aprox. 3500ppm), and remained more or less stable from the third day of analysis onwards (Fig. 2). SO_2 adsorption of both almonds and rice steadily increased from the first day until the end of the study (to aprox. 2300ppm). After aeration, a high degree of desorption was observed for wheat flour treated with 3% SO_2 (Fig 3). Values dropped from more than 2500ppm just after the treatment to around 400ppm after 7 days ventilation.

When treated with a low SO_2 content (150ppm and 50ppm), initial residual contents of SO_2 after the treatment were low. After 3 h and 24 h ventilation, the residual limits also decreased quickly even to the minimum detection limit of the technique.

In conclusion, the addition of a low concentration of SO_2 (from 35 ppm to 3%) in a MA of 70% CO_2 increased the mortality of *S. oryzae* adults and eggs compared to the mortality obtained with the same MA without SO_2. The highest level of SO_2 tested (3%) had a strong effect on mortality, while concentrations of 150ppm SO_2 or lower had less impact on mortality. In comparison, when increasing the CO_2 concentration form 70% to 95%, adult mortali-

ty and the reduction of egg emergence of *S. oryzae were much less influenced by the addition of* SO_2. Residual concentrations of SO_2 in wheat flour were low after the 150ppm and 50ppm SO_2 treatments. After 7 days aeration residual levels in all treatments were low or undetectable.

Acknowledgements

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Table 1. Total mortality (mean ± SEM) of *S. oryzae* adults exposed to 70% and 95% CO_2 (+5% O_2) alone or in combination with different SO_2 concentrations (35ppm, 50ppm, 150ppm and 3%) during 20 h and 24 h, respectively

Content of SO_2	Mortality (%) 70% CO_2	Mortality (%) 95% CO_2
0ppm	7.8 ± 1.22 d	54.4 ± 3.71 b
35ppm	28.1 ± 5.93 c	–
50ppm	62.7 ± 11.35 b	52.0 ± 3.13 b
150ppm	–	66.5 ± 3.04 a
3%	100 ± 0 a	–

Means followed by the same letter in a given column indicate no significant difference between treatments (Tukey, n = 5, a = 0.05)

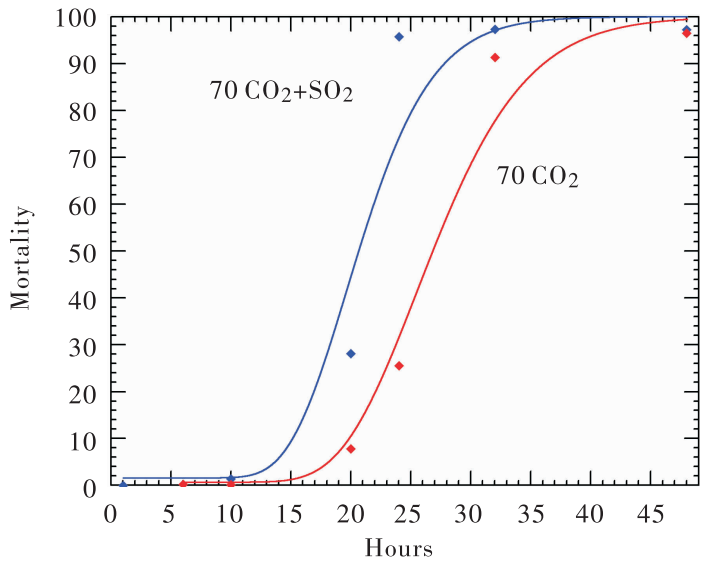

Fig. 1 Time-to-death curves for 70% CO_2 (70 CO_2), and 70% CO_2 and 35 ppm SO_2 (70 CO_2 + SO_2) applied to adults of *S. oryzae*.

Table 2. Mortality responses (LT_{50}) of *S. oryzae* adults to 70% CO_2 (+5% O_2) and 70% CO_2 and 35 ppm SO_2 (+5% O_2) resulting from laboratory fumigations at 25℃

Treatment	χ^2 (d. f.)	Slope ± SE	LT50 (hours)[a]
70% CO_2	805.93 (88)	10.71 ± 0.33	26.8 (25.7 – 28.0)
70% CO_2 + 35ppm SO_2	3202.3 (103)	8.99 ± 0.36	20.4 (17.5 – 22.4)

a: numbers in brackets give the 95% confidence range

Table 3. Adult emergence reduction (means ± SEM) of *S. oryzae* eggs exposed to 70% and 95 % CO_2 (+5% O_2) alone or in combination with different SO_2 concentrations (35ppm, 50ppm, 150ppm and 3%) during 5 days and 3 days, respectively.

Content of SO_2	Emergence reduction (%) 70% CO_2	Emergence reduction (%) 95% CO_2
0ppm	97.2 ± 0.22 b	56.8 ± 3.10 a
35ppm	99.9 ± 0.03 a	–

Content of SO_2	Emergence reduction (%) 70% CO_2	Emergence reduction (%) 95% CO_2
50ppm	–	57.2 ± 1.36 a
150ppm	–	64.8 ± 2.87 a
3%	100 ± 0 a	–

Means followed by the same letter in a given column indicate no significant difference between treatments (Tukey, n = 5, α = 0.05)

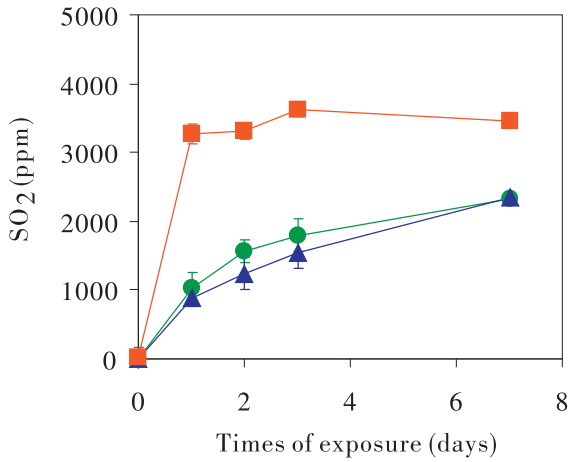

Fig. 2 SO_2 residual content (mean ± SEM; n = 3) in polished rice (circle), almonds (triangle) and wheatflour (square), after fumigations in desiccators (200 mm diameter) with 70% CO_2, 5% O_2 and 3% SO_2 at 25℃.

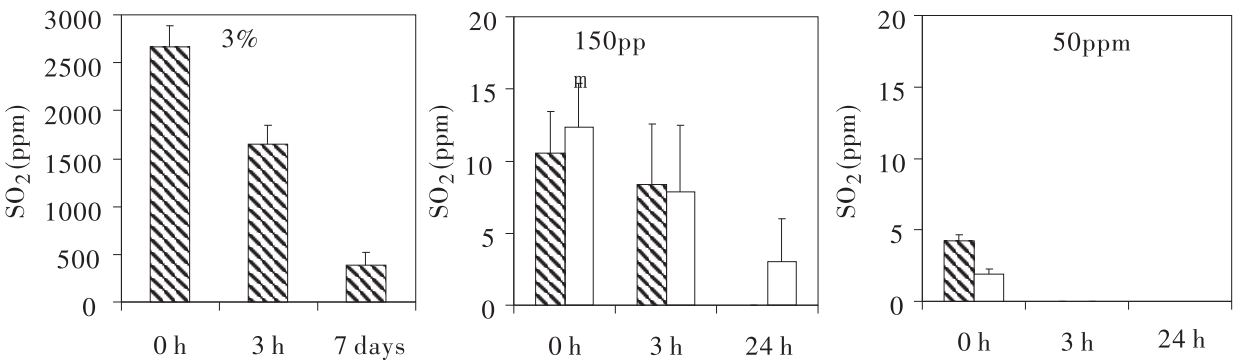

Fig. 3 SO_2 residual content (mean ± SEM; n = 3) from wheat flour fumigated in desiccators (200 mm diameter) with 70% CO_2, 5% O_2 and 3%, 150ppm and 50ppm SO_2 during 24 h (striped bars) and 7 days (white bars) at 25℃. Measurements were made at after the treatment (0 h), and after 3 h, 24 h and 7 days aeration.

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Application of Controlled Atmospheres and Fumigation in Control of Psocids

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Abstract: Small, polyphagous insects are generally ignored in most grain storage systems; however, psocids have recently become a serious problem in food facilities and stored products in many countries and regions. Fumigant methyl bromide has been used around the world since the 1930s as a quarantine treatment for plants and to control insects in buildings and commodities. With the ban of methyl bromide for controlling stored product pests, numerous chemicals are being considered as alternative fumigants. Candidate fumigants include phosphine, sulfuryl fluoride, carbonyl sulfide, carbon disulfide, ethyl formate, ethylene oxide, hydrogen cyanide, methyl iodide, sulphur dioxide, methyl formate, and acetaldehyde. Owing to the lack of alternatives that match the combined advantages of phosphine, it is likely that phosphine will remain the major grain fumigant for the foreseeable future. As a friendly and rapid fumigant, ethyl formate also promised controlling stored products pests. Controlled atmospheres with elevated carbon dioxide, low oxygen levels, or a combination of both have been tested and become useful and practical for the control of stored product insects. The potential use of these and other nontoxic and environmentally benign materials as alternatives to currently used fumigants has been widely recognized. Despite numerous reports of resistance to many insecticides, chemical control will continue to play an important role in psocids control programs. Studies on population ecology and resistance development to control treatments are fundamental to the development Integrated Pest Management (IPM) strategies for psocids. This review is mainly to summarize the last decade's application of CA and fumigation in control of psocids and expect to assist in formulating strategies to control these rapidly proliferating pests.

Key words: controlled atmospheres, fumigation, phosphine, ethyl formate, *Liposcelis*

Introduction

Psocids, or booklice, mainly belonging to the genus *Liposcelis*, are serious pests of stored grain in tropical and subtropical Asia [1-3] and have emerged in the 1990s as major pests in Australia [4,5] and China [6]. Their ability to exist for long periods without food and their small size makes them extremely invasive of storage structures. In recent years, there has been a gradual worldwide recognition that psocids pose a series of distinct pest problems in the area of grain storage and stored products [7]. In the last two decades, *Liposcelis* species have been reported as pests in Europe, Canada, Australia, New Zealand and Asia in commercial and domestic situations [3,6,7].

Methyl bromide has been used around the world since the 1930s as a quarantine treatment for plants and to control insects in buildings and commodities effectively. However, routine fumigations of warehouses and storage facilities with methyl bromide have failed to control psocids [8]. The adverse effects of methyl bromide on the atmospheric ozone layer and worker safety issues prompted the enactment of the Clean Air Act by the US Federal government which requires all developed countries eliminate the bulk of their methyl bromide consumption by 2005 [9]. The loss of this important fumigant has forced researchers to find alternatives. Numerous chemicals are being considered as alternative fumigants to control stored product pests, but none are as fast acting as methyl bromide. Candidate fumigants include phosphine, sulfuryl fluoride, carbonyl sulfide, carbon disulfide, ethyl formate, ethylene oxide, hydrogen cyanide, methyl iodide, sulphur dioxide, methyl formate, and acetaldehyde [9-13].

Controlled atmospheres (CA) are also al-

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ternative treatments to the use of methyl bromide for post-harvest insect control. CA with elevated carbon dioxide, low oxygen levels, or a combination of both have been tested and become increasingly useful and practical to control stored product insects. Although commercial use is still limited to a few countries, the potential use of non-toxic and environmentally benign materials as alternatives to currently used fumigants has been widely recognized.

In the last decade, the development of resistance to the most commonly used fumigants such as phosphine and methyl bromide has been well documented [3]. Similarly, extensive use of CA in insect control could lead to selection of insect populations resistant to hypercarbia and hypoxia [6,14,15]. This paper summarizes the past decade's application of CA and fumigation in control of psocids and will present strategies to control these rapidly proliferating pests.

Occurrence and Pest Status of Psocids

Psocoptera is a relatively small order of insects with approximately 4400 species worldwide. Some are adapted to live in food stores, food processing facilities, bulk grain, houses, etc. [16]. Indoors, their occurrence is more frequent in humid environments [17]. There are reports of their presence in various products: coffee and cocoa, tobacco and cereal-derived foods [18]. They are regarded as secondary pests, often overlooked due to their small size and the existence of other more damaging primary pests especially in cereal grains. Nonetheless, when population explosions occur, they can cause considerable economic damage and present a permanent hygienic danger for transferring microorganisms and contaminating material with their excrement and dead bodies. They also cause serious deterioration of the quality, especially of products in the food processing industries. The specific biological traits (small body size, short generation period) contribute to rapid and substantial psocids population growth. The pest status of these insects can be broadly categorized and the mainly three items are as follows: a nuisance as food/commodity contaminant; direct feeding damage of food/commodity; and health risk.

One of the most widely spread members of the order Psocoptera is a minute apterous species *Liposcelis bostrychophila* Badonnel, commonly, but erroneously, known as booklouse. *L. bostrychophila* is commonly found in various processed and unprocessed dry foods in households, granaries and warehouses [7]. Apart from causing measurable damage to stored grains [19], infestations of this psocid can also cause health problems among storage and warehouse workers. This insect feeds continually and reproduces rapidly by obligatory thelytokous parthenogenesis.

Outbreaks of *L. bostrychophila*, together with *L. entomophila* (Enderlein) have been reported in humid tropic countries such as Indonesia, Malaysia, Singapore, The Philippines, Thailand, China and India [3,20,21]. Unlike the above two species of cosmopolitan distribution, *L. paeta* Pearman is prevalent in the tropics and has also been focused in a series of reports [1,22,23].

In China, *L. bostrychophila* and *L. entomophila* have posed an alarming threat to stored products, especially in the storage facilities where CA and insecticide combined treatments are commonly applied. Routine fumigation of warehouses and storage facilities with methyl bromide or the use of insecticides have failed to control these two pests, which can readily reinfest grain in storage after treatments [24]. In addition, rapid development of resistance to chemical and physical treatments has also been reported for *L. bostrychophila* and *L. entomophila* [20].

The distribution and abundance of psocids in Australia's grain storage system has been on the increase over the past decade. *Liposcelis* species have been particularly successful inhabitants of grain stores and are now specifically targeted by integrated control programs. Although considerable effort has been invested in chemical control, psocids continue to thrive in older, unsealed stores. *Liposcelis* infestations are notorious for contributing to violations of Australian grain export legislation that prescribes a zero tolerance to the export of live insects in grain, resulting in serious legal and monetary ramifications. Recently, as a new pest species, *L. decolor* (Pearman) has emerged in eastern and southern Australia, with frequent infestations occurring in both the central bulk-handling systems and in grain stored on farm [22]. Phosphine fumigation that is targeted to control all major stored-grain pests in this country has failed against this species. A search of the literature revealed no published information on the effectiveness of any chemical treatment against this pest, although it has been reported as an e-

merging pest in several countries around the world, such as China, Croatia, Spain and the Czech Republic [17,21].

Fumigation for Psocids Control

Fumigants hold a specific place in storage of feed and food, especially for pest control. High volatility and penetrability through bulks of stored goods and quick lethal effect on organisms within these products render gaseous substances suitable tools in the scope of integrated pest control. When insect pests survive all human precautions and prevention efforts, large bulks of stored products can be disinfested by use of appropriate fumigants and fumigation techniques. No other approach leads to such instantaneous and feasible pest control without moving the produce, or building up undesired residues.

As a common fumigant of stored products, methyl bromide acts rapidly, controlling insects in less than 48 h in space fumigation, however, because it depletes ozone in the atmosphere, this chemical was banned in 2005 in developed countries. Many alternatives have been tested as replacements for methyl bromide. Among these, phosphine, ethyl formate and essential oils are three promising representatives in psocids prevention [11-13].

Phosphine

With the decreasing use of contact insecticides in recent years due to consumer sensitivity towards residues, fumigation with phosphine has become the worldwide dominant disinfestation treatment and its application in post-harvest grains amounts to 80% in Australia [24]. Owing to the lack of alternatives that match the combined advantages of phosphine, it is likely that phosphine will remain the major grain fumigant for the foreseeable future. Lethal effects of phosphine on egg, nymph and adult stages of *L. bostrychophila* in laboratory showed that phosphine fumigation had different efficacy on the different stages. After 24, 72 and 120 h exposure, LC_{50} of egg were 0.137, 0.045, and 0.035 mg/L, respectively; after 24 h treatment, LC_{50} of nymph varied in the range of 0.004 – 0.007 mg/L, and that of adults was 0.020 mg/L. Exposed to 0.025 mg/L phosphine for 24 h every 10 d, the population was completely controlled [25].

Like many other fumigants, a serious problem with the extensive use of phosphine has been the evolution of resistance in several pest species including psocids [26,27]. In Australia, protocols for phosphine application have failed to control several species of psocid pest and nation-wide surveys have revealed the occurrence of resistance to phosphine in at least four *Liposcelis* psocid species, with the detection of strong resistance in *L. bostrychophila* [22]. While investigating response to phosphine in *L. bostrychophila* populations, an apparent delay in the development of eggs in a resistant strain under fumigation was observed. From the investigation on this delay in development of *L. bostrychophila* eggs as a mechanism of resistance, Nayak et al. [27] discovered that the most successful strategy to control resistant *L bostrychophila* is to apply relatively low concentrations of phosphine for extended exposure times that allow all eggs to hatch to the much less tolerant nymph stage.

Ethyl Formate

Ethyl formate (EF) is being investigated as an alternative to phosphine and methyl bromide for the fumigation of stored products. The compound has been found to have a rapid action against stored product insects and shows promise as a fast-acting toxicant [11]. Unlike phosphine which takes days to kill insects, EF kills target insects rapidly. Field trials have shown that EF has good potential as a fumigant in unsealed farm bins. Residues disappear without aeration by degrading to non-poisonous, naturally occurring products (formic acid and ethanol) [10]. Sealed jar fumigation experiments showed that treatment time and temperature significantly affected the fumigation effects of EF on *L. bostrychophila* adults. Within 24 h of treatment, EF showed a fairly high fumigation activity. Fumigation at 16℃ expressed a even better result than at 31℃. The LC_{50} s of *L. bostrychophila* adults fumigated by EF with an exposure time of 12, 24, 36, 48 and 60 h were 15.882, 15.676, 14.011, 13.154 and 10.495 μL/L, respectively. Fumigation at 20, 25 and 30℃ with a treatment time of 24 h indicated the LC_{50}s of 11.372, 13.283 and 15.676 μL/L, respectively [12]. The investigation on the fumigation activities of EF against *L. bostrychophila* in simulated storehouses with wheat, maize and paddy showed that fumigation activities of EF in the wheat storehouse were the best, followed by the maize warehouse, and then the paddy storehouse. Besides, EF can control *L. bostrychophila* effectively [13].

Essential Oils

Natural plant extracts are commonly used in many developing countries to control insect pests in storage because of economic conditions on small farms [28]. The use of plant extracts, including allelochemical compounds such as essential oils, is a feasible alternative to the use of synthetic insecticides [29]. The activities of natural plant extracts are manifold, and they induce fumigant and topical toxicity as well as antifeedant or repellent effects [29]. Six plant essential oils (extracted from the leaves of six source plants: *Citrus tangerina* Tanaka, *Citrus aurantium* L., *Citrus bergamia* Risso et Poiteau, *Pinus sylvestris* L., *Cupressus funebris* Endl., and *Eucalyptus citriodora* Hook) as repellent and fumigant against *L. bostrychophila* were assessed in the laboratory. The repellency test indicated that *L. bostrychophila* adults were repelled by filter paper strips treated with six essential oils. Of these essential oils, the *C. funebris* oil was most effective followed by that of *P. sylvestris*, *C. tangerina*, *C. bergamia* and *E. citriodora*. The average repellency of the *C. aurantium* oil against *L. bostrychophila* adults was significantly lower than other five test oils by day 14. These essential oils had a high level of toxicity in the fumigation assay against *L. bostrychophila* adults at both 10 and 20 ppm (v/v) [30].

Controlled Atmospheres Studies on Psocids

Controlled atmospheres (CA) are alternative treatments to the use of methyl bromide for post-harvest insect control. The insecticidal properties of CAs have been generally classified into two main types: (a) low oxygen and (b) CO_2 enriched atmospheres. The efficiency of CA, with elevated carbon dioxide, low oxygen levels, or a combination of both, for stored-product insect control has been confirmed by various laboratory and field studies, and several studies on the acute lethal response of psocids to CA have also been reported [3,20].

Although CA possess non-toxic and environmentally benign materials, several researchers have revealed that stored products pests are capable of adapting to these two stresses, resulting in the resistance development to hypoxia or hypercarbia [20]. In the last decade, a series of experiments about CA effects on psocids have been carried out in Chongqing Key Laboratory of Entomology & Pest Control Engineering, China [6,14,15,20,25,31,32]. In the laboratory, *L. bostrychophila* developed and reproduced successfully under CA exposures, but the development and reproduction of *L. bostrychophila* were inhibited by CA exposures. Egg development was more sensitive to high CO_2 than low O_2 concentration due to relatively low respiration rate of the egg stage. Leong and Ho [3] reported that when eggs of *L. entomophila* were exposed to hypercarbia for 24 h, the development of egg was delayed. Although no delays in nymphal development times were observed after hatching, the adult emergence of *L. entomophila* was inversely related to the CO_2 concentration to which the eggs were exposed.

Temperature is one of the most important factors affecting the development and reproduction of psocids [6] and also modifying the effect of CA [3]. Some studies reveal that the influence of CA on the mortality of *L. bostrychophila* is highly correlated to temperature. Similarly, the acute lethal effect of CA in relation to temperature on *L. bostrychophila* was also reported by Bell *et al.* [33]. Under CA exposures, the preoviposition period of *L. bostrychophila* was prolonged and the adult longevity and fecundity were reduced.

The effects of CA and dichlorvos (DDVP) on population growth and resistance development by *L. bostrychophila* indicated that the population of the psocid increased rapidly under natural conditions; after 11 weeks, this population had increased 48.1 – fold at 28.8℃, 80% relative humidity. Exposure to CA (35% CO_2, 1% O_2) or DDVP (0.3 mg/mL) alone failed to control the population growth. However, alternating exposure to CA and DDVP provided a significant increase in mortality as compared to those exposed to only CA or DDVP. The results of bioassay showed that both populations exposed only to CA and DDVP developed a low but significant resistance to CA and DDVP, respectively. Probit analysis did not show an appreciable increase in slope value of either population in spite of continuous exposures, indicating considerable heterogeneity of these psocids in response to CA or DDVP and suggesting a greater potential for the development of higher levels of resistance. It is suggested that alternating CA with insecticides could be an important management measure for psocids in storage [15].

Conclusions

There is no single replacement for all the

uses of methyl bromide. Each insect problem will require its own solution, mainly revolving around integrated pest management. The most promising alternative fumigants are currently phosphine and EF for psocid control. Although alternatives to methyl bromide are often more expensive and labor intensive, they are practical and do not deplete the ozone layer.

Despite numerous reports of resistance to many insecticides, chemical control will continue to play an important role in psocids control programs. Studies on population ecology and resistance development to control treatments are fundamental to the development Integrated Pest Management (IPM) strategies for psocids.

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0106

Efficacy of Ethanedinitile(C_2N_2) against Some Cereal Pathogens

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Abstract: Ethanedinitrile (EDN) is being investigated by CSIRO Australia as an alternative to methyl bromide for a range of uses, including soil and timber fumigation.

EDN is an effective devitalisation agent of cereals and is being evaluated for devitalising feed grain imports into Australia. Part of this process involves assessment of efficacy against target pathogens of concern including *Tilletia indica* Mitra (Karnal bunt), *Peronosclerospora sorghi* Weston & Uppal (sorghum downy mildew), *Tilletia controversa* K hn (dwarf bunt) and *Ustilago maydis* (*DC.*) *Corda* (boil smut), which is being conducted in collaboration with the USDA ARS.

This fumigant was tested on: 1) naked spores; 2) bunted seed, when this is a propagule in the life cycle of the pathogen; 3) spores dusted on maize. It was applied at 120 mg/L over a period ranging from a few minutes to 5 days at 5, 17 and 22℃.

The naked teliospores of the three smut fungi were more easily controlled than spores still contained within the fungal structure. (sorus) or those spores that were dusted onto maize seed, which were the most difficult to control. Oospores of *S. sorghi* germinate poorly, if at all, on artificial medium. Results of the bioassay showed trace infection of sorghum in the untreated controls, and in one plant that was treated for 1 hr at 17℃.

Introduction

The Australian intensive livestock industry periodically suffers shortfalls in cost effective feedstuff during droughts. Importation of feed into Australia presents quarantine concerns as many serious pests and pathogens carried on feed grains are not established in Australia.

Ethanedinitrile (EDN) is being investigated by the CSIRO Australia as an alternative to methyl bromide for a range of uses, including soil (Ren et al, 2002 [1]; Rosskopf, 2007[2]) and timber fumigation (Wright et al 2002[3], Waterford, 2004[4]). The feasibility of using EDN to sterilise imported commodities of quarantine risks was tested (Waterford, 2004[5]) and was successful in devitalising barley, maize, sorghum and wheat. This present study reports results from assessments of efficacy against target pathogens of quarantine concern including *Tilletia indica* Mitra (Karnal bunt), *Peronosclerospora sorghi* Weston & Uppal (sorghum downy mildew), *Tilletia controversa* K hn (dwarf bunt) and *Ustilago maydis* (*DC.*) *Corda* (boil smut), which is being conducted by the CSIRO in collaboration with the USDA ARS.

EDN is a colourless gas with an almond-like odour; its chemical and physical properties are listed in Table 1. EDN has been patented by the CSIRO (Desmarchelier and Ren 1996[6]) as a new fumigant effective against insects and micro-organisms. It has a threshold limit value (TLV) of 10 ppm, which compares favourably with 5 ppm for methyl bromide.

Table 1. Chemical and physical properties of cyanogen compared to other fumigants

Formula	CH_3Br	C_2N_2
Molecular weight	95	52
Boiling point @1 atm	3.6℃	-21.17℃
Specific gravity (gas), air =1.0	3.3	1.82
Flammability limits in air, v/v%	13.5-14.5	6-32
Solubility in water, v/v%	3.4	Highly soluble
Conversion factor mg/L to ppm, v/v@1 atm	260	480

Materials and Methods

The efficacy of EDN was tested on naked spores, bunted seed, when this is a propagule in the life cycle of the pathogen and spores dusted on maize. Three replicates of each were put into open Ependorf tubes and placed into open des-

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iccators of measured volume, allowed to equilibrate to the 75% relative humidity overnight. The lids then closed to lids seal, and injected with EDN through a gas septum port, having first withdrawn an equivalent volume of air to prevent desiccator lids from popping. In the case of P. sorghi, homogenised infected leaf material with oospores was placed into small Nitex bags made of 20 m pore-size polyester screen and placed into racks in the desiccator.

EDN was applied at 120 mg/L and held at 5, 17 and 22℃. Times of exposure were 10, 25, 60, 120, 250, 500, 1000, 1750, 3000, 4500, 7000 minutes. After treatment spores of treated material and untreated controls were plated out for assessment of efficacy. In the case of sorghum downy mildew, seed of a susceptible variety of sorghum was inoculated with treatment and control spores and planted out as a bioassay of efficacy.

The EDN was generated in the laboratory in a fume hood by slowly injecting saturated KCN into hot (95℃) $CuSO_4$. The air in an inverted bell, fitted with a gas sampling septum, was first withdrawn filling the bell with the hot $CuSO_4$ The generated gas was then transferred by syringe into a Tedlar gas sampling bag and more EDN generated until sufficient for the days doses was made. After cooling to room temperature percent purity was analysed using a Thermal Conductivity Detector (TCD) fitted to an SRI model 8610C gas chromatograph using a 3 foot 1/8 inch column packed with Porapak Q 80/100 mesh, run at 100℃ with a carrier gas (He) 20 mL – 1. Purity was measured from 78 to 89 % which reflected the temperature of the $CuSO_4$.

The quantity of EDN needed to achieve target concentrations in each desiccator was calculated based on percent purity from the TCD analysis and desiccator volume. Exposure concentrations were then measured by taking samples with a gastight syringe through a gas sampling septum and analysing them with a Flame Ionisation Detector (FID) using the same column and GC. Concentrations for the longer exposures were topped up from time to time to maintain the concentration as near to 120 mg/L as possible. The mg · h/L dosage, Ct product achieved, was calculated from the FID results for each exposure.

Treated material and untreated controls spores of *T. indica*, *T. controversa* and *U. maydis* were seeded onto water agar medium to assess viability based on spore germination. Treated oospores where mixed into the upper 5 cm layer of soil in a 2 × 2 inch plastic pot and planted with seeds of a highly susceptible sorghum cultivar and placed in a growth chamber for disease development.

Results and Discussion

Figures 1 to 3 present the response of treated spores to the range of doses and temperatures. These data indicate that naked teliospores of the three smut fungi (*T. indica*, *T. controversa* and *U. maydis*) were more easily controlled than spores still contained within the fungal structure or sorus of *T. indica* and *T. controversa*. Spores that were dusted onto corn were the most difficult to control. This would indicate that surface interactions on the corn seeds and penetration of EDN into the fungal structures reduce the effective dose.

All three smut species treated at 22℃ were controlled to a high level at dosages less than 2 000 mg · h/L. As this is the likely treatment temperature of the commodity and the proposed dosage would be greater than this experimental treatment, using EDN should provide good control of these pathogens.

In general the data indicate that EDN was more toxic at higher temperatures. Overall, *T. indica*, with its large teliospore, was the most tolerant of the smut fungi.

Oospores of *S. sorghi* germinate poorly, if at all, on artificial medium hence this was not a feasible method to check efficacy for this pathogen. No vital stains were shown to be effective with oospores of *P. sorghi*. However, the treated oospores mixed into soil and planted with seeds of susceptible sorghum also proved problematic as an assessment of efficacy. Trace infection was observed in the untreated controls, and in one replicate of the 1 hr treatment at 17℃ at a dose of 120mg · h/L. No other infection was recorded in the remaining 44 treatments however, cross-contamination of the treated oospores cannot be ruled out. Most likely, given that initial inspection of the infected material indicated a high number of spores, is that the newly acquired oospores may have been exhibiting yearly season dormancy, which would explain the low levels on infection in the inoculated control plants and near absence of infection in any of the treatments (Pratt, 1978[7]).

Acknowledgements

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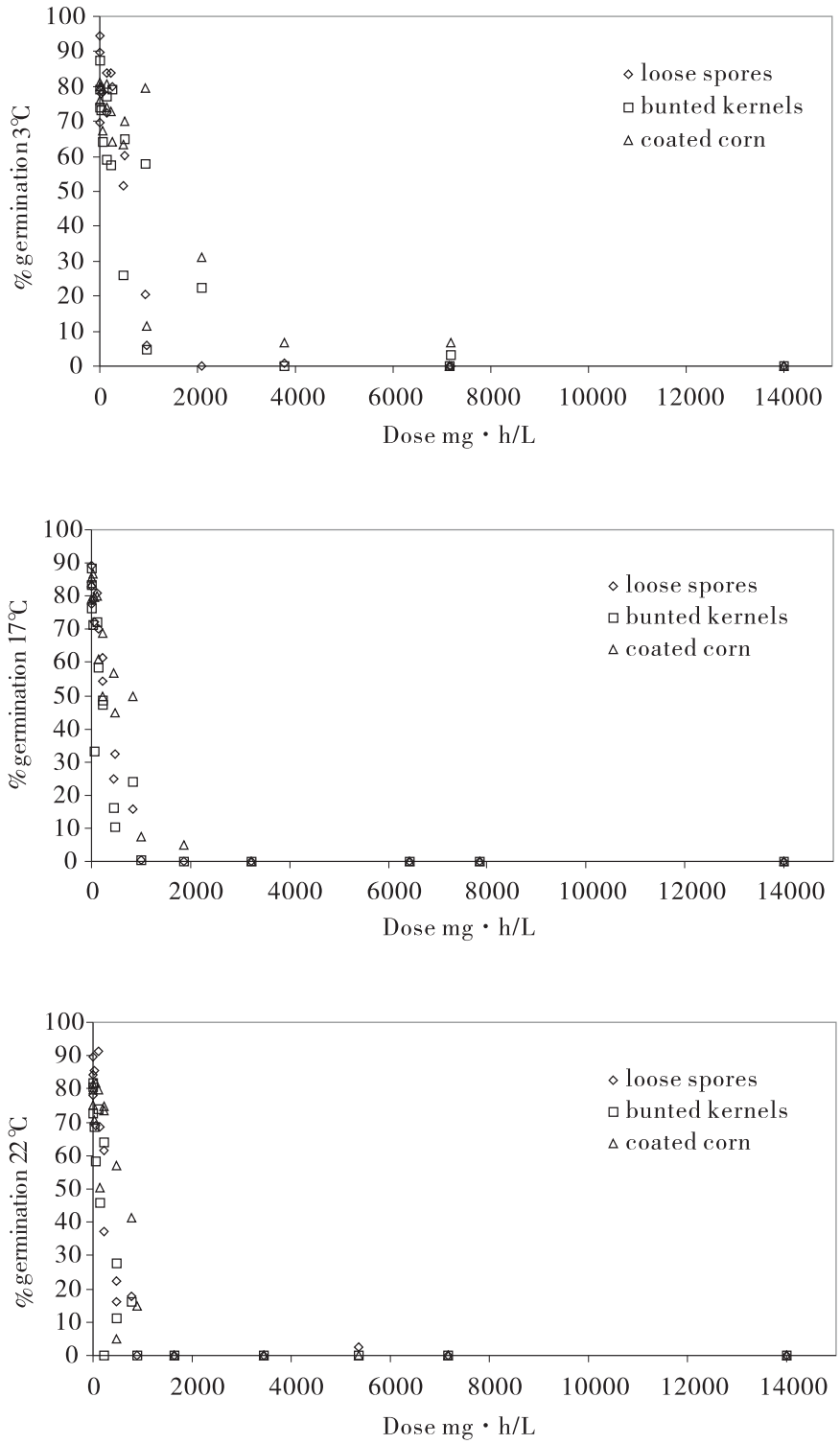

Fig. 1 Efficacy of ethanedinitrile (C_2N_2) at 120 mg/L against teleospores of *Tilletia controversa* treated as loose spores, bunted kernels and spores dusted onto corn for 10, 25, 60, 120, 250, 500, 1000, 1750, 3000, 4500, 7000 minutes of exposure at 3, 17 and 22℃

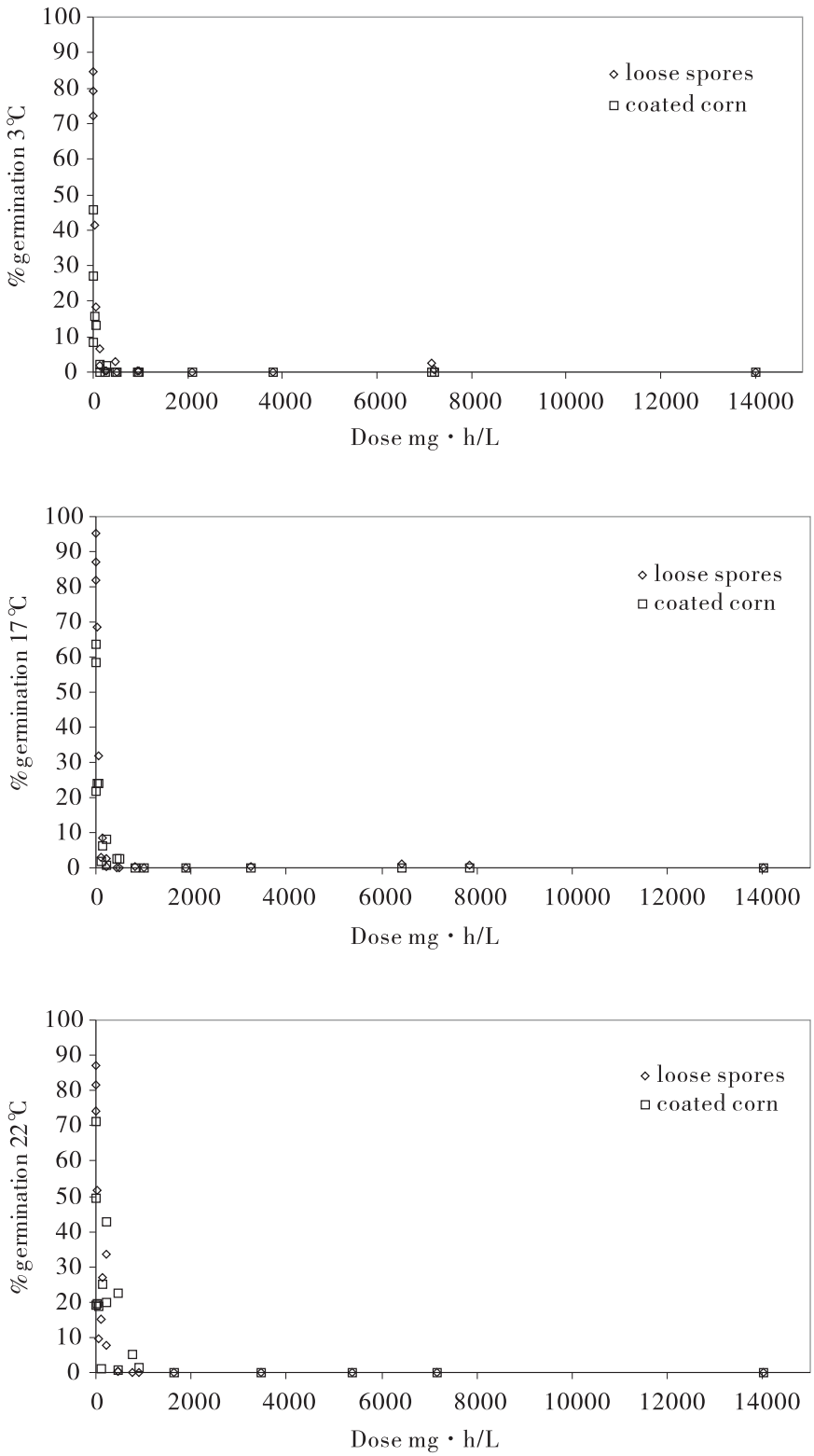

Fig. 2 Efficacy of ethanedinitrile (C_2N_2) at 120 mg/L against teleospores of *Ustilago maydis* treated as loose spores and spores dusted onto corn for 10, 25, 60, 120, 250, 500, 1000, 1750, 3000, 4500, 7000 minutes of exposure at 3, 17 and 22℃

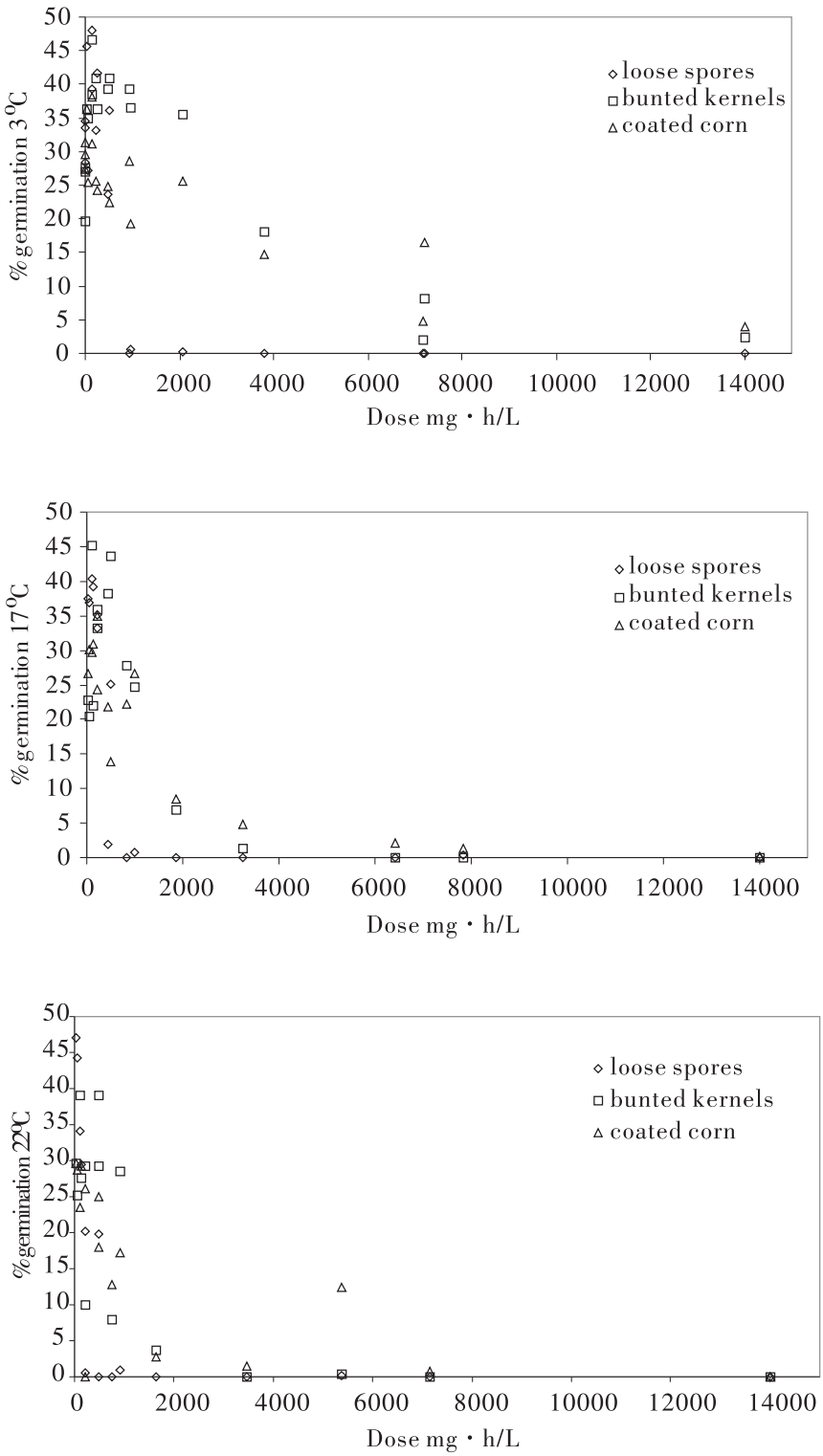

Fig. 3 Efficacy of ethanedinitrile (C_2N_2) at 120 mg/L against teleospores of *Tilletia indica* treated as loose spores, bunted kernals and spores dusted onto corn for 10, 25, 60, 120, 250, 500, 1000, 1750, 3000, 4500, 7000 minutes of exposure at 3, 17 and 22℃

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0107

The Inhibition Effect of Low Oxygen on Four Species of Stored Grain Insect Pests

Cao Yang[1], Li Guangtao[1], Zhou Jia[2], Li Yanyu[1] and Qu Guiqiang[1]

Abstract: Four kinds of representative stored grain pests; *Tribolium castaneum* Herbst, *Oryzaephilus surinamensis Linne*, *Sitophilus zeamais* Motschulsky and *Sitotroga cerealella* Olivier, were placed into the low oxygen environments attemperature of 30℃. The oxygen contents were 5%, 10% and 15%. Rresearch was performed the on the life history, growth and development and the inhibition effects under the different low oxygen environments…The results showed that when compared with the same kinds of test pests grown and developed under oxygen content of 21%, the eggs of these kinds of test pests could not be incubated under a low oxygen environment of 5%, and they could not finish their life history. A low oxygen environment of 10% can inhibited the incubation of the eggs of *T. castaneum*, *O. surinamensis* and *S. cerealella* obviously, and it also had a certain inhibition on the developments of larvae of *T. castaneum* and *O. surinamensi* s This results in prolongation of the larva period. . The larva of *S. cerealella* could not pupate under a low oxygen environment of 10%. A low oxygen environment of 10% has no obvious effect on the eclosion rate of pupae and pupal period of *T. castaneum*, but it can prolong the pupal period of *O. surinamensis* obviously inhibiting the growth of S. zeamais effectively. Except for certain inhibitory effects on growth and developments of larvae of *S. cerealella*, a low oxygen environment of 15% caused certain prolongations of development periods of other test pests, but had no obvious effect on hatchability, eclosion rate and etc. Low oxygen has an obvious inhibition effect on stored grain pests This inhibition effect is more obvious with the reduction of the oxygen content and prolongation of sealing time, . Therefore, a low oxygen environment of 5% – 10% oxygen can inhibit growths and developments of *T. castaneum*, *O. surinamensis*, *S. zeamais* and *S. cerealella* effectively.

Key words: low oxygen, stored grain pests, population inhibition, life history, different stages

Preface

At present, chemical fumigants are still used extensively as major pesticides for stored products (including stored grains), although they have advantages such as high efficiency, low residual and low cost, their operational safety and environmental safety are of high concern by the public; The rapid increase of drug resistance of the pests[1-4] and the problem of its effect on the ecological environment of the world has become very highlighted [5-6]. For example, the PH_3 resistances of stored product pests is very serious. It results in failure of fumigation. This has been often been reported and threatens the service lives of the fumigants;. Bromomethane's destructive effect on ozonosphere will result in global obsolescence in 2015. Therefore, it is necessary to develop green, environmental friendly and effective newtype fumigants and fumigation technologies. Gas adjustment pests controlling technology means changing the components of gas and their ratios artificially, and thus achieve effective control of pests[7]; It mainly includes reducing the oxygen concentration in an air tight space [8-11] and increasing the CO_2 concentration in the air tight space [12-20], to achieve the goal of pests killing. These technologies belong to the green grain storage technologies which are favored by more and more enterprises, and they have been used in the grain storage industry of our country recently. In order to reduce the application cost the low oxygen grain pests controlling technology has been highly [8-9]. Reducing the oxygen concentration in a grain pile to 2% and below through nitrogen introduction, chemical deoxidizing agents, oxygen-removal by burning or air tight biological oxygen-reducing application for more than 15 days, can kill various stages of grain pests effectively [21]. If the oxygen concentration is more than 2%, there are few reports about the effect on growths and developments of stored gain pests. Although our country has performed film sealing on grain surface and

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five-face or six-face film sealing on a grain pile which is called " double low" or " three low" grain storage technology separately for an extended time, and has found that low oxygen had effects on controlling of pests in grain pile the key parameters for controlling growths and developments of pests such as degree of air airtighness tof the grain pile, oxygen concentration and holding time have not been be determined This influences the extensive promotion of these technologies. The present research was performed to study t the effects of different low oxygen environments on growths and development of four kinds of stored grain pests under the laboratory conditions. They confirmed the need for concentration and time for controlling of the pest population, initially, providing a basis for the establishment of process parameters for controlling of the stored grain pests by low oxygen.

Materials and Method

Materials

Kinds and Sources of Test Pests

The following test pests were cultured in the Academy of State Administration of Grain for dozens of generations *Tribolium castaneum* Herbst (Yiyang strains), *Oryzaephilus surinamensis* Linne (Xinshi strains), *Sitotroga cerealella* Olivier (Xinshi strains) and *Sitophilus zeamais* Motschulsky (Guangzhou strains). Hereinafter, the denotation of test pests are: TC, OS, SC and SZ respectively.

Reagents and Materials

Medical oxygen; purity ≥ 99. 7% ; Beijing Beiwen Gas Production Factory

High-purity nitrogen; purity ≥ 99. 9996% ; Beijing Beiwen Gas Production Factory

Poly tetrafluoroethylene solution; Shanghai Sanaifu New Materials Co. , Ltd.

NaCl; Beijing Chemical Factory

Acid fuchsin; purity ≥ 94% ; Guangdong Xilong Chemical Factory

High activity dried yeast; Angle Yeast Co. , Ltd.

Main instruments and equipments

Electric thermostatic incubator; Shanghai Precision Instrument Co. , Ltd.

Rotary flow-meter, LZB - FB type; Flow rates: 25 - 250mL/Min, 100 - 1000mL/min, 1 - 6L/min; Jiangsu Chemical Engineering Instrument Co. , Ltd.

Vacuum dryer (Φ210mm); Beijing Longyuan Glass Co. , Ltd.

Continuous varyiing power stereo microscope XTS20 series; Beijing Fukai Instruments Co. , Ltd.

Orsat gas analyzer; Shangdong Leling Xinghua Glass Instruments Factory

Culture Plate: self-made. Organic glass of which diameter is 10mm and height is 8mm; there are 54 holes of which diameters are 5mm distributed uniformly and filter paper sticks at the bottom of the cultivation plate, while the covered was made of polyester plate; see Fig 1.

Method

Preparation of Test Pest

Obtaining of the eggs of TC and OS

Place 1000 of TC adults hatched after 2 weeks, into culture bottle and added feed which had passed a 80 - mesh screen (whole wheat flour: yeast, 95 :5). Three days after egg laying, the adults were sieved out with a 40 - mesh screen and remaining feed was sieved with an 80 - mesh screen. Eggs remained on the 80 - mesh screen. The eggs of OS were obtained similarly.

Obtaining the Eggs of SC

500 adults were selected and placed into a culture bottle, the mouth ealed with 18 mesh screen, hung upside down above the egg-receiving culture dish, then placed into an incubator at 30 ± 1℃ and 75% ± 1% relative humidity. After three days, the eggs of adults fell into the culture dish through the 18 mesh screen. The eggs were then placed into the receiving apparatus to perform the test.

Obtaining the Eggs of SZ

500 adults were selected and put into a clean culture bottle with wheat. The bottle was then placed into an incubator for 3 days at a temperature of 30 ± 1℃ and 75% ± 1% relative humidity, The adults were then removed and the wheat was treated with acid fuchsin. Red spots on the wheat, indicated there were eggs.

Preparation of Test

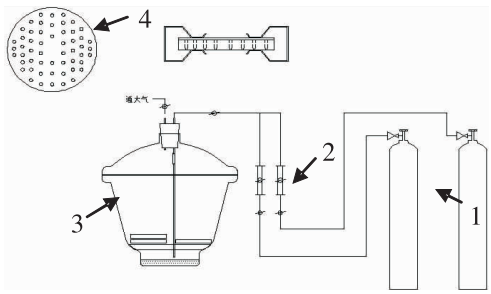
Eggs of TC, OS and SC were sought out the with a small brush the wheat with eggs of SZ were sought out under a stereo microscope and put into the holes of culture plates One test group was used for each oxygen concentration, three repetitions for each group and treatment. Each treatment in the culture plates contained 54 test eggs.

Controlling of the test temperature and humidity

The incubator test temperature was 30 ± 1℃. A saturated NaCl solution o controlled the humidity [2].

Controlling of the low oxygen condition

See Fig 1. According to the different requirements of oxygen concentration for the different tests, oxygen and nitrogen were mixed at different flow rates under the precondition that the outlet pressures of pressure reduction gauge coincided. The mixtures flowed into the dryer (the humidity had been adjusted) in which the test pests had been placed. Tthe outlet gas concentration of the dryer was tested using an Orsat gas analyzer. When the oxygen concentration reached to the desired value and become constant, gas filling was stopped and the outlet. was sealed. The dryer was placed into an incubator of which the temperature had been adjusted. An air – pressure balance bottle with distilled water was used to balance the pressure in the dryer with the pressure outside.



1. gas source; 2. flow – rate controlling device; 3. test container; 4. culture plate

Fig. 1 Schematic diagram of the research equipments for inhibiting of growth and developments of pests by low oxygen

Designs and observations of tests for the treatment group and the reference group

The volume contents of oxygen in the treatment group were 15% ±1%, 10% ±1% and 5% ±1% and the volume contents of oxygen in the reference group was 21% of that in the normal atmospheric environment.

The culture plates were taken out for testing the pests at fixed time every day, and the hatches observed and recorded, Exuviations, pupations and eclosions of the test pests were examined under a stereo microscope. The treatment groups were exposed to the gas environments after observations were finished every day to create the same low oxygen environment, and the dryer then seaed after each inspection.

Dada processing method

An SAS data processing software was used, and analysis of significance was performed with with the anova program. .

Result and Analysis

Inhibition effect of the low oxygen environment on TC, OS and SC in various stages

Under the conditions of temperature of 30 ±1℃ and 75% ±1% humidity is, the development history, hatch rate, pupation rate and eclosion rate of TC, OS and SC are shown in tables 1 to 3.

2.1.1 Hatch and development of eggs

In table 1, we can see that with the reduction of the environmental oxygen concentration, hatch rates of these three test pests were gradually reduced, and were reduced to 0 when the oxygen concentrationreached 5%. When the oxygen concentration was 15%, hatch rates of TC and OS were not effected, but the hatch rate of SC was inhibited. Table 1 also shows that the egg stages of these three test pests were prolonged under the low oxygen environment. When the oxygen concentration was 15%, it prolonged the egg stage of SC. When the oxygen concentration was 10%, the egg stages of TC and SC were prolonged ($a = 0.01$), but the prolongation of the egg stage of OS was not obvious. It is obvious that the low oxygen environments of 15%, 10% and 5% have certain lethal effects on eggs of these three test pests, and with the reduction of the oxygen concentration, the death rates of the eggs increased and reached 100% under the low oxygen environment of oxygen concentration of 5%. At the same time, with the reduction of the environmental oxygen concentration, the egg stages were gradually prolonged.

Table 1. Inhibition effect of the low oxygen environment on the eggs of TC, OS, and SC

O_2 (%)	Hatch rate (%)			Egg stage (d)		
	TC	OS	SC	TC	OS	SC
21	90.7 ±2.0A	75.0 ±5.0A	66.7 ±2.3A	3.5 ±0.1A	4.02 ±0.09A	5.5 ±1.0A
15	86.0 ±1.0A	73.3 ±3.3A	24.1 ±3.9B	3.6 ±0.1A	4.21 ±0.06A	6.7 ±0.5B
10	79.6 ±3.2A	20.0 ±3.3B	23.5 ±4.1B	4.0 ±0.1B	4.30 ±0.15A	7.4 ±0.7C
5	0	0	0	–	–	–

Note: The letter in the table represents the difference is very significant under the level of $a = 0.01$.

Pupation Rate of Larva and Larva Stage

From table 2, we can see that when the environmental oxygen concentrations were 15% and 10%, in comparison with the data of the reference group, the pupation rates of the larvae of TC were inhibited, but there was no significant difference between the results under these two concentrations; For OS when compared with the reference group, the oxygen concentration of 10% inhibited the pupation rates of OS. When the oxygen concentration was 15%, it inhibited the pupation rates of SC, and the larvae could not pupate. when the oxygen concentration was 10%. At the same time, with reduction of the environmental oxygen concentration, the larva stage was gradually prolonged. Therefore, with low oxygen environments of 15% and 10%, the larva stage of TC is prolonged for 1 day and 5 days respectively and there is a significant difference ($a = 0.05$). The effects on larva stages of OS and SC were relatively small. Therefore, the low oxygen environments of oxygen concentrations of15% and 10% have lethal effects on the larvae of these three test pests. With the reduction of the oxygen concentration, the pupation rates of larvae were reduced, i. e. the death rates increased.

Table 2. Inhibition effect of the low oxygen environment on the larvae of TC, OS and SC

O_2 (%)	Hatch rate(%)			Egg stage(d)		
	TC	OS	SC	TC	OS	SC
21	81.7 ±5.1a	97.2 ±1.9a	66.7 ±2.4a	28.3 ±1.7a	21.91 ±0.05a	16.2 ±0.9a
15	50.0 ±7.8b	89.4 ±2.0a	31.9 ±3.1b	29.1 ±1.7a	20.00 ±0.09a	18.1 ±0.9a
10	43.5 ±8.1b	66.7 ±0.1b	0c	33.1 ±1.6a	22.25 ±0.63a	–
5	–	–	0	–	–	–

Note: The letter in the table represents the difference is very significant under the level of $a = 0.05$.

Eclosion Rate of Pupa and Pupal Stage

From table 3, we can see that with the reduction of the environmental oxygen concentrations, the effects on eclosion rates and pupal stages of these three test pests are the same basically as with that the eggs and larvae; eclosion rates are reduced and pupal stages are prolonged. The obvious effect only occurs on pupae of OS treated with low oxygen environment of oxygen concentration of 10%.

Table 3. Inhibition effect of the low oxygen environment on the pupae of TC, OS and SC

O_2 (%)	Hatch rate(%)			Egg stage(d)		
	TC	OS	SC	TC	OS	SC
21	91.4 ±5.1a	85.1 ±2.9a	91.7 ±4.1a	5.3 ±0.3a	4.06 ±0.04a	5.2 ±0.6a
15	91.6 ±8.3a	71.1 ±2.0a	71.4 ±4.6a	5.5 ±0.7a	4.07 ±0.05a	5.9 ±0.6a
10	88.7 ±6.6a	57.1 ±3.1B	–	6.0 ±0.2a	4.50 ±0.50B	–
5	–	–	–	–	–	–

Note: The letter in the table represents the difference is very significant under the level of $a = 0.01$

Effects on Generations of TC, OS, SZ and SC

From Table 4, we can see that with the reduction of the environmental oxygen concentrations, the generation completion rates of these four test pests were gradually educed, and the development times of all the generations were gradually prolonged. . Under the low oxygen environment of 15%, the reductions of the generation completion rates of TC, OS, and SZ were not obvious. The generation completion rate of SC was reduced significantly; by 86. 5%; in comparison with the reference group. There were no significant inhibitions of generations in these four kinds. The generation completion rate of SC was 0 when the oxygen concentration was 10%, and the generation completion rates of other three kinds of the test pests were reduced significantly. Their generation were prolonged ($a = 0.05$). This shows that under the low oxygen environments of which oxygen concentrations are 15% and 10%, the growths and developments of these four kinds of test pests were inhibited, and under low oxygen environments of oxygen concentrations of 5%, these four kinds of the test pests could not complete their life histories. Under the low oxygen environment of oxygen concentrations of 10%, SC also could

not complete its life history.

Table 4. Inhibition effect of the low oxygen environment on the generations of TC, OS, and SC

O_2 (%)	Generation completion rate (%)				Generation (d)			
	TC	OS	SC	SZ	TC	OS	SC	SZ
21	56.3 ± 8.1a	66.7 ± 7.8a	40.8 ± 5.5a	48.0 ± 4.6a	34.7 ± 1.9a	29.91 ± 0.08a	26.9 ± 0.6a	34.2 ± 0.1a
15	37.5 ± 9.9ab	53.3 ± 5.7a	5.5 ± 1.6b	46.0 ± 4.3a	37.3 ± 2.3ab	30.33 ± 0.11a	30.7 ± 0.6a	34.4 ± 0.2a
10	29.6 ± 4.2b	6.7 ± 3.1b	0	27.0 ± 3.1b	41.4 ± 1.6b	31.00 ± 1.00b	–	36.5 ± 0.4b
5	0	0	0	–	–	–	–	–

Note: The letter in the table represents the difference is significant under the level of a = 0.05

Discussion

Many scholars have performed researches on pest killing by low oxygen [23,24]. Low oxygen not only can kill the pests, but also can effectively inhibit the growths and developments of pests. By observation on the development status of SZ under the environments of O_2 being 10%, CO_2 10% and N_2 80%, we found that the whole development period was prolonged by 10 – 11 days [25]. The low oxygen environment of oxygen concentration of 10% can result in the prolongation of the development period of the TC non – adult stage[26]. The research result of this article also shows the lethal effect and inhibition effect on growths and developments of the test pests. The ATP yields of pests are not enough under the low oxygen environment [27,28], and this is more obvious at the stages of hatch, exuviation, pupation and eclosion, since at these stages, the insects have high energy requirements. Thus the test pests accumulate enough energy through prolongation of the development period in the growth process; this may be the major reason for the prolongations of the development periods of the test pests.

Abroad, the process parameters of rapid stored grain pest killing by low oxygen are that when the oxygen concentration is less than 3%, the pests will be killed rapidly (above 15 days)[29], but the reports on the inhibition of growths and developments of stored gain pests by the low oxygen environment are few. The results of the present research are that, under the condition of keeping oxygen concentration in grain pile for a long time, such as keeping the oxygen concentration at 5% – 10% for more than 2 months, there is an inhibition effect on increasing populations of the stored grain pests in the grain pile, or the pests die gradually to realize the goal of pest controlling. Therefore, when low oxygen technology is extended and applied to inhibit stored grain pest populations, airtighness should be enhanced. Research and development should be performed on methods and technologies of keeping the low oxygen concentration in grain pile for a long time. This would reduce the application cost of the technology and stored grain pest control by low oxygen will have good development prospects in our country.

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Epicarp of *Citrus sinensis* (Osbeck): A Potential Source as A Fungitoxic and Insecticidal Fumigant for the Management of Storage Fungi and Pests

Neeta Sharma[1*] and Abhishek Tripathi[2]

Abstract: The essential oil extracted from the epicarp of *Citrus sinensis* was used as a potential source for the management of storage fungi and pests. The essential oil exhibited absolute fungitoxicity as fumigant against the storage fungal pathogens viz., *Alternaria alternata*, *Aspergillusniger*, *A. fumigates*, *A. ochraceous*, *Cladosporium cladosporiodes*, *Penicillium chrysogenum*, *P. citrinum*, *P. italicum*, *P. oxalicum*, *Rhizopus arrhizus* and *R. nigricans* in the 7.0 l/l air to 10.0 l/l air, range of concentration.. The oil also exhibited absolute insecticidal activity as fumigant against stored pests viz., *Sitophilus oryzae*, *Trogoderma granarium* and *Tribolium castaneum* at LD_{50} 15.0 l/l, 12.8 l/l and 10.9 l/l air concentration of oil respectively. The activity of the oil did not changed even at exposure to 100℃ temperature or autoclaving the oil. The oil also retained its activity after 24 months of storage. GC – MS studies of the oil revealed the presence of 10 chemical constituents. Limonene was found to be the major component (84.2 %). *Citrus sinensis* natural volatile could therefore be a safer fumigant than those currently used, to control storage fungi and pests.

Key words: *Citrus sinensis*, fungitoxic, insecticidal, fumigant, GC – MS.

Introduction

Storage fungi and pests are the major cause of production losses in stored commodities. Storage fungi are generally present as mycelia below the pericarp, or as dormant spores on the surface of seeds. They cause spoilage of stored foods through discoloration, loss of viability, heating and mustiness, biochemical changes leading to quality loss and production of toxins[1]. The postharvest losses and quality deterioration caused by storage pests are major problem throughout the world[2]. The intensification of food production has led to several problems in the postharvest phase including the major concern of pest infestation during storage. This is further aggravated by the increased attention paid to maintenance of buffer stocks to provide food security for a country. Pest problems have increased side by side with the increase in the quantity of stockpiled food and the longer duration of storage. Such pest problems are more acute in the tropics than in temperate zones because the environment in the former is more conducive to the growth and development of pests.

To control storage fungi, fungicide application is the usual practice. However, using synthetic chemicals to control these pathogens can cause carcinogenicity, teratogenicity, high and acute residual toxicity and other side effects on humans[3,4]. The development of resistance is also becoming a significant problem within the populations of the pathogen. due to the application of the synthetic fungicides[5,6].

Like fungicides synthetic insecticides have been successfully used to protect stored grains from insect infestations but their indiscriminate and massive use have created serious problems such as hazards to the environment including human health and non-target organisms[7], residues in food grains[8], environmental pollution[9,10], and development of resistant strains[11,12]. It would be highly desirable to find safe alternatives to synthetic insecticides to protect stored grains and grain products from insect infestations.

The negative consumer perception of chem ical preservatives drives attention towards natural alternatives. Particular interest is focused on the potential application of plant essential oils. Exytracts from plants have recently been of great interest. Their possible use as natural additives emerged from a growing tendency to replace synthetic antimicrobial agents with natural

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ones. Phyto-compounds are expected to be far more advantageous than synthetic pesticides due to their sheer magnitude of complexity, diversity and novelty of chemicals, reactions and phenomenon[13]. They are also bio-degradable in nature, non-pollutant and posses no residual or phytotoxic properties[14,15,16]. The general antifungal activity of essential oils is well documented[17,18]. Some of the essential oils have been reported to inhibit postharvest fungi under *in vitro* conditions[13,19,20,21,22]. In recent years, some pesticidal plants, e. g. *Azadirachta indica*, *Chrysanthemum cinerariaefolium* and *Carum carvi* have been receiving global attention and their secondary metabolites have been formulated as botanical pesticides in plant protection[23].

There are very few reports on the antifungal activity of the *Citrus sinensis* essential oil against different microbial species[22,24,25,26]. Effects of the citrus oils and some spices on the growth and aflatoxin production by *Aspergillus parasiticus* was reported by Karapinar[27]. Ernestina et al. [28] also reported fungicidal activity of citrus oil against the causal agent of the anthracnose disease on tropical fruits. An added advantage of some of the essential oils is their bioactivity in the vapour phase, a characteristic which makes them attractive as possible fumigants for stored product protection[16]. The findings thus indicate the possibility of exploiting *Citrus sinensis* essential oil as an effective inhibitor against the storage fungi and pests.

Recently, attention has been drawn to the possible use of plant products or plant derived compounds as promising alternatives to synthetic insecticides in controlling insect pests of stored products[29,30,31,32]. The effectiveness of many plant derivatives for use against stored grains pests has been reviewed by Golob and Webley [33] and Jacobson[34,35].

No reports have appeared are using *Citrussinensis* as fumigants against storage fungi and stored pests. The present findings thus, indicate the possibility of exploiting *Citrus sinensis* essential oil as a fumigant against storage-contaminating fungi and pests.

Materials and Methods

Extraction of Essential Oil

Fresh epicarp of *Citrus sinensis* (L.) Osbeck (Musambi) was collected from various juice shops of Lucknow, India during the months of May to October. The essential oil was extracted from collected material by hydro-distillation for 5h using a Clevenger-type apparatus[36]. A clear, light yellow, oily layer was obtained on the top of the aqueous distillate which was separated from the latter and dried with anhydrous sodium sulphate. The extracted essential oil was stored at 4℃ in air-tight sealed glass vials, covered with aluminum foil until further analysis.

GC – MS Analysis of Essential Oil

The GC – MS of essential oil was analysed on a Shimadzu QP – 2000 instrument at 70 eV and 250℃. GC Column: ULBON HR – 1 equivalent to OV – 1, utilizing a fused silica capillary – 0. 25mm 50M with film thickness – 0. 25. The GC – MS was operated at an initial temperature of 60℃ for 5 minutes and then heated at the rate of 5℃ per minute to 250℃. Carrier gas (helium) flow was 2 ml per minute. The identification of components was based on comparison of their mass spectra fragmentation patterns with those of Mass Spectrometry Data Centre, the Royal Society of Chemistry. U. K. (Eight Peak Index of Mass Spectra, 3rd Ed. 1983) and with those reported in the literature[37].

Fungal Species

Strains of organisms used were: *Alternariaalternata* MPPLU 01 (Aa), *Aspergillus niger* MPPLU 05 (An), *A. fumigates* MPPLU 07 (Af), *A. ochraceous* MPPLU 09 (Ao), *Cladosporium cladosporioides* MPPLU 14 (Cc), *Penicillium chrysogenum* MPPLU 27 (Pch), *P. citrinum* MPPLU 31 (Pci), *P. italicum* MPPLU 29 (Pit), *P. oxalicum* MPPLU 33 (Pox), *Rhizopus arrhizus* MPPLU 43 (Ra) and *R. nigricans* MPPLU 45 (Rn) from the collection of Mycology and Plant Pathology Division, Botany Department, University of Lucknow. The cultures of the phytopathogenic organisms were maintained on Potato Dextrose Agar (PDA) at 4℃.

Stored Pests

Stored pests viz. Rice weevil *Sitophilus oryzae* L. (Coleoptera: *Curculionidae*), Khapra beetle *Trogoderma granarium* Everts (Coleoptera: Dermestidae) and Red flour beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) were collected from private store houses of Lucknow district,. Mass cultures were maintained in earthen pots (2 kg) and/or large plastic containers (1. 5 kg) and sub – cultures in beakers (500 g) or small plastic containers (100 g) with the wheat as food medium. The beetles were allowed to grow in a natural environment as occurs in a traditional storehouse and were checked at regular intervals. A huge

number of beetles were thus reared to set a continuous supply of newly formed adults. The wheat seeds were thoroughly washed with tap water to remove dusts and other insect contamination present in the materials and carefully dried under sun-light, having 13% – 14% moisture content. Then the foods were kept in an incubator for about 24 hours at 60℃ to disinfest them. The sterile foods were then preserved in airtight glass jars (1000 ml) in order to impede further infestation.

Volatile Activity Assay of Essential Oil against Storage Fungi

Tests for the volatile activity of oil were carried out by inverted Petri dishes method in 90mm Petri dishes (Borosil) containing 20 ml of solidified PDA. A 5 mm diameter disc of the test fungus, cut from the periphery of an actively growing culture, was placed on the agar in each Petri dish and the dishes were kept in an inverted position. Sterilized cotton swab was placed on the upper lid of each of the inverted dishes. Different concentrations of oil were pipetted on cotton swab and were sealed by parafilm to check the release of volatile oil. For each corresponding control an equal amount of water was pipetted onto the sterilized cotton swab. The inverted Petri dishes were incubated at 25 ± 1℃ for 7 days. Fungitoxicity was expressed in terms of percentage of mycelia growth inhibition and calculated according to the formula of Pandey et al.[38] (1982).

Percentage of mycelial growth inhibition = [dc – dt/dc 100], where dc = Average diameter of fungal colony in control and dt = Average diameter of fungal colony in treatment.

Volatile Activity Assay of Essential Oil against Storage Pests

Glass vials (5.5 cm long by 2 cm in diameter), capped with polypropylene stoppers were used for the bioassay. Pests were transferred to the vials in groups of 15 adults along with the food material. The vials were covered with fine nylon cloth secured with adhesive tape. The vials containing the insects were then turned upside down over the vials containing the oil such that the oil vapours saturated the atmosphere of the containers containing the pests. The control consisted of a similar setup but without essential oil. This procedure was replicated three times. The vials were placed at room temperature with a photoperiod of 14 h light and 10 h dark. Mortality was determined after 24 h of treatment. The LD_{50} and LD_{95} values were calculated by Probit analysis[39]. Control mortality was accounted by Abbott's[40] formula.

Effect of Temperature and Autoclaving on Fungitoxicity and Insecticidal Activity of Oil

Experiments were performed to determine the thermostable or thermolabile nature of the oil. Different glass vials containing five ml oil each were subjected to different temperature treatments for three hours in incubators previously adjusted to 40, 60, 80 and 100℃. Antifungal activity of oil was also tested after autoclaving it at 121℃ for 15 min. The glass vials were then allowed to cool down to room temperature and the fungitoxicity of the treated oil from each set was tested at its MIC against the test fungus using the volatile activity assay. Insecticidal activity of oil was determined in a same manner.

Effect of Storage or Self-life on Fungitoxicity and Insecticidal Activity of oil

Experiments were undertaken to ascertain the duration for which the oil can be stored without loosing its fungitoxicity. Five ml of essential oil was stored in an air tight glass vial at room temperatures (20℃ to38℃ ± 2℃). The fungitoxicity of the stored oil at its MIC was tested at regular intervals of 2 months using the volatile activity assay. The insecticidal activity of oil was determined in a similar manner.

Results and Discussion

Extract from epicarp (waste product) of *C. sinensis* by hydro-distillation yielded 1.8 % essential oil. GC – MS analysis of the oil led to identification of 10 components. The main components of the *C. sinensis* epicarp essential oil studied and their percentages are presented in Table 1, the major constituents were Limonene (84.2 %), Linalol (4.4%) and Myrcene (4.1%).

The activity of the oil was tested against storage fungi by the volatile activity assay. In this assay the oil shows better activity[22]. The *Citrus sinensis* essential oil exhibited absolute fungitoxicity as fumigant against the storage fungal pathogens viz., *Alternaria alternata*, *Aspergillusniger*, *A. fumigates*, *A. ochraceous*, *Cladosporium cladosporiodes*, *Penicillium chrysogenum*, *P. citrinum*, *P. italicum*, *P. oxalicum*, *Rhizopus arrhizus* and *R. nigricans* in the 7.0 L/L air to 10.0 L/L air, range of concentration. (Table, 2), which is less than many of essential oils tested reviously by different workers[38,41,42,43]. The findings of our studies indicate that the essential oil of *C. sinensis* is an effective fungitoxicant a-

gainst storage fungi.

The oil also exhibited absolute insecticidal activity as fumigant against stored pests viz., *Sitophilus oryzae*, *Trogoderma granarium* and *Tribolium castaneum* at LD_{50} 15.0 L/L, 12.8 L/L and 10.9 L/L air concentration of oil respectively (Table 5). A large number of essential oils extracted from various spice and herbaceous plants have already been screened for toxicity as potential fumigants. Toxicity of a number of monoterpenes has been evaluated against various stored-product insects. Coats et al. [44] found that exposure of *S. oryzae* for 24 h to linalool and *d*-limonene had an LC_{50} of 14 and 19 L/L air whereas the LC_{50}'s for myrcene and α-terpineol were >100 μL/L. Citrus sinensis essential oil showed potent toxicity to all the stored pests tested. The primary component of the essential oil was limonene which was found to be the principal toxic constituent. Therefore this oil may show a promise as an alternative to fumigants currently used to control storage-grain insect pests.

The thermostability of the oil was tested by the volatile activity assay at MIC against storage fungi. It was found that at temperature ranging from 40 – 100℃ and even after autoclaving the oil, at 121℃ for 15 min, its activity was not altered (Table, 3). The thermostability of the oil was also tested against stored pests and it was found that the oil was effective even after receiving treatments of temperature (data not shown). The efficacy of essential oil was determined in terms of percent inhibition of mycelial growth of storage fungi after various storage periods using the volatile activity assay at MIC. It was observed that the oil retained its fungitoxicity even after 24 months of storage of (Table, 4). Similarly the oil did not alter its activity against all the stored pests tested (data not shown).

Table 1. Components of *Citrus sinensis* (L.) Osbeck epicarp essential oil identified by GC – MS

Peak No.	Components	Retention time (scan)	Percentage in total oil
1	α – pinene	12.36(192)	0.9
2	β – pinene	13.70(232)	0.6
3	Myrcene	14.60(259)	4.1
4	Limonene	16.60(319)	84.2
5	Linalol	18.60(379)	4.4
6	Citral	19.96(420)	0.5
7	α – Terpineol	21.10(454)	0.8
8	Terpinolene	21.56(468)	1.3
9	Citronellal	22.93(509)	1.9
10	Geraniol	23.86 (537)	1.3

Table 2. Effect of different concentrations of *Citrus sinensis* oil on per cent radial growth inhibition of different storage fungi at 25 1℃ using volatile activity assay (VA).

Conc. of oil (μL/L air)	Percent radial growth inhibition										
	Aa	An	Af	Ao	Cc	Pch	Pci	Pit	Pox	Ra	Rn
1.0	12.3	12.9	8.9	10.7	9.6	11.3	15.8	13.6	16.7	9.4	12.6
2.0	20.1	26.5	19.6	23.6	15.6	18.9	23.1	25.3	28.9	18.7	19.7
3.0	35.6	41.2	31.3	34.9	31.3	30.6	33.9	36.5	39.6	29.7	30.4
4.0	59.3	59.6	45.8	51.0	43.6	49.8	53.8	58.9	55.6	43.2	39.8
5.0	73.5	80.1	66.9	63.7	54.6	71.5	70.4	73.1	71.3	57.5	53.7
6.0	97.8	96.3	81.5	77.9	83.6	85.9	81.3	88.7	84.6	69.3	67.2
7.0	100	100	95.6	86.9	100	100	96.7	100	97.7	74.8	76.4
8.0	100	100	100	98.6	100	100	100	100	100	88.6	90.6
9.0	100	100	100	100	100	100	100	100	100	97.8	98.2

Conc. of oil (μL/L air)	Percent radial growth inhibition										
	Aa	An	Af	Ao	Cc	Pch	Pci	Pit	Pox	Ra	Rn
10.0	100	100	100	100	100	100	100	100	100	100	100
11.0	100	100	100	100	100	100	100	100	100	100	100

Table 3. Effect of different temperature treatments and autoclaving on fungitoxicity of *Citrus sinensis* oil (at MIC) against different storage fungi incubated at 25 1℃ using volatile activity assay (VA).

Temperature (℃)	Percent inhibition of mycelial growth										
	Aa	An	Af	Ao	Cc	Pch	Pci	Pit	Pox	Ra	Rn
40	100	100	100	100	100	100	100	100	100	100	100
50	100	100	100	100	100	100	100	100	100	100	100
60	100	100	100	100	100	100	100	100	100	100	100
70	100	100	100	100	100	100	100	100	100	100	100
80	100	100	100	100	100	100	100	100	100	100	100
90	100	100	100	100	100	100	100	100	100	100	100
100	100	100	100	100	100	100	100	100	100	100	100
autoclaving (121℃for 15 min)	100	100	100	100	100	100	100	100	100	100	100

Table 4. Effect of different storage periods on fungitoxicity of *Citrus sinensis* oil at MIC against different storage fungi incubated at 25 ± 1℃ using volatile activity assay.

Storage period (months)	Percent inhibition of mycelial growth										
	Aa	An	Af	Ao	Cc	Pch	Pci	Pit	Pox	Ra	Rn
6	100	100	100	100	100	100	100	100	100	100	100
8	100	100	100	100	100	100	100	100	100	100	100
10	100	100	100	100	100	100	100	100	100	100	100
12	100	100	100	100	100	100	100	100	100	100	100
14	100	100	100	100	100	100	100	100	100	100	100
16	100	100	100	100	100	100	100	100	100	100	100
18	100	100	100	100	100	100	100	100	100	100	100
20	100	100	100	100	100	100	100	100	100	100	100
22	100	100	100	100	100	100	100	100	100	100	100
24	100	100	100	100	100	100	100	100	100	100	100

Table 5. Fumigant toxicity of *Citrus sinensis* essential oil against the three stored pests.

Stored pest	LD_{50} (95% FL[b]) (μL/L air)	LD_{95} (95% FL) (μL/L air)	Slope Chi – Square (χ^2)
Sitophilus oryzae 15.0(10.5 – 19.6)	26.4 (16.9 – 35.9)	1.26	0.22
*Trogoderma granarium*12.8(9.4 – 16.2)	21.8(18.3 – 25.3)	0.98	2.11
*Tribolium castaneum*10.9(8.6 – 13.2)	17.7(12.2 – 23.2)	1.830.78	

[b]FL indicates fiducial limits.

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0109

Development of the Red Flour Beetle *Tribolium castaneum* (Herbst) at a Reduced Oxygen Atmosphere

Qu Guiqiang, Cao Yang* and Li Yanyu

Abstract: In the present study, the development of the red flour beetle Tribolium castaneum (Herbst) was conducted at 10% oxygen in nitrogen. The length of survival of the various stages of the insect, were recorded. Following a statistical analysis, , the results indicated that at 10% oxygen atmosphere can suppress the development of red flour beetles effectively, especially the egg and the larva. But the pupa stage showed more tolerance to reduced oxygen atmosphere. By comparing the amount of males and females we can conclude that the female red flour beetle is more sensitive to the reduced oxygen atmosphere than the male.

Key words: red flour beetle, reduced oxygen atmosphere, development, survival

Introduction

Controlled atmosphere (CA) disinfestation technology involves the alteration of the proportions of the natural storage gases – CO_2, O_2 and N_2 to render the atmosphere in the stores unfavorable to pests. CA does not involves use of fumigants such as phosphine or methyl bromide, nor alteration of the humidity and temperature of the environment [1].

Most researchers have concentrated on the acute mortality effect on insects under a low concentration of oxygen, and obtained a satisfactory results that a prolonged low oxygen atmosphere can kill most stored grain insects. [2,3,4] However, the sub lethal effects of CAs on insects, such as delayed development, impaired metamorphosis and altered fecundity, have not been well documented [3]. It is possible to arrest insect pest development and minimize damages [4,5]. Information on insects under a low oxygen atmosphere development is important, since this information can be referred to a number of ways such as calculating and deciding the sealing time in a field treatment of CA.

Reports indicate that insect development under a low oxygen concentration atmosphere is slower and weaker as compared with those developed in a normal atmosphere[6]. Spratt (1979) reported that under an atmosphere of 10% O_2, 10% CO_2 and 80% N_2, the development of maize weevil was about 10 – 11 days longer than the normal one [7].

As an important stored grain insect, the development of the red flour beetle can cause serious damage. The present study was carried out to study the development of the red flour beetle at a reduced oxygen atmosphere.

Materials and Methods

Insect Culture

About 500 – 800 adult red four beetles were placed in several 1 L jars which contained approximately 300 g of flour and 5% yeast (by weight). Every three days the jars were sieved for eggs removal. The adults were kept for oviposition for up to one month.

Experimental method

Gas mixing equipment is shown in Fig. 1. The development of the red flour beetle was carried out in atmospheres containing 10 1% O_2 by volume with N_2 as a balance at 30℃. The flow rates of O_2 and N_2 were 180 mL/h and 1600 mL/h respectively. Normal air atmosphere served as control.

The red flour beetle eggs were obtained from 2000 adults placed in 1 L jars that contained approximately 300 g of flour and 5% yeast (by weight). After one day of oviposition, the jars were sieved for egg removal.

The egg exposure device was constructed of plastic; the height and diameter were 8 mm and 100 mm respectively. A plate with 54 holes (diameter of 5mm). was glued on the bottom of each device to prevent insect escaping. The

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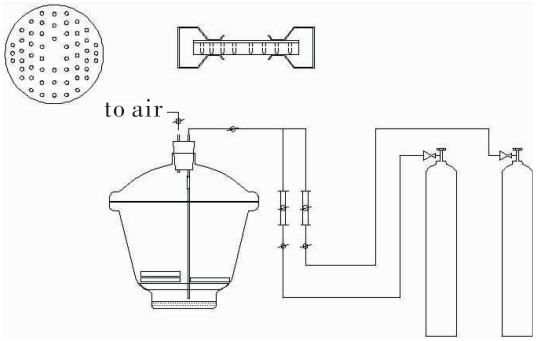

Fig. 1 **the experimental equipment**

eggs were placed one by on in each hole.

The treated and control group both contained 3 devices with eggs for each replicate. After putting them in the relevant desiccators which contained saturated NaCl solution to create a 75% relative humidity at 30℃, the desiccators were sealed and the atmosphere of treated group were modified and the concentration of oxygen was measured by gas chromatography.

After 24 h exposure, the insects were checked by using a binocular microscope.

Results

Insect Development

Table 1**. Development times required for the three life stages of the red flour beetle at** 10% O_2 **and** 30℃**.**

Stage	Group	(%)	Period (d)	p – value for period
		Hatching ratio		
egg	treated	69.0	4.13 ±0.47	0.0007
	control	79.6	3.96 ±0.26	
		Pupation ratio		
larva	treated	31.5	48.17 ±13.98	0.0076
	control	43.0	41.51 ±9.41	
		Emergence ratio		
pupa	treated	80.0	5.79 ±0.74	0.4042
	control	96.0	6.02 ±1.37	

Observation on 162 eggs placed in both treated and control group showed that 111 eggs hatched in the treated group, and 129 eggs hatched in the control group. The egg hatch ratio of treated group was 69.0%, and in the control group was 79.6%. This indicates that an atmosphere of 10% oxygen concentration has suppressing affect on egg hatch of the red flour beetle.

The average development period of egg stage in the treated group (4.13 ± 0.47 d), was longer than the control group (3.96 ± 0.26d) in. P – values = 0.0007 < 0.05 indicated that low oxygen atmosphere can effectively prolong the period of egg development of the red flour beetle.

There were 35 larvae pupating in the treated group containing 111 larvae. There were 56 larvae pupating in the control group containing 129 larvae. The pupation ratio of the treated group was (31.5%) lower than in the control group (43.4%). This indicates that an atmosphere of 10% oxygen can suppress the pupation ratio of the red flour beetle.

The average development period of the larval stage in the treated group was (48.17 ± 13.98d) longer than in control group (41.51 ± 9.41 d). P – values = 0.0076 < 0.05 indicated that a low oxygen atmosphere can effectively prolong the period of larval development of the red flour beetle.

There were28 pupae that emerged e in the treated group, and 54 pupae that emerged in the control. The emergence ratio of treated group was (80.0%) lower than in the control group (96.0%). This indicates that an atmosphere of 10% oxygen concentration can effectively suppress the red flour beetle emergence.

The average development period of the pupal stage in treated group was (5.79 ± 0.74 d) shorter than n the control group (6.02 ± 1.37 d). But the P – values = 0.4042 > 0.05 indicated that low oxygen atmosphere did not affect pupal development period of the red flour beetle.

Sex Ratio

In the treated group, 29 pupae emerged.

There were 7 females and 22 males, 25% and 75% of the total amount individually, which indicates a sex ratio of approximately 1: 3. In the control group, the number of females and the males were 25 and 29 individually, 46% and 54% of the total amount of emergence respectively, which indicated a sex ratio of about 1: 1.

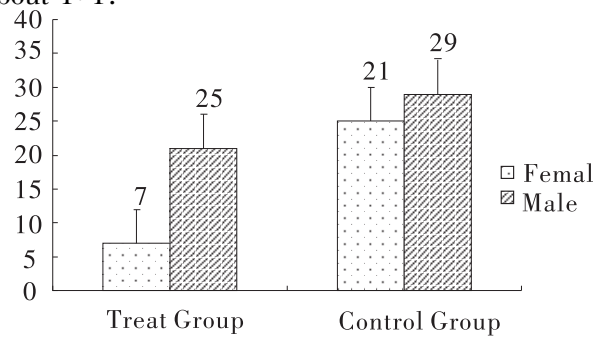

Fig. 2 **Variations of sex ratio between treated** (10% O_2) **and control groups at** 30℃

These results indicate that if the red flour beetle can survive and develop in a a reduced oxygen concentration atmosphere, more females will be killed before reaching the adult stage, than males. , This indicates the female is more susceptible than the male, and that a low oxygen atmosphere obviously has an cumulative lethal effect on the female red flour beetle.

Discussion

According to the comparison of the development periods in the treated and control group, there was a prolonged egg and larval period in the treated group. The approximate length of the pupal period in both groups showed a significant resistance of pupae under the reduced oxygen concentration atmosphere in comparison with the other two stages which was already shown in previous research[8,9]. The above differences can be explained by the pupa which is in a static stage of metamorphosis. This stage is considered as a less energy demanding period. Thus a relatively low respiration rate could affect the need for oxygen. Contrarily, the egg stage which is also a static stage during metamorphosis was more susceptible than pupae in this study.

The explanation may be found in the work of Emekci et al. , (2002) who showed that the RQ in a 10% oxygen atmosphere for the red flour beetle egg, larva and pupa were 0. 96, 1. 00 and 0. 78, respectively. This reflects a protein-carbohydrate metabolism for egg, carbohydrate metabolism for larva and protein-lipid metabolism for pupa [10]. Lipid is an effective energy resource; a complete lipid metabolism can produce 129 ATP, which is more than by carbohydrate or protein metabolism. , The lipid metabolism of pupae probably ensures its survival in a reduced oxygen atmosphere, without being significantly affected by the lack of oxygen.

During the present study, the female of the red flour beetles showed a higher sensitivity than the males, from which we can conclude that there are some dissimilarities in development between the sexes of red flour beetle in a reduced oxygen atmosphere. Further research is needed to explain these results.

In the treated group, unusual moulting of the larva was observed. Skin separation from the tail was earlier compared to control. It was also observed that many old instar larvae remained with a smaller body size compared with the control. More research on the impact of a reduced oxygen atmosphere on tissue development, endocrine system and cell development is needed.

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0110

Biochemical Mechanisms of Phosphine Action and Resistance

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Abstract: Global grain shortages highlight the strategic requirement for dependable storage of food reserves. While phosphine fumigation has been the mainstay of grain protection for many years, resistance in pest insects threatens the continued effectiveness of phosphine. Effective management of phosphine resistance requires an understanding of how phosphine acts as well as how organisms can overcome its action. Despite the importance of phosphine, its mode of action has not been determined. To resolve this problem, we have investigated the toxicology of phosphine in the model organism, *C. elegans*, which is ideal for laboratory studies. We see that mitochondrial activity is directly related to the effectiveness of phosphine, as mutation, chemical and physical treatments that reduced respiration cause resistance. In contrast, chemicals that activate mitochondrial electron transfer enhance phosphine toxicity, allowing resistance to be completely overcome. We have found that key metabolic regulatory pathways that determine food abundance or deprivation are likewise capable of enhancing sensitivity toward phosphine, even in otherwise resistant organisms, apparently through regulation of metabolic rate. Our studies reflect and extend previous work with insects that demonstrated a requirement for oxygen and a role for mitochondria in phosphine toxicity. These results will assist us in the development of strategies to monitor resistance or enhance the efficacy of phosphine fumigation.

Key words: Phosphine, mitochondria, oxygen toxicity, reactive oxygen species, *Caenorhabditis elegans*.

Introduction

Phosphine, hydrogen phosphide (PH_3), is a poisonous gas used for fumigation of stored commodities. Currently it is the only fumigant with worldwide registration (MBTOC 2006). Its continued and widespread use is due to its low cost, rapid diffusion through a grain mass, ease of generation and use, and lack of production of toxic by-products which leaves grain free of harmful residues (Chaudhry 1997).

Despite the importance of phosphine, the mechanism of its toxicity remains poorly understood. It has been demonstrated that phosphine toxicity is dependent on the presence of molecular oxygen in both insects and nematodes (Bond 1963; Cheng *et al.* 2003; Kashi 1981). Aerobic respiration is also shown to be inhibited in a variety of species following phosphine administration (Pimentel *et al.* 2007). It is hypothesized that complex IV of mitochondrial respiratory chain (MRC), *cytochrome c oxidase*, is the primary site of action of phosphine (Chefurka 1976; Nakakita 1976). The inhibition of this mitochondrial enzyme induces the elevated production of reactive oxygen species (ROS) which caused damage to DNA, lipids, and protein molecules (Salvioli *et al.* 2001).

Phosphine exposure, both *in vitro* and *in vivo*, results in lipid peroxidation, which is believed to be the consequence of superoxide(s) generated from mitochondria. Furthermore, this lipid oxidation process was enhanced by the presence of transition metal ions such as iron (Cha'on *et al.* 2007; Qian and Buettner 1999). It is therefore not surprising that glutathione (GSH), a cellular antioxidant that can protect cells against oxidative damage has been shown to protect cells against phosphine (Hsu *et al.* 1998; Quistad *et al.* 2000).

The mechanisms whereby insects become resistant to phosphine are even less well understood than the mode of action of phosphine. Resistance to phosphine was first reported in pest insects of stored products in 1977 (Champ and Dyte 1977). Recent studies reveal that the situation is getting worse, as extremely resistant pest insects have been reported from around the world (141 in China (Li *et al.* 1994); 380 in India (Rajendran and Narasimhan 1994); 600

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in Australia (Collins *et al.* 2002); > 770 in Japan (Hori and Kasaishi 2005); and 1160 in Brazil (Athie *et al.* 1998)). Increasing phosphine resistance forces grain handlers to increase the fumigation time and/or phosphine concentration, which increases occupational health risks and results in increased cost. Greater understating of the phosphine toxicity and resistance mechanisms will allow more effective management of pest insects and containment of the spread of resistance.

In order to gain more understanding, we initiated the investigation of phosphine action in the model organism, a nematode, *Caenorhabditis elegans*. *C. elegans* was selected due to its rapid lifecycle, small size, small genome, ease of culture, and availability of genetic resources. We have created a phosphine-resistant line (*pre* – 33) in *C. elegans*, which is 9 times more resistant to phosphine than is the wild-type strain (Cheng *et al.* 2003) and as resistant to phosphine as most highly resistant insect lines.

Phosphine Toxicity Requires Oxygen

One of the first tasks in developing *C. elegans* as a model for the study of phosphine toxicity was to confirm that phosphine acts in *C. elegans* as it does in insects. A key feature of phosphine toxicity in insects is that oxygen not only enhances toxicity, but in atmospheres of less than 2% oxygen, phosphine is completely harmless (Bond 1963; Kashi 1981). As with insects, we found that oxygen enhances phosphine toxicity in *C. elegans* as wild-type animals showed a dose-dependent increase in mortality with increasing oxygen concentration even though the phosphine concentration was maintained at a low level (0.1mg/L) (Cheng *et al.* 2003). Very interestingly, the *pre* – 33 mutant line that was resistant toward phosphine was also completely resistant to the synergistic enhancement of phosphine toxicity by high oxygen levels (Fig 1). Even when the phosphine concentration was increased to 0.6mg/L, there was no significant increase in mortality. This result suggests that phosphine toxicity might be mediated through oxidative stress since it is dependent on the presence of oxygen.

Synchronized 42 h – old nematodes were treated with 0.1 mg/Lphosphine at 25 C for 24 h at the indicated concentrations of oxygen. The effect of hyperoxia on *pre* – 33 under 0.6mg/L phosphine is also shown as a dashed line. All results are the average of two experiments.

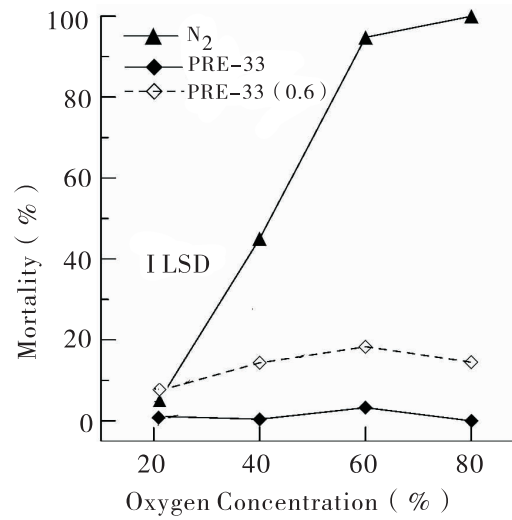

Fig. 1 The effect of oxygen concentration on phosphine – induced mortality in *C. elegans*

Differences between data points greater than the LSD bar on the left side of the figure are considered to be significant. Note 1 mg/L phosphine equals approximately 700ppm.

Phosphine Inhibits Mitochondrial Activity

Mitochondria are the proposed target of phosphine as demonstrated by *in vitro* studies of mitochondria isolated from pest insects (Chefurka 1976). We took advantage of *C. elegans* to test whether mitochondrial function is likewise disturbed by phosphine in live animals (Zuryn *et al.* 2008). We found that the respiration rate was inhibited by 70% within 1 hour of phosphine exposure in wild-type animals (Fig. 2). Paradoxically, the respiration rate of the *pre* – 33 mutant is much lower than in wild-type animals in the absence of phosphine exposure. Phosphine exposure, however, failed to lower the respiration rate any further than was observed for the wild type strain (Fig. 2). This presented the unusual situation that the metabolic disruption caused by phosphine exposure in wild type animals was also observed in mutant animals that were resistant to phosphine exposure. To resolve this apparent contradiction, we then measured the mitochondrial membrane potential in these animals. In the wild type strain, phosphine caused a decrease in mitochondrial membrane potential (MMP) after 5 hours as determined by fluorescence of the dye tetramethylrhodamine ethyl ester (TMRE; Sigma) (Fig. 3). TMRE is a cell permeable cationic dye which accumulates in intact mitochondria in proportion to the MMP (Ehrenberg *et al.* 1988). A similar decrease in MMP was ob-

served in mutant animals, but because they had a higher basal MMP, the net result was that membrane potential was preserved in these animals relative to wild type under equivalent exposure to phosphine (Fig. 3). Thus, the reduction in mitochondrial respiration in mutant animals was likely a side-effect of a metabolic change elsewhere that allowed them to survive despite reduced mitochondrial activity.

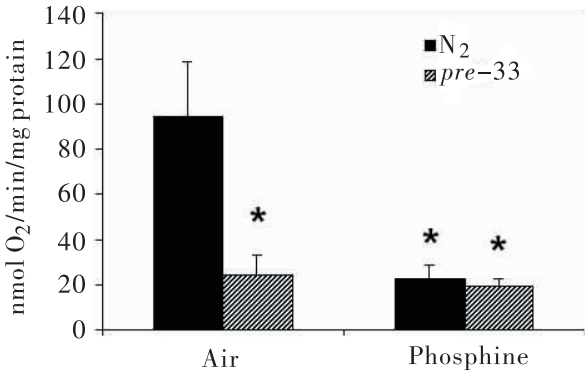

Fig. 2 Oxygen consumption rates in phosphine – treated nematodes.

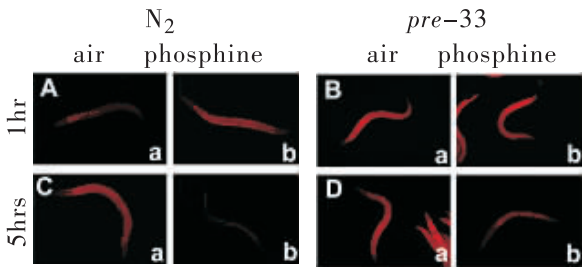

Fig. 3 Photographs of TMRE – stained nematodes exposed to phosphine.

The mutant phenotype strongly supports the metabolic theory of phosphine toxicity, by which factors that reduce the flow of electrons through the mitochondrial electron transport chain result in phosphine resistance, whereas factors that increase electron flow are proposed to enhance phosphine toxicity (Schlipalius *et al.* 2006). Preliminary work in our lab has indeed identified powerful phosphine synergists that completely overcome the resistance of *pre*-33 (unpublished). Another interesting point in the study of Zuryn *et al.* is that maintenance of the MMP despite reduced electron flow through the mitochondrial electron transport chain seems to be critical for the survival of phosphine resistant nematodes. Thus, the apparent paradox referred to above can be explained by proposing two distinct mechanisms of phosphine toxicity, the first of which is oxidative stress resulting from the interaction between phosphine and a high rate of electron flow through the mitochondrial electron transport chain, and the second of which is phosphine-induced collapse of the mitochondrial membrane potential. Collapse of the membrane potential is known in other organisms to trigger cellular suicide (apoptosis). In a multi-cellular organism, this serves to sacrifice individual cells that are metabolically defective in an effort to preserve other cells of the organism. In the case of phosphine exposure, the apoptotic response would be triggered in every cell of the organism, resulting in cataclysmic cell death.

Exposure to phosphine at 20℃ for 1 h reduces the respiration rate in N_2 nematodes *in vivo*. Phosphine resistant mutant *pre*-33 have lower respiration rates than normal and are therefore less affected by the phosphine exposure. Phosphine treatment was 70 ppm (sublethal for 24 h) on 48 h – old nematodes. $^{*}p < 0.05$ is significant differences compared with N_2 animals in air. There was no significant difference between *pre*-33 nematodes exposed to air alone and *pre*-33 nematodes exposed to phosphine. Columns represent means of three independent experiments with error bars representing the SEM. Note 1 mg/L phosphine equals approximately 700ppm.

The dye TMRE was used to qualitatively assess the mitochondrial membrane potential (MMP) of nematodes exposed to 350ppm phosphine at 20℃. Wild type (N_2) animals (A, C) exposed to phosphine (b) had a much lower MMP than their counterparts in air (a). This was a similar scenario to what was observed in *pre*-33 mutant nematodes (B, D), which are resistant to phosphine-induced mortality. Note that *pre*-33 nematodes have a higher basal MMP compared with N_2 nematodes both in air and following phosphine exposure in most cases. Note 1 mg/L phosphine equals approximately 700ppm.

Phosphine and Diethyl Maleate Synergism

Glutathione (GSH) is a cellular antioxidant compound that protects against oxidative stress. Valmas and Ebert (2006) first reported the synergistic effect of phosphine and the GSH depleting compound diethyl maleate, clearly implicating oxidative stress in phosphine toxicity in *C. elegans*. The LD_{50} of phosphine and diethyl maleate co-treatment is doubled compared to diethyl maleate alone (Fig. 4). It has been reported phosphine is able to inhibit the activity of the antioxidant enzymes, catalase and peroxi-

dase in both resistant and sensitive insects (Chaudhry and Price 1992). Such a situation would leave GSH as one of the primary remaining defences against phosphine-induced oxidative stress. This finding supports the hypothesis that phosphine exposure results in lethal oxidative damage. Phosphine has actually been shown to exacerbate the problem of mitochondrially-produced reactive oxidants because phosphine and hydrogen peroxide can interact to form the much more highly reactive hydroxyl radical that readily reacts with lipids and other key molecules in the cell (Quistad *et al.* 2000).

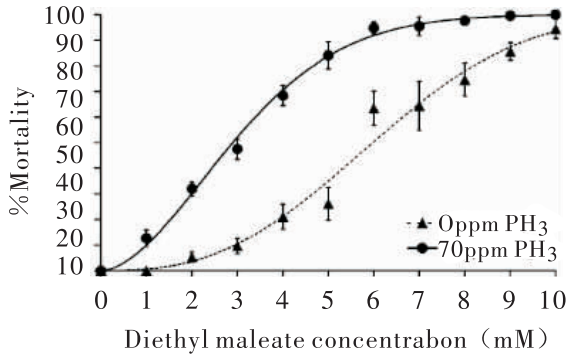

Fig. 4 Diethyl Maleate Interaction with Phosphine.

Mortality of wild-type (N_2) *C. elegans* when exposed to diethyl maleate and two different doses of phosphine: 0 ppm (▲); and 70 ppm (·); at 20℃ for 24 h. Regressions lines are based on complementary log – log/log relationships, and data points are weighted means from biological replicates ± weighted SEM. The LC50 of DEM in the absence of phosphine at 20℃ for N2 is 5.98 mM; and with 70 ppm PH_3 is 2.9 mM. All plates were counted after 24 h recovery. Note 1 mg/L phosphine equals approximately 700ppm.

Disruption of Iron Homeostasis Incre Ases Phosphine Toxicity

Metabolic disruption is not the only possible mechanism of action of phosphine, because phosphine also interacts strongly with specific metal ions, such as iron which are essential for the activity of a large number of cellular enzymes. Because iron is toxic when it is free in the cell as opposed to bound up in enzymes, a special storage protein, ferritin, ensures that iron is available while preventing it from doing harm. We supposed that phosphine-mediated release of iron from the ferritin store might contribute to the toxicity of phosphine. This hypothesis is supported by phosphine-mediated induction of ferritin gene expression. Fig. 5 clearly shows the dose-dependent induction of ferritin mRNA in response to phosphine exposure (Cha'on *et al.* 2007).

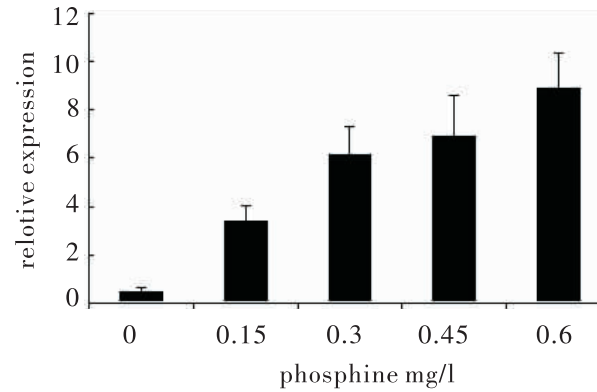

Fig. 5 Ferritin – 1 induction by phosphine.

42 h old adults N_2 nematodes were exposed to phosphine at different concentrations (0, 0.15, 0.3, 0.45 and 0.6 mg/L) for 10 h at 20℃. The level of ferritin – 1 mRNA is analysed by quantitative real-time PCR. Columns are means of five biological replicates. Error bars are SEM. Note 1 mg/L phosphine equals approximately 700ppm.

Furthermore, *C. elegans* is hypersensitive to phosphine under conditions of iron overload (Fig 6). Whereas an increase in iron concentration in the absence of phosphine caused no significant increase in mortality, the same iron concentrations resulted in dose-dependent mortality in the presence of an otherwise sub-lethal dose of phosphine. Finally, the iron released from ferritin in response to phosphine exposure results in lipid peroxidation, a hallmark of phosphine toxicity (Cha'on *et al.* 2007).

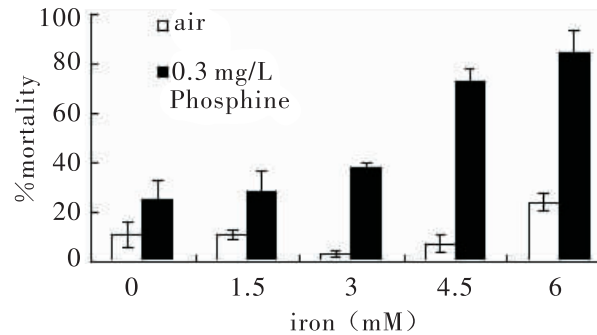

Fig. 6 Iron overload increased phosphine toxicity in *C. elegans*.

24h – aged N_2 nematodes were transferred to normal medium and iron-rich medium (1.5, 3, 4.5, 6 mM). Animals were left over night before exposed to either air or air with 0.3 mg/L phosphine for 24 hr at 20℃. The mor-

tality of those exposed to air was investigated immediately after 24 h exposure, whereas those under phosphine exposure were examined after a 24 h recovery period. Columns are means of three biological replicates. Error bars are SEM. Note 1 mg/L phosphine equals approximately 700ppm.

Conclusion

The mode of action of phosphine toxicity has been unclear and the mechanism of phosphine resistance is even less well studied. We have confirmed and extended our understanding of the biochemical mechanisms using the model organism *C. elegans*, in combination with genetic analysis of phosphine resistance in both insects and *C. elegans*. We have confirmed that phosphine toxicity is dependent on the availability of molecular oxygen and that the toxicity is elevated in response to increasing concentrations of oxygen as is consistent with the oxidative stress model of phosphine toxicity. We also demonstrated that mitochondrial function is disrupted by phosphine and that a key distinction between sensitive and resistant animals is the ability of the latter to maintain mitochondrial membrane potential in the face of phosphine exposure. Compounds that greatly enhance the toxicity of phosphine were identified that exploit each of these toxicity mechanisms. We also found that iron homeostasis has an impact on phosphine toxicity. Even though the influence of iron release on phosphine toxicity was much smaller than the other two mechanisms, the existence of high level genetic resistance among pest insects indicates that the primary mechanisms of phosphine action have been overcome. It is entirely likely that we now rely on secondary modes of action of phosphine for pest control in the field. Thus, understanding both primary and secondary mechanisms of phosphine action will be essential if we are to maintain the effectiveness of phosphine for years to come.

Acknowledgements

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0111

Insecticidal Effect of Ozone against *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium confusum* Jacquelin Du Val (Coleoptera: Tenebrionidae): Influence of Commodity

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Abstract: Laboratory experiments were carried out in order to assess the insecticidal efficacy of ozone, against three major stored-grain beetle species, the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and the confused flour beetle *Tribolium confusum* Jacquelin Du Val (Coleoptera: Tenebrionidae). The insects were exposed for 2, 4, 6 and 8 h, in vials treated with ozone at two dose rates, 115 and 55 ppm. At the highest dose rate, mortality of *R. dominica* and *S. oryzae* adults was close to 60% after 2 h of exposure, while 2 h later, all adults were dead. For *T. confusum* adults, mortality was negligible after 2 h of exposure, but reached 100% at the 6 h exposure interval. For *T. confusum* larvae, approx. 10% of the exposed individuals were dead after 2 h of exposure, while at the 4 h interval reached 70%. In vials treated with the low concentration, mortality was low, especially for *T. confusum* adults, where almost all the exposed individuals survived. The presence of wheat or maize in the vials reduced mortality, for all species tested, but there were no differences in mortality levels between these two commodities. Moreover, for all species, mortality levels varied at different distances from the ozone input point.

Key words: ozone, *Rhyzopertha dominica*, *Sitophilus oryzae*, *Tribolium confusum*

Introduction

The range of insecticides that are currently used on stored grain and related commodities is becoming narrower, due to newer regulatory and legislation issues, aiming to reduce risk from pesticide residues on food, occupational exposure risks to workers and adverse effects on the environment. Among these pesticides, methyl bromide, a widely used fumigant for stored-product insect control, has been identified as an ozone depleter by the Montreal Protocol, resulting in its phase out. Phosphine (aluminium phosphide), the main alternative to methyl bromide, it is extremely toxic to mammals, and cannot be used in all types of facilities, since it is highly corrosive to certain mineral materials. On the other hand, other alternative insecticides, such as organophosphorus compounds, are slow acting in comparison with methyl bromide, and many of these substances are toxic to mammals and leave residues on food. Moreover, many stored product insect species are now resistant to phosphine and other substances[3], while several species are now considered as allergens and mycotoxin carriers, which seriously endanger human health[1,5,8]. Loss of fumigants, resistance to remaining fumigants and the consumers' demand for residue and contaminant-free food require the use of new, reduced – risk substances.

Ozone can be generated by electrical discharges in air and is currently used in the medical industry as a disinfectant against microorganisms, as a means of reducing odor, and for removing taste, colour, and environmental pollutants in industrial applications[7,9]. McKenzie et al.[11] noted that ozone treatment reduced the toxic effect of aflatoxin-contaminated maize fed to turkey pullets. Also, at 5 ppm, ozone inhibited surface growth, sporulation, and mycotoxin production by cultures of *Aspergillus flavus* and *Fusarium moniliforme*[10]. One other attractive characteristic of ozone is that it decomposes rapidly (half-life of 20 50 min) to molecular oxygen without leaving a residue. The use of ozone generators eliminates the need for handling, storage, and disposal problems of conventionally used fumigants and other pesticides. These characteristics make ozone an attractive candidate for controlling insects and fungi in stored grain. Erdman[4] observed mortality of

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larvae of the red flour beetle, *Tribolium castaneum* (*Herbst*) (*Coleoptera*: *Tenebrionidae*), *and* the confused flour beetle, *Tribolium confusum* Jacquelin Du Val (Coleoptera: Tenebrionidae), after exposure to 45 ppm of ozone. In the laboratory, 5 ppm of ozone resulted in 100% mortality of adult saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), and *T. confusum*[9,10]. In the present study, we tested ozone against three major stored – grain beetle species, in empty containers, and in containers that contained grain.

Materials and Methods

Insects Commodities and Ozone Generator

The species tested were the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) and *T. confusum*. The first two species were reared in the laboratory on whole wheat kernels, while the third was reared on wheat flour plus brewer's yeast (5% by weight). All species were kept at 25℃ and 65℃. Adults were used in the tests with *S. oryzae* and *R. dominica*, while for *T. confusum*, both adults and larvae were tested. Untreated wheat (var. Mexa) and maize (var. Dias) were used in the tests. The generator type used was Ambiozon, Ser. 2000F (Ambiozon SL, Madrid, Spain). Two dose rates 115 and 55 ppm of ozone were used.

Bioassays and Analysis

The tests were carried out in cylindrical columns, which could be separated in 12 equal segments. These columns were 70 cm high and 8 cm in diameter. Between two adjacent segments, there was a hole of 2.5 cm in diameter, covered with fine mesh, in order to prevent insects from moving among the segments. The ozonation was conducted from a small hole at the bottom of the column, from the first segment (segment 1). In each segment, 30 insects were placed separately for each species or life stage. These individuals were exposed for 2, 4, 6, and 8 h, and after the termination of each interval, mortality was counted. There were three types of bioassays. In the first bioassay, the columns were without grain and contained only insects, while in the other two bioassays the columns contained maize or wheat, respectively. The entire procedure was repeated three times., For the data analysis, the GLM-proc or SAS[13] was used separately for each species/stage. Insect mortality was the response variable and segment, exposure, dose and column containment were the treatment variables.. Means were separated by the Tukey-Kramer HSD test.

Results

Mortality of *R. dominica* Adults

From the main effects, only dose and exposure were significant, while interactions were not significant (Table 1). In the empty columns, at the high dose, after 2 d of exposure, mortality was similar in all segments, and ranged between 60 and 79% (Table 2). After 4 h of exposure, mortality was high (>93%), while all adults were dead at the 6 – h exposure interval. At the same dose, in the columns that contained maize or wheat, mortality was notably reduced, especially in the last segments. Hence, after 2 h of exposure, more than two thirds or half of the exposed individuals were still alive in segment 1, for maize and wheat, respectively, while mortality was negligible in the last segment (segment 12). At longer exposure intervals, mortality was increased, and reached 100%, but reduced mortality levels were also noted in the last segments. At the low dose, mortality was notably lower in comparison with the respective figures for the high dose, and did not exceed 65%. Nevertheless, mortality was also reduced when the column contained the two types of grains.

Mortality of *S. oryzae* Adults

For *S. oryzae* adults, only dose was significant (Table 1). The mortality levels noted were similar to those recorded for *R. dominica* adults (Table 3). At the high ozone concentration, after 2 h of exposure in the empty columns, mortality ranged between 63 to 74%, and was similar in all segments. All adults were dead after 6 d of exposure. At the same dose, in columns that contained grains, mortality was reduced after short exposures (2 – 4 h), especially in segments 6 – 12. After 8 h of exposure, survival occurred in segments 10 – 12 and 9 – 12, for maize and wheat, respectively. At the low ozone concentration, mortality was generally lower than the respective figures of *R. dominica*, especially in the case of the two types of grains, where all adults survived in most of the segments examined.

Mortality of *T. confusum* Larvae

All main effects, regardless of the segment, were significant (Table 1). Moreover, dose x exposure and treatment x dose were also

significant. In the empty columns, at the high dose and after 2 d of exposure, mortality was lower in comparison with the previous species. Mortality was similar in all segments (Table 4). All larvae were dead after 6 d of exposure. On the other hand, at the 2 h exposure interval, mortality was negligible in the columns that contained grains, and did not exceed 65% at the 6 h interval. At the 8 h exposure, with the exception of segments 1 – 3 with maize, survival occurred in all segments examined. At the low ozone concentration, for all three treatments, all adults survived after 4 h of exposure. At the 8 h exposure, mortality did not exceed 33, 10 and 14%, in empty columns, columns with maize and columns with wheat, respectively.

Mortality of *T. confusum* Adults

As in the case of larvae, treatment, dose, dose x exposure and treatment x dose were significant (Table 1). Mortality was generally lower in comparison with *T. confusum* larvae (Table 5). At the high concentration, after 2 h in empty columns, mortality was extremely low, but 2 h later ranged between 60% – 78%. All adults were dead at the 8 h exposure. In columns that contained grains, at the 8h exposure, with the exception of segments 1 – 3 in maize, survival occurred in all segments examined, while it was only 30 and 21% in segment 12, for maize and wheat, respectively. At the low concentration, practically, no mortality was recorded.

Discussion

The present study indicates that the efficacy of ozone is greatly affected by the target species, life stage, exposure interval, dose rate and the presence of commodity. Furthermore, we examined relatively shorter exposure intervals in comparison with the majority of the previous available studies. For instance, Kells et al. [6] reported 92% – 100% mortality at 50 ppm after 3 d of exposure, for adults of the maize weevil, *Sitophilus zeamais* (Motsch.), adults of *T. castaneum* and larvae of the Indian meal moth, *Plodia interpunctella* (H bner) (Lepidoptera: Pyralidae). In that study, the authors tested ozone in maize, and noted that ozone fumigation had two phases: rapid degradation and slow movement in the grain mass, and reduced degradation with free movement. The latter phase is considered as a direct result of saturation[6]. In our study, regardless of the species tested, the presence of grain caused a significant reduction of mortality, suggesting that the rate of degradation was high, despite the fact that ozone flow was continuous. On the other hand, in the empty column, mortality was high, especially in the case of *R. dominica* and *S. oryzae*. Also, the presence of fine material may also negatively affect ozone efficacy[6,12]. Based on the present results, at least in some of the combinations tested, 115 pm of ozone can be effective even after 8 h, and in some cases, even after 6 h of exposure. In contrast, the reduction of concentration to 55 ppm was not effective, indicating that longer exposures are needed at this ozone level.

Apart from mortality in the entire column, ozone efficacy was notably varied among different column parts. Thus, the efficacy of ozone was reduced with the increase of distance from the ozone introduction point. This trend was expressed more vigorously in the case of maize and wheat, while there was little or no effect in the empty column. Generator performance is affected by several factors, and, at least in the present study, any fluctuations were not 'corrected'' by the continuous ozone flow. Ozone concentration is decreased with depth[6]; therefore the introduction of ozone from the lowest part of a grain mass may cause delayed effectiveness of ozone. However, most insect populations exist at the top layer of bulked grains[2], and for this reason, the introduction of ozone from the top may be advantageous. Nevertheless, populations can often be established in lowest layers[14]. From a practical standpoint, the use of fans or other air circulation techniques can assist ozone penetration[12]. Generally, ozone mortality was similar for the two types of grains, suggesting that the mechanisms that reduce ozone efficacy are similar for both maize and wheat. This is in accordance with previous reports by Kells et al. [6,] and Mendez et al. [12], where penetration was similar for both maize and wheat, despite the fact that the porosity of these grains is different.

From the species tested, adults of *R. dominica* and *S. oryzae* was much more susceptible than adults and larvae of *T. confusum*. Also, the rate of reduction in mortality level of *T. confusum* larvae was increased from segment 1 to segment 12 in comparison with *R. dominica* and *S. oryzae*. The latter two species are characterized as primary pests, which mean that they are able to damage sound grain kernels, and allow the secondary pests, such as *T. confusum* to

continue the infestation. For this reason, the increased susceptibility of *R. dominica* and *S. oryzae* to ozone can be considered as an important characteristic, since fast mortality reduces the concomitant damage by the secondary colonizers. However, the immature stages of these species develop inside the grain kernel, and, given that ozone is not as penetrating as other gases, such as phosphine, it is expected that these life stages will remain unaffected. Maier et al. [9] noted that a carefully designed ozonation system could be compatible with other fumigation techniques, such as phosphine, at the same exposure interval. This system should provide proper penetration, and continuous standardized flow to adjust variations in gas concentration. The results of the present work show that ozone, at increased concentrations, is effective at shorter exposures, especially in empty areas, but also into the grain mass. This characteristic, in combination with the other advantages of ozone, such its fungicidal effect and the fact that ozonation does not affect the final product[9], makes ozone a good candidate for further evaluation. For its wider use, additional experimental work is required to establish the feasibility of ozonation, in conjunction with cost considerations.

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Table 1. ANOVA parameters for main effects and interactions for the species tested (total df = 863)

		R. dominica		S. oryzae		T. confusum (larvae)		T. confusum (adults)	
Source	*df*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Treatment	2	0.43	0.6464	0.22	0.8025	11.58	<0.0001	7.15	0.0009
Dose	1	118.47	<0.0001	130.56	<0.0001	84.30	<0.0001	77.84	<0.0001
Treatment x dose	2	0.62	0.5372	0.17	0.8393	11.27	<0.0001	5.85	0.0030
Segment	11	0.22	0.9960	0.18	0.9983	0.39	0.9581	0.40	0.9553
Treatment x segment	22	0.03	0.9999	0.03	0.9999	0.07	0.9999	0.05	0.9999
Dose x segment	11	0. ,05	0.9999	0.09	0.9999	0.16	0.9991	0.24	0.9937
Exposure	3	3.86	0.0093	1.69	0.1667	8.04	<0.0001	12.69	<0.0001
Treatment x exposure	6	0.19	0.9773	0.01	0.9999	0.19	0.9769	0.95	0.4567
Dose x exposure	3	2.53	0.0562	1.27	0.2812	5.98	0.0005	11.42	<0.0001
Segment x exposure	33	0.01	0.9999	0.01	0.9999	0.08	0.9999	0.06	0.9999

Table 2. Mean mortality (% ± SE) of *R. dominica* adults after exposure to the ozone – treated column (within each row, for each column containment and dose, means followed by the same letter are not significantly different; where no letters exist, no significant differences were noted; HSD test at 0.05)

Segment (1 input, 12 output)	Empty column (115 ppm)				Maize (115 ppm)				Wheat (115 ppm)			
Exposure (h)	2h	4h	6h	8h	2h	4h	6h	8h	2h	4h	6h	8h
1	63.2 ±7.3a	100 ±0.0b	100 ±0.0b	100 ±0.0b	66.6 ±7.3a	100 ±0.0b	100 ±0.0b	100 ±0.0b	50.4 ±8.9a	100 ±0.0b	100 ±0.0b	100 ±0.0b
2	73.4 ±3.4a	100 ±0.0b	100 ±0.0b	100 ±0.0b	62.2 ±4.3a	100 ±0.0b	100 ±0.0b	100 ±0.0b	50.9 ±7.9a	100 ±0.0b	100 ±0.0b	100 ±0.0b
3	72.3 ±2.6	96.4 ±2.1b	100 ±0.0b	100 ±0.0b	60.0 ±5.4a	97.4 ±1.2b	100 ±0.0b	100 ±0.0b	42.7 ±10.4a	98.4 ±1.1b	100 ±0.0b	100 ±0.0b
4	79.0 ±4.5a	100 ±0.0b	100 ±0.0b	100 ±0.0b	47.9 ±7.8a	94.3 ±3.9b	100 ±0.0b	100 ±0.0b	30.2 ±5.4a	90.3 ±3.9b	100 ±0.0c	100 ±0.0c
5	62.5 ±7.4a	96.5 ±2.3b	100 ±0.0b	100 ±0.0b	53.4 ±6.5a	90.4 ±8.1b	100 ±0.0b	100 ±0.0b	26.3 ±7.8a	90.2 ±7.1b	100 ±0.0c	100 ±0.0c
6	71.3 ±5.3a	97.4 ±2.4b	100 ±0.0b	100 ±0.0b	50.4 ±4.8a	95.6 ±3.2b	100 ±0.0b	100 ±0.0b	21.5 ±9.5a	87.4 ±5.9b	100 ±0.0c	100 ±0.0c

Segment (1 input, 12 output)	Empty column(115 ppm)				Maize(115 ppm)				Wheat (115 ppm)			
7	60.4 ±12.6a	95.4 ±3.0b	100 ±0.0b	100 ±0.0b	46.2 ±8.3a	80.0 ±5.9b	100 ±0.0c	100 ±0.0c	24.5 ±7.5a	70.4 ±5.6b	100 ±0.0c	100 ±0.0c
8	65.4 ±3.2a	96.5 ±2.1b	100 ±0.0b	100 ±0.0b	36.4 ±5.4a	77.6 ±7.9b	100 ±0.0c	100 ±0.0c	20.3 ±5.8a	73.7 ±7.9b	100 ±0.0c	100 ±0.0c
9	63.5 ±7.9a	96.5 ±2.0b	100 ±0.0b	100 ±0.0b	36.5 ±4.5a	80.9 ±10.4b	100 ±0.0c	100 ±0.0c	13.4 ±4.4a	63.9 ±5.9b	96.7 ±2.2c	100 ±0.0c
10	70.3 ±4.0a	100 ±0.0b	100 ±0.0b	100 ±0.0b	20.3 ±6.5a	72.5 ±8.9b	97.4 ±2.1c	100 ±0.0c	6.8 ±3.0a	56.9 ±9.9b	93.0 ±4.3c	100 ±0.0c
11	63.6 ±4.4a	93.5 ±3.9b	100 ±0.0b	100 ±0.0b	10.9 ±4.3a	47.9 ±8.5b	84.3 ±10.9c	90.4 ±5.5c	3.5 ±2.1a	41.4 ±8.9b	90.2 ±5.1c	92.6 ±5.2c
12	62.8 ±6.9a	100 ±0.0b	100 ±0.0b	100 ±0.0b	3.3 ±2.3a	36.0 ±8.0b	68.4 ±8.3c	83.3 ±6.4c	0.0 ±0.0a	40.3 ±9.0b	89.3 ±9.0c	100 ±0.0c

Segment (1 input, 12 output)	Empty column(55 ppm)				Maize(55 ppm)				Wheat (55 ppm)			
Exposure (h)	2h	4h	6h	8h	2h	4h	6h	8h	2h	4h	6h	8h
1	0.0 ±0.0a	16.9 ±5.8b	30.5 ±5.4b	53.4 ±7.8c	0.0 ±0.0a	6.5 ±4.3b	15.7 ±6.7bc	30.4 ±8.9c	0.0 ±0.0a	3.5 ±2.1ab	13.5 ±5.4bc	23.4 ±7.5c
2	0.0 ±0.0a	13.9 ±4.3b	40.5 ±5.5c	60.0 ±8.5d	0.0 ±0.0a	3.4 ±2.3ab	12.4 ±4.4bc	25.5 ±5.9c	0.0 ±0.0a	3.6 ±2.5ab	13.4 ±6.5bc	23.3 ±6.5c
3	0.0 ±0.0a	23.3 ±5.4b	40.0 ±6.4c	65.0 ±8.4d	0.0 ±0.0a	3.5 ±1.4ab	10.6 ±5.6bc	22.3 ±6.5c	0.0 ±0.0a	0.0 ±0.0a	6.5 ±2.3b	14.6 ±7.0b
4	0.0 ±0.0a	10.3 ±5.4b	36.9 ±8.4c	60.4 ±10.5d	0.0 ±0.0a	3.5 ±1.3ab	11.5 ±7.6bc	20.3 ±4.5c	0.0 ±0.0a	0.0 ±0.0a	6.5 ±2.5b	13.4 ±5.6b
5	0.0 ±0.0a	10.3 ±4.6b	35.4 ±7.4c	63.0 ±4.5d	0.0 ±0.0a	3.0 ±2.1ab	6.7 ±2.6b	16.9 ±4.4c	0.0 ±0.0a	0.0 ±0.0a	3.7 ±1.4ab	10.3 ±5.1b
6	0.0 ±0.0a	12.1 ±4.7b	36.6 ±8.4c	62.4 ±8.4d	0.0 ±0.0a	0.0 ±0.0a	3.0 ±2.0ab	10.3 ±5.1b	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	5.5 ±3.2
7	0.0 ±0.0a	16.4 ±6.5b	37.5 ±6.5c	60.3 ±5.5d	0.0 ±0.0a	0.0 ±0.0a	3.3 ±1.7a	13.5 ±4.5b	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	2.5 ±2.0
8	0.0 ±0.0a	12.3 ±6.5b	23.0 ±7.0b	40.5 ±7.4c	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
9	0.0 ±0.0a	13.9 ±7.5b	30.3 ±8.9b	56.5 ±9.0c	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
10	0.0 ±0.0a	16.5 ±7.4b	40.3 ±6.8c	65.1 ±7.8d	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
11	0.0 ±0.0a	20.2 ±6.4b	43.4 ±4.3c	70.3 ±9.6d	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
12	0.0 ±0.0a	16.4 ±5.4b	36.5 ±7.0c	60.4 ±9.0d	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0

Table 3. Mean mortality (% ± SE) of S. *oryzae* adults after exposure to the ozone – treated column (within each row, for each column containment and dose, means followed by the same letter are not significantly different; where no letters exist, no significant differences were noted; HSD test at 0.05)

Segment (1 input, 12 output)	Empty column (115 ppm)				Maize (115 ppm)				Wheat (115 ppm)			
Exposure (h)	2h	4h	6h	8h	2h	4h	6h	8h	2h	4h	6h	8h
1	71.3 ±9.0a	100 ±0.0b	100 ±0.0b	100 ±0.0b	73.6 ±5.6a	100 ±0.0b	100 ±0.0b	100 ±0.0b	76.5 ±9.5a	100 ±0.0b	100 ±0.0b	100 ±0.0b
2	64.3 ±5.4a	95.6 ±3.5b	100 ±0.0b	100 ±0.0b	69.6 ±8.7a	100 ±0.0b	100 ±0.0b	100 ±0.0b	73.5 ±8.6a	100 ±0.0b	100 ±0.0b	100 ±0.0b
3	73.4 ±8.5a	100 ±0.0b	100 ±0.0b	100 ±0.0b	70.5 ±7.6a	100 ±0.0b	100 ±0.0b	100 ±0.0b	62.4 ±5.4a	100 ±0.0b	100 ±0.0b	100 ±0.0b
4	63.4 ±7.4a	96.4 ±3.2b	100 ±0.0b	100 ±0.0b	63.2 ±8.6a	100 ±0.0b	100 ±0.0b	100 ±0.0b	63.2 ±5.4a	96.8 ±2.1b	100 ±0.0b	100 ±0.0b
5	63.3 ±7.9a	100 ±0.0b	100 ±0.0b	100 ±0.0b	69.6 ±7.6a	100 ±0.0b	100 ±0.0b	100 ±0.0b	63.9 ±6.5a	95.4 ±2.3b	100 ±0.0b	100 ±0.0b
6	56.4 ±4.5a	95.5 ±2.3b	100 ±0.0b	100 ±0.0b	53.4 ±10.0a	100 ±0.0b	100 ±0.0b	100 ±0.0b	60.4 ±7.9a	90.4 ±5.4b	100 ±0.0c	100 ±0.0c
7	65.4 ±7.9a	100 ±0.0b	100 ±0.0b	100 ±0.0b	49.5 ±5.4a	93.4 ±5.5b	97.6 ±1.2b	100 ±0.0b	60.9 ±10.5a	86.5 ±6.8b	100 ±0.0c	100 ±0.0c
8	74.6 ±11.6a	100 ±0.0b	100 ±0.0b	100 ±0.0b	44.4 ±6.5a	90.3 ±5.4bc	97.6 ±1.2cd	100 ±0.0d	56.5 ±8.9a	83.4 ±7.0b	92.4 ±6.3bc	100 ±0.0c
9	74.3 ±8.5a	100 ±0.0b	100 ±0.0b	100 ±0.0b	38.7 ±7.5a	90.0 ±5.7bc	93.4 ±4.0cd	100 ±0.0d	55.5 ±7.5a	80.5 ±10.5b	91.5 ±3.4b	94.6 ±2.9b
10	63.5 ±8.5a	96.5 ±3.0b	100 ±0.0b	100 ±0.0b	25.4 ±8.5a	60.5 ±8.9b	73.5 ±5.4b	88.7 ±6.5c	53.4 ±10.4a	70.5 ±6.5ab	88.4 ±7.4bc	90.4 ±4.6c
11	63.4 ±8.9a	96.6 ±2.2b	100 ±0.0b	100 ±0.0b	30.4 ±7.6a	60.9 ±5.6b	69.8 ±9.3b	75.4 ±7.5b	50.4 ±8.9a	69.8 ±12.5ab	75.4 ±6.8b	82.2 ±5.7b
12	73.2 ±11.5a	100 ±0.0b	100 ±0.0b	100 ±0.0b	20.3 ±5.9a	50.0 ±6.5b	65.6 ±5.2c	73.4 ±6.5c	49.5 ±9.5a	64.5 ±5.4ab	73.4 ±6.7b	77.7 ±8.0b
Segment (1 input, 12 output)	Empty column (55 ppm)				Maize (55 ppm)				Wheat (55 ppm)			
Exposure (h)	2h	4h	6h	8h	2h	4h	6h	8h	2h	4h	6h	8h
1	0.0 ±0.0a	3.4 ±2.1ab	3.4 ±2.3ab	8.7 ±4.3b	2.1 ±1.3a	5.4 ±3.2ab	13.4 ±5.4bc	23.4 ±7.9c	0.0 ±0.0a	5.4 ±3.5ab	12.4 ±3.5bc	22.7 ±7.0c
2	0.0 ±0.0a	6.5 ±3.1b	13.4 ±4.5bc	20.0 ±5.3c	0.0 ±0.0a	3.2 ±2.0a	5.4 ±3.7ab	9.5 ±4.3b	0.0 ±0.0a	7.5 ±3.5b	8.5 ±4.3b	15.4 ±5.6b

Segment (1 input, 12 output)	Empty column(115 ppm)				Maize(115 ppm)				Wheat (115 ppm)			
3	0.0 ±0.0a	3.5 ±2.1a	16.5 ±5.5b	32.2 ±5.9c	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	3.5 ±2.4	0.0 ±0.0a	0.0 ±0.0a	2.5 ±1.5a	12.0 ±5.1b
4	0.0 ±0.0a	10.5 ±5.1b	13.0 ±5.9b	22.5 ±4.8b	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	3.4 ±1.4
5	0.0 ±0.0a	6.9 ±3.4b	17.4 ±5.4bc	30.0 ±8.5c	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
6	0.0 ±0.0a	6.0 ±3.1b	10.5 ±4.3bc	25.8 ±8.5c	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
7	0.0 ±0.0a	6.7. ±2.8b	12.3 ±5.1bc	22.1 ±5.9c	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
8	0.0 ±0.0a	4.8 ±3.1b	17.9 ±7.8c	25.4 ±6.5c	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
9	0.0 ±0.0a	9.5 ±5.4b	19.7 ±4.8bc	32.6 ±12.4c	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
10	0.0 ±0.0a	5.4 ±2.5b	13.4 ±5.4bc	37.7 ±5.8c	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
11	0.0 ±0.0a	6.1 ±3.2b	18.5 ±5.9c	29.8 ±7.5c	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
12	0.0 ±0.0a	3.0 ±1.4a	15.4 ±5.7b	24.6 ±6.8b	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0

Table 4. Mean mortality(% ± SE) of *T. confusum* larvae after exposure to the ozone – treated column(within each row, for each column containment and dose, means followed by the same letter are not significantly different; where no letters exist, no significant differences were noted; HSD test at 0.05)

Segment (1 input, 12 output)	Empty column(115 ppm)				Maize(115 ppm)				Wheat (115 ppm)			
Exposure (h)	2h	4h	6h	8h	2h	4h	6h	8h	2h	4h	6h	8h
1	42.5 ±5.9a	70.9 ±5.4b	100 ±0.0c	100 ±0.0c	0.0 ±0.0a	30.4 ±5.7b	60.4 ±12.5c	100 ±0.0d	0.0 ±0.0a	25.4 ±6.5b	57.4 ±8.5c	98.9 ±0.8d
2	52.9 ±8.0a	77.4 ±8.5b	100 ±0.0c	100 ±0.0c	5.4 ±3.2a	32.5 ±7.0b	64.6 ±10.6c	100 ±0.0d	0.0 ±0.0a	27.8 ±6.5b	59.5 ±7.5c	90.4 ±7.4d
3	44.5 ±6.5a	70.3 ±5.4b	100 ±0.0c	100 ±0.0c	0.0 ±0.0a	25.7 ±7.9b	55.5 ±7.9c	100 ±0.0d	0.0 ±0.0a	22.5 ±9.0b	53.3 ±9.1c	85.4 ±5.9d
4	55.4 ±6.8a	78.9 ±5.4b	100 ±0.0c	100 ±0.0c	0.0 ±0.0a	25.8 ±8.6b	58.6 ±6.9c	92.1 ±5.4d	3.2 ±2.1a	22.4 ±6.5b	50.2 ±5.4c	83.2 ±6.0d
5	54.5 ±6.8a	88.0 ±5.7b	100 ±0.0c	100 ±0.0c	0.0 ±0.0a	20.5 ±6.0b	48.0 ±8.4c	84.5 ±5.4d	3.0 ±1.7a	23.5 ±7.4b	44.3 ±5.1c	78.5 ±7.0d

Segment (1 input, 12 output)	Empty column(115 ppm)				Maize(115 ppm)				Wheat (115 ppm)			
6	50.6 ±8.9a	89.0 ±6.0b	100 ±0.0c	100 ±0.0c	0.0 ±0.0a	23.2 ±5.6b	44.4 ±7.6c	80.8 ±5.9d	0.0 ±0.0a	19.5 ±6.9b	40.9 ±5.4c	65.4 ±8.5d
7	49.7 ±7.7a	87.5 ±7.8b	100 ±0.0c	100 ±0.0c	0.0 ±0.0a	18.3 ±6.3b	33.8 ±7.5b	63.5 ±5.0c	0.0 ±0.0a	17.5 ±8.4b	33.4 ±5.1b	62.5 ±5.4c
8	44.5 ±6.8a	79.6 ±6.6b	100 ±0.0c	100 ±0.0c	0.0 ±0.0a	10.7 ±5.4b	25.4 ±5.4c	63.0 ±10.3d	0.0 ±0.0a	15.4 ±7.3b	30.9 ±5.4b	52.5 ±7.8c
9	42.6 ±7.6a	81.4 ±8.7b	100 ±0.0c	100 ±0.0c	2.4 ±1.5a	18.5 ±5.8b	30.4 ±7.4b	62.8 ±7.4c	0.0 ±0.0a	12.5 ±4.6b	27.5 ±5.4c	41.5 ±8.2c
10	44.6 ±7.6a	78.7 ±4.5b	100 ±0.0c	100 ±0.0c	2.1 ±1.2a	12.5 ±5.1b	25.4 ±6.5b	53.0 ±8.9c	0.0 ±0.0a	6.2 ±3.2b	19.5 ±5.4c	34.4 ±4.5d
11	52.5 ±8.0a	72.5 ±8.0b	100 ±0.0c	100 ±0.0c	0.0 ±0.0a	11.3 ±3.4b	15.4 ±5.7b	34.1 ±6.5c	0.0 ±0.0a	5.4 ±3.9b	16.1 ±6.7bc	30.4 ±7.5c
12	50.6 ±7.4a	74.6 ±5.7b	100 ±0.0c	100 ±0.0c	0.0 ±0.0a	6.5 ±2.9b	11.4 ±3.9b	31.1 ±9.4c	0.0 ±0.0a	2.3 ±1.4a	5.5 ±3.9a	20.0 ±5.9b

Segment (1 input, 12 output)	Empty column(55 ppm)				Maize(55 ppm)				Wheat (55 ppm)			
Exposure (h)	2h	4h	6h	8h	2h	4h	6h	8h	2h	4h	6h	8h
1	0.0 ±0.0a	0.0 ±0.0a	2.3 ±1.5a	32.4 ±6.5b	0.0 ±0.0a	0.0 ±0.0a	2.3 ±1.3ab	9.5 ±5.4b	0.0 ±0.0a	0.0 ±0.0a	12.1 ±3.4b	12.5 ±4.5b
2	0.0 ±0.0a	0.0 ±0.0a	5.0 ±2.3a	29.5 ±5.4b	0.0 ±0.0a	0.0 ±0.0a	0.0 ±0.0a	8.5 ±3.2b	0.0 ±0.0a	0.0 ±0.0a	8.9 ±3.4b	11.2 ±3.2b
3	0.0 ±0.0a	0.0 ±0.0a	7.5 ±4.7b	32.5 ±5.4c	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	4.3 ±2.3	0.0 ±0.0a	0.0 ±0.0a	7.7 ±3.4b	13.3 ±3.5b
4	0.0 ±0.0a	0.0 ±0.0a	3.5 ±2.3a	29.4 ±4.3b	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	3.2 ±2.3	0.0 ±0.0a	0.0 ±0.0a	5.4 ±2.7ab	13.3 ±3.5b
5	0.0 ±0.0a	0.0 ±0.0a	3.4 ±2.3a	32.4 ±5.4b	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0a	0.0 ±0.0a	9.0 ±3.6b	9.8 ±4.4b
6	0.0 ±0.0a	0.0 ±0.0a	10.1 ±4.6b	20.4 ±5.4b	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	3.1 ±2.0
7	0.0 ±0.0a	0.0 ±0.0a	9.0 ±5.4b	22.4 ±8.6b	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
8	0.0 ±0.0a	0.0 ±0.0a	7.4 ±4.3b	21.0 ±9.0b	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
9	0.0 ±0.0a	0.0 ±0.0a	9.0 ±4.5b	18.9 ±8.1b	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
10	0.0 ±0.0a	0.0 ±0.0a	5.4 ±2.9a	23.3 ±6.7b	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
11	0.0 ±0.0a	0.0 ±0.0a	0.0 ±0.0a	20.2 ±5.8b	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
12	0.0 ±0.0a	0.0 ±0.0a	1.5 ±0.9a	22.4 ±6.5b	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0

Table 5. Mean mortality (% ± SE) of *T. confusum* adults after exposure to the ozone – treated column (within each row, for each column containment and dose, means followed by the same letter are not significantly different; where no letters exist, no significant differences were noted; HSD test at 0.05)

Segment (1 input, 12 output)	Empty column (115 ppm)				Maize (115 ppm)				Wheat (115 ppm)			
Exposure (h)	2h	4h	6h	8h	2h	4h	6h	8h	2h	4h	6h	8h
1	2.5 ± 1.4a	77.7 ± 6.5b	100 ± 0.0c	100 ± 0.0c	0.0 ± 0.0a	32.6 ± 6.5b	64.5 ± 7.5c	100 ± 0.0d	0.0 ± 0.0a	27.8 ± 5.6b	56.4 ± 5.6c	97.8 ± 0.9d
2	3.2 ± 1.5a	72.5 ± 8.9b	100 ± 0.0c	100 ± 0.0c	5.5 ± 2.3a	34.5 ± 6.5b	61.6 ± 4.9c	100 ± 0.0d	0.0 ± 0.0a	25.6 ± 7.1b	54.5 ± 4.3c	92.5 ± 3.6d
3	0.0 ± 0.0a	74.6 ± 6.3b	100 ± 0.0c	100 ± 0.0c	0.0 ± 0.0a	27.5 ± 8.4b	52.3 ± 5.8c	100 ± 0.0d	0.0 ± 0.0a	22.5 ± 7.4b	52.1 ± 6.5c	86.6 ± 6.7d
4	0.0 ± 0.0a	74.3 ± 8.0b	100 ± 0.0c	100 ± 0.0c	0.0 ± 0.0a	25.4 ± 6.9b	54.4 ± 8.4c	91.5 ± 6.0d	3.2 ± 2.0a	24.6 ± 8.1b	49.0 ± 6.5c	80.4 ± 7.5d
5	3.2 ± 1.5a	69.5 ± 6.5b	100 ± 0.0c	100 ± 0.0c	0.0 ± 0.0a	22.2 ± 5.4b	44.4 ± 7.0c	84.6 ± 5.4d	3.3 ± 2.3a	22.5 ± 5.4b	47.7 ± 7.6c	76.9 ± 11.6d
6	0.0 ± 0.0a	62.5 ± 10.3b	100 ± 0.0c	100 ± 0.0c	0.0 ± 0.0a	21.5 ± 4.0b	42.5 ± 6.5c	80.5 ± 8.7d	0.0 ± 0.0a	22.5 ± 6.9b	42.5 ± 6.3c	66.7 ± 5.9d
7	0.0 ± 0.0a	62.5 ± 7.5b	100 ± 0.0c	100 ± 0.0c	0.0 ± 0.0a	11.5 ± 5.4b	32.0 ± 6.5c	65.4 ± 9.0d	0.0 ± 0.0a	17.9 ± 5.4b	36.6 ± 6.3c	60.4 ± 9.5d
8	0.0 ± 0.0a	72.5 ± 8.9b	100 ± 0.0c	100 ± 0.0c	0.0 ± 0.0a	11.1 ± 3.5b	22.5 ± 6.5b	63.5 ± 6.5c	0.0 ± 0.0a	12.5 ± 4.1b	30.3 ± 7.3c	53.1 ± 6.0d
9	0.0 ± 0.0a	73.3 ± 7.8b	100 ± 0.0c	100 ± 0.0c	4.4 ± 1.3a	17.6 ± 6.7b	32.5 ± 6.9b	65.0 ± 7.0c	0.0 ± 0.0a	12.3 ± 4.3b	25.4 ± 7.1b	45.4 ± 6.4c
10	0.0 ± 0.0a	62.0 ± 6.5b	93.3 ± 4.3c	100 ± 0.0c	0.0 ± 0.0a	7.5 ± 3.3b	19.6 ± 7.8b	55.5 ± 10.3c	0.0 ± 0.0a	7.5 ± 3.4b	15.4 ± 6.5bc	32.3 ± 8.5c
11	0.0 ± 0.0a	63.5 ± 6.7b	97.1 ± 1.5c	100 ± 0.0c	0.0 ± 0.0a	8.0 ± 3.1b	15.4 ± 6.1b	32.3 ± 4.0c	0.0 ± 0.0a	4.6 ± 2.3ab	13.4 ± 4.3bc	30.5 ± 7.8c
12	0.0 ± 0.0a	59.9 ± 10.2b	93.2 ± 3.5c	100 ± 0.0c	0.0 ± 0.0a	5.4 ± 2.7ab	13.3 ± 5.4bc	30.2 ± 10.3c	0.0 ± 0.0a	3.5 ± 1.5a	6.7 ± 2.3a	20.9 ± 4.7b
Segment (1 input, 12 output)	Empty column (55 ppm)				Maize (55 ppm)				Wheat (55 ppm)			
Exposure (h)	2h	4h	6h	8h	2h	4h	6h	8h	2h	4h	6h	8h
1	0.0 ± 0.0	0.0 ± 0.0	3.2 ± 1.7	3.2 ± 1.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.2 ± 1.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.7 ± 2.1
2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.9 ± 2.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.4 ± 1.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.1 ± 1.4

Segment (1 input, 12 output)	Empty column(115 ppm)				Maize(115 ppm)				Wheat (115 ppm)			
3	0.0 ±0.0a	0.0 ±0.0a	3.5 ±2.4ab	6.7 ±3.3b	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
4	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
5	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
6	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
7	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
8	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
9	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
10	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
11	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
12	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0

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Increased Fecundity in Strains of *Tribolium castaneum* (Herbst.) and *Sitophilus zeamais* (Motsch.) after Ten Generations of Selection with Methyl Bromide

C. J. Waterford [1] and R. G. Winks[2]

Abstract: Five strains of *Tribolium castaneum* (Herbst.) and two strains of *Sitophilus zeamais* (Motsch.) were selected with methyl bromide for ten generations. Investigation into the reproductive capacity of the progeny compared with that of the parent strains showed increased fecundity in all strains of *T. castaneum* and in one of two strains of *S. zeamais*.

Changes in tolerance of the selected strains were only of the order of x1.3 at the LD_{50}. However, in quarantine and pre-shipment treatments with methyl bromide this increased fecundity has implications for the re-establishment of infestations in marginal' treatments thereby increasing the risk of fumigation failure. This could become an important consideration, for example, in fumigation of poorly sealed shipping containers.

Key words: fecundity, resistance, fitness, methyl bromide

Introduction

Methyl bromide remains the fumigant of choice for quarantine fumigation against insect infestations in international cargo [1]. Requirements to fumigate shipments with methyl bromide have taken on an added level of complexity with an estimated 400 million container movements occurring every year worldwide. This increased shipment of cargo in containers and the commercial belief that containers are gastight has created a weakness in the quarantine treatments that relies on fumigation with methyl bromide.

Ball and van S. Graver[2] showed that reaching the required dose in unsheeted containers filled with hay for export is correlated with half life pressure decay time. When control of infestations to quarantine standards is the goal, data presented here indicate that the need for gastight enclosures with methyl bromide now becomes more important. This is emphasised by the recent suspension of fumigation of "tarpless containers" by USDA APHIS [3].

Materials and Methods

Test insects used were strains of *T. castaneum* and *S. zeamais* collected during the FAO survey of pesticide susceptibility during 1972 – 1973 by Champ and Dyte [4] and held in laboratory culture. Culturing and general handling techniques follow those described in Winks [5]. Selection followed the exposure protocols of the FAO resistance method for methyl bromide [6] with selection doses targeted approximately at 70% mortality. Survivors were cultured as parents of the next generation.

The concentration of methyl bromide was confirmed at 99.9% purity by gas chromatography using the response of a Gowmac (gas density detector. All dosing and handling was carried out at 25℃. The insects were starved and conditioned in an incubator at 25℃, 57% r. h. overnight prior to exposure.

The parent and final selection for each strain was assessed for mortality response when exposed to a range of concentrations for a five hour exposure period in 2.5 L desiccators. Groups of 200 adults were used at each exposure time. End-point mortality response was determined from successive observations using the method recommended by Winks [7] and results were analysed using the method of Finney [8]. Parameters of the response lines for strains tested are shown in Tables 1 and 2.

Fecundity of strains was assessed by culturing 200 parents in 150 g of wholemeal flour supplemented with a small amount of brewers yeast. Parents were removed after seven days oviposition and sexed. After six weeks progeny were removed and the total weight estimated from the weight of 200 parents. The number of progeny produced per female was calculated.

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Results and Discussion

Parameters of the response lines for strains tested are shown in Table 1 and changes in fecundity shown in table 2.

Though the levels of resistance developed over ten generations of selection are not large, up x1.6 for *T. castaneum*, this could impact on marginal fumigations in fumigation enclosures where leaks, combined with the prevailing ambient conditions, dilute the applied fumigant in critical areas such as near the doors of containers. This is highlighted by Ball and van S. Graver [2] who reported several containers between 10 and 20 seconds pressure half life pressure decay times that did not achieve the target Ct product. Given that higher levels of resistance would lead to higher survival in these under dosed areas, and combined with the increased fecundity reported here (up to 193% for one strain of *S. zeamais* and 122% for *T. castaneum*) an unpleasant surprise may await when the doors of such a container are opened. Clearly this poses significant implications in quarantine treatments.

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Table 1. Dosage estimates and parameters of regression of probit mortality on log dosage for methyl bromide selections of adults of *Tribolium castaneum* and *Sitophilus zeamais* at 25℃, 57% r. h. with resistance changes at the LD_{50} and LD_{99} compared to the parent strain.

Strain	LD_{50} mg. hr L^{-1}	LD_{99} mg. hr L^{-1}	Resistance compared to parent strain at		Slope	Mean probit	Heterogeneity	
			LD_{50}	LD_{99}		response(Y)	X^2	*d. f.*
TC12	32.73	46.66			19.9	7.05	5.03	5
TC12m10	45.5	65.7	x 1.4	x 1.41	13.0	4.9	17.3	12
TC367m4	50.21	65.05			20.7	4.9	12.7	5
TC367m11	59.56	82.15	x 1.19	x 1.26	16.6	4.8	8.76	5
TC369	42.2	51.5			43.3	5.3	.69	3
TC369m10	59.6	71.4	x 1.4	x 1.37	60.2	5.1	18.9	5
TC408	36.7	52.5			18.5	4.6	57	2
Tc408m14	63.25	84.5	x 1.7	x 1.6	15	5.6	34	2
TC411	41.6	56.4			17.6	5.2	5.8	5
TC411m10	51.6	70.0	x 1.24	x 1.24	17.6	4.9	20.6	5
SZM9	20.3	25.3			19.2	5.2	4.1	5
SZM9m10	26.8	32.6	x 1.25	x 1.22	21.3	5.1	36	5
SZM23	19.9	25.2			22.9	5.2	8.54	5
SZM23m10	26.6	32.1	x 1.27	x 1.21	28.7	5.3	16.6	5

Table 2. Comparison of fecundity of parent and methyl bromide selected strains of *Tribolium castaneum* and *Sitophilus zeamais* at 25℃, 57% r. h.

Strain	Sex ratio ♀/♂	Number of progeny		% increase
		Total	Per ♀	
TC12	119/81	2540	20.5	
TC12m10	103/97	2806	27.2	33
TC367m4	111/88	1286	11.6	
TC367m11	97/102	1551	16	38
TC369	107/93	2004	18.7	
TC369m10	97/100	1937	20	7
TC408	96/104	2799	29.2	

Strain	Sex ratio ♀/♂	Number of progeny		% increase
		Total	Per ♀	
TC408m14	81/118	3047	37.6	29
TC411	55/44	708	12.9	
TC411m10	48/53	1379	28.7	122
SZM9	108/92	2799	25.9	
SZM9m10	106/94	1875	17.7	-33
SZM23	107/93	1447	13.5	
SZM23m10	119/81	4703	39.5	193

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SESSION 2

SUBSTITUTES FOR METHYL BROMIDE AND REPLACEMENT TECHNOLOGIES

Chairpersons:
Jonathan Banks, Australia
Wang Yuejin, China
Tom Batchelor, Belgium

0201

Development of an Organic Stored Product Pest Control Treatment Station Utilizing Nitrogen for Shipment Containers

Dale Jude, P. Moog[1] & Dirk E. Maier[1,2*]

Abstract: There are few non-chemical alternatives existing for pest control in stored organic grains and grain-based products. One available technology that appears timely to develop and demonstrate is nitrogen-based modified atmosphere. This study was undertaken to demonstrate that nitrogen treatment works utilizing a transportable nitrogen-based modified atmosphere treatment station by conducting a full-scale trial with bagged grain in a shipping container. Initial tests were conducted using 0.71m^3 bags made from a five-layer oxygen barrier extruded plastic film filled with soybeans and purged with 99.9% nitrogen until the oxygen level was near zero. Stored product insect bioassays were placed inside the bags containing adult maize weevil (MW), red flour beetle (RFB), lesser grain borer (LGB), and Indian meal moth (IMM) adults and larvae. Bioassays were kept for 3, 7, and 21 days inside the nitrogen – purged bags. Results showed 100% mortality for all insects as oxygen level inside a bag was below 0.13% after 3 days of exposure, which was maintained for 7 and 21 days. Maintaining a good seal on the bag, in addition to the proper selection of plastic film material, were essential contributors to the success of this study. Preparing a larger bag for the 6.1m long container was a substantial challenge. Nevertheless, trials with a shipping container using corn-filled tote bags and purged for 7 days with 99.9% nitrogen showed 100% mortality of RFB, MW and LGB adults.

Introduction

To maintain agricultural competitiveness, emerging value-added opportunities such as organic grains and grain-based products need to be supported through applied research and demonstration. Organic crop production is one diversification strategy to enhance the viability of U. S. producers and organic crops are an innovative source to meet the demand for nutritious and healthy foods. The U. S. organic farming system has expanded rapidly since the 1990's in response to a demand increase from local and national markets. As of 2001 (latest data), Indiana had at least 2000 certified organic hectares with 82% planted with field crops and hay. About 500 hectares each were utilized for corn and soybeans and almost another 500 hectares for wheat and oats[1]. Additionally, hectares are dedicated to organic popcorn and tofu soybeans for export primarily to Japan[2]. One key challenge facing Indiana producers and processors of organic grains is pest control consistent with organic criteria during post-harvest handling to ensure quality and avoid costly rejection at the point of sale/receipt. At least one major snack food manufacturing plant is faced with this challenge since it began receiving organic food corn from a supplier[3].

Few non-chemical alternatives exist for pest control in stored organic grains and grain-based products[4]. Available technologies that have been explored include refrigeration of product to −18℃ for 6 days (effective but costly and time consuming), heating of equipment and structures to 45℃ for 16 – 24 h (product cannot be heated as it reduces quality), ozonation of bulk product (controls external pests but neither internal infesters nor bagged product), use of insect growth regulators (works for certain pests only), pheromones (limited effectiveness as it only attracts male insects into traps), PyGanic (a pyrethrin derived from a natural product; a grain protectant that cannot be used for control at the time of shipment or receipt), Diatomaceaos earth (a silica-based grain protectant that cannot be used for control at the time of shipment or receipt), and modified atmosphere using 45% – 60% CO_2 for 4 – 21 days (effective but costly and time consuming; and the primary treatment competing with the nitrogen-based technology proposed in this project).

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In sealed containers purged with nitrogen, a progressive hypoxia or anoxia causes mortality in stored product pests[5]. Navarro[6] documented 95% mortality of almost all life stages of *Ephesia cautella*, *Sitphilus oryzae*, and *Tribolium castaneum* when exposed to 99% nitrogen (1% oxygen) for at least 6.5 days and 99.9% nitrogen (0.1% oxygen) for 9 days. Interestingly, pupae of *Sitphilus oryzae* required at least 20 and 14 days at 0.1% and 1% oxygen, respectively. A number of additional studies showed similar efficacy using nitrogen-based modified atmosphere treatment for a range of durable and perishable products such as for pears[7]; for snow pea[8]; for cacao[9]; and for Dhakki dates[10].

Based on the successful research results documented in the literature, discussion with other researchers[11] who have conducted some of this successful research recently, current advancements in the development of nitrogen generators, plastic liners and gas monitoring equipment as well as the current needs of Indiana producers and processors and interest by the leading Indiana pest control service supplier, the time appeared right to develop and demonstrate a nitrogen-based modified atmosphere pest control treatment system for organic grains and grain-based products in Indiana.

This project focused on the development and demonstration of a nitrogen-based modified atmosphere treatment station for the control of stored product pests in bagged organic grains and grain-based products grown in Indiana (and surrounding states) and designated for sale in domestic and overseas markets. The objectives of this study were:

1 To construct a transportable nitrogen-based modified atmosphere treatment station and conduct a pilot-scale trial with bagged grain in sealed stacks.

2 To demonstrate the transportable nitrogen-based modified atmosphere treatment station by conducting a full-scale trial with bagged grain in a shipping container.

Materials and Methods

Materials and Procedure for Constructing the Oxygen Barrier Plastic Liner

Key to a successful modified atmosphere treatment with nitrogen is the availability of an airtight enclosure. Typical shipping containers are not sufficiently airtight to meet this need. Specially designed plastic material that acts as a barrier to oxygen can be used to create brick-shaped liners that fit inside a shipping container. The liner material for this project was obtained from Germany. The plastic material is 5m wide extruded 6mm thick sheeting that consists of five layers with the middle layer designed as an oxygen barrier.

For both objective experiments, the plastic film was cut into sheets just large enough to minimize waste and the number of seams that had to be welded. Each sheet was folded once across its width to allow welding of the two longer sides. This created a brick-shaped bag that could be filled with product before welding the third seam and sealing the bag. Before the final seam was welded, two inexpensive plastic valves were installed on each bagone was used for pumping in the nitrogen and the other for bleeding out the air. For the Objective 1 experiments, the 5m wide plastic liner was cut into 2m long sheets; for the Objective 2 experiments, the liner was cut into 8.5m long sheets. A commercially available plastic belt sealer was modified by adding 2.54cm diameter wheels that run along a 10.2cm wide and 2.5m long aluminum channel that served as a guiding track. After initial folding, each plastic sheet to be sealed was laid on a table with the aluminum track fastened in parallel to the table. The plastic sealer self-propelled along the track while welding the first seam of the bag. Then the bag was turned over on the table and the sealer was allowed to weld the second seam. Once a bag was in place and filled with product, excess material was trimmed and the sealer was used manually to weld the final seam and seal the bag. Creating and welding the smaller bags was relatively easy while creating and welding the large bags to fit inside the container proved to be a substantial challenge. Handling of all bags was done carefully to minimize folds and seal breakage in order to avoid any source for air leakage.

Objective 1: Construction and Testing of Transportable Treatment Station with Sealed Stacks

*Treatment Station Construction*A Pressure Swing Adsorption Nitrogen generator Model HPN – 25 was made available by Innoventor (Maryland Heights, Missouri) that only required electrical power. Castor wheels were added to make the unit transportable so that it could be placed at a pest control service supplier for treatment of infested organic products shipped to their location, or the unit could be shipped via truck to the location of an organic producer,

marketer or processor for the treatment of infested organic products before shipping or upon receipt.

*Sealed Product Stacks*Two stacks of soybean-filled bags were used. One open-top cube-shaped plastic liner was placed on each pallet and stacked full with 0.5kg product bags. Subsequently, temperature sensors and insect cages were placed among the soybean bags. Then the top of the cube-shaped plastic liners were sealed. Access valves for the inlet purging and outlet recirculation lines were incorporated into the seam as the liners were welded shut.

*Nitrogen Treatment*Nitrogen was introduced on one side of the liner enclosing each product stack to purge its content with 99.9% nitrogen which was then maintained for 3, 7 and 21 days. Once achieved, the generator was shut off, and the supply hose was removed. A treatment was considered complete once the required exposure time had been reached. Each treatment was repeated three times. An additional three stacks without any liners were used as control.

*Temperature and Oxygen Monitoring*Temperature was monitored continuously at several points in each bagged stack. Oxygen concentration in each stack was monitored using zirconium type oxygen analyzers. The AirDac data acquisition and control software developed by the Purdue Agricultural Air Quality Laboratory was adapted for daily gas concentration monitoring.

*Insect Bioassays*Bioassays of live insects (adults of red flour beetles (RFB), lesser grain borer (LGB), maize weevil (MW), and Indian meal moth (IMM) and larvae of Indian meal moth) were placed among the bags in each stack. For each sealed and control stack of soybeans, insect bioassays were placed in three locations each inside and outside the product bags. These were collected at the end of each treatment and live *versus* dead insects were counted.

Objective 2: Demonstration of Transportable Treatment Station with A Shipping Container

Shipping Container Preparation A 6.1m long shipping container was used to hold 14 maize-filled 455kg tote bags placed inside the large brick-shaped plastic liner bag. The shipping container doors were left cracked open for the nitrogen supply line to connect to the inlet valve installed on the sealed liner bag. The container was filled to allow access to the totes for data monitoring. The shipping container remained on site at Purdue University and was not transported.

*Sealed Product Stacks*Before filling the container, carpet padding was placed on the floor of the container and then the large brick-shaped liner bag was placed on the floor at the far end of the container. The top-portion of the liner was attached to the ceiling with two-sided tape. The open front of the liner bag was aligned with the front doors of the container. A second layer of carpet padding was placed on the floor of the liner and then a layer of 1.27cm thick plywood was placed on the carpet padding. This permitted a fork lift to drive in and out of the container to place pallets carrying the totes inside the container. Tote bags were stacked two high side-by-side and from back to front in the container. The container was not completely filled to allow access around the totes and placement of the data monitoring equipment and laptop computer. Subsequently, temperature sensors, gas monitors, and insect bioassays were placed among the tote bags.

Nitrogen Treatment Nitrogen was introduced to the back of the liner by an extended inlet tube to purge its content with 99.9% nitrogen from back to front and then maintain near zero oxygen for seven days. A bleeder valve at the seam was used to control the purging process. The generator was continuously operating when the liner was sealed. A treatment was considered complete once the required exposure time of seven days at < 0.1% oxygen had been reached.

Temperature and Nitrogen Monitoring, and Insect Bioassays Preparation and Placement were similar as described in Objective 1, except no IMM larvae and adults were used in the bioassays.

Results and Discussion

Objective 1: Construction and Testing of Transportable Treatment Station with Sealed Stacks

Purging the 0.71m^3 bag stacks with 99.9% nitrogen took about 3.5h to reduce oxygen to near zero. The nitrogen generator was operated to supply 6.8m^3/s and two sealed bag stacks were purged at the same time. An essential part of each experiment was maintaining the seal of the liner in order to achieve and maintain a near-zero oxygen level inside each bag. Figures 1 to 3 show changes in oxygen level inside the plastic liners after 3, 7, and 21 days of storage. Based on these figures, oxygen level in-

creased with length of storage from 0.25% for 3 days to 2.25% after 21 days.

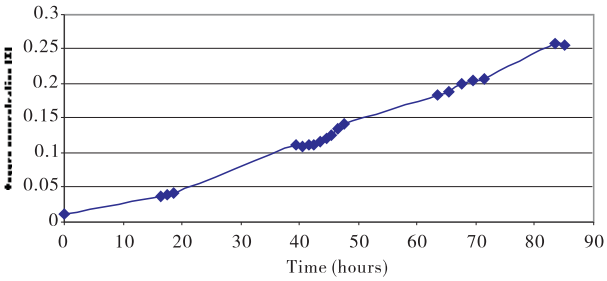

Fig. 1 Change in oxygen level for 3 days inside asealed bag liner initially purged with nitrogen.

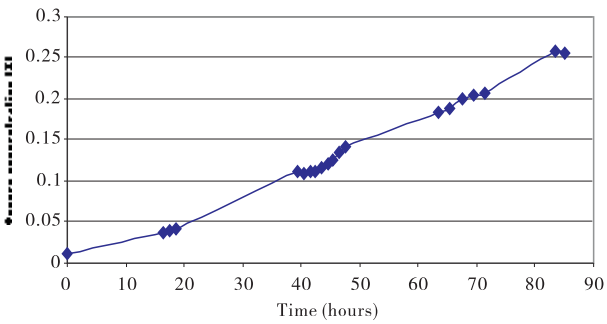

Fig. 2 Change in oxygen level for 7 days inside a sealed bag liner initially purged with nitrogen.

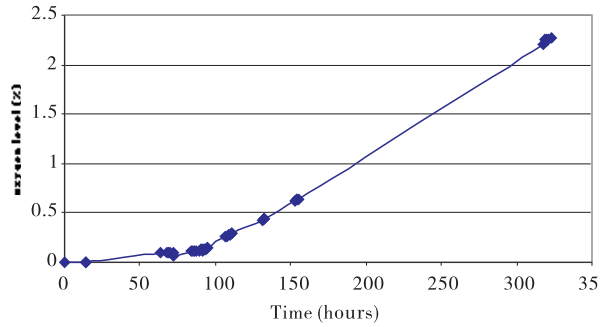

Fig. 3 Change in oxygen level for 21 days inside a sealed bag liner initially purged with nitrogen.

Insect mortality of adult MW, LGB, RFB, IMM and IMM larvae exposed for the different treatment times is presented in Table 1. All treatments yielded 100% mortality for all insects considered except for the MW in one of the replications in the 3 day exposure. In that replication, only 67% mortality was observed but oxygen level in that bag liner at the end of the 3 day period was 0.24%. When oxygen level was maintained below 0.13% for the first three days of any treatment period, mortality reached consistently 100% for all species and life stages. No dead insects were found in the control bioassays.

Table 1. Percent mortality of insects in cages for different treatment times under MA with nitrogen.

Storage Time	MW adult	LGB adult	RFB adult	IMM adult	IMM larvae
3 days (trial 1)	100	100	100	100	100
3 days (trial 2)	67 *	100	100	100	100
3 days (trial 3)	100	100	100	100	100
7 days(3 trials)	100	100	100	100	100
21 days (3 trials)	100	100	100	No data	No data

* Oxygen level was 0.24% for 67% mortality compared to ≤ 0.13% for 100% mortality.

Part of the success of this system depends on using plastic material impervious to oxygen. The plastic obtained from Germany performed well in terms of maintaining low oxygen levels. Other plastics tested were purchased from local hardware stores. These plastic materials were thinner(i.e.,4 mm vs 6 mm) and not as strong as the extruded 5 – layer German plastic.

We conducted a simple test which consisted of placing a fast green dye solution inside small plastic bags and then placing them in a 1 liter beaker filled with water. In each case, the dye passed through these inexpensive plastics, indicating that they would also be more permeable to the smaller oxygen molecules. On the other hand, performing this test with the 5 – layer German plastic did not show any leak of the dye.

Another plastic material tested was one used for silage bags. This material is thicker (9mm) than the German liner material and was designed to be a barrier for oxygen. The thickness of the material made it harder for the sealer to produce a good seal along the seams. At the same time, extra care in handling of the plastic during folding and sealing was needed to prevent creases that reduced the sealing integrity of the material. Testing a small cube made from the silage bag material and filling it with soybean sample bags showed the oxygen level reaching 0.55% at 30 hours after nitrogen purging ended. This oxygen level was sufficient to allow insects such as MW to survive.

Objective 2: Demonstration of Trans-

portable Treatment Station with a Shipping Container

The initial trial with the 6.1m shipping container required 4.5 days or about 108 hours to purge the brick-shaped liner with 99.9% nitrogen. Lining the container took skill and ingenuity in order to properly secure the plastic material along the ceiling and walls of the container. A fan was used to blow air into the container to inflate the plastic liner and double-sided tape was used to keep the liner attached to the ceiling and walls. Care was taken in loading the tote bags to make sure that the plastic did not tear. Unfortunately, the large liner was not perfectly sealed as leakage was detected after the generator was turned off. As a result, it was run continuously to maintain the desired near zero oxygen treatment effect.

After seven days of near zero oxygen exposure, no live adult MW, RFB, and LGB were found in the insect bioassays. As a result, replicates two and three were also purged continuously with nitrogen, which also resulted in 100% mortality of all adult insects.

Conclusions

This study shows that the application of nitrogen for modified atmosphere storage proved to be effective in controlling maize weevil, red flour beetle, Indian meal moth, and lesser grain borer. Its effectiveness relies on the utilization of specially designed oxygen barrier plastic material and the integrity of sealing the plastic liner in order to maintain near-zero oxygen concentrations for the duration of the treatment. After 3 days of treatment, oxygen had to be less than 0.13% to achieve 100% mortality. For longer treatment periods, the oxygen level could be as high as 0.4% oxygen for 7 days and up to 2% for 21 days.

Acknowledgements

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0202

Ethyl Formate Plus Methyl Isothiocyanate Is A Potential Liquid Fumigant for Stored Grains

Yonglin. Ren*, T. Van Emmerik, D. A. Mahon, ByungHo Lee and B. Padovan

Abstract: A new fumigant designed for the grains industry consists of 95% ethyl formate (EF) and 5% methyl isothiocyanate (MITC) as a synergistic additive. The formulation is stable at 25, 30 and 45℃ during 2, 3 and 4 months storage. The formulation shows a high level of efficacy in controlling the major grain pests at all life stages of the insects. The dose to kill insects at all stages of *Rhyzopertha dominica*, *Sitophilus granerium*, *Sitophilus oryzae* and *Tribolium castaneum* in infested wheat and barley is 80 mg L^{-1} for 5 days and 25℃. After 8 days holding period, residues of EF and MITC were marginal or below the experimental permit level of 0.2 mg kg^{-1} and 0.1 mg kg^{-1} respectively, without the need for forced aeration of the wheat.

Introduction

Ethyl formate (EF) is an old fumigant which had been successfully used for individual package fumigation of dry fruits since 1929 (Simmons and Fisher 1945) and evaluated for grain protection in the 1980s (Muthu 1984). For the past few years, CSIRO Entomology has evaluated the use of EF as a fumigant for control of stored product insects. In previous commercial-scale trials with EF (90 gm^{-3}) on wheat (Desmarchelier *et al.* 1998), complete kill of mixed aged cultures of *Rhyzopertha dominica*, and adults and larvae of *Tribolium castaneum*, was achieved. Complete control of adult *S. oryzae* and 99% of progeny were also killed relative to the control. Results from all previous laboratory and commercial – scale trials with EF on wheat, barley, oats, field peas and canola (Desmarchelier *et al.* 1998; Ren & Mahon 2003; Mahon *et al*, 2003) have shown that the internal larval stages of *Sitophilus oryzae* are difficult to control. The dosage of EF required to completely kill the mixed aged cultures of *S. oryzae* was > 160 gm^{-3}, which is well above the flammable threshold of 84 – 91 gm^{-3}.

Methyl isothiocyanate (MITC) is colourless solid which is slightly soluble in water. It has a high boiling point (117 – 180℃) and a density of 1.069 g/mL. MITC occurs naturally at levels of 0.001 – 0.05 mg/kg in brassicas (Sarwar and Kirkeaard 1998a, b & c) where it has an important role in protecting these crops against pests (Sarwar and Kirkeaard 1998c). MITC is used as a soil fumigant for nematodes, fungi, and other diseases in vegetables and fruit (Gaetano & Matta 1992). Metam sodium degrades in the soil to MITC which is play the role of fumigant function (Ajwa, *et al.* 2003).

Previous research has shown that MITC can significantly reduce the dosage of EF to below the flammable level. In addition, MITC can synergise the toxicity of EF. Ren *et al.* (2005) reported that adults of *S. oryzae* were unaffected by 5.9 mg/L of EF for 24 hours at 25℃, but the addition of 5% MITC resulted in a 99% mortality of *S. oryzae* adults at the same EF dose, indicating significant synergism between EF and MITC.

This paper reports on an evaluation of a new formulation of EF consisting of 95% EF plus 5% MITC, which was developed by CSIRO Entomology, as a candidate fumigant for use on stored commodities.

Materials and Methods

Materials

The wheat used in this study was Australian Standard White (ASW). The moisture content of the wheat was 10.5, 12.5 and 14.5% determined by using a Graintec HE 50 electronic moisture meter and verified by use of the oven method. The results obtained were expressed as a percentage calculated from replicates.

The EF used was the formulation Eranol, supplied by Orica Australia and has an active ingredient of 97.1% EF. The MITC was supplied by Aldrich Chemical Company Inc. The formulation used was 95% EF + 5% MITC, v/w.

Measuring Stability of Formulation

The stability of the formulation was deter-

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mined during storage at 25, 30, and 45℃ for 2, 3 and 4 months. Confirmation of identity was obtained by Gas Chromatography/Mass Spectroscopy (GC/MS) on a Finnigan Ion Trap, after separation on a capillary DB – 624 column (J & W, 122 – 1334). The formulation was determined on a Tracor 220 GC, equipped with a flame photometric detector, after separation on a 1m glass column packed with HayeSep Q (Alltech, 2801). A Bruker Tensor 37 FTIR spectrometer equipped with a Deuterated L – α – Alanine doped Triglycine Sulphate, DLATGS detector, was used to collect all spectra. Samples were analysed using a diamond with ZnSe lens single reflection ATR MIRacle accessory (Pike Technologies). For both the background and samples, 32 scans were collected over the wavelength range 600 – 4000 cm^{-1} at a spectral resolution of 4 cm^{-1}. Two drops of the liquid formulation were placed on the ATR crystal and the sample covered with a volatiles cover (Pike Technologies). ATR – FTIR spectra were collected from the liquid formulation during storage at different times and temperatures in 10ml glass micro flasks (Alltech) sealed with mininert valves (Alltech).

Measuring Concentrations of EF and MITC

The concentration of the EF and MITC components was determined using a Varian STAR 3400CX gas chromatograph (GC) equipped with a flame ionisation detector (FID) after isothermal separation on a 30m (0.53mm (i. d.) megabore capillary column ZBWAX (B13844) at the oven temperature of 95 or 140℃. The concentrations of EF were calculated on the basis of peak areas against external standards, prepared by dilution in 250 ml bottles with a Mininert valve equipped with septa (Alltech Australia, Cat. No. 95326). A sample volume of 50 – 100 l was injected into the GC – FID.

Insects and Bioassays

Toxicity studies of EF alone compared with EF + MITC were conducted on adult and mixed aged cultures (an unknown quantity of eggs, larvae and pupae from four cultures of different start dates). Wheat and barley columns (1.5m tall (24.5cm inside diameter (i. d.)) with a capacity of 52kg and 95% full were used for bioassays. Steel cylindrical cages (50mm (30mm i. d.) containing mixed aged cultures of *T. castaneum*, *S. granerium*, *S. oryzae* and *R. dominica* where placed at various points within the grain bulk. The application rates used were 80 and 2 × 80 = 160 g/t (double injection of 80 g/t, after 4 hours, the second dose was injected) for EF and 80 g/t for EF + MITC was applied to the top of column and subjected to a low rate of recirculated air (1 gas exchange/hour) at 25℃ and for 5 days exposure. Bioassay samples were retrieved at the end of the fumigation period, the adult insects were counted and removed and the remaining mixed – age cultures incubated at 25℃ and 70% RH. Subsequent emerging adult insects were counted weekly for a period of 6 weeks, with live and dead adults removed at each count.

Measuring Sorption/Desorption of Formulation on/from Wheat

The sorption of the formulation on wheat was measured as disappearance of EF and MITC in the headspace of grain filled Erlenmeyer flasks. The concentration of EF and MITC was measured by GC at timed intervals. Ethyl formate and MITC standards were prepared for calibration of the GC and calculation of the concentration of EF and MITC in the fumigation flasks. At the end of the fumigation, the Erlenmeyer Flasks were opened and the grain was placed in 2.5L glass jars in a fume cupboard for initial aeration, but later removed to a constant temperature room and humidified where necessary. For the desorption study, a fumigated sample was taken immediately after opening the flask (called 0 day aeration) and on the following 1, 3, 8 and 14 days after the start of aeration. The desorption sample was placed into a 100mL flask equipped with a ground-glass joint and a septum, using a 90% fill ratio. As with the sorption procedure, the headspace readings were taken as soon as possible, and following readings were taken on 1, 3, 8 and 14 days after sampling for desorption, until the headspace concentration of EF and MITC no longer increased or decreased. Residue samples were taken at the same time as the desorption study (immediate after opening called Day 0, and on Day 1, 3, 8 and 14 of aeration), and where possible analysed immediately. If they were not able to be analysed immediately they were stored in a – 20℃ freezer and thawed to room temperature during analyses.

Analysis of EF and MITC Residues in Wheat

Residues of EF in wheat were analysed following the procedure as described by Vu and Ren (2004). A wheat sample of 100g and 100mL of 70% (w/w) ammonium nitrate were

placed in a sealed 250 mL flask and the levels of the residue were determined by sampling the headspace of the flask. Levels of EF residue were determined against spiked standards, prepared by adding EF to untreated commodity plus extraction solvent. The MITC residues were analysed by headspace Solid Phase Micro-extraction (HS – SPME). The SPME fibre used was an 85 m Polyacrylate (PA) (Sigma – Aldrich Australia, Cat. 57304). A wheat sample of 50g in a 250 ml flask fitted with a sample port was immersed in a heated oil bath for 45m. The SPME fibre was inserted through the septa into the sample and exposed for 5m and then taken to the GC. Levels of MITC residue were determined against spiked standards, prepared by adding MITC to methanol. MITC was determined by a Varian *CP*-3800 gas chromatograph (GC) equipped with a flame ionisation detector (FID) after isothermal separation on a 25m (0.53 mm i. d. Varin Capillary column CP – Pora-BOND Q at the oven temperature of 150℃.

Results and Discussion

Stability of Formulation

The stability of EF and MITC in a ratio of 95:5 v/w % (EF:MITC) after storage for 2, 3 and 4 months at 25, 30 and 45℃ was determined using GC/FID, with the peak areas compared with those of a standard formulation. The percentage of MITC and EF present was then calculated.

No significant decrease in the level of MITC or EF was found at any of the temperatures and times investigated. IR GC/MS spectra of the liquid formulation were collected by FTIR and GC/MS. There was no presence of interconversion, or breakdown products (e. g. methylamine, formic acid, and ethanol) (Fig. 1 and 2) whose bands are associated with EF, and MITC (aliphatic, carboxylic, and MITC functionalities) at the temperatures and times studied. The formulation therefore appears stable at the times and temperatures tested.

Table 1. Percentage of MITC/EF at different storage times and temperatures

emperature (℃)	MITC/EF at different storage time (months)		
	2	3	4
25	100/100	100/100	109/97
30	97/99	102/103	97/98
45	101/98	100/92	93/94

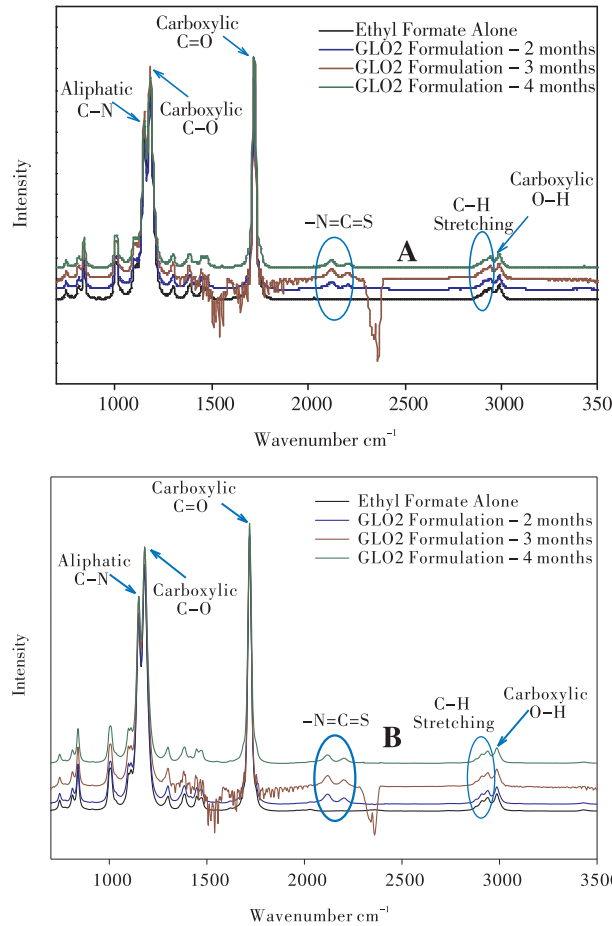

Fig. 1 IR spectra of the liquid formulation stored for 3m at 25℃(A) & 45℃(B)

Bioassays

The bioassay results from a 52kg wheat and barley column showed that all stages of the four insect species (*T. castaneum*, *S. granerium*, *S. oryzae* and *R. dominica*) and adults of three insect species (*T. castaneum*, *S. oryzae* and *R. dominica*) were completely killed by EF + MITC of 80 g/t and 160 g/t of EF alone respectively.

A dose of 80 g/t of EF without MITC killed all adult *T. castaneum* and *R. dominica*, but only 98% – 100% of *S. oryzae* adults. These results are consistent with previous commercial-scale trials that showed EF used alone controlled adult *T. castaneum* and *R. dominica*, but not *S. oryzae* adults, fumigated in wheat, split faba beans and sorghum (Mahon *et al.* 2003; Ren *et al.* 2003).

Sorption and Desorption

The sorption of the formulation (for both EF and MITC) on wheat decreased with an increase in the moisture content of wheat (Fig. 3 and Fig. 4). Within the first 3h after application, almost 70% of the formulation was ab-

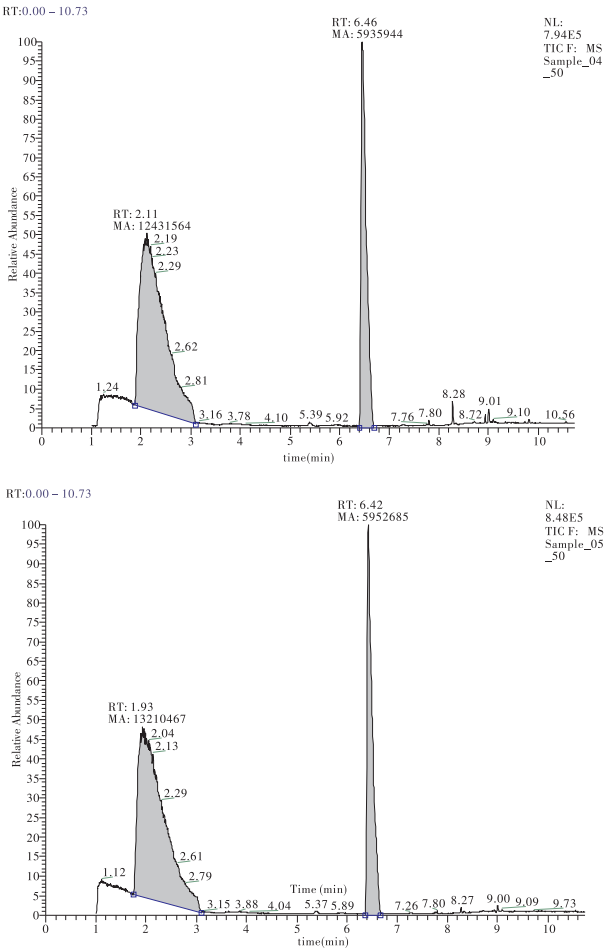

Fig. 2 GC/MS spectra of the liquid for mulation stored for 3m at 25℃(A) and 45℃(B)

sorbed by the wheat. Desorption rate of EF and MITC from the wheat was affected by the moisture content, exposure time and holding period. The desorption rate decreased with increasing moisture content, e. g. more EF and MITC were desorbed from 10.5% mc wheat than from 12.5 and 14.5% mc wheat (Fig. 5). More EF and MITC were desorbed from wheat which was treated with a short exposure period. The first day holding period removed 70% – 80% of the EF and MITC from wheat. The levels of EF and MITC which were desorbed from wheat at different moisture content and exposure time were significantly below the TLV of 300ppm EF and 0.1 ppm MITC.

Residues of EF and MITC in Wheat

During the period of exposure, both EF and MITC declined, e. g. MITC residue levels of 0.6, 0.5, 0.2 and 0.1mg/kg in wheat (12.5% mc, at 25℃) after 1, 3, 8 and 14 days fumigation (Fig. 6). During the holding period, both EF and MITC were further reduced, e. g. 12.5% mc wheat fumigated for 1 day, MITC

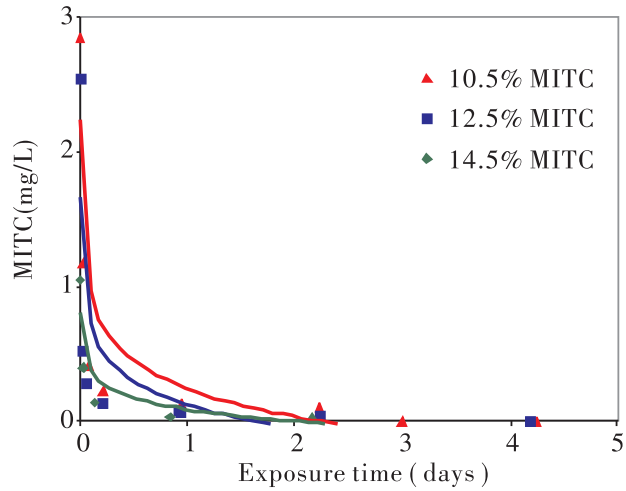

Fig. 3 Sorption of MITC on wheat at 25℃

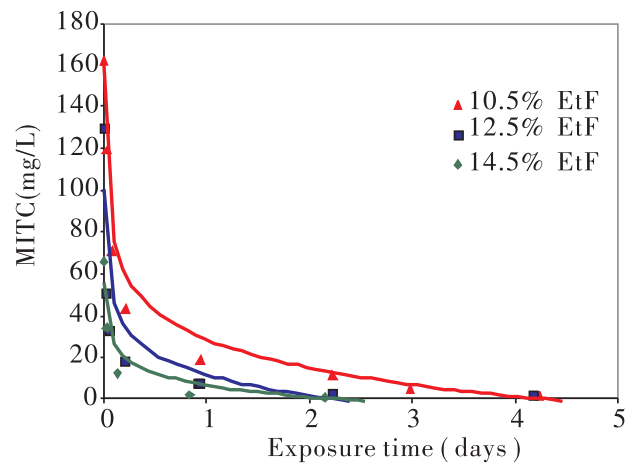

Fig. 4 Sorption of EF on wheat at 25℃

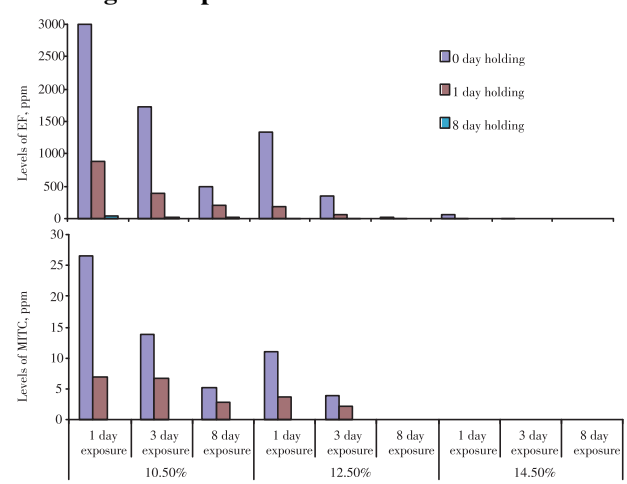

Fig. 5 Desorption of EF and MITC from wheat at 25℃

residue levels were 0.5, 0.1 and 0.07 mg/kg after 1, 8 and 14 days holding. The increasing moisture content appeared to accelerate the decrease in both EF and MITC residues. After 8 days holding period, residues of EF were above those in the control grain sample, but below the experimental permit level of 0.2 mg/kg, without

the need for forced aeration. The levels of the MITC in the same wheat after 8 days holding period had also declined to marginal or below the experimental permit level of 0.1 mg/kg without the need to use forced aeration. The EF results are consistent with previous commercial-scale trials with EF on wheat, barley, oats and peas (Desmarchelier *et al.* 1998; Mahon *et al.* 2003; Ren *et al.* 2003).

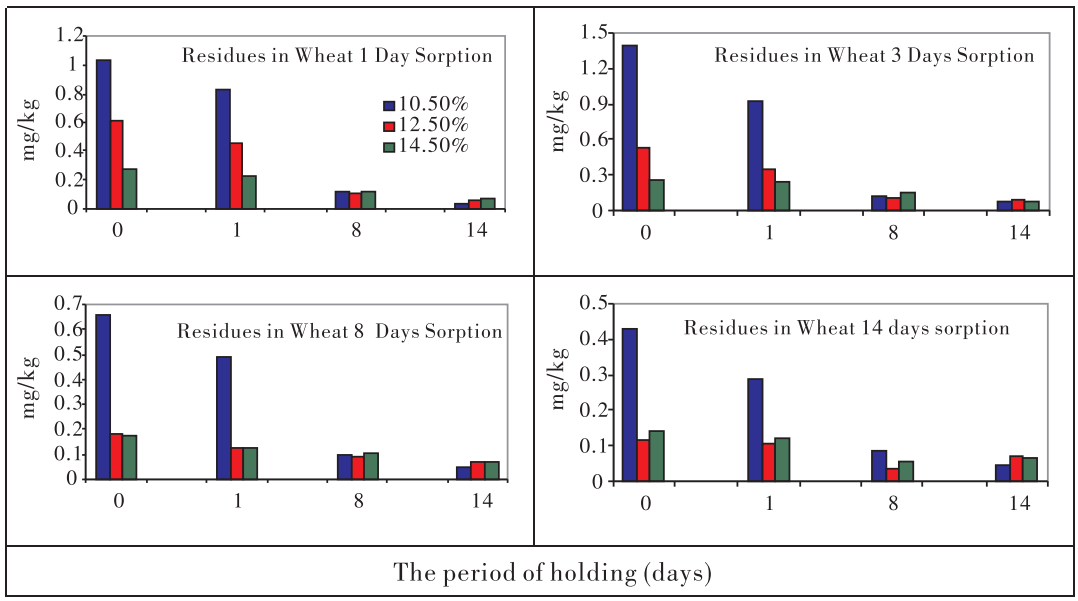

Fig 6. MITC residues in wheat treated with increasing exposure periods and with varying moisture content and holding periods (days).

Conclusions

All stages of the four insect species (*T. castaneum*, *S. granerium*, *S. oryzae* and *R. dominica*) and adults of three insect species (*T. castaneum*, *S. oryzae* and *R. dominica*) were completely killed by EF + MITC of 80 g/t, compared to EF alone which failed to achieve complete mortality of *S. oryzae* adults. EF + MITC desorbed over an 8 day period to levels that were within the experimental permit level for residues of both fumigants. MITC appears to have a synergistic effect against pests allowing EF to be effective at lower doses than EF used alone. We conclude that EF + MITC formulation has potential as a fumigant for controlling stored product pests in commodities.

Acknowledgements

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0203

The Role of the Montreal Protocol in Reducing Quarantine and Pre-shipment Uses of Methyl Bromide

Tom Batchelor and Melanie Miller

Abstract: Global production of methyl bromide for quarantine and pre-shipment (QPS) in 2006 was 10,275t which is about 34% of global methyl bromide production in 2006. It is now the single largest emissive use of any ozone-depleting substance that is not subject to reduction and phase out. Many countries have not reduced their consumption of QPS – MB in response to Protocol Decisions that encourage actions by governments to reduce use and emissions. Protocol decisions are not considered mandatory, unlike production and phase out schedules which are contained in Articles of the Montreal Protocol and compliance by countries is mandatory. Consequently, the global consumption of QPS – MB is declining very slowly and, at the current rate of reduction, we estimate it will approach zero by 2063. In order to create consistency between QPS – MB and the reduction in consumption of other ozone-depleting substances, the Montreal Protocol meetings are expected to discuss options for strengthening the controls on QPS – MB. Wood (including timber and whole logs, 49%), soil (mainly for strawberry runner production in two countries, 19%) and grain and cereals for consumption (15%) account for 83% of the QPS – MB use. Technical reports since 1994 have identified a wide range of alternative treatments. A worldwide survey in 2004 concluded that alternatives are available for 65% of QPS – MB uses. Several countries have unilaterally ceased consumption of QPS – MB, while others are now developing strategies to reduce use and emissions. Any future agreement on a global reduction in QPS – MB would likely result in financial support for the adoption of alternatives for QPS – MB in developing countries.

Introduction

The Montreal Protocol

The Montreal Protocol was originally agreed in 1987 to reduce the consumption of only eight ozone-depleting substances (ODSs), and at that time by only 24 countries and the European Economic Community. Today, 191 governments have signed the Protocol and production and consumption of all ODSs has been reduced significantly over the last 20 years. This achievement results in the Montreal Protocol being widely acknowledged as the most successful of all the environmental treaties.

The Protocol traditionally operates by agreement on control schedules which are timetables for the reduction and phase out of production and consumption of groups of ODS. These control schedules are sometimes weak initially, but they are strengthened over time on the basis of the most recent review of the scientific, environmental, technical and economic information on alternatives to ODS. Technical bodies such as TEAP and MBTOC provide annual updates on progress in the development and adoption of ODS alternatives, including information on alternatives for quarantine and pre – shipment uses of methyl bromide (QPS – MB).

This paper describes the current controls in the Montreal Protocol relating to QPS – MB, the quantities consumed according to use and provides examples of activities that have been undertaken to reduce its use.

QPS, non – QPS, Control Schedules and Definitions

QPS – MB is the only ODS consumed in significant volume that does not have a reduction and phase out schedule in the Montreal Protocol. In 1992 the Protocol adopted initial controls for most uses of methyl bromide. QPS was exempt control because governments at the time considered that few if any alternatives were available to disinfest where necessary imported and exported food and materials.

Fig 1. illustrates the differences between QPS and other (non – QPS) uses of methyl bromide, as defined by the Montreal Protocol. It shows the types of target pests, items treated, controls under the Protocol and percentage of total consumption in 2006. QPS is shown on the right side of Figure 1.

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1. The Methyl Bromide Technical Options Committee reports to the Technology and Economic Assessment Panel

Fig. 1 QPS uses and non – QPS uses of methyl bromide showing the types of pests targeted, examples of uses, and applicability to developed and developing countries.

Category		Non – QPS			QPS
Developed countries	% use	30%	2%	6%	34% Quarantine and Preshipment Exempted from reduction and phase out
	Controls	Only permitted for critical uses which must be phased out as soon as possible			
Developing countries	% use	22%	1%	5%	
	Controls	Phase out by 1 January 2015			

Descriptor	Soils	Stuctures	Durables	Durables	Perishables
Examples of treated items	Soil treatment prior to planting high value cropssuch as strawberries, tomatoes, sweet peppers, aubergine, melons, flowers, seedlings, nursery plants	Flour mills, food processing facilities, empty ship holds	Stored grains, cocoa beans, coffee, nuts, dried fruit, timber, wood products	Stored grains, cocoa beans, coffee, nuts dried fruit, timber, wood products	Fresh fruit vegetables and cut – and cut
Target pests	Soil pests, nematodes, fungi, weeds	Stored product pests and rodents	Stored Product pests	Stored and fresh product quarantine and officially regulated insects	
Exported or imported	No.	Some exports and imports		Yes	

Phase-out schedules for non – QPS uses were adopted in 1995. In 1997, stronger controls were agreed that resulted in MB consumption being banned in industrialised countries from 1 January 2005, except for QPS and specific circumstances where alternatives are considered not to be technically and economically feasible (so called 'critical uses'). Developing countries are permitted to consume non – QPS – MB until 2015. Economic assistance from the Protocol for alternatives has significantly reduced methyl bromide consumption in developing countries[3]. Non – QPS consumption globally continues to fall substantially each year.

MB is classified as a QPS use if it meets the criteria forquarantine' orpre-shipment'. *Quarantine applications* are MB treatments applied to prevent the introduction, establishment and/or spread of *quarantine* pests (including diseases), or to ensure their official control, where "official control" is that performed by, or authorised by, a national plant, animal, environmental protection or health authority; and "quarantine pests" are pests of potential importance to the areas endangered thereby and not yet present there, or present but not widely distributed and being officially controlled[4]. *Pre – shipment applications* are *non-quarantine* MB applications applied within 21 days prior to export to meet the official requirements of the importing country or existing official requirements of the exporting country[5]

Quantities of Methyl Bromide Consumed for QPS

Production for QPS

The Montreal Protocol requires governments to report data on QPS annually. The years 1999 and 2002 to 2006 represent the most complete data[6] reported by Parties on QPS production. These data, together with the production for 1992 estimated by TEAP[3], are shown in Figure 2. Global production for QPS in 2006 was reported to be 10,275t.

The trend is generally downwards apart from a sharp increase in 2005 to almost 14000t, which could be due to additional QPS production necessary to satisfy uses "... *that were not previously considered as QPS, the impact of the adoption of ISPM* – 15, *and stock issues*"[3]. At the current rate of reduction of QPS production we estimate that QPS – MB will approach zero in about 55 years or 2063.

Figure 2: Reported production of methyl bromide by Parties for QPS in 1999 and from 2002 to 2006 (Ozone Secretariat 2008). TEAP estimate of production for 1992.

The Use of QPS – MB by Category

The use of QPS – MB reported by Parties for 15 categories was 5 273 tonnes (TEAP 2006)[7]. However, TEAP cautioned that Parties had reported less than 25% of more than 4,000t estimated by TEAP to be used annually for whole logs and timber[7]. Based on the available data, Table 1 indicates that the top seven categories account for 98.8% of the total

quantity used for QPS – MB.

The single largest category comprises wood (including sawn timber and whole logs), which accounts for almost 50% of the total. Two surveys undertaken recently in Australia and the Asia-Pacific region also identified timber and wooden materials as major users of QPS – MB[3]. These data have implications for the deployment of resources for the adoption of relevant alternatives (Section 7).

Table 1. Quantity of methyl bromide used for QPS by 32 Parties reporting quantities in various categories of QPS use (data from 2002, 2003 and 2004)[7]

Categories of QPS Use	Quantity (tonnes)	Percent of total	Number of Parties Reporting
Wood, including sawn timber & whole logs	4,000[1]	48.80%	Note[2]
Soil (pre – plant)	1,527	18.63%	2
Grain and cereals for consumption	1,262	15.40%	14
Fresh fruit and vegetables	722	8.81%	11
Wooden packaging materials	335	4.09%	19
Dried foodstuffs	160	1.95%	11
Cotton and fibre	91	1.11%	10
Other[3]	99	1.21%	
TOTAL	8,196		

TEAP (2006) estimate, as Parties did not report; 2 Not all Parties reported; 3 Other = Equipment; cut – flowers and branches; personal effects; bulbs, corms, tubers and rhizomes; nursery stock; hay, straw and fodder; seeds for planting

Decisions that aim to Minimise the Use and Emissions of QPS – MB

Since 1994, six Decisions[4] on QPS – MB have been agreed in the Montreal Protocol, summarised in Table 2. They aim to promote the adoption of alternatives, minimise QPS – MB use, and reduce emissions. Decisions in the Montreal Protocol are not legally binding, unlike control schedules which are legally binding.

To date most Parties have generally not taken significant and concerted action to implement these Decisions, particularly in regard to adopting available alternatives, installing recovery equipment, and reviewing national regulations with a view to removing the requirement to use MB for QPS.

Table 2. Summary of Montreal Protocol decisions that encourage reduction in the use and release of QPS – MB

Decision	Parties are encouraged to…
VI/11 (1994)	· Use containment, recovery and recycling technologies more widely
VII/5 (1995)	· Refrain from the use of QPS – MB where possible · Use non – ozone – depleting technologies where possible · Minimise emissions and use of MB through containment, recovery, re cycling to the extent possible, which should be more widely applied
X/11 (1998)	· Submit to the Ozone Secretariat a list of regulations that mandate the use QPS – MB · Report volumes of QPS – MB consumed
XI/13 (2001)	· Report volumes of QPS – MB consumed · Review their national plant, animal, health and stored product regulations with a view to removing the requirement for the use of MB for QPS where technically and economically feasible alternatives (TEFAs) exist · Monitor the use of QPS – MB by commodity and quantity to target efficient use of resources to develop and implement TEFAs · Identify TEFAs early, where they exist
	· Encourage the use of recovery and recycle technology, where technically and economically feasible, until alternatives for QPS – MB become available

Decision	Parties are encouraged to…
XVI/10(2004)	· Provide best – available data to a "QPS Task Force" that identifies QPS – MB, by commodity and application · Provide information on applying TEFAs for MB uses that are more than 10% of QPS consumption or highest volume uses · Provide information on known QPS – MB uses, where data are available
XV1/11(2004)	· Apply heat or use alternative packaging materials rather than MB, in response to ISPM – 15 · Accept imported wood packaging treated without MB

Adoption of Alternatives

The Protocol urges governments to identify and adopt technically and economically feasible alternatives(TEFAs), where possible, which are discussed in the last Section.

Methyl Bromide Recovery and Recycle Technology

In cases where alternatives are not available, the Protocol asks governments to reduce MB emissions by encouraging the use of recovery technology. TEAP (2006) estimates that 90% of the QPS – MB is emitted[7] to the atmosphere.

Some countries have attempted to reduced these emissions by attaching recapture technology to fixed fumigation facilities. Deployment of recapture technology is increasing but is still low[8]. Canada and the USA have developed and installed MB recovery equipment for fixed fumigation facilities over the past 10 years. The relatively high cost of installing and operating this equipment[9] has limited investment in this technology. In addition, recovery technologies do not reduce reliance on MB, and still allow substantial emissions in most cases. For this reason, some countries have preferred to invest in research on MB alternatives, rather than recovery technology, to provide a permanent solution.

A second approach which has been developed recently captures MB emissions before shipping containers are opened to unload fumigated materials. Although these have been installed mainly to address health and worker safety, a co-benefit is enhanced protection of the ozone layer protection.

Companies are operating in the Netherlands and Belgium. Belgium, for example, has two companies with a combined capacity to service up to 75 000 containers per year[3]. Similar recovery units are also reported to be operating in Australia (10 units), India (1), Malaysia(2), and USA(1). Recent legislation in New Zealand requires recovery equipment to be used within a local port on all fumigations using more than 3 kg of MB.

List of Regulations that Require the Use of Methyl Bromide

In 1998 Decision X/11 requested governments to submit to the Ozone Secretariat a list of regulations that require the use of MB. In 1999 MBTOC requested 96 countries to provide information on the use of QPS – MB, including this list of regulations. Only one third of countries provided some information on uses[9], and few provided a list of regulations.

In 2001, Decision XI/13 requested governments review their national plant, animal, health and stored product regulations, with a view to removing the requirement for the use of MB for QPS where technically and economically feasible alternatives exist. Countries were not asked to report on this activity, so there is no information on the number of countries that implemented this Decision.

Monitoring QPS Uses to Target Resources for Alternatives

Decision XI/13 in 2001 encouraged governments to monitor the use of QPS – MB, by commodity and quantity, to target efficient use of resources to develop and implement TEFAs. In general, countries have not put in place procedures to monitor the use of QPS – MB by commodity and quantity, as evidenced by the difficulty government's face in reporting on use by sector. TEAP (2006) surmised that Parties may have not have had sufficient time between 2001 and 2003/2005 to implement such procedures[7]. As a result, there is little information available to governments on targeting resources to develop and implement alternatives.

Alternatives to QPS – MB

MBTOC reports since 1994 have identified alternatives for a large portion of QPS – MB uses, including many non-MB phytosanitary treatments approved by national authorities. Forty-two Parties, in responding to an earlier

survey, reported that 65% of the QPS – MB could be replaced with commercially-available alternatives, but they considered that cost, location and lack of acceptance by trading partners were major impediments to their implementation[10]. TEAP has noted that many approved alternatives are available for major uses, but there has been little incentive for their adoption[11].

Activities that Reduce the Use of QPS – MB

Despite the lack of response to Montreal Protocol decisions, which are non-binding on governments, a number of countries have undertaken activities to reduce or eliminate QPS – MB, or are in the process of doing so, as illustrated by the following examples.

Legislative Action Targeting all Uses of QPS

The EC was the first region to limit QPS – MB by capping[ ] the annual consumption of QPS – MB to 1012t from 1 January 2001. EC Member States are required to report annually to the European Commission on the quantities of QPS – MB authorised in the previous year, the purposes for which it was used, and progress in evaluating and using alternatives for QPS. The quantity authorised for consumption was in practice about one third less than the cap each year since 2001 and, moreover, the cap did not increase when the EU expanded from 15 to 27 countries beginning on 1 May 2004.

The total QPS – MB used in the EC in 2004, 2005 and 2006 was 400, 354 and 362t respectively. Ten countries used no QPS – MB, 11 averaged less than 20t per year and 6 averaged more than 20t (but five of them were reducing annually)[13]. Legislation in some EC countries prohibits the use of QPS – MB and has done so for many years[14].

Logs and Wood Products

New Zealand recently placed a levy on MB imports to finance work to reduce emissions and use[15]. An international symposium in New Zealand in 2008 discussed 28 possible alternatives to QPS – MB. Research is underway on the use of generated phosphine on key exports crops including logs, sawn timber, apples and onions. QPS – MB use in 2006 was 74% of the total used of 177 tonnes and is increasing at 14% per year due to exports to China, India and Malaysia[7]. Trials are underway that aim to gain approval for in-transit fumigation of logs with phosphine to India, following the success of similar shipments to China that eliminated some 200 tonnes per year of QPS – MB. In the longer term, an ecologically-based, risk assessment system is under development for forestry exports that ensures that quarantine pests are treated only when necessary and with the most environmentally benign fumigant.

Uptake of Alternatives for Wood Packaging Materials (ISPM – 15)

ISPM – 15 standard was endorsed by the IPPC in March 2002, and most countries had implemented legislation by mid – 2005. The standard requires the use of either MB or heat for wood packaging material (WPM). Now three years later, heat or MB treatment of WPM, compliant with the ISPM – 15 standard, is required in 37 countries. Many independently-certified heat treatment facilities exist in developing and developed countries including Australia (56 facilities), the EU[16] (>1500), India (41), Malaysia (30), NZ (146), and the US (>4000). Canada and China (Taiwan) no longer use QPS – MB to meet ISPM – 15 requirements and instead rely on heat treatments[3]. There has also been an increase in non-wood packaging material made from recycled plastic, which completely avoids the disinfestation requirement. The large increase in MB use from 2002 predicted by TEAP (2005) is therefore unlikely to eventuate, given the significant global deployment of heat treatment facilities over the past 4 years[17].

In regard to imported logs typically fumigated on arrival in Japan, that country may soon register two new fumigants (sulfuryl fluoride/methyl isothiocyanate mixture and methyl iodide), following successful trials that concluded in March 2008[3].

Soil Fumigation

According to estimates by TEAP, the second largest user of QPS – MB is for "Soil (preplant)", which refers to soil fumigation with MB in the USA and Chile[7] for strawberry runner production and certain other nursery crops. Live plant material is certified by the government as "pest-free" on the basis of soil fumigation several months earlier, with the intention of reducing the risk of soil nematodes as pests being shipped to importing countries and regions.

Discussions in the Montreal Protocol in 2006 questioned whether soil fumigation with MB can control pests several months later to applicable quarantine standards[18], and noted that QPS – MB used for this purpose does not

appear to comply with the definition of QPS. Approximately 1,500t of MB are used annually for this purpose. TEAP reported that fumigation with MB was highly effective in reducing soil-borne pathogens but did not consistently eradicate them[19]. Moreover, alternatives are available to replace QPS – MB for pre-plant soil fumigation[19,7]. It appears that this use should be replaced by alternatives which would reduce QPS – MB uses by almost 20% ,or be evaluated by MBTOC again as a potential critical use.

Conclusions

Several countries put in place legislation that prohibited QPS – MB in the 1990s, without adverse effects. The EC placed limits on the volume that can be used, and the use of QPS – MB has fallen. Other countries have also adopted alternatives in specific sectors and have targeted sectors that consume the most QPS – MB such as logs and stored grain. More countries are now focusing on the high-volume sectors and have begun to take action to reduce QPS – MB. However, many countries have taken little action to date. QPS – MB use has declined very slowly relative to the other uses of methyl bromide. QPS – MB is the largest uncontrolled emissive use of any ozone-depleting substance. For this reason the Montreal Protocol is likely to explore other options that could result in greater controls on the use of QPS – MB.

Developing countries would benefit from controls on QPS – MB because controlled uses in the Montreal Protocol enable countries to apply for financial assistance to comply with the controls.

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0204

Toxic Activity of Allicin on Several Stored Product Pests

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Abstract: The fumigant effect of allicin was tested on the larvae, pupae and adults of *T. castaneum*, *Oryzaephilus surinamensis* and *Cryplotestes ferrugineus*. The LC_{50} of 3 – days exposure of adults to allicin was 0.68, 0.86 and 0.99 L/L respectively. The LC_{50} of 3 – days exposure of the larvae to allicin was 0.11, 0.12 and 0.36 L/L respectively, indicating that the larvae were much more sensitive than adults to allicin. Trials in the laboratory using allicin with wheat that imitated a commercial f resulted in LC_{50} values for adults of the three species of 8.29, 7.61 and 8.26 L/L respectively. An allicin concentration of 5 L/L suppressed pupal emergence of *T. castaneum*, *O. surinamensis* and *C. ferrugineus* by 85.56%, 94.44% and 100.00 % respectively. Low concentrations of allicin were effective in controlling these stored product pests, but micro-encapsulation may be necessary to conceal its odour and to allow future commercialisation of this promising fumigant.

Introduction

Phosphine (PH_3) is a toxic gas used to protect stored commodities against insect pest infestation. It is by far the most widely used fumigant of stored products worldwide, because it is inexpensive to apply and leaves little or no residue (Cao *et al.* 2006). With the emergence of high level phosphine resistance in insect populations in various regions of the world, there is increasing interest in determining the mode of action and the mechanisms whereby insects acquire resistance to this fumigant (Cao 2000; Zhang *et al.* 2004). The future use of phosphine as a commercial fumigant could be threatened by the further development of resistant strains.

Many alternatives have been tested to replace methyl bromide fumigation for stored product and quarantine uses. Recently, the secondary metabolites of some plants have been formulated as botanical pesticides for plant protection, since they do not leave residues toxic to the environment, they have lower toxicity to mammals and medicinal properties for humans (Catherine 1995).

Crude extracts and a steam-distilled oil fraction of garlic were found to be larvicidal against several species of mosquitoes and could be used as a potential grain protectant against *Tribolium castaneum* and *S. zeamais* (Lu *et al.* 2003). Hexane extracts of garlic were also found to be insecticidal to *Liposcelis entomophlia*, *R. dominica* and *T. castaneum* (Lv *et al.* 2006). Chemical analysis showed that the major chemical component of garlic oil is allicin (Yin 2002).

In our research, the fumigant effect of commercial allicin was tested on the stored product insects *T. castaneum*, *Oryzaephilus surinamensis* and *Cryplotestes ferrugineus*.

Materials and Methods

Insects

T. castaneum, *O. surinamensis* and *C. ferrugineus* colonies was obtained from Qihe storage of Shandong province, Jiyuan storage and Zhidian storage of Henan province respectively. These insects were reared for several generations in our laboratory. *T. castaneum* was reared on wheat flour mixed with yeast (7∶1, w/w) while *O. surinamensis* and *C. ferrugineus* were reared on wheat flours mixed with oatmeal and yeast (7∶3∶1) and maintained in the dark in incubators at 30 ± 1℃ and 70% – 80% RH.

Commercial Allicin

Commercial allicin was purchased from Sudong Chemical Plant Laboratory in Nantong of Jiangsu Province. The purity (98.5%) was confirmed by gas chromatography.

Toxicity Bioassay in Laboratory

Fumigation toxicity of allicin was tested in the laboratory using a sealed jar (Zhang *et al.* 2001). Three adults were put into the 300mL jar and a filter paper (9cm Longth, 1cm width) soaked with different concentrations of allicin

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was hung in the jar. Fifteen adults were used in each treatment and three replicated in this assay. The control jar was similarly treated but without allicin in the filter paper. The jars with treated and control were kept in a controlled climate room at 28 ± 2℃, 24 h darkness and 75% 5% RH. The phosphine was dissipated on time and the numbers of dead adults counted after exposure periods of 6h, 12h, 24h, 48h, 72h, 96h, 120h and 144h respectively.

The toxicity of allicin against larvae and pupae were carried out with the same methods described for the adults. The phosphine was released and the number of dead larvae counted at the times described above. The other conditions were same as those described for the adults. The number of adults that emerged was observed every day until the adult number remained constant. The emergence rate of pupae was calculated.

Imitation of a Commercial Fumigation

The methods for imitating a commercial fumigation in the laboratory were the same as those used in the toxicity bioassay, but the difference was that there was 110g of wheat in each sealed jar (300mL). The fumigation time was 24h, 48h, 72h, 96h, 120h and 144h.

Statistical Analysis

Every experiment was replicated at least five times and values were expressed as mean SE. LC_{50} and LC_{95} values were calculated by Probit Analysis (SAS Institute 2004). The modified mortality of larval and pupae were calculated by the following formula: The modified mortality = (treatment mortality control mortality)/(1 − control mortality) * 100%.

Results and Analysis

Toxicity of Allicin Against Adults

The toxicity of allicin to adults of *T. castaneum*, *O. surinamensis* and *C. ferrugineus* was determined using different exposure times and concentrations. The relationship between logarithm of concentration (x) and probability of death (y) was fitted a with linear regression equation (Table 1).

The LC_{50} and LC_{95} of adults exposed to allicin were significantly reduced with extended fumigation times. The LC_{50} and LC $_{95}$ of allicin against *T. castaneum* was 1.51 and 4.23μL/L respectively when the time was 6h. When the time was 72h, the LC_{50} and LC_{95} of allicin for *T. castaneum* were 0.68 and1.27μL/L, respectively. The results indicate that for the same LC the concentration of allicin could be reduced if the fumigated time was prolonged.

The LC_{50} and LC_{95} of allicin against *T. castaneum* were less than that of *O. surinamensis* and *C. ferrugineus* when the fumigated time was held constant e. g., LC_{50} was 79.07% and 68.69% that of *O. surinamensi* and *C. ferrugineus* when the exposure time was 72h. This suggested that *T. castaneum* was the most sensitive to allicin of the three species of adults.

Table 1. Comparison of toxicity of allicin against three species of adult grain pests

Adults	time /h	Regression equation/y =	LC_{50}/μL. L^{-1} (95% confident range)	LC_{95}/μL. L^{-1} (95% confident range)	r	χ^2
T. castaneum	6	4.345 +3.671x	1.51(1.413 −1.619)	4.23(3.612 −5.205)	0.991	5.169
	12	4.892 +3.720x	1.07(0.993 −1.144)	2.96(2.648 −3.403)	0.972	18.733
	24	5.227 +4.605x	0.89(0.823 −0.958)	2.03(1.886 −2.217)	0.917	14.317
	48	5.662 +5.841x	0.77(0.700 −0.834	1.47(1.391 −1.566)	0.950	7.030
	72	6.014 +6.170x	0.68(0.604 −0.756)	1.27(1.188 −1.346)	0.949	5.155
O. surinamensis	6	3.981 +1.759x	3.80(2.930 −5.862)	32.74(16.199 −111.675)	0.985	1.897
	12	4.224 +1.670x	2.92(2.373 −4.038)	28.19(14.74 −84.679)	0.974	3.788
	24	3.671 +1.856X	2.28(1.919 −2.955)	26.64(14.014 −79.535)	0.959	11.510
	48	4.760 +1.715x	1.38(1.215 −1.581)	12.57(8.048 −25.600)	0.948	19.339
	72	5.108 +1.656x	0.86(0.704 −1.000)	8.48(5.775 −15.723)	0.930	10.058
C. ferrugineus	6	3.819 +2.447x	3.04(2.540 −4.002)	14.32(9.110 −29.170)	0.959	9.207
	12	4.124 +2.255x	2.45(2.117 −2.995)	13.11(8.679 −24.573)	0.917	18.461
	24	4.269 +2.217x	2.14(1.885 −2.530)	11.79(8.037 −20.935)	0.994	1.458
	48	4.656 +1.954x	1.50(1.344 −1.697)	10.43(7.207 −18.137)	0.963	7.532
	72	5.012 +1.883x	0.99(0.853 −1.111)	7.37(5.379 −11.771)	0.969	5.998

Imitation of a Commercial Fumigation in the Laboratory

In order to test the fumigation effect of allicin in storage, an imitation of a commercial fumigation was carried out using wheat. Perhaps allicin could be absorbed by the wheat and the toxicity could be proportional to the rate of absorption. The rate of adsorption is the ratio of LC_{50} of insect in bottles with bottles and blank bottles (Table 2).

The adsorption rate was the lowest when allicin fumigation time was extended to the fourth day for adults of *O. surinamensis* and *C. Ferrugineus*; and to the sixth day for adult is *T. castaneum*. The results indicate that the optimum fumigation time for allicin against stored product insects was 4 to 6 days. The adsorption rate decreased when the fumigation time was longer. The fumigated time could be adjusted in the future according to the known adsorption rate.

Table 2. Comparison adsorption and toxicity and mimicked toxicity effect of allicin with three species of adults

Adult	LC50 and adsorption rate	1d	2d	3d	4d	5d	6d
	Blank bottle $LC_{50}/\mu L.\ L^{-1}$	0.89	0.77	0.68	0.59	0.51	0.38
T. castaneum	Wheat bottle $LC_{50}/\mu L.\ L^{-1}$	13.49	10.17	8.29	6.98	6.02	4.92
	Adsorption rate	15.16	13.21	12.19	11.83	11.80	12.95
	Blank bottle $LC_{50}/\mu L.\ L^{-1}$	2.28	1.35	0.86	0.76	0.62	0.51
O. surinamensis	Wheat bottle $LC_{50}/\mu L.\ L^{-1}$	28.74	15.99	9.24	5.10	4.41	4.06
	Adsorption rate	12.61	11.84	10.74	6.71	7.11	7.96
	Blank bottle $LC_{50}/\mu L.\ L^{-1}$	2.14	1.50	0.99	0.61	0.57	0.51
C. ferrugineus	Wheat bottle $LC_{50}/\mu L.\ L^{-1}$	23.02	15.87	12.36	7.56	4.19	3.38
	Adsorption rate	10.76	10.58	12.48	12.39	7.35	6.63

Fumigant Toxicity of Allicin Against Larvae

The toxicity of allicin to larvae of *T. castaneum*, *O. surinamensis* and *C. ferrugineus* was determined using different exposure times and concentrations. The relationship between logarithm of concentration (x) and probability of death (y) was fitted a with linear regression equation (Table 3). The LC_{50} and LC_{95} of larvae could be decreased as the fumigated time was prolonged. Compared with the LC_{50} and LC_{95} achieved in 6h, in 72h they declined to 85.14% and 87.99% respectively. The quantity of allicin could be reduced for the same level of mortality if the fumigation time was correspondingly prolonged.

Table 3. Toxicity of allicin against larval of *T. castaneum*, *O. surinamensis* and *C. ferrugineus*.

Adults	time /h	Regression equation/y =	$LC_{50}/\mu L.\ L^{-1}$ (95% confident range)	$LC_{95}/\mu L.\ L^{-1}$ (95% confident range)	r	χ^2
	6	5.348 + 2.650x	0.74 (0.649 – 0.884)	3.08 (2.112 – 5.799)	0.971	4.662
	12	5.985 + 3.075x	0.48 (0.428 – 0.531)	1.64 (1.310 – 2.291)	0.956	9.122
T. castaneum	24	6.558 + 2.668x	0.26 (0.202 – 0.311)	1.08 (0.900 – 1.403)	0.996	24.229
	48	7.542 + 3.293x	0.17 (0.111 – 0.220)	0.53 0.465 – 0.616)	0.985	9.177
	72	8.028 + 3.213x	0.11 (0.046 – 0.176)	0.37 (0.278 – 0.446)	0.978	3.796
	6	4.656 + 1.647x	1.62 (1.257 – 2.397)	16.11 (8.004 – 53.770)	0.972	3.799
	12	5.129 + 1.624x	0.83 (0.700 – 1.020)	8.57 (5.073 – 19.959)	0.957	6.557
O. surinamensis	24	5.440 + 1.717x	0.55 (0.461 – 0.653)	5.03 (3.361 – 9.363)	0.964	6.020
	48	5.890 + 2.018x	0.36 (0.290 – 0.430)	2.36 (1.833 – 3.402)	0.967	8.690
	72	6.891 + 3.060x	0.24 (0.180 – 0.296)	0.83 (0.730 – 0.965)	0.989	14.915

Adults	time /h	Regression equation/y =	LC_{50}/μL. L^{-1} (95% confident range)	LC_{95}/μL. L^{-1} (95% confident range)	r	χ^2
C. ferrugineus	6	3.979 +2.728x	2.37(1.918 –3.376)	9.49(5.752 –23.109)	0.963	5.448
	12	4.308 +2.625x	1.83(1.573 –2.306)	7.76(5.131 –15.343)	0.954	8.179
	24	6.081 +2.802x	1.05(0.919 –1.195)	5.44(3.851 –9.485)	0.959	5.544
	48	5.875 +2.976x	0.51(0.406 –0.598)	1.83(1.573 –2.197)	0.936	14.850
	72	6.553 +3.541x	0.36(0.254 –0.460)	1.06(0.935 –1.208)	0.929	11.183

Toxicity of Allicin to Pupae

The effects on emergence of pupae exposed to different concentrations of allicin fumigant are shown in Table 4. Emergence was completely prevented in *T. castaneum* using a concentration of 5μL/L. An allicin concentration of 4μL/L reduced emergence of *T. Castaneum*, *O. surinamensis* and *C. Ferrugineus* pupae to 67.78, 80.00 and 93.33% respectively. The results suggest that, of the three species, *C. ferrugineus* pupae were the most sensitive to allicin.

Table 4. Modified mortality of pupae fumigated with different concentrations of allicin

Pupae	Modified morality/%				
	1/μL. L^{-1}	2/μL. L^{-1}	3/μL. L^{-1}	4/μL. L^{-1}	5/μL. L^{-1}
T. castaneum	10.00 ±3.85 b	25.56 ±4.01 c	52.22 ±4.01 c	67.78 ±1.11 c	85.56 ±1.11 c
O. surinamensis	32.22 ±2.94 a	50.00 ±5.77 b	68.89 ±4.01 b	80.00 ±1.92 b	94.44 ±1.11 b
C. ferrugineus	41.11 ±4.84 a	66.67 ±3.85 a	86.67 ±1.11 a	93.33 ±1.92 a	100.00 ±0.00 a

Discussion

This study showed that allicin had a significant fumigant effect on different life stages of three species of insects, especially on *T. castaneum*. These species were sensitive to allicin at low fumigant concentrations, perhaps because this is their first exposure to this fumigant. Research on the how each species metabolises allicin may provide further insight on how to optimise its use as a fumigant.

Papachr and Stamopulosd (2002) showed that the fumigation concentration should be increased or the fumigated time should be extended in order to optimise the fumigant's affect when used with grains that differ in their ability to absorb allicin. Our research showed that the fumigation effect was rapid and mortality increased with concentration. When the fumigant time was prolonged, the concentration of allicin could be reduced.

The fumigated concentration of allicin in the laboratory was very low, but it needed to be much higher when allicin was used with wheat. Absorption rate in wheat decreased gradually over time. Wheat absorbed significant quantities of allicin rapidly. Toxic concentrations of allicin diffused into the grain interior showing that it was highly permeable reaching insects in the interior of the grain. The lower absorption rate indicated good permeability and a better fumigant effect (Bai *et al.* 2002). Allicin with wheat required a fumigation period of 4 to 6 days.

The results indicate that allicin had a highly toxic effect on three species of stored product insects. Allicin is neither reported to have a toxic to humans and animals, nor does it contribute to environmental pollution. Allicin could be a substitute for existing fumigants to control stored product insects, particularly for the disinfestation of grain where synthetic chemicals are becoming less preferred.

However, allicin's strong odour may limit its application but micro-encapsualtion (Hyun *et al.* 2000; Kavindra *et al.* 2000) may conceal the odour, as it has done with other botanical insecticides. Previous studies have shown its odour can be concealed without reducing its toxicity (Ankri *et al.* 1999). Allicin therefore shows promise as a safe and effective replacement for existing stored product fumigants.

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0205

Impact of Sulfuryl Fluoride Fumigation and Heat Treatment on Stored – Product Insect Populations in German Flour Mills

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Abstract: Comparative treatments of sulfuryl fluoride (SF) and heat were evaluated in Germany as replacements for methyl bromide (MB), an ozone – depleting fumigant which has now been banned in developed countries for most of its uses. A study was carried out in two flour mills in Germany to determine the impact of SF fumigation (ProFume™ Dow AgroSciences LLC) and heat treatment on populations of flour beetles: *Tribolium castaneum* (Herbst) and *T. confusum* Jacquelin du Val and stored product moths: *Plodia interpunctella* (Hübner) and *Ephestia elutella* (Hübner). One mill was selected for fumigation with ProFume? the other for the heat treatment. Traps baited with an aggregation pheromone lure and an oil – based food attractant were used to monitor populations of flour beetles. For the monitoring of flour moths, sticky traps baited with a pheromone lure were applied. Traps were placed inside the mill buildings within the areas selected for treatment. In the mill that was fumigated with SF, populations of stored product insects were monitored for 6 weeks prior to, and 16 weeks following, the treatment. In the mill that underwent a heat treatment, the monitoring started 15.5 weeks prior to, and ended 6.5 weeks following, the treatment. Both SF fumigation and heat treatment provided successful control of the target pests during the monitoring period. After the SF fumigation only 3 *Tribolium* individuals could be detected and no moths. After heat treatment 20 *Tribolium* beetles and 2 moths were detected. Thirteen beetles were found in the basement which is the most difficult place to heat while the remaining ones were scattered throughout the building. We conclude that both SF and heat are both suitable replacements for MB for controlling stored product pests in German flour mills.

Introduction

In Germany, MB use for fumigation of flour mills was phased out completely by the end of the year 2004 following the stipulations of the Montreal Protocol (Reichmuth, C., *pers. comm.* 2004). Among the alternatives studied in order to replace MB in flour mills, only fumigation with sulfuryl fluoride (SF) and heat treatments have shown sufficient action on stored product pests and suitability to be used in practice. Insecticide treatments are limited to special purposes such as pirimiphos-methyl application on surfaces or fogging with pyrethroids against flying moths. Such treatments should preferably be included in individually designed Integrated Pest Management (IPM) concepts which are based on components such as pest prevention by means of structural design, sanitation and monitoring of stored product pests using traps and lures. Additional features of IPM schemes for mills are proper documentation of pest occurrence and control measures, training and motivation of staff [1].

SF was developed in the 1950s by the Dow Chemical Company in the USA as a fumigant to control drywood termites and wood-boring beetles. Under its trade name Vikane (Dow Agrosciences LLC) it has been in use for this purpose until this day. In 2003, its proven efficacy against stored product insects[2] led to registrations as a fumigant for post-harvest applications under the trade name ProFume (Dow Agrosciences LLC) in the USA, followed by registrations for different post-harvest applications in the European Union from 2004 onwards[3].

Heat treatment has been previously described for controlling stored-product insects in the early 1900s[4]. It has been included in the first textbook on stored product protection ever published in Germany[5] and continued to play a substantial role for treating empty rooms to date[6].

The work described here was completed between August and December 2007. Its objective was to assess the impact of a SF fumigation and a heat treatment (both applied separately) on the post-treatment development of stored-product insect populations in German flour mills and to provide a comparison of both types of treatment. The design of the study was chosen in a way which allows easy comparison with a simi-

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lar study undertaken in the United Kingdom (Small, G. unpublished, pers. comm. 2006).

Materials and Methods

Both flour mills which were selected for this trial are situated in the North of Germany. The mill which was fumigated with SF (Mill A) consists of two parts for milling oats and maize, respectively. The buildings are not fully separated. The volume of this mill was 23,000 m^3 plus a loading hall with a volume of 9,000 m^3 and silos with a total volume of 1.850 m^3. The mill was built in 1848 and has been extended on three occasions. It is constructed of bricks and ferro-concrete, with mainly wooden floors and some parts in concrete.

The mill which was treated with heat (Mill B) is a flour mill for wheat and rye. The facility has two main buildings: the mill itself has a volume of about 40,000 m^3 and the flour silo 60,000 m^3. The mill-building was constructed 70 to 80 years ago with bricks for the outside frame and concrete plus wood inside. The inner building consists of steel-girder, ferro-concrete, pitch-pine floors and a wooden roof construction. Windows are made of plastic material. The flour silo is approximately 25 years old. Construction material is concrete plus a metal-sheet frame outside.

The SF fumigation at Mill A was carried out by a commercial fumigator using the ProFume Fumiguide computer program from Dow AgroSciences LLC. Fumigation started on 31 August 2007 and concluded on 2 September 2007. The period of exposure was 50 h with a CT (product of concentration and time) of 1 013 g · h/m^3.

The heat treatment was carried out by milling staff with 12 years of experience using this technology. It was completed using heaters located outside the building. The heat was conducted into the interior via tubes. The treatment started on 2 November 2007 and was concluded on 4 November 2007. The total duration of the treatment was 48 h. Exposure to lethal temperatures around 50℃ in all treated areas lasted 24 h.

In both mills, the presence of stored product insect pests was monitored using commercially available traps and pheromone lures which were distributed in all treated areas of the buildings. In order to detect the rust – red flour beetle *Tribolium castaneum* (Herbst) and the confused flour beetle *T. confusum* Jacquelin du Val, Dome Traps loaded with specific pheromones for detecting *Tribolium* spp. were used (20 traps in Mill A and 25 traps in Mill B). *Plodia interpunctella* (H bner) and *Ephestia elutella* (H bner) were detected with Delta Traps and a pheromone designed to attract *P. interpunctella* and different species of *Ephestia* (10 traps in Mill A and 12 traps in Mill B). These materials were obtained from Trece, Inc. (USA).

Trap catches were recorded immediately before the treatments and at monthly intervals after the treatment. All traps and pheromone lures were replaced during all inspections. Insect samples collected were sent to a Government laboratory for identification (LAVES, Mr. Stelling, Stade, Germany).

Results and Discussion

In order to provide an overview of infestation before and after the treatment, trap catches were pooled for the different floors or areas of both mills. For Mill A the respective *Tribolium* spp. numbers are shown in Table 1:

Table 1. Total numbers of *Tribolium confusum* and *T. castaneum* trapped before and after fumigation at mill A.

	Numbers of *Tribolium* spp. counted					
	07/07	08/07		09/07	10/07	12/07
Basement	2	8	Fumigation with sulfuryl fluoride	0	0	0
1st floor	12	4		0	0	0
2nd floor	13	2		0	0	0
3rd floor	51	4		0	0	0
4th floor	42	5		1	0	1
5th floor	31	7		0	0	0
Maize mill	4	5		1	0	0

Except for the three *Tribolium* spp. specimens that were detected after treatment, the percentage reduction of flour beetles was 100% in all areas of the fumigated Mill A throughout the three post-fumigation monitoring periods. Percentage reduction was calculated using the

formula:

$$(CT) \times 100/C$$

In this formula C = number of insects trapped during the pre-treatment monitoring period (09/07), and T = number of insects trapped during the single post-treatment monitoring periods.

The number of moths in the traps is shown in Table 2.

Table 2. Total numbers of *Plodia interpunctella* and *Ephestia elutella* trapped before and after fumigation at mill A

	Numbers of moths counted					
	07/07	08/07		09/07	10/07	12/07
Basement	2	4		0	0	0
2nd floor	0	0	Fumigation with sulfuryl fluoride	0	0	0
3rd floor	0	0		0	0	0
4th floor	4	3		0	0	0
Maize mill	28	9		0	0	0

No moths were detected throughout the three post-fumigation monitoring periods. Therefore, the percentage reduction of stored product moth species was 100% in all areas of Mill A, which were infested before the fumigation.

Table 3 shows the number of *Tribolium spp* trapped in Mill B which underwent a heat treatment.

Table 3. Total numbers of *Tribolium confusum* trapped before and after heat the treatment in Mill B

	Numbers of *Tribolium* spp. counted						
	07 – 08/07	08 – 09/07	09 – 10/07	10 – 11/07		11/07	12/07
Basement	13	20	25	12		0	13
1st floor	2	6	6	1		0	1
2nd floor	8	12	6	5	Heat treatment	0	0
3rd floor	3	13	17	2		1	0
4th floor	2	2	1	2		1	2
5th floor	0	5	4	0		0	2

Out of the two flour beetle species, only *T. confusum* was found in Mill B. At the time of the first post-treatment monitoring, only one confused flour beetle was detected on each of floors 3 and 4. During the second post-treatment monitoring period, a small beetle population had started to build up again. Out of the 18 individuals caught by that time, 13 were detected in the basement. As this cool area was the most difficult place to heat, there is a probability that some eggs survived the heat treatment in hidden places of the basement. They may have given rise to the small population discovered 6.5 weeks after treatment.

Moth catches in Mill B are shown in Table 4.

Table 4. Total numbers of *Ephestia elutella* trapped before and after heat treatment in Mill B

	Numbers of *Ephestia elutella* counted						
	07 – 08/07	08 – 09/07	09 – 10/07	10 – 11/07		11/07	12/07
Basement	6	20	4	7		0	0
1st floor	2	3	0	5		0	0
2nd floor	8	14	19	26	Heat treatment	1	0
3rd floor	2	3	2	2		0	0
4th floor	0	1	5	6		0	0
5th floor	0	4	3	0		0	0

In Mill B, only *E. elutella* could be detected but no *P. interpunctella*. Apart from one individual detected in the 2nd floor immediately after treatment, percentage reduction of *E. elute-*

lla was 100 % in all infested areas throughout the two post-fumigation monitoring periods.

The results of the trap catches indicate that both treatments, SF fumigation (Mill A) and heat treatment (Mill B), successfully controlled the target pests during the monitoring period. Compared with the SF fumigation, heat treatment appears to be slightly inferior for controlling flour beetles as 20 *Tribolium* beetles were detected after the treatment. All but two of them appeared 6.5 weeks after treatment. Thirteen of these were found in the basement which is the most difficult area to heat while the remaining ones were distributed all over the building. It is possible that eggs survived heat treatment in hidden cool places of the basement which gave rise to the small population discovered 6.5 weeks later. Apart from the basement, however, very few single insect specimens appeared elsewhere in the mill treated with heat.

These results confirm the findings of previous studies which reported excellent control of stored product pests under field conditions using SF[7]. In that research, infestation remained below pre-fumigation levels three months after fumigation in all parts of the mill. The reduction of infestation levels of *T. confusum* achieved by heat treatment varied between 95% and less than 70%, depending on the treated area/floor. The research concluded that the initial impact of SF fumigation on *T. confusum* populations was much higher than with heat treatment.

Our excellent pest control results using both types of treatments in Germany were certainly due to the fact that all of them were conducted by professionals that were experienced in using their respective technologies. In addition, our work was carried out in mill buildings which were constructed 25 to 150 years ago. The older parts of these mills provide a more rigorous test of heat and SF than relatively modern and well-sealed mills which have been constructed more recently.

We conclude that application of heat as well as SF fumigation can be considered as valid replacements for MB for controlling stored product pests in German flour mills.

It must be emphasized that freedom from pests is not permanent. Therefore, the heat and SF applications work best within a framework of elaborated IPM schemes, with emphasis on thorough inspection of incoming grain, pest exclusion and sanitation.

Acknowledgements

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0206

Gasified Pirimiphos – methyl Control of Stored – grain Insects

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Abstract: We investigated the ability of gasified 50% pirimiphos – methyl EC to control stored-grain insects in a depot with stored-grain, and the ability of 5% pirimiphos-methyl AS to control insects in an empty depot. The results showed that there was no difference in chemical properties between gasified and EC pirimiphos-methyl. A concentration of 5.6 mg/kg gasified pirimiphos-methyl had better penetration into the grain and could effectively control the development of the offspring of stored-grain insects for one year. The effects of 0.5 g/m^3 pirimiphos-methyl AS controlling insects in an empty depot with different sprayers were compared. The gasification applicator was significantly more effective than the common high pressure sprayer. Based on the detection of fatty acid and other residues in stored – grain at different depths, controlling stored-grain insects by pirimiphos-methyl had no significant effect on quality.

Introduction

Pirimiphos-methyl or Chlorthiophos is a broad-spectrum, rapid-acting, and long – lasting pesticide. Organophosphate insecticides and acaricides with dermal and inhalation toxicology are extensively used as repellents for insect and acarid pests in all parts of grain storage (Hong Wang *et al.* 2006).

Other pest control chemicals were considered as candidates. Methyl bromide, a fumigant used for controlling stored-grain insects, was not selected because of its ozone-depleting properties. Phosphine is still the main fumigant to control stored-grain insects, but resistance of stored-grain insects to phosphine is developing rapidly.

The ability of pirimiphos-methyl to control stored-grain insects by gasification was investigated as a potentially effective, low toxicity and pollution-free method to control stored-grain insects while reducing the risk of insect resistance development following the work that had been initiated by others in this field (Pengcheng Fu &Kai Xu 2001; Jing Xie *et al.* 2007).

Materials and Methods

Test Depots

Depot with no stored-grain: Yueyang Depot of State Grain. No. 17 – 1 (common high pressure sprayer), No. 18 – 1 (gasification applicator produced by Yueyang Jinniu Biotechnology Co. Ltd). Horizontal silo, brick-concrete structure, generally sealed and leak-proof: Volume 7480 m^3.

Depot with stored-grain: No. 18 – 1 Yueyang Depot of State Grain. Horizontal silo made of brick and concrete, generally sealed and leak-proof. Volume 4210 m^3.

Test Grain

Harvested in 2005, early long-grain non – glutinous rice, 2452 t, moisture content 12.8 %, volumetric weight 577 kg/m^3, impurity content 1.5 %, brown rice yield 75.1 %, fatty acid value 22.5. Yueyang Depot of State Grain is responsible for routine tests related to grain quality. Hunan Chemical Industry Research Institute is responsible for detection of pesticide residue.

Test Insects

Rhizopertha dominica Fabricius, *Sitophilus zeamais* (Motschulsky), *Cryptolestes ferrugineus* (Stephens) (Chengdu Grain Storage Research

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Institute, State Administration of Grain, P. R. China). The insects were buried to a depth of 30cm in the center and four corners of the grain stack. The population density of wild insects in-depot were lesser grain borer 15 per kilogram grain, maize weevil 3 per kilogram grain, rust red grain beetle 13 per kilogram grain, and many booklice.

Insecticides

50% pirimiphos-methyl EC, 5% Pirimiphos-methyl AS (Hunan Haili Chemical Industry Co., Ltd).

Controlling Insects in Empty Depot

Both the Common High Pressure Sprayer and Gasification Applicator were used in this research. The liquor was sprayed uniformly and as high as possible in the air from Common High Pressure Sprayer.

Controlling Insects in Depot with Stored-grain Using Gasified 50% Pirimiphos-methyl

The liquor was sprayed using the Gasification Applicator under the condition of phosphine recirculation device, with 4 high – pressure centrifugal [fans] [pumps] operating for 48 hours.

Test of Pirimiphos-methyl Chemical Property

The 50% pirimiphos-methyl was evaporated at 190℃ ±10℃ and then liquefied. To verify whether the chemical property of 50% pirimiphos-methyl changed under high temperature condition, the 50% pirimiphos-methyl was evaporated at 190(10℃ and then liquefied. The chemical property of 50% pirimiphos-methyl before and after evaporation was determined using vapour-phase chromatography at the Hunan Chemical Industry Research Institute.

Results and Analysis

Test of Pirimiphos-methyl Chemical Property

Table 1 shows there was almost no change in active ingredient content of 50% pirimiphos-methyl, as the 50% pirimiphos-methyl remained stable at all three temperatures.

Table 1. Active ingredient content change of 50% pirimiphos-methyl before and after evaporation

Evaporation temperature(℃)	EvaporationTime(s)	Active ingredient content(%)	
		Before evaporation	After evaporation
180	0.5	48.4	48.2
	1	48.4	48.2
	2	48.4	48.3
190	0.5	48.4	48.3
	1	48.4	48.3
	2	48.4	48.3
200	0.5	48.4	48.3
	1	48.4	48.3
	2	48.4	48.2

Controlling Insects in Empty Depot

The test started on 6 June 2006 with concentration of 0.5 g/m^3 in depot No. 17 – 1. The effect of the treatment was evaluated on 8 June 2006 (Table 2).

Table 2. Effect of controlling insects in empty depot by common high pressure sprayer

	Number of living insects per square meter			
	Cryptolestes pusillus (*Schonherr*)	*Sitophilus zeamais* (Motschulsky)	Booklice	*Rhizopertha dominica* Fabricius
Before test	2	6	7	10
After test	0	1	2	2
Mortality	100%	84%	72%	80%

Controlling Insects in Empty Depot by Gasification Applicator

The test started on 25 July 2006 with concentration of 0.5 g/m^3 in depot No. 18 – 1. The

effect of the treatment was evaluated on 8 August 2006 (Table 3).

Table 3. Effect of controlling insects in empty depot by gasification applicator

	Number of living insects per square meter		
	Rhizopertha dominica Fabricius	*Sitophilus zeamais* (Motschulsky)	*Cryptolestes pusillus* (Schonherr)
Before test	10	8	7
After test	1	0	0
Mortality	90%	100%	100%

The effects of controlling insects in an empty depot using 0.5g/m^3 pirimiphos-methyl by common high pressure sprayer and gasification applicator were investigated. Compared to common high pressure sprayer, the gasification applicator treatment was better.

Controlling Insects in Depot with Stored-grain Using Gasified 50% Pirimiphos-methyl

The pest density in the stack of grain was evaluated 10 days, 20 days 30 days and a year after application. It took 10 hours to spray all 31 kilograms of 50% pirimiphos-methyl. The results are shown in Table 4.

Table 4. Effect of 50% pirimiphos – methyl controlling insects in Depot with stored – grain by gasification applicator

		Pest density per kilogram grain *														
		Rhyzopertha dominica (Fabricius)					*Cryptolestes ferrugineus* (Stephens)					*Sitophilus zeamais* (Motschulsky)				
		0d	10d	20d	30d	1year	0d	10d	20d	30d	1year	0d	10d	20d	30d	1year
Test depot	1#	4	3	3	2	0	2	2	1	1	1	2	0	0	0	0
	2#	30	22	17	15	0	26	18	14	13	1	2	0	0	0	0
	3#	20	17	12	10	0	10	8	6	5	0	2	0	0	0	0
Control depot	1#	5	2	1	0	10	3	1	1	1	12	1	0	0	0	12
	2#	29	7	2	1	3	27	8	3	1	3	3	0	0	0	12
	3#	18	0	1	1	3	11	5	2	2	13	3	0	0	0	4

* The pest density was determined at the temperature beyond 20℃ at which the large proportion of pests can grow and develop normally.

The pest density of test depot and control depot were determined. Compared to the control depot, firstly, the pest density in different fixed-point of test depot decreased apparently with time. One year later, the pest density of *Rhyzopertha dominica* and *Sitophilus zeamais* decreased to zero. This suggested pirimiphos-methyl could effectively control stored-grain pest for up to one year. Secondly, pirimiphos-methyl controlled three main stored-grain pests well: *Rhyzopertha dominica*, *Cryptolestesferrugineus*, *Sitophilus zeamais*.

Homogeneity of Spraying by Gasification Applicator

The pirimiphos-methyl residues in different point of the stack of grain, which could reflect the homogeneity of spraying by gasification, were determined before spraying and 14 days after spraying, respectively. The sampling points are shown in Figure 1. The results are shown in Table 5.

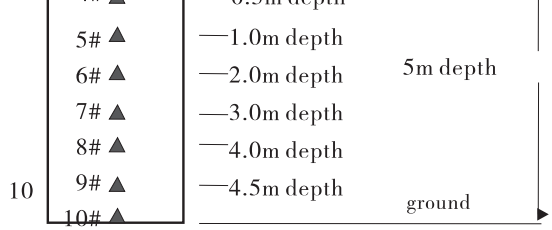

Fig. 1 Samples of resdues of Pirimiphos-methyl

Table 5. Residues of 50% Pirimiphos-methyl controlling insects

Sampling points	Residual quantity of Pirimiphos – methyl (mg/kg)		
	Before spraying	14 days after spraying	1 year after spraying
Surface layer	0	11.0	3.70
0.2m	0	5.10	/
1m	0	3.60	0.07
2m	0	0.50	<0.02
3m	0	0.20	0.04
4m	0	0.10	0.00

The Results Showed

1. According to the distribution of residues, we found pirimiphos-methyl that was sprayed by the gasification applicator to penetrate to a depth of 4 meters;
2. The residual quantity of pirimiphos-methyl decreased with increasing depth in the stack of grain;
3. The highest residual quantity was 11.0 mg/kg 14 days after spraying. It decreased one year later to 3.7 mg/kg, which is lower than the Maximum Residue Limits (MRL 5mg/kg).

Comparison of Side Effects of Pirimiphos-methyl & Aluminum Phosphide on Grain Quality (fatty acid)

The fatty acids of grain from insects treated in the depot with different pesticides were compared. Pirimiphos-methyl was used in depot No. 17 – 1 and aluminum phosphide was used in depot No. 18 – 1. The fatty acids of grain from these two depots were determined before the test and one year later. The results are shown in Table 6.

Table 6. Comparison of the side effects of pirimiphos-methyl and aluminum phosphide on grain quality (fatty acids)

	Value of fatty acids	
	Aluminum phosphide	Pirimiphos – methyl
Before test	22.5	22.5
One year later	27.2	25.2

Cost Comparison of Pirimiphos-methyl & Aluminum Phosphide

The results showed that the application cost of pirimiphos-methyl was higher than aluminum phosphide, but pirimiphos-methyl was easier to apply and less labour-intensive (Table 7).

Discussion

The efficacy of controlling insects in an empty depot using 0.5g/m^3 pirimiphos-methyl using a common high pressure sprayer and a gasification applicator were investigated. Compared to the common high pressure sprayer, the gasification applicator resulted in better pest control and was easier to operate. Furthermore, the cost of using pirimiphos-methyl was relatively low, about 0.05 yuan/m^3.

Table 7. Test cost of controlling insects with pirimiphos-methyl and aluminum phosphide (per tonne grain)

	Test cost (yuan/tonne)	
	Pirimiphos – methyl	Aluminum phosphide
Pesticides	0.83	0.43
Power consumption	0.04	0.02
Depreciation of equipment	0.08	0.08
Total	0.95	0.53

Pirimiphos-methyl was evaporated at 190 (10℃ and then liquefied. The results showed there was almost no change in the active ingredient content of pirimiphos-methyl in this temperature range. Pirimiphos-methyl was very effective for controlling stored-grain insects and inhibiting the growth of offspring, and had no side effects on grain quality.

Pirimiphos-methyl penetrated the grain to a depth of 4 meters when sprayed by gasification. Residues of pirimiphos-methyl decreased with the increase of depth of stack of grain. The highest residual quantity of pirimiphos-methyl was 11.0 mg/kg 14 days after spraying by gasification applicator. One year of grain storage was required for this residue level to reduce to less than the accepted MRL of 5 mg/kg.

As chemical protective additive, pirimiphos-methyl can be applied to grain without insects or with low pest density. However, the potential to control insects using pirimiphos-methyl decreases at high pest density.

As a kind of chemical protective additive of stored – grain, in aqueous solution or as an e-

mulsifiable concentrate, pirimiphos-methyl can be used for controlling insects in stored-grain and in an empty depot. The phosphine recirculation device was effective. The use of the Gasification Applicator combined with the phosphine recirculation device has the potential to be used more widely with other chemical protectants of stored-grain, such as 0.5% celangulin and deltamethrin.

The method of spraying liquor using a Gasification Applicator offers a new way to apply chemical protectants to stored-grain. Further refinements to the application methods could be made to reduce the costs of this new technique.

Recently, the rapid development of resistance to grain protectants or phosphine fumigations in stored grain pest has been reported. In this light, it is essential to take measures to control the increase of resistance. Controlling grain pests by spraying liquor using a Gasification Applicator may be a good choice.

Acknowledgements

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0207

The Use of Gaseous Ozone to Control Pests in Export Commodities

James G. Leesch * and J. Steven Tebbets

Abstract: Ozone was tested against several insects and arachnids infesting stored products (coffee beans, navel oranges and grapes). Tests were conducted 1) to determine the usefulness of ozone gas treatments to replace methyl bromide for the control of pests on exports to other countries, 2) to determine any phytotoxic response of the commodity upon which the pest resided and 3) to determine the parameters that provided the highest efficacy and least phytotoxic response of the system. Tests were conducted with all life stages of the coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari), adult bean thrips (BT), *Caliothrips fasciatus* (Pergande), mature female adult black widow spiders (BW), *Latrodectus hesperus* Chamberlin and Ivie and three species of late stage citrus mites, citrus flat mite (CFM), *Brevipalpus lewisi*, citrus rust mite (CRM), *Phyllocoptruta oleivora*, and two-spotted mite, *Tetranychus urticae* (TSM). Results were highly variable dependent on both species and life stage tested. Ozone gas controlled all life stages, except eggs of CBB, at concentrations from ranging from 2,500 to 10,000 ppm (v/v). Insects were tested as well as the commodity on which they are a problem.

Key words: grain storage, controlled atmosphere, ozone, coffee beans, navel oranges, grapes.

Introduction

With the loss of methyl bromide for postharvest pest control in perishable and durable commodities [1], new methods to control or eliminate postharvest pests are urgently needed. Ozone in gaseous form has not received much attention as a replacement for methyl bromide. Ozone has many advantages as a fumigant; 1) it is short lived in most situations and likes to return to its less reactive, more stable form, oxygen; 2) it has been declared a Generally Regarded as Safe (GRAS) compound meaning that it does not require registration to be used; 3) it is highly reactive against living organisms; 4) with the proper equipment, it can be generated on site from either air or oxygen; 5) it is easily converted back into oxygen so that none is emitted into the atmosphere following fumigation; and 6) it leaves no residues except the products of oxidation.

The major disadvantage of ozone is its oxidative action on many materials including some commodities. The advantages of using ozone prompted us to look at four situations where ozone fumigation might solve pest control problems. The first situation involved the use of ozone as a quarantine treatment to eliminate the coffee berry borer (CBB) from coffee beans being imported into Hawaii for roasting and blending. The second was the elimination of adult bean thrips (BT) from the navel of 'Navel' oranges. The thrips overwinter in this winter crop and can be found in the navel when the oranges are exported to Australia. The third situation involved the use of ozone to eliminate 3 species of mites from citrus being exported. And finally, the fourth situation attacked was the killing of adult black widow spiders (BW) from table grapes being exported to Europe and particularly the U. K. Trials with ozone against these pests and the host commodity were undertaken at the San Joaquin Valley Agricultural Sciences Center from 2001 to 2008 and here we give the results of those tests.

Materials and Methods

Exposure of Insects

A small fumigation chamber was used for exposures to ozone. The chamber was constructed from a stainless steel jacketed cylinder, solid on one end and having a 1.5 cm thick polycarbonate circular closure on the other end, held in place by bolts tapped into a flange around the end of the cylinder. The chamber was 56.5 cm long × 26.7 cm diameter with a volume of 31.6 L. Ozone was generated from oxygen in a ClearWater model CD12 unit and a continuous injection of ozone was necessary to

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keep the concentration constant. To monitor and control the concentration of ozone gas within the chamber a Hankin model HA – 100 – GTP – 12 ozone gas analyzer was used. Temperature was controlled by using a cooler circulating a water/antifreeze mixture in the jacket of the chamber when a temperature lower than 21℃ was required. The chamber was equipped with a vacuum pump and needle valves to maintain a negative pressure of about 460 to 508 mm Hg(absolute) during the exposure period. When carbon dioxide was used in combination with ozone, the CO_2 was introduced into the chamber using a rotameter to a concentration of 82% CO_2. Carbon dioxide has often been shown to have a synergistic effect in combination with other fumigants as shown by research done to decrease bacteria in meat[2] and also the fact that CO_2 increases insect respiration[3,4,5,6]. Relative humidity in the chamber followed that of the room at 35% ± 10%. The source, rearing, and exact exposure of the pests investigated follows.

Coffee Berry Borer(CBB)

CBB were supplied by Dr. Maribel Portilla, USDA, ARS, Mississippi State University. All stages of the insect were exposed to an ozone schedule of 10 000 ppm(v/v in air) for 6 h at 10℃ and without additional CO_2. (atmospheric CO_2). Negative pressure in these tests was about 460 mm Hg(absolute). The insects, that were treated, were internal pests infesting their natural host, inside galleries within green parchment coffee berries. Tests were conducted to determine efficacy of the treatment only.

Bean Thrips(BT)

BT were reared at our laboratory in Parlier, CA. Only the adult stage of thrips was of concern. Tests were conducted at 625, 1 250, 2 500, and 5 000 ppm(v/v) for 2 hr at 5℃ and with 102% CO_2. The insects treated were "external" pests infesting the navel of navel oranges. Although not always or easily visible inside the navel, the navel affords no barrier to penetration of the ozone gas reaching the target pest. Tests were conducted to determine both efficacy of ozone to control thrips and phytotoxicity of the oranges to exposure to ozone gas.

Citrus Mite Species(CM)

Three species of mites found on California-grown citrus were provided by Dr. Joseph Morse, U. C. Riverside, CA. The mites were grown on small, green lemons and different stages of development(egg to adult) were treated as external pests on the young, green fruit. The mites were exposed to three schedules: Treatments were conducted as described for BT above at schedules of 5 000 ppm(v/v) for 2 h, 10 000 ppm for 2 h, and 10 000 ppm for 4 h, providing CT products of 10 000, 20 000, and 40 000 ppm h.

Black Widow Spiders(BW)

Black widow spiders were collected from the field during nighttime hours in and around Fresno, California. They were brought into the laboratory, transferred individually to 7 – dram plastic vials with snap-cap lids. The vials had many 1 mm holes in the top, bottom, and sides to allow for free exchange of gas during treatment. The spiders were fed lepidopterous larvae and tests were conducted within one to two days from field collection. Mature or nearly mature female spiders were fumigated. All tests were conducted at a 1 – hour exposure time at 31℃ at several concentrations of ozone gas and with or without the addition of 102% CO_2.

Results

All stages of the CBB, except eggs, were controlled when exposed to 10 000 ppm ozone gas under a vacuum of 460 mm Hg, at 13℃ for 6 hours without additional CO_2. Visual tests and taste tests of green parchment coffee beans treated with the same ozone schedule were done earlier at a different facility and showed no adverse effects from the ozone treatments. BT were controlled from 1 250 to 2 500 ppm ozone in combination with 82% CO_2 for 2 hr at 5℃. Damage was minimal, less than 10% at all dosages even up to 5 000 ppm(CT = 10 000 ppm hr) when waxed (carnauba or shellac) and packed oranges were exposed to ozone gas. However, the severity of damage increased with increasing gas concentrations with field run oranges that had not been waxed. Tests with BWs showed ozone to be more efficacious alone, without the addition of CO_2 gas. Exposures of one hour provided 95% control at CT products of about 10 000 ppm hr and 7 000 ppm hr with or without 10% CO_2, respectively. Grapes were quite tolerant to ozone gas. The only response of grapes to ozone was the observation that the rachis of the berries sometimes showed small, hairline streaks running parallel with the rachis after a period of cold storage following treatment. Citrus mites were extremely tolerant to ozone gas in air as discussed below.

Coffee Berry Borer Infesting Green Coffee Beans

Ozone gas was shown to be an effective treatment controlling all life stages of CBB, except eggs, with an exposure schedule of 10 000 ppm for 6 hours at 13 ±3℃ (55 ±5°F) or above and at 508mm Hg. (absolute). Tests were replicated until ≥35 000 larvae, pupae, or adults were treated and observed. Since all stages of CBB are very prone to desiccation in dehydrated green parchment coffee, it is presently unclear if the eggs pose a threat to the coffee industry and thus may not be important to control with fumigation. In observations of eggs that we used in our tests, it was clear that eggs desiccate quickly and might not survive the shipping of berries to another country. Coffee tasting tests with coffee made with ozone-treated beans indicated that no off-flavor or odours resulted from the ozone treatment (Jack Armstrong USDA – ARSpersonal communication, 2006).

Table 1. Survival of coffee berry borer, *Hypothenemus hampeI* (Ferrari) (Coleoptera: Scolytidae) exposed to ozone gas at 10 000 ppm for 6 hours at 13℃ and –12" Hg.

Stage	Total[1]	Live	P	95% LL	95% UL
Egg	6086	887	0.146	0.137	0.155
Larva[2]	46,782	0	0	—	—
Pupa[3]	39,947	0	0	—	—
Adult	35,222	0	0	—	—

[1] Estimated number treated.
[2] First and second instar larva pooled.
[3] Pupa and prepupa pooled

Bean Thrips in Navel of 'Navel' Oranges

Ozone gas effectively controlled BT infesting the navel of 'Navel' oranges. In small chamber tests, we observed 100% mortality after 2 hours at 1 250 ppm ozone (2 500 ppm hr) at 20℃ or at 2 500 ppm (5 000 ppm hr) at 5℃ under vacuum (508 mm Hg-absolute) and in combination with 8% – 10% CO_2. However, experiments on a commercial scale required 5 000 ppm for 2 hours (10 000 ppm hr) (all else the same) to obtain 100% control of BT.

There are two reasons the test failed at lower exposures in the commercial chamber: 1) the ozone generator was undersized resulting in a prolonged ramp time to setpoint, and 2) the air circulation system of the chamber was not designed to force the ozone atmosphere through the large palletized load and to efficiently distribute and penetrate the load with ozone gas. We observed minimal or no damage to navel oranges in years of testing with ozone gas until tests conducted in 2005. This crop year was classified as a "weak rind" year and samples of packed, waxed navel oranges taken from various packinghouses in the central San Joaquin Valley were damaged more severely than previously observed as a result of exposure to ozone gas at 5 000 ppm for 2 hours at 5℃ at 508 mm Hg (absolute) with CO_2. Several different types of symptoms were recorded and described.

Tests were conducted in 2006 to determine if low or high rates of application of wax will protect (diminish or eliminate) the oranges from damage due to exposure to ozone gas. Results are shown in Figure 1. All the processing waxes added to the field oranges, except one, were effective in reducing phytotoxic effects on theNavel' oranges. This indicates that the oranges should be treated after waxing in the processing procedures. We hope to revisit this project in the future with better designed large chambers in the hopes of demonstrating efficacious treatments at lower doses of 1 250 to 2 500 ppm for 2 hr and alleviating the problem of damage to the commodity as well.

Citrus mites on Navel Oranges

Mites proved to be quite tolerant to ozone gas in air even at very high doses. Three species of CM were exposed to ozone gas: 1) citrus flat mite (FM), *Brevipalpus lewisi*, 2) citrus rust mite (RM), *Phyllocoptruta oleivora*, and 3) two-spotted spider mite (TM), *Tetranychus urticae*. Mites infesting bean plants were exposed to ozone gas at 5 000 ppm for 2 hours, 10 000 for 2 hours, or 10 000 ppm for 4 hours (CT product = 10 000, 20 000, or 40 000 ppm hr, respectively). Other parameters were the same as described above for BT. Mortality data showed CFM and CRM most tolerant with TSM most susceptible to ozone. Based on 95% mortality as an acceptable level of control at a schedule of 5 000 ppm for 2 hours, only the two-spotted mite is likely to be controlled using ozone gas.

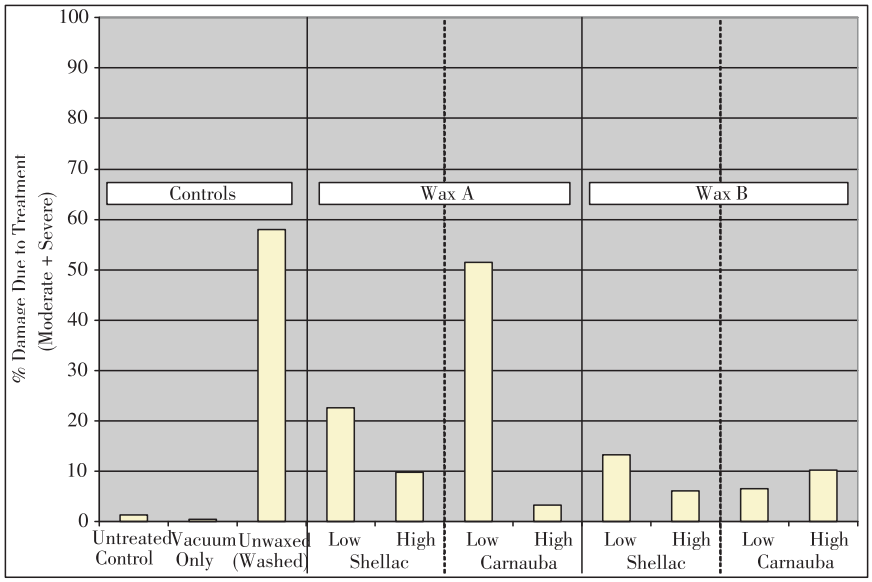

Fig. 1 Phytotoxic response of navel oranges following a 2 – hour exposure to ozone gas (ppm) and 8 2% CO_2 and 508 mm Hg (absolute) at 5 or 20℃; After 21 days storage at 1℃.

Table 3. Mortality of selected species of citrus mites exposed to ozone gas

Dose (ppm v/v)	Time (h)	CT (ppm h)	Percent mortality		
			Flat Mite (CFM)	Rust Mite (CRM)	2 – spotted Mite (TSM)
5,000	2	10 000	83.7	89.8	97.7
10 000	2	20 000	92.5	96.8	99.2
10 000	4	40 000	91.4	99.4	99.8
0 (control)	0	0	9.7	20.0	12.5
Total number treated/observed (estimated):			2 000 – 2 500	100 000 – 150 000	300 – 500

Black Widow Spiders on Table Grapes

The dosage-response curve was established for the adult females of the black widow spider. Mature adult female BWs were exposed to varying concentrations of ozone gas for 1 hour at 5℃ (40 F) at 508 mm Hg (absolute) with or without the addition of 10% CO_2. Lethal dosages to kill 50 or 95 percent of the BW population were calculated using regression analysis from probit percent mortality and log dose transformed data. Ozone gas performed better against BW without the presence of additional CO_2, i. e., at atmospheric CO_2 ($<0.1\%$). It was determined that a CT treatment of about 7 000 ppm hr with ozone alone would kill all BW in grape clusters. In the presence of 10% CO_2, a CT of 10,000 ppm hr was needed to obtain the same level of control at 95% mortality (LD_{95}) (Table 4). Grapes were quite tolerant to ozone gas. In experiments with grapes in these and other tests, the only response of grapes observed was a slight streaking of the rachis of the berries characterized by small, hairline streaks running parallel with the rachis after a period of cold storage following treatment. We observed no berry shatter or other adverse effects in grapes from the ozone treatments.

Table 4. Estimated lethal dosages (CT = ppm hr) of ozone gas to control adult black widow spiderswith or without the addition of CO_2 gas.

	With CO_2 (10%) (±95% CL)	Without CO_2 (0.1%) (±95% CL)
LD50	1 950 (1 630 – 2 290)	1 455 (1 216 – 1 717)
LD95	10 131 (7 538 – 15 657)	7 144 (5 387 – 10 691)

Discussion

Ozone gas has promise as an alternative fumigant against surface pests, possibly on a wide range of commodities, but with limited distributional, penetrative and ovicidal properties[7,8,9]. There are many unanswered questions concerning the interaction of the different physical parameters associated with an ozone treatment schedule and the optimal levels and hierarchy of each to enhance the effectiveness of the treatment and diminish any phytotoxic response of the commodity due to exposure to ozone gas.

In our testing of BT in oranges, and BW in grapes, where only adults are the target, ozone proved to be a good choice as a fumigant. In the studies with CBB, ozone gas was effective against all stages at a reasonable treatment schedule. However, ozone was ineffective against CBB eggs. The lack of toxicity to insect eggs was found earlier with stored product insects and may be a deficiency of ozone against insects[8,10]. For protecting grain against insect infestation, it has been shown that ozone used in a flow-through system over a period of days may be very efficacious while not damaging the nutritional characteristics of the grain[11,12,13]. One study tested the chronic effects of ozone on grain insects and found a definite efficacy over a period of days or months[14]. However, use of fumigants in most horticultural crops is for export and quarantine purposes. That means that the time a commodity spends under fumigation can only be a matter of hours, not days, so that these fumigations must be designed to be efficacious in a short time without damaging the commodity being treated. Other variables included time of exposure, temperature, pressure (vacuum), and the presence or absence of carbon dioxide (CO_2) need further investigation to determine the contribution of each to the toxicity of ozone to arthropods.

There are a few disadvantages associated with ozone, including its strong oxidizing power and its short half-life in situations where it can oxidise biotic materials which requires that ozone be continually applied during treatment. However, ozone has several advantages as a fumigant. It can be generated on site, is easy to catalyze back into oxygen so that no toxic material is being emitted to the atmosphere, leaves no residue, is considered a GRAS (Generally Considered As Safe) compound, at treatment levels discussed in this paper, ozone reduces microbial load of fungal and mold spores, increasing shelf-life of fresh produce, and is not flammable at concentrations and temperatures used in commodity fumigation. Clearly, ozone has a place in commodity protection and quarantine treatments and further research will define its role as a fumigant.

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Fumigant Activities of Three Plant Powders against Stored Grain Beetles

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Abstract: The insecticidal properties of *Eugenia aromatica*, *Dennettia tripetala* and *Piper guineense* were assessed in the laboratory for their potential to protect stored grains against insect depredation using their fumigant action. Results from fumigant bioassay revealed that responses of test beetles to plant materials was dependent upon susceptibility of insect species, application rate and exposure time. *E. aromatica* powder was the most toxic; evoking the highest mean percentage mortality on the test beetles and this was significantly different ($P \leqslant 0.05$) from the mortalities obtained in treatments with two other plant powders and control. At the highest concentration of 0.47mg/cm^3, *E. aromatica* gave 63.75, 82.5, 67.5, 43.75, and 48.75% mean percentage mortalities on *S. zeamais*, *C. maculatus*, *T. castaneum*, *L. serricorne* and *O. mercator* respectively at four days after treatment. The plant powders used in this study have bioactive components, which are toxic to stored product insects, thus could serve as good substitute for synthetic chemical insecticides like Methyl Bromide. Resource-poor farmers in developing countries could harness the use of these plant materials for protecting their produce against insect attack.

Introduction

Developing countries in tropical regions are faced with problems of malnutrition, food shortage and scarcity due to their inability to protect crops from quality and quantity deterioration caused by the activities of microbes, rodents, and insects pests[1,2]. Food crops particularly cereals and grain legumes form the main diet and protein source among the people in developing countries[3]. However, insect pests usually attack all facets of the crops both in the field and soon after harvest[4].

The production of cereals and leguminous crops by peasant farmers in developing countries in the tropics suffer a setback due to their inability to afford the high cost for procuring synthetic chemical insecticides or effective and efficient storage facilities, which are used for protecting stored produce from insect pest attack[5,6,7]. Apart from the high cost of procuring chemicals and irregular supplies, synthetic insecticides leave residual toxicity on protected food and this could be harmful to man and his livestock [4,8]. Alternative controls aimed at reducing the use of synthetic insecticides are earnestly being sought and in recent decades[9], traditional pest control methods such as the use of plant derived insecticides have attracted researchers as good alternative control agents[10,11]. This paper examines the fumigant property of powders from three plant species and their contact toxicities against coleopterous pests of cereals and grain legumes.

Materials and Methods

Insect Cultures

They were disinfested by a method described by Adedire and Lajide (1999)[12], while the storage beetles (*Callosobruchus maculatus* (Fabricius); *Tribolium castaneum* (Herbst); *Sitophilus zeamais* (Mots.); *Oryzaephilus mercator* (Fauvel) and *Lasioderma serricorne* (Fabricius)) were obtained from established laboratory cultures raised in the storage research laboratory, Federal University of Technology Akure, Nigeria. The insect pests were originally obtained from International Institute for Tropical Agriculture Ibadan (IITA), before rearing in our laboratory. The diets used in rearing the test beetles

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and their bioassay are whole maize (*S. zeamais*), whole cowpea (*C. maculatus*), maize grits (*T. castaneum*), cocoa seed (*L. serricorne*), wheat (*O. mercator*) 750g food media were measured into 1litre Kilner jars. Those grains were obtained from Erekesan market, Akure, Nigeria during October, 2002. Twenty unsexed adults of each insect species were introduced into their respective culturing medium and covered with muslin cloth held tightly in place by rubber bands and was kept in the rearing chamber with a 12 – h photoperiod at ambient condition (28 2℃ and 755% R. H.). Newly emerged (teneral, 1 – 7 days) adult insects were used for each test. Beetles were certified dead when there are no movements after gently probing their abdomen several times with a sharp forceps.

Preparation of Plant Powders

Fruits of *Piper guineense* Thonn and Schum., *Dennettia tripetala* Baker, and cloves of *Eugenia aromatica* (*Syzygium aromaticum*) Baillon used for this study were obtained fresh from Erekesan market in Akure. The fruits of the plants collected were first dried naturally on laboratory benches at prevailing tropical storage condition. The dried plant materials were pulverized into fine powder using Kenwood electric blender and sieve through a 10 m size mesh. The powder was kept in brown airtight bottle.

Contact Activity of Plant Powders on Beetle Mortality

Different concentrations (0. 1, 0. 2, 0. 3, 0. 4, 0. 5g per 20g food medium) of plant powder/grain mixture were prepared in 9cm diameter Petri dishes and twenty newly emerged adult insects were introduced into the treated grains in the dishes and covered. Weevil mortality was observed on a daily basis for four days and 50% mortality (LD_{50}) was determined. A control experiment was set up without powder treatment. All the treatments were replicated six times.

Fumigant Effect of Plant Powders on Beetle Mortality

The fumigant effects of powders of *E. aromatica*, *D. tripetala* and *P. guineense*, at dosages of 0. 8, 2. 5, 4. 2mg/cm^3 were evaluated on adults *S. zeamais*, *C. maculatus*, *T. castaneum*, *L. serricorne* and *O. mercator* in the laboratory at ambient conditions. Five pairs of newly emerged adult beetles were introduced inside a sac made of muslin cloth containing 20g food medium (Fig. 1). The bag was tied and suspended inside a Glass fumigating chamber (height = 12cm, diameter = 8cm) containing specified concentrations of plant powder. The fumigating chamber was corked and sealed. Each treatment was replicated six times. Beetle mortality was recorded at four days after treatment and the numbers of dead adult beetles were recorded. Beetles were certified dead if they did not respond to forceps probe.

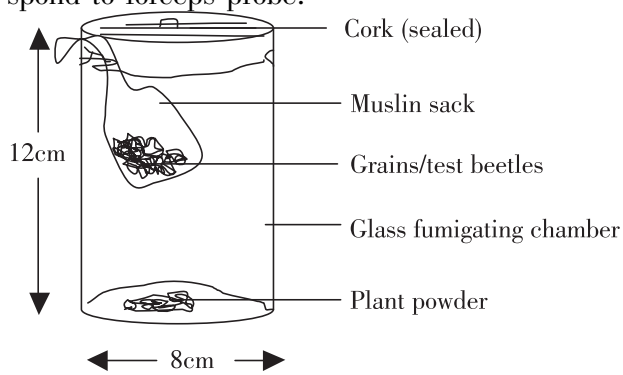

Fig. 1 Showing the fumigating chamber set up

Statistical Analysis

The data from contact toxicity bioassay were analyzed for LD_{50} values and their 95% confidence limits (95% CL) by probit analysis using DPS data processing system. The analysis of variance and Tukey's test for mean separations were also calculated using the DPS (version 3. 01) data processing system[13].

Results

Contact Toxicity

The powder of *E. aromatica* caused the highest mortality to *C. maculatus* (i. e. least LD_{50} 0. 22) at one day after treatment, followed by *D. tripetala* (LD_{50} 0. 24) and *P. guineense* (LD_{50} 0. 30) in that order (Table 1). *D. tripetala* and *P. guineense* gave similar LD_{50} (0. 26) but different confidence limits and slopes in *S. zeamais* infested food media at one day post treatment period. At day two after treatment, *E. aromatica* was still the most potent powder with the least LD_{50} followed by *D. tripetala* and *P. guineense* respectively. Similar trends of plant powder activities were observed at days 3 and 4 in treatments infested with *S. zeamais*, *C. maculatus*, *T. cataneum*, *L. serricorne* or *O. mercator*. The LD_{50} of each plant powder on test beetles decreases as the exposure period increases which is indicative of increase in potency of plant powders with time of exposure. All plant powders tested were effective, however the susceptibility of the insect species used varied with different plant materials. At four days after treatment *C. maculatus* appears the most sus-

ceptible insect to *E. aromatica* powder treatment (LD_{50} = 0.08g) while the least susceptible beetle was *L. serricorne* LD_{50} = 0.24.

Fumigant Effect of Plant Powders on Beetle Mortality

Fumigant effect of plant powders on the beetles is presented in Table 2. The lowest concentration (0.8mg/cm^3) of *E. aromatica* was not effective on test beetles, mortalities ranged between 516.25% at day four after treatment, while the highest concentration (4.2mg/cm^3) caused significant ($P \leqslant 0.05$ level, Tukey's test) mortality 43.75% – 82.50% across all the test beetles. Adult mortality in the controls was 0%. The mortality recorded in treatments with *P. guineense* was low (15% – 37.50%) compared to treatments with *E. aromatica* (43.75% – 82.50%) and *D. tripetala* (45% – 65%) at the highest concentration. *D. tripetala* had higher fumigant activity on *L. serricorne* than other plant materials tested. Based on analysis of variance (ANOVA), all the plant powders were effective fumigants at 4.2mg/cm^3 with percentage mortality significantly ($P \leqslant 0.05$) different from the control (Table 2).

Discussion

Results of this study have revealed that powders of *D. tripetala*, *E. aromatica*, and *P. guineense* were effective as botanical insecticides against all the test beetles. However, their effectiveness was dependent on application rates and exposure periods. The test beetles showed varying degrees of susceptibility. *E. aromatica* had the highest contact toxic effect on the test beetles followed by *D. tripetala* powder while the least effective was *P. guineense*. The observed toxicity of *E. aromatica* is in agreement with findings of Lajide *et. al.* (1998)[14] who observed that *E. aromatica* evoked high contact toxicity on *S. zeamais*. The high beetle mortality observed in *E. aromatica* treated media could be due to the pungent asphixiating smell of its volatile components. In addition to direct toxic effect, plants powder could also produce odours that may confuse of repel beetles Boeke *et al.* (2004)[15].

Since particle size affects dispersion, powders of plant materials have the tendency of coating seeds more uniformly than whole plant or plant parts, thereby enhancing contact with the target pests[16]. The action of *E. aromatica* on these beetles may be as a result of stomach poisoning[14] or contact action. High toxic effect of *E. aromatica* on *S. zeamais* and *T. castaneum* may be as a result of the feeding habits of these pests during which lethal dose of the plant material were ingested[17]. *E. aromatica* contains eugenol, sesquiterpene and caryophylline[18]. Eugenol is toxic and could inhibit growth in insects[18,19].

The volatile components of *E. aromatica* could also result in blockage of spiracles and evoke respiratory impairment or reduce oxygen carrying capacity of the haemolymph. The effectiveness of *D. tripetala* on adult mortality of *C. maculatus*, *S. zeamais* and *T. castaneum* agrees with the reports of Okonkwo and Okoye (1996)[20] who observed that powders of *D. tripetala* resulted in 100% mortality of maize weevil *S. zeamais* and *C. maculatus*. According to Agbakwuru *et. al.* (1978)[21], *D. tripetala* contains (*phenylnitroethane* which is known to have insecticidal activity hence, the action of *D. tripetala* on these storage beetles could be attributed to this active principle found in the plant.

Although *P. guineense* evoked high percentage mortality on the test beetles, its efficacy is lower than those of *E. aromatica* and *D. tripetala*. Similar observations have been made by other workers[22,20,14]. The observed mortality could be ascribed to the presence of amides piperine, Chavicine, N – iso – butyloctadeca-trans – 2 – trans – 4 – dienamide, sylvatine, a (dihydro piperine and trichostachine in the fruits of *P. guineense*[23]. The biological activities of the powder have been linked to the presence of these active principles in the plant because some of these compounds, especially chavicine and piperine have contact toxicity and fumigant action on insects. Mbata *et al.* (1995)[24] had reported that 0.4g/5.0g of powdered seeds of *P. guineense* when admixed with maize, resulted in 50% adult *S. zeamais* mortality. In related study with plant powders Adedire and Akinneye (2004)[25] observed that powder of *Tithonia diversifolia* at 5% concentration impaired oviposition adult emergence and evoked 98% mortality on *C. maculatus*. These results agreed with the observations made in this study where it was observed that though *P. guineense* had some lethal effect on all the test beetles, it was however not as effective as *E. aromatica* and *D. tripetala*.

When used as fumigant, the powders of *E. aromatica* was the most effective of all the three plant materials evoking 82.50% adult mortality

in *C. maculatus* followed by *D. tripetala*, while *P. guineense* was the least effective. The effectiveness of *E. aromatica* as bio fumigant may be due to its volatile component and pungent smell, which result in asphyxiating effect on the beetles. This is in line with the observations of- who reported [26,27] that powders of some botanicals and aromatic plants have some fumigant or lethal effect on storage beetles.

This study has revealed *E. aromatica* as a botanical with high fumigant activity against stored product beetles. This property could be exploited and used to replace synthetic fumigants such as methyl bromide, phosphine gas, which are ecologically intolerable.

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Table 1. Contact toxicity effect (LD_{50} and Confidence limit) of three plant powders on five post harvest insect pests at days post treatment.

Days post treatment	Plant materials	*S. zeamais* LD_{50} (95% CL) * Slope ± SE	*C. maculatus* LD_{50} (95% CL) * Slope ± SE	*T. casternum* LD_{50} (95% CL) * Slope ± SE	*L. serricorne* LD_{50} (95% CL) * Slope ± SE	*O. mecartor* LD_{50} (95% CL) * Slope ± SE
1	*E. aromatica*	0.25 (0.23 – 0.28) * 3.06 ± 0.31	0.22 (0.07 – 0.35) * 3.48 ± 0.32	0.31 (0.28 – 0.34) * 4.03 ± 0.40	0.58 (0.46 – 0.85) * 2.47 ± 0.38	0.47 (0.40 – 0.59) * 3.34 ± 0.44
	D. tripetala	0.26 (0.24 – 0.28) * 3.64 ± 0.34	0.24 (0.22 – 0.26) * 3.13 ± 0.31	0.35 (0.31 – 0.40) * 2.95 ± 0.34	0.63 (0.44 – 5.89) * 11.29 ± 4.12	0.51 (0.43 – 0.70) * 2.58 ± 0.37
	P. guineense	0.26 (0.24 – 0.29) * 3.17 ± 0.32	0.30 (0.28 – 0.34) * 3.19 ± 0.34	0.44 (0.37 – 0.58) * 2.03 ± 0.31	0.81 (0.58 – 1.60) * 2.55 ± 0.48	0.88 (0.54 – 8.31) * 4.85 ± 1.60
2	*E. aromatica*	0.19 (0.17 – 0.21) * 3.90 ± 0.34)	0.13 (0.03 – 0.20) * 3.01 ± 0.32	0.23 (0.21 – 0.25) * 3.73 ± 0.34	0.40 (0.35 – 0.47) * 3.66 ± 0.43	0.32 (0.29 – 0.35) * 3.40 ± 0.35
	D. tripetala	0.23 (0.12 – 0.32) * 3.66 ± 0.33	0.20 (0.01 – 0.36) * 3.24 ± 0.31	0.28 (0.25 – 0.32) * 2.71 ± 0.31	0.48 (0.40 – 0.63) * 2.37 ± 0.39	0.37 (0.32 – 0.44) * 2.61 ± 0.32
	P. guineense	0.23 (0.21 – 0.26) * 3.11 ± 0.31	0.22 (0.12 – 0.31) * 3.48 ± 0.32	0.29 (0.19 – 0.60) * 2.67 ± 0.31	0.64 (0.45 – 2.81) * 9.90 ± 3.15	0.77 (0.54 – 2.01) * 4.41 ± 1.05
3	*E. aromatica*	0.14 (0.12 – 0.17) * 3.39 ± 0.33	0.09 (0.07 – 0.12) * 3.18 ± 0.38	0.17 (0.06 – 0.25) * 3.63 ± 0.33	0.30 (0.27 – 0.33) * 3.53 ± 0.35	0.24 (0.22 – 0.26) * 3.46 ± 0.33
	D. tripetala	0.21 (0.15 – 0.33) * 3.88 ± 0.34	0.16 (0.00 – 0.26) * 2.95 ± 0.30	0.22 (0.19 – 0.25) * 2.39 ± 0.28	0.34 (0.30 – 0.39) * 2.59 ± 0.31	0.28 (0.25 – 0.31) * 3.00 ± 0.31
	P. guineense	0.22 (0.13 – 0.30) * 3.50 ± 0.32	0.20 (0.07 – 0.29) * 3.81 ± 0.34	0.23 (0.21 – 0.25) * 3.27 ± 0.32	0.75 (0.53 – 1.82) * 4.42 ± 1.02	0.78 (0.56 – 1.64) * 3.25 ± 0.66
4	*E. aromatica*	0.11 (0.08 – 0.13) * 3.27 ± 0.37	0.08 (0.05 – 0.12) * 3.81 ± 0.55	0.14 (0.12 – 0.16) * 3.90 ± 0.36	0.24 (0.22 – 0.26) * 3.85 ± 0.35	0.17 (0.08 – 0.23) * 3.51 ± 0.33
	D. tripetala	0.20 (0.06 – 0.29) * 3.86 ± 0.34	0.12 (0.03 – 0.17) 3.38 ± 0.35	0.17 (0.14 – 0.19) * 2.57 ± 0.29	0.26 (0.23 – 0.29) * 2.85 ± 0.30	0.21 (0.19 – 0.23) * 3.14 ± 0.31
	P. guineense	0.20 (0.18 – 0.23) * 3.50 ± 0.32	0.18 (0.10 – 0.24) * 4.14 ± 0.35	0.18 (0.09 – 0.25) * 3.40 ± 0.323	0.75 (0.55 – 1.40) * 2.99 ± 0.56	0.61 (0.49 – 0.93) * 3.67 ± 0.60

Table 2. Fumigant effect of three plant powders on five post harvest insect pests at four days after treatment.

Plants Materials Used	Conc. (mg/cm^3)	Percentage mean mortality ± standard error (% Mean ± S. E) *				
		S. zeamais	*C. maculatus*	*T. casternum*	*L. serricorne*	*O. mecartor*
E. aromatica	0.0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	0.8	5.00 ± 2.04^{ab}	12.50 ± 2.04^{bc}	8.75 ± 1.25^{ab}	15.00 ± 1.25^{cd}	16.25 ± 2.0^{4b}
	2.5	32.50 ± 1.25^{d}	40.00 ± 1.44^{e}	41.52 ± 2.39^{de}	30.00 ± 2.50^{e}	30.00 ± 2.39^{c}
	4.2	63.75 ± 1.25^{e}	82.50 ± 1.39^{g}	67.50 ± 2.04^{f}	43.75 ± 3.75^{f}	48.75 ± 2.04^{d}
D. tripetala	0.0	1.25 ± 1.25^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	0.8	12.50 ± 1.25^{bc}	21.25 ± 1.25^{cd}	11.25 ± 2.39^{ab}	12.50 ± 3.15^{bc}	3.75 ± 1.25^{a}
	2.5	18.75 ± 3.15^{c}	25.00 ± 2.39^{d}	25.00 ± 3.15^{c}	21.25 ± 2.04^{d}	18.75 ± 2.04^{b}
	4.2	61.25 ± 1.44^{e}	65.00 ± 2.04^{f}	50.00 ± 2.04^{e}	45.00 ± 1.25^{f}	48.75 ± 2.04^{d}
P. guineense	0.0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
	0.8	2.50 ± 1.25^{a}	6.25 ± 2.04^{ab}	7.50 ± 2.04^{ab}	2.50 ± 1.25^{a}	1.25 ± 1.25^{a}
	2.5	5.00 ± 2.04^{ab}	18.75 ± 2.39^{cd}	12.50 ± 1.44^{b}	6.25 ± 1.25^{ab}	2.50 ± 1.44^{a}
	4.2	28.75 ± 3.75^{d}	37.50 ± 1.25^{e}	35.00 ± 1.25^{d}	15.00 ± 1.44^{cd}	15.00 ± 2.39^{b}
CK	0.0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

* Each value is the percentage mean ± standard error of six replicates. Means followed by different letter(s) vertically are significantly different at $P \leqslant 0.05$ by Tukey's test.

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0209

Efficacy of Ozone Fumigation to Control Some Stored Product Insects

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Abstract: Ozone is a toxic gas with potential to replace methyl bromide for controlling stored product pests. The efficacy of an ozone fumigation treatment on beetles (*Sitophilus oryzae*, *Rhyzopertha dominica*, and *Tribolium confusum*), and moths (*Cadra cautella*, *Corcyra cephalonica*, *Ephestia kuehniella*, and *Plodia interpunctella*) was evaluated. Insects at various stages of development (eggs, larvae, pupae, and adults of *S. oryzae* and *R. dominica*; eggs, third instar larvae and adults of *T. confusum*; eggs, and third instar larvae of moths) were treated with ozone at 600 ppm v/v, 20 ± 2℃, for different time periods (from 30 min to 3 hours). Treatments were carried out in a column (10 cm dia. × 110 cm) containing 4 kg of rough rice (paddy). Mortality was assessed at 24 and 48 hours after treatment, and weekly thereafter.

The egg stage was the most tolerant to ozone fumigation treatment for all the species tested. Ozone fumigation for 30 min killed 100% of the third instar larvae of the four species of moths and of *S. oryzae* adults. More than 50% of the adults of *R. dominica* and of *T. confusum* and 30% of third instar larvae of *T. confusum* were alive 24 hour after a 30 minute treatment; but they were found dead after 48 hours. After a 1 hour treatment, some third instar larvae and adults of *T. confusum* were alive after 24 hours; but they were found dead after 48 hours. To obtain a 100% mortality of all the stages of the species tested, a 3 hour ozone fumigation treatment was required.

Key words: *Sitophilus oryzae*, *Rhyzopertha dominica*, *Tribolium confusum*, *Cadra cautella*, *Corcyra cephalonica*, *Ephestia kuehniella*, *Plodia interpunctella*, fumigation, ozone

Introduction

Ozone is a highly oxidising agent and widely used in food industry for disinfection of surfaces, for hygiene in processing plants and to treat some foods for controlling insects and fungi [1,2,3,4,5,6,7]. Ozone is highly reactive and damages cell membranes of organisms by causing oxidative stress[8], but does not affect intrinsic grain properties or seed germination [9].

In 1965, Beard[10] indicated that prolonged exposure to high levels of ozone was lethal to adult house flies and caused flies to lay fewer eggs per female. Levy et al.[11] observed that eggs and larvae of *Musca domestica* (L.) and *Stomoxys calcitrans* (L.) are less susceptible to ozone compared to pupae and adults.

Both laboratory tests[9,12,13] and field trials have been carried out to verify the efficacy of ozone fumigation on stored product insects with different concentrations and different exposure times. Kells et al.[14], treating 8.9 tonnes of maize with 50 ppm ozone for 3 days, obtained 92% – 100% mortality of some insects of stored products. Field experiments on strains of *Rhyzopertha dominica* (F.) with a high level resistance to phosphine showed susceptibility to ozone fumigation[15].

Although low pressures and CO_2 may improve efficacy of some fumigants, requiring shorter fumigation periods, the efficacy of high concentration ozone on *Ephestia kuehniella* (Zell.) is not enhanced under low pressure of 100 mmHg and 92% CO_2[16].

The efficacy of an ozone fumigation treatment on beetles and moths was evaluated at high concentrations of ozone with short exposures.

Materials and Methods

Tests were performed on *Cadra cautella* (Walk.), *Corcyra cephalonica* (Staint.), *Ephestia kuehniella* (Zell.), *Plodia interpunctella* (Hbn.), *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.) and *Tribolium confusum* J. du Val. The procedure adopted was a) to place insects at different stages of development with paddy rice (rough rice) inside a column, and b) to treat with ozone and observe their survival.

Tests were carried out by SAPIO produzione idrogeno ossigeno srl., Via Malcontenta 49, Porto Marghera (VE) Italy.

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Test insects were reared in thermostatically controlled incubators at 26 ± 1℃; 70 ± 5% r. h. at the Istituto di Entomologia agraria, Universitdegli Studi, Milan, Italy.

The moth species were reared on a diet used for mass rearing [17].

For the moth species, the eggs were collected from Petri dishes placed under a special plexiglass egg-laying cylinder, 15 cm dia. x 40 cm, with the base fitted with an 18 mesh metal net through which the eggs fall. Eggs were collected from 50 moths in each cylinder. Tests were carried out on eggs laid 30 – 48 hours previously, and held at the laying conditions.

T. confusum was reared on soft wheat flour, maize flour and bran (in equal parts). To obtain Eggs and larvae of *T. confusum* were obtained by sieving (60 mesh) a 50 g sample of sieved soft wheat flour, previously infested for a period of 5 days by 100 adults.

Eggs of *S. oryzae* and *R. dominica* were obtained by exposing rice samples (50 g) to 100 adults for 5 day. Larvae were obtained from 50 g samples of cereals containing eggs laid 8 – 11 days, 13 – 16 days and 18 – 23 days before the treatment. For pupae, the period prior to exposure was 26 – 30 days.

The fumigation system was made of an ozone generator connected to a polycarbonate cylinder (10 cm dia., 110 cm high). The generator produced ozone from purified oxygen (0.8 L/min). In the cylinder, ozone concentration was 600 ppm v/v. Ozone was introduced from the bottom of the cylinder through a perforated plate. The top of the cylinder had a lid with holes used to regulate the ozone exit. The exit gas stream was heated to 80℃ to decompose the ozone present to oxygen.

Insects were added to 4 kg of paddy rice in the column: groups of 20 larvae for each moth species and for *T. confusum*, while for the other beetles, 20 g of infested material for each tested stages or instars were added. Groups of a 100 eggs for each moth species and for *T. confusum*, were placed in gauze bags (70 mesh). In tests with adult beetles, groups of 20 individuals of *T. confusum* and 50 of *R. dominica* and of *S. oryzae* were introduced in the column at 20 ± 2℃ for different time periods (from 30 min to 3 hours). Each test was repeated 4 times, each with an untreated control batch.

The mortality adults of all the species and the third instar larvae of the moths and of *T. confusum* were assessed after 24 and 48 hours. In the other cases, the biological samples were maintained at a temperature of 26 ± 1℃ and 70 ± 5% r. h. and checked weekly until adult emergence. Mortality was corrected according to Abbott's formula [18].

Results

One hundred percent mortality of all the tested stages of moths and beetles was obtained with a 3 hours ozone fumigation.

Eggs of moths were the most tolerant stage to ozone fumigation. With 30 minutes fumigation, a corrected mortality of < 50% for eggs of *Cadra cautella*, *Corcyra cephalonica*, and *Ephestia kuehniella* was obtained (Table 1). *Plodia interpunctella* (79.7% corrected mortality) was the least tolerant species.

Table 1. Effect of ozone fumigation at 600 ppm on eggs of *Cadra cautella*, *Corcyra cephalonica*, *Ephestia kuehniella* and *Plodia in terpunctella* for different exposure times, expressed as percentage mortality (percentage values corrected according to Abbott's formula).

Species	Corrected mortality %			
	30 min	1 h	2 h	3 h
Cadra cautella	49.1	85.6	91.6	100
Corcyra cephalonica	31.6	67.5	80.6	100
Ephestia kuehniella	34.7	69.9	98.7	100
Plodia interpunctella	79.7	88.7	92.1	100

After a 1 hour fumigation, mortality of moth eggs was between 67.5 (*C. cephalonica*) and 88.7% (*P. interpunctella*). After 2 hours fumigation, mortality was > 90%, except for *C. cephalonica* (80.6%). Third instar larvae of moths were most susceptible to ozone fumigation: a 30 minutes fumigation was enough to obtain a 100% corrected mortality.

As far as the beetles were concerned, with a 2 hours fumigation, eggs of *T. confusum* were the least susceptible stage (Table 2). Mortality of eggs of *R. dominica* after 30 minutes and 1 hour were 87.7 and 97.5% mortality. For eggs of *S. oryzae* these values were 41.7 and 72.5%, respectively. With a 2 hours treatment, a mortality higher than 95% was obtained for eggs of both *R. dominica* and *S. oryzae*.

32.5% of third instar larvae and 63% of adults of *T. confusum*, and 64.5% of adults of *R. dominica* survived for > 24 hours after an ozone fumigation of 30 minutes (Table 3), but all

were dead (100% mortality) after 48 hours. All adults of *S. oryzae* were dead after 24 hours succeeding the fumigation treatment. Larvae and pupae of *R. dominica* and of *S. oryzae* were susceptible to a 30 minutes fumigation. After 24 hours following a 1 hour fumigation, 2.5% of third instar larvae and 1% of adults of *T. confusum* were still alive, but all were dead after 48 hours.

Table 2. Effect of ozone fumigation at 600 ppm on eggs of *Sitophilus oryzae*, *Rhyzopertha dominica* and *Tribolium confusum* for different exposure times, expressed as percentage mortality (percentage values corrected according to Abbott's formula).

Species	Corrected mortality %			
	30 min	1 h	2 h	3 h
Rhyzopertha dominica	87.7	97.5	100	100
Sitophilus oryzae	76.0	77.4	97.2	100
Tribolium confusum	41.7	72.5	92.1	100

Table 3. Percentage of adults of *Rhyzopertha dominica* and larvae and adults of *Tribolium confusum* surviving a 30 min exposure at 600 ppm ozone at 24 hours and 48 hours after the fumigation.

Species	Survival %	
	24h	48h
Rhyzopertha dominica adults	64.5	0
Tribolium confusum Ⅲ *instar larvae*	32.5	0
Tribolium confusum adults	63.0	0

Adult insects, derived from eggs surviving the ozone fumigation, displayed the postembryonal development period observed to those untreated (controls).

Discussion

With 600 ppm ozone concentration, the egg was the most tolerant stage. To obtain a 100% mortality, 2 hours fumigation were required for *Rhyzopertha dominica* and 3 hours for *Sitophilus oryzae*, *Tribolium confusum*, *Cadra cautella*, *Corcyra cephalonica*, *Ephestia kuehniella* and *Plodia interpunctella*.

Ozone, unlike nitrogen and carbon dioxide, was lethal at short periods of fumigation to stages of insects that develop inside the kernels, such as *Rhyzopertha dominica* and *Sitophilus oryzae*. In this research, with a high concentration of ozone, a complete mortality was observed after a 30 minutes fumigation.

Erdman[19] fumigated insects at 300 ppm ozone and observed a complete mortality of adults of *T. castaneum* and *T. confusum* during few days after the treatment. Also in this research, with the 30 minutes fumigation, third instar larvae and adults of *T. confusum* and adults of *R. dominica* were alive after 24 hours and dead after 48 hours.

With high concentration of ozone the fumigation period is reduced. With low concentrations it was observed, in a laboratory study, that 5 ppm of ozone resulted in a 100% mortality of adult *Oryzaephilus surinamensis* (L.) and *T. confusum* after exposure times of 3 and 5 days respectively[20]. Laboratory studies have indicated that 50 ppm ozone for 3 days killed adult insects commonly found in stored grain[1]. Kells et al.[14] fumigated insects at 25 ppm and 50 ppm ozone for 3 days and observed that adults of *S. oryzae* are more susceptible than adults of *T. confusum*. In our tests, we observed the same response. Besides, Erdman[19] showed that *T. castaneum* was consistently more ozone – sensitive than *T. confusum*.

Ozone fumigation does not leave toxic residues and requires, at high concentrations, a short period of treatment. It is an interesting alternative to the use of fumigants and of physical methods that require a long period of application, such as nitrogen and carbon dioxide. An attractive aspect of ozone is that it decomposes rapidly (half life in air of 20 – 50 min) to molecular oxygen without residues, and ozone can be generated on – site, eliminating the need to store or dispose of chemical containers. A high content of dust in cereals can negatively affect the efficacy of CO_2 treatment[21,22] and the same result was observed also for ozone fumigation[23]. A disadvantage of ozone application is its corrosive properties towards most metals[9,12]. Nevertheless, the use of ozone fumigation is interesting in the disinfestation of stored products such as cereals stocked in concrete silos and in steel silos after coating with specific resins. It is noteworthy that inox steel (AISI 316) silos is not susceptible to ozone corrosion.

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0210

Fumigation Efficacy of Ethyl Formate against *Tribolium Castaneum* (Herbst)

Tang Peian[1], Deng Yongxue*[1], Wang Jinjun[1], Yang Longde[2], Yang Zili[2] and Jiang Tianke[2]

Abstract: The lethal effects of ethyl formate (EF) on eggs, early larvae, late larvae, pupae and adults of *T. castaneum* were systematically studied in the laboratory under different dosages, treatment times and temperatures. The results indicate that treatment time and temperature significantly affect fumigation efficacy of EF on *T. castaneum*. There was a significantly higher fumigation activity of EF within 48h, and the fumigation efficacy at lower temperatures was better than that at higher temperatures. At 20, 25 and 30℃ and 24h fumigation, the LC_{50} values of adults were 24.24, 27.52 and 29.95 μL/L, respectively; whereas 48h of fumigation produced corresponding LC_{50} values of 22.18, 25.74 and 27.30 μL/L, respectively. EF was most effective against eggs and least effective against pupae. EF was most effective against *T. castaneum* in a simulated wheat storehouse and least effective in a simulated paddy rice storehouse. The corrected mortalities of *T. castaneum* in upper, middle and lower layers of simulated wheat and maize storehouses were 100% with a dose of 70 g/m^3 after 24 h treatment at 30℃, whereas in a paddy storehouse *T. castaneum* can survive at all layers. When the EF dose was 90 g/m^3, the corrected mortality in the upper layer was 100%, in the middle layer 19.3% and in the lower layer it was zero.

Introduction

Grain storage is a key method to use the grain resources properly. However, grain can be infested by insects, mites and other organisms during storage, which cause heavy losses in both quality and quantity[1-3]. *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) is a worldwide storage pest. It feeds on many kinds of grains and their products, oil crops, dry fruits and herbs. Adults have an odour gland which can cause a mouldy smell when populations are large, causing problems to grain store owners and significant grain loss[4].

Grain storage facilities depend mainly on PH_3 and methyl bromide to control *T. castaneum*. Resistance of stored products insect pests to PH_3 has been recorded. Methyl bromide will be phased out because it depletes the ozone-layer. Scientists around the world are looking for alternatives that overcome insect resistance and that are not ozone depleters [2-5].

Ethyl formate is an environment-friendly fumigant. It is a colorless liquid with a boiling point of 54.1℃ under normal temperatures. It was registered as fumigant for dried fruit in Australia in 2002[6-8].

EF has some inherent advantages as fumigant: firstly, it has wide natural occurrence in a range of foods such as vegetables, fruit, grain and animal products. Secondly, fumigation with EF does not adversely affect product quality or seed germination. Thirdly, it breaks down on the commodity after fumigation rather than being desorbed, and its breakdown products are also naturally occurring components of food[7-10].

This study aimed to understand the fumigant activity of EF on adult and immature stages of *T. castaneum* under different conditions. This research can provide information for using EF commercially for controlling stored product insects as an alternative to methyl bromide.

Materials and Methods

Insects and Ethyl Formate

Insects were obtained from the Applying Insects and Mites Laboratory of SWU (Southwest University, Chongqing, China), and grown on a mixture of whole wheat and brewer's yeast (whole wheat: yeast is 20:1). One week after oviposition, the adults were removed from the food and the off-spring were grown at 32 ± 1℃, RH75% ± 5%, and 24h dark. Newly emerged and healthy adults were selected for the experi-

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ments.

Ethyl formate (AI > 98.00%) was obtained from Shanghai Chemical Reagent Group of China.

Effect of EF Concentration and Fumigation Time on the Effectiveness

One liter jars were used in the fumigations. At 25℃ the treatment times were 12, 24, 36, 48 and 60h at EF concentrations of 30, 35, 40, 45 μL/L, respectively. Adults of *T. castaneum* were placed at the bottom of the 1 000mL jars, then measured amounts of EF were dropped onto the filter paper at the bottom of the fumigation box (d = 2cm, h = 1cm). Nylon gauze was used to wrap the box as quickly as possible so that *T. castaneum* would not make direct contact with EF. Plastic film was used to seal the jars. The jars were incubated in the dark at the designated temperature. Each treatment contained 50 adults, and there were 3 replications. Controls were identical except unfumigated. Mortality was checked after 24h fumigation.

Temperature Effect on the EF Efficacy

The same fumigation method as described above was used. The EF concentrations were 26, 28, 30, 32 μL/L at temperatures of 16, 19, 22, 25, 28, 31 & 34℃ respectively. Fumigation time was 24h.

LD_{50} of EF Against *T. castaneum*

The same fumigation method as described above was used. *T. castaneum* adults were treated for 24h at 20℃ at concentrations of 21, 22, 23, 24, 25, 26 & 27 μL/L; at 25℃ at 24, 25.5, 27, 28.5, 30, 31.5 & 33 μL/L; at 30℃, 26, 28, 30, 32, 34 & 36 μL/L. Fumigation for 48h at 20℃ at concentrations of 20, 21, 22, 23, 24, 25 & 26 μL/L; at 25℃ at concentrations of 22, 24, 26, 28 & 30 μL/L; at 30℃ at concentrations of 22, 24, 26, 28, 30 & 32 μL/L. These concentrations and related time/temperature combinations were chosen to achieve mortality from 16% to 84%. Each treatment contained 50 adults and there were 3 replications.

Fumigation Activity Comparison of EF against Immature Stages of *T. castaneum*

The methods used were similar to those used by Obeng-Ofori (2005)[11]. 3000 adults of mixed sex were reared on 8.4kg culture media with whole wheat: yeast = 20: 1. Seven days after oviposition, the parent adults were sieved out. Every 100g of the culture media with eggs were separated and put into jars. Nylon gauze was used to seal the jars which were incubated at 32 ± 1℃. After 1d, the jars were randomly placed into dryers, and the jars with the eggs were fumigated with EF at 20℃ & 30℃ for 24h in the dark in the incubators. Then after 8, 16 & 23d, similar experiments were carried out to test the efficacy of EF against early larvae, late larvae and the pupae. Immature insects were taken out and reared in the chamber. The numbers of adults were checked 7 weeks later. Each treatment had three replications. The controls were set up without EF fumigation.

Fumigation Activity of EF to Adults of *T. castaneumn* Simulative Storehouse

The big glass dryers with 18L volume were used as to simulate a storehouse. Ten kg of wheat, 10kg maize and 7.5kg of paddy rice were placed in each dryer respectively. Four stages of *T. castaneum* were put into a special film box, in which both sides were covered with nylon gauze to prevent the insects escaping. The boxes each with 50 insects were assigned to the upper, middle or lower layer in the dryer, which was kept at 30 ± 1℃ for 12h. EF was added to each dryer for 24h. The mortality was checked after fumigation. Each treatment and controls had three replications.

Data Analysis

All the data concerning mortality were corrected by using Abbott's formula (Abbott 1925). Mortality data were transformed using arcsine ($x^{0.5}$) and ANOVA was carried out using SPSS software. Duncan's multiple range test was used to test the difference significance and IRM software (developed by Southwest University) was used to obtain LD_{50} values and regression equations.

Results

Effect of EF Concentration and Fumigation Time on the Activities of EF

EF showed satisfactory results under the EF concentration and fumigation time (Table 1). Corrected mortality increased with the fumigation time for the same concentration of EF. Under the same fumigation time, the corrected mortality also increased as the EF concentration increased. Two – way ANOVA indicated that fumigation time and concentration affected the corrected mortality significantly ($P < 0.01$). Under the conditions of fumigation time of 36h and an EF concentration of 30 μL/L, the corrected mortality was 81.88%. The corrected

mortality even reached 93.96% when concentration was 35 μL/L, and the corrected mortality was 100% when the concentration was 40 μL/L. When the concentration reached 45 μL/L, the corrected mortality was 100% after 12h fumigation. This indicated that EF as fumigant killed adults quickly.

Table 1. The fumigation activities of ethyl formate against *T. castaneum* adults in different treatment time and concentrations (25℃)

Treatment time/h	Corrected mortality/%			
	30μL/L	35μL/L	40μL/L	45μL/L
12	60.00 ± 7.21 a	78.00 ± 5.77 a	92.67 ± 1.76 a	100.00
24	70.67 ± 2.91 ab	87.33 ± 5.21 ab	96.67 ± 1.76 b	100.00
36	81.88 ± 3.49 bc	93.96 ± 2.33 bc	100.00 c	100.00
48	89.19 ± 2.44 c	97.97 ± 1.17 c	100.00c	100.00
60	87.16 ± 4.73 c	98.65 ± 0.68 c	100.00 c	100.00
F	6.716	6.373	10.646	–
df	4,10	4,10	4,10	–
P	0.007	0.008	0.001	–

Temperature effect on EF Fumigation Activity

The temperature affected the fumigant activity on adults of *T. castaneum* significantly ($P < 0.01$, Table 2). Lower temperatures can provide better fumigant activity than higher temperatures. The corrected mortality of adults of *T. castaneum* decreased significantly ($P < 0.01$) when temperature increased over the range 16 to 34℃.

Table 2. The effects of treatment temperature on ethyl formate activity (24h)

Temperatures/℃	Corrected mortality/%			
	26μL/L	28μL/L	30μL/L	32μL/L
16	75.52 ± 3.13 d	83.67 ± 3.53 d	91.84 ± 3.12 d	100.00 e
19	69.39 ± 4.71 cd	81.63 ± 7.07 d	88.44 ± 5.93 d	95.24 ± 2.97 de
22	59.73 ± 3.08 c	71.81 ± 3.49 cd	81.88 ± 6.97 cd	86.58 ± 5.24 cd
25	41.61 ± 4.19 b	55.70 ± 4.19 bc	67.79 ± 4.19 bc	77.85 ± 4.65 bc
28	36.00 ± 4.62 b	50.67 ± 5.21 ab	60.00 ± 6.93 ab	65.33 ± 2.91 ab
31	30.00 ± 5.29 ab	40.00 ± 6.43 ab	50.00 ± 3.46 ab	58.00 ± 8.08 a
34	21.48 ± 2.01 a	36.24 ± 4.08 a	43.62 ± 3.08 a	53.02 ± 5.24 a
F	25.110	12.864	10.780	18.535
df	6,14	6,14	6,14	6,14
P	0	0	0	0

LC_{50} Values of EF Against adults of *T. castaneum*

Table 3 shows the LC_{50} values of ethyl format against adults of *T. castaneum* using different fumigation times and temperatures. The LC_{50} value is smaller at lower temperatures than at higher temperatures when treatment time is the same. It confirmed that EF displayed better fumigation activity at lower temperature than higher temperature. LC_{50} value was lower at 48h fumigation time than that under 24 h fumigation time, which indicated that increased fumigation time could improve EF efficacy.

Fumigation Activity to the Immature Stages of T. *castaneum*

Table 4 shows that EF was toxic to the immature stages of *T. castaneum* and its fumigant activity varied significantly ($P < 0.01$) according to life stage. EF was most toxic to eggs and least toxic to pupae. The corrected mortality is

higher at 20℃ than at 30℃. A concentration of 35 μL/L led to more than 90% mortality of all stages. Egg and younger larvae were all killed at 20℃. The controls produced more than 800 adults and 1 000 pupae, which also showed the good fumigation activity of EF against immature stages of *T. castaneum*.

Table 3. The LC_{50} values of ethyl format against adults of *T. castaneum*

Time/h	℃	Regression equation Y =	r	LC_{50} (μL/L)	LC_{95} (μL/L)
24	20	−18.24 +16.79x	0.9785	24.24 ±0.14	32.20 ±0.85
	25	−13.27 +12.69x	0.9974	27.52 ±0.21	37.08 ±0.80
	30	−12.77 +12.03x	0.9862	29.95 ±0.26	41.03 ±1.02
48	20	−13.42 +13.68x	0.9795	22.18 ±0.16	32.51 ±1.07
	25	−13.55 +13.15x	0.9962	25.74 ±0.22	34.33 ±0.86
	30	−9.94 +10.40x	0.9892	27.30 ±0.27	39.28 ±1.26

Table 4. Ethyl formate activity against immature stages of *T. castaneum*

Stages	Temperatures (℃)	Mortality/%		
		25 μL/L	30 μL/L	35 μL/L
Eggs	20	87.10 ±0.63 e	98.08 ±0.52 f	100.00 f
	30	81.90 ±0.93 d	93.89 ±0.62 e	100.00 f
Early larvae	20	83.08 ±1.07 d	95.18 ±0.66 e	100.00 f
	30	77.98 ±1.01 c	90.01 ±1.02 d	96.94 ±0.27 d
Late larvae	20	74.01 ±1.83 c	86.10 ±1.58 c	98.23 ±0.31 e
	30	67.35 ±1.74 ab	81.02 ±1.49 b	92.28 ±1.13 b
Pupae	20	69.08 ±1.79 b	82.41 ±0.90 bc	95.09 ±0.55 c
	30	63.30 ±1.52 a	74.95 ±2.39 a	90.27 ±0.88 a
	F	39.936	47.992	135.316
	df	7,16	7,16	7,16
	P	0	0	0

The Fumigation Activities of EF to *T. castaneum* in Simulative Storehouses

The fumigation activities of EF when simulated in a storehouse showed the fumigant was most effective in a wheat storehouse and least effective in a paddy storehouse. The corrected mortalities of *T. castaneum* in the upper, middle and lower layers in the wheat and maize storehouses were 100% with a dose of 70 g/m^3 after 24 h fumigation at 30℃, whereas in paddy storehouse *T. castaneum* survived in all layers. In the paddy rice simulated warehouse, using an EF dose of 90 g/m^3, the corrected mortality in the upper layer was 100%, in the middle layer 19.3% and zero percent in the lower layer.

Discussion

Our results showed that EF is effective for controlling adults of *T. castaneum*. At 40 μL/L for 36h or 45μL/L for 12h, the corrected mortality of adults of *T. castaneum* reached up to 100%. EF showed its rapid activities as a fumigant. EF was more toxic at relatively low temperatures, in contrast to previous publications[citations] which were carried out under different conditions. Further work will be undertaken to confirm our results.

Our research indicated that EF showed good fumigation activity against eggs of *T. castaneum*. Fumigant activity varied according to the insect stage. The susceptibility to EF of *T. castaneum* life stages decreased from the egg, to young larvae, to old larvae and to the pupal stage. The respiration rate of insects is known to be relatively low when they are in the egg and pupal stage and hence the reduced efficacy of common fumigants against eggs and pupa[16]. In contrast to these previous studies, EF was toxic to the egg stage but less toxic to the pupal stage.

Phosphine and methyl bromide are the most commonly used chemicals used today for grain storage disinfestation. However, methyl bromide will be phased out in China because of

its damage to the ozone layer and resistance of stored grain insect pests to PH_3 develops fast because of the inappropriate use[2,10]. It is very urgent to find some kinds of new fumigants for the stored product protection to all the researchers. EF is a relatively new chemical, which shows promise as an alternative to PH_3 and methyl bromide[12-15]. Based on the results above, our research indicates that EF can be used as a potential alternative fumigant in grain storage in the future.

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Table 5. The fumigation activity of ethyl formate against adults of T. *castaneum* in a simulated storehouse

	Wheat, Ethyl Formate (g/m^3)			Maize, Ethyl Formate (g/m^3)			Rice, Ethyl Formate (g/m^3)		
	50	60	70	50	60	70	70	80	90
upper	100 b	100 a	100	100 b	100 b	100	91.3 ±5.7 b	99.3 ±0.7 c	100 c
middle	98.7 ±1.3 b	100 a	100	96.0 ±3.1 b	100 b	100	7.3 ±6.4 a	13.3 ±4.8 b	19.3 ±9.3 b
lower	90.6 ±3.3 a	98.7 ± 1.3 a	100	74.0 ±2.3 a	94.7 ± 2.4 a	100	1.3 ±1.2 a	0 a	0 a
F	10.076	1	–	24.220	17.557	–	40.864	219.661	121.245
df	2,6	2,6	–	2,6	2,6	–	2,6	2,6	2,6
P	0.012	0.422	–	0.001	0.003	–	0	0	0

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tion growth and resistance development by the psocid, *Liposcelis bostrychophilia* Badonnel (Psocoptera: Liposcelididae). *Journal of Stored Products Research*, 2002, 38: 229 – 237

[34] Table 5 The fumigation activity of ethyl formate against adults of *T. castaneum* in a simulated storehouse

0211

Observations on the Activity of Insect Pests inside and outside two Flour Mills

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Abstract: Two Italian mills were selected for fumigation with sulfuryl fluoride or methyl bromide. The impact of fumigations upon populations of flour beetles and moths was evaluated. Traps baited with aggregation pheromone lure and an oil-based food attractant were used to monitor populations of flour beetles. Sticky traps baited with a pheromone lure were used to monitor the Mediterranean Flour Moth and the Indian Meal Moth. Traps were placed inside the mill buildings, within the areas selected for fumigation, and outside. The purpose of monitoring outside the mill buildings was to detect possible sources of reinfestation.

After fumigation, several stored product insects were detected in traps placed outside the mill buildings. The results showed that sulfuryl fluoride performed similarly to methyl bromide and was therefore a suitable alternative to control stored product pests in Italian mills. The re – infestation of mills could be directly attributed to undetected foci of infestation outside the fumigated area or to infested products brought into the mill.

Key words: stored-product pests, monitoring, Indian Meal Moth, *Tribolium*, fumigation, sulfuryl fluoride, methyl bromide

Introduction

At this time, the need of replacing methyl bromide with other techniques or alternative products in the traditional annual mill fumigation is meeting several difficulties in Italy. This is due both to the need of organizing pest management in an innovative way, in food industries characterised by inadequate structures, and to the habit of relying exclusively on methyl bromide, wrongly considered to be the safest product for killing pest insects. Among the possible alternatives, sulfuryl fluoride is at present the only active ingredient usable in Italy for fumigation.

In this work, we examined in particular the possibility of reinfestation after a fumigation treatment, as a consequence of the presence of pests outside the mill. Our results emphasise that pest management treatments, carried out with fumigations, must be integrated, with prevention and monitoring practices and with localised treatments outside the fumigated buildings. In fact, many stored-product pest species can be trapped outside grain storage and processing structures[1,2,3,4]. Some authors found high numbers of some pest species immediately outside food processing facilities and speculated that immigration could be important in pest dynamics inside the mill[5,6]. Other authors have shown, with the mark-recapture technique, that *Plodia interpunctella* was capable of entering the building from outside, and that this movement is primarily at the basement, first and top floor levels[7]. It was also observed that rebound of stored-product insect populations detected during post-fumigation monitoring could be directly attributed either to infested product being brought into the mill or to undetected foci of infestation outside the mills[8].

Materials and Methods

Two mills (A and B), located in the North of Italy, were studied. In mill A, fumigation was scheduled traditionally with methyl bromide; in mill B, disinfestation was carried out with sulfuryl fluoride. The main characteristics of the mills are shown in Table 1.

Table 1. Main characteristics and treatments of the fumigated mills.

	Mill A	Mill B
Date of fumigation	24/05/07 – 27/05/07	14/07/07 – 16/07/07
Volume (m^3)	14,000	6,900
Building material	concrete	bricks, wooden floor
Fumigant	methyl bromide	sulfuryl fluoride
Dosage ($g\ m^{-3}$)	66.7	20

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	Mill A	Mill B
Exposure time(h)	48	56
Internal temperature(℃)	22 – 27	24 – 28

Efficaly Determination of Salfaryl Fluoride Fumigation

This was the first time that mill B had been treated with sulfuryl fluoride. To check the results of fumigation, twenty – six bioassays (with insects reared at the Institute of Agricultural Entomology, University of Milan) were placed in the mill prior to the fumigation. One untreated control was kept in a cool box in a non – fumigated area of the mill. Insect species included in the bioassays were *Tribolium confusum* (50 eggs, 10 larvae, 10 adults), *Plodia interpunctella* (50 eggs and 10 larvae), *Sitophilus oryzae* (10 adults and mixed population of eggs, larvae and pupae).

Insect cultures were contained in 50 – mL polystyrene jars closed with metallic mesh lids. Food consisted of: rice for *S. oryzae*; white flour for *T. confusum*; a laboratory diet made up of bran, wheat, yeast, corn flour, white flour, honey and glycerol for *P. interpunctella*. Following fumigation, the bioassays were removed and maintained at 26℃ and 65% r. h. in the laboratory of the Institute of Agricultural Entomology, University of Milan, for mortality assessment.

Monitoring

Dome™ Traps Design CFB/RFB (containing an aggregation pheromone lure and an oil-based food attractant) were used to monitor population of *Tribolium* spp. Pheronet Meal Moth traps (Russel IPM, UK) baited with an IMM + 4 pheromone lure were used to monitor the Mediterranean Flour Moth and the Indian Meal Moth.

Traps were placed inside the mill buildings, within the areas selected for fumigation, and outside (Table 2). Where possible, Pheronet traps were placed in approximately the same locations as Dome traps.

Table 2. Number of DOME and Pheronet traps inside and outside the treated mills.

Location	Number of traps – Mill A		Number of traps – Mill B	
	DOME	Pheronet	DOME	Pheronet
Inside mill	24	26	10	10
Outside mill	6	7	2	2

In mill A, infestation levels of the stored product pests were monitored 2 weeks prior to fumigation and 6 weeks post – fumigation, while in mill B 1 week prior and for a total of 7 weeks post – fumigation.

Results and Discussion

Mill A

The overall percentages of reduction in moths and *Tribolium* spp., after fumigation, were 94 and 91%, respectively.

However, a steady and high number of moths was present in an area external to the mill that contained milling by-products (Fig. 1). Some conveyors link this area with the internal part of the mill. This could explain the rapid recolonisation of the mill by *P. interpunctella* and *E. kuehniella*. The moth population decreased significantly in mill A after fumigation and then it gradually increased in the weeks following the treatment (Fig. 1).

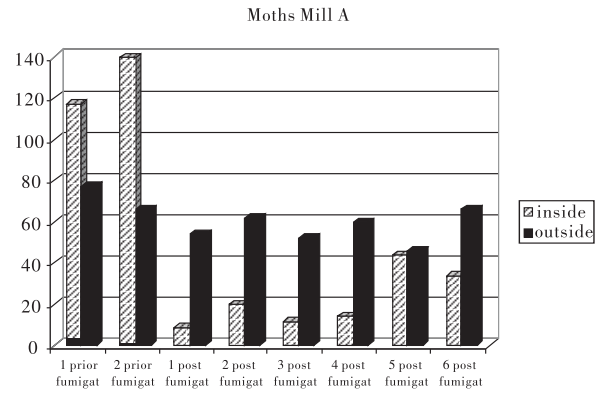

Fig. 1 Number of IMM and MFM trapped in and outside mill A, prior and after fumigation with methyl bromide.

Also, fumigation reduced the internal populations of *Tribolium* spp. remarkably, whereas their numbers remained almost constant outside the mill (Fig. 2). Campbell & Arbogast[7] pointed out that *Tribolium castaneum* trap captures tended to be lower outside compared to inside a mill. They suggested that population rebound after fumigation may result both from persistence of individuals within some patches within the mill and also the movement of new individuals into the mill either actively or in infested products. In mill A, after fumigation, we observed a gradual increase in *Tribolium* captures throughout the mill, whereas outside it, captures remained low.

Mill B

In mill B, it was not possible to estimate the reduction within the mill since no *Tribolium*

spp. and no moths were trapped inside the mill prior to fumigation. Since the owners had decided to test the use of sulfuryl fluoride as alternative to MB, bioassays were placed in the different areas of the building to verify fumigation efficacy. The results show the overall mortality of all the used species, in the different life stages (Table 3).

No infestation of *E. kuehniella* or *P. interpunctella* was detected inside mill B throughout the duration of the study (Fig. 3). *P. interpunctella* was detected only outside, namely in the trap placed near the loading area for flour into bulk flour trucks. Despite its continued presence in the external area, the mill itself never became infested. The loading area for flour is

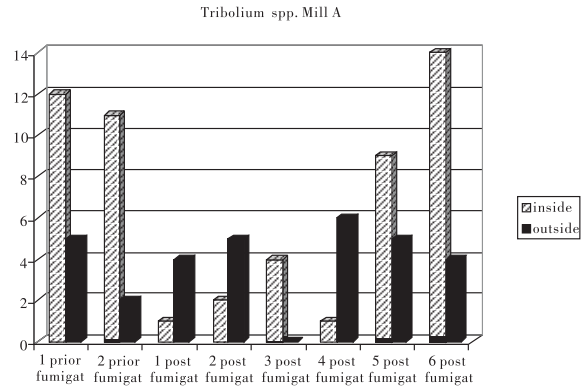

Fig. 2 Number of *Tribolium* spp. trapped in and outside the mill A, prior and after fumigation with methyl bromide.

Table 3. Mortality of bioassays used in the mill B to verify fumigation efficacy with sulphuryl fluoride.

Species	Life stage	Total number	Alive aftertreatment	Mortality rate (%)	Mortality rateof untreatedcontrol (%)
T. confusum	eggs	1300	000	100	36
	larvae	260		100	0
	adults	260		100	0
P. interpunctella	eggs	1300	0	100	6
	larvae	260	0	100	0
S. oryzae	adults	260	0	100	10

located adjacent to the mill. Mill doors and windows are kept closed when not in use. All the mill floors are thoroughly cleaned and polished every day. In such a situation, larvae of pest insects usually do not find food to grow.

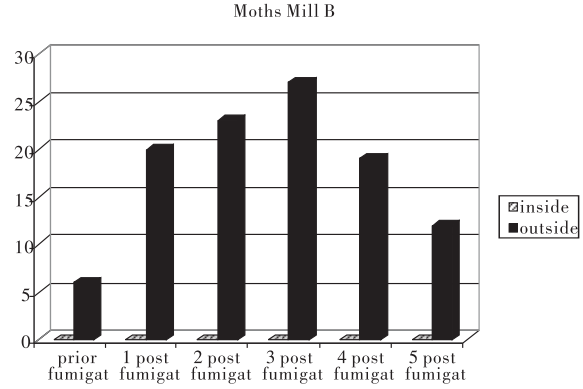

Fig. 3 Number of IMM trapped in and outside the mill B, prior and after fumigation with sulphuryl fluoride.

During inspections, neither present nor previous signs of infestation by Lepidoptera were detected inside the mill. As far as beetles are concerned, before fumigation, *Tribolium* spp. was not present either inside nor outside the mill. After fumigation, some individuals were occasionally found inside the mill (Fig. 4). As already mentioned, the cleaning conditions of the mill were excellent.

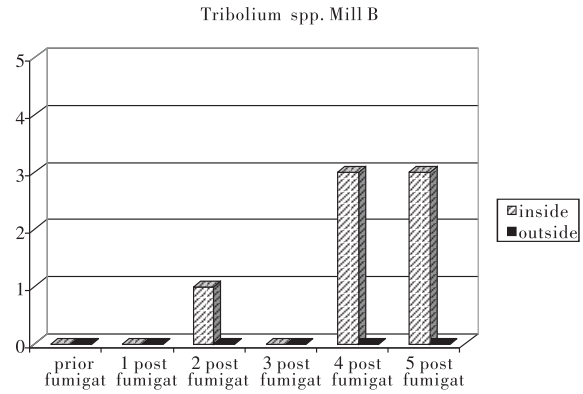

Fig. 4 Number of *Tribolium* spp. trapped in and outside the mill B, prior and after fumigation with sulphuryl fluoride.

Conclusions

Effectiveness of the sulphuryl fluoride fumigation of mill B was verified through use of bioassays. This supports the use of sulphuryl fluoride as an effective alternative to methyl bromide to control stored product pests in Italian mills.

It is important to underline that the distri-

bution of insects outside of food processing and storage facilities has a significant influence on the population dynamics and spatial distribution of pests inside facilities.

The collected data show how it is fundamental to monitor the external area to the mill itself for presence of pests. The area must be considered an integral part of the mill structure for pest control. It is thus necessary to include this area in the monitoring and infestation management program and when planning disinfestation treatments. Otherwise there is a risk that pest populations will not be eliminated, due to pest survival there. In only a few days, the presence of these foci of infestation would nullify the results of even a drastic disinfestation treatment such as fumigation with toxic gases. IMM and MFM populations will be difficult to destroy with fumigation unless better measures are taken to prevent entry of moths into the mill. This is what happened in mill A.

Furthermore, a good management of the mill is very important in terms of cleaning and rationalisation of any openings towards the outside. This prevents pests from entering the milling areas, despite the presence of insects in the field, as in mill B. In this mill there was a high risk of infestation by *P. interpunctella* in the mill products to be sold, because of a focus of infestation around the loading door for flour, which had been ignored by the miller.

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Toxicity of Ethyl Formate on Adults of *Liposcelis entomophila*

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Abstract: The fumigation activities of ethyl formate (EtF) against adults of *Liposcelis entomophila* (Enderlein) were researched by the sealed-jar fumigation method under the conditions of different EtF dosages, temperatures and exposure times in laboratory. The results indicated EtF dosage, temperature and exposure time affected the fumigation efficacy significantly. EtF showed a high fumigation activity within short exposure time and EtF performed better at relatively low temperature than at relatively high temperature. At 20℃, 25℃ and 30℃ after 24 h fumigation, the LC_{50} values were 7.769、8.964 and 9.900 μL/L, respectively.

Key words: ethyl formate, *Liposcelis entomophila*, fumigation activities

Liposcelis spp, also named as booklice, paperlice and psocids, is a kind of small insect which belongs to Liposcelididae, Psopotera. *Liposcelis* spp has been becoming one of the most important stored product insect pests in the tropical and subtropical regions all over the world (Rejendran, 1994; Chen et al., 2003). The five important species among *Liposcelis* in the world are *Liposcelis bostrychophila* Badonnel, *L. entomophila*, *L. paeta* Pearman, *L. decolor* (Psocoptera), and *L. pearmani* Lienhard. Economic importance of *Liposcelis bostrychophila* and *L. entomophila* was reported in many Asian countries, such as China, India, Thailand, Philippine, Singapore, Malaysia, and Indonesia (Leong et al., 1995; Wang et al., 1999). There are five kinds of booklice damage (Chen et al., 2003). The first is booklice's direct feeding, which cause weight and quality losses of stored grain. The germination rate of wheat seed decreased significantly as the result of booklice damage. 4% – 5% weight loss of stored paddy rice was suffered because of psocid occurrence. Secondly, dead psocid, excretion, ecdysis, and psocid fragment contaminated the stored grain and other stored products. Thirdly, psocid was the vector of some diseases and it was a potential anaphylactogen, which led to skin allergic action for the allergic people. Fourthly, high population density of psocid cause grain moisture increase, temperature increase and grain molding. Fifthly, high psocid population density caused the psychological pressure for most people and also made the people feel uncomfortable. In Europe, existence of psocids in food production facility was the reason of consumer's complaint (Tuner, 1987). In Australia, psocid was reported as small bugs vs. big problems (Reuss et al., 1994). In China, *Liposcelis bostrychophila* and *L. entomophila* had become the dominant species in "Two Low Storage of oxygen and phosphine" and "Three Low Storage of oxygen, phosphine, and temperature" national warehouses (Wang et al., 1999).

The main chemicals to control psocids are phosphine and methyl bromide. But Resistance of psocids to chemicals was very serious because of irrational chemical use. Due to small psocid size and strong resistance to chemicals, it was easy to neglect its existence. Hence, it is becoming more and more difficult to control them. Furthermore, Methyl bromide has been phased out in 2005 in developed countries and will be phased out in 2015 in developing countries including China, so it is urgent to find new fumigants to be used as alternatives to methyl bromide and phosphine. Ethyl formate (EtF) is a promising and environmental friendly fumigant (Muthu et al., 1984; Ren et al. 2000; Damcevski et al., 2000), which was registered as dry fruit fumigants in 2002 in Australia (Ren et al. 2003, 2006; Damcevski et al., 2006). The purpose of this research was to evaluate the fumigation activity of EtF on the adults of *L. entomophila* under the conditions of different temperature, fumigation time and EtF concentration and expected to provide data information for developing EtF as an alternative of Methyl bromide and phosphine to control *L. entomophila*.

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Material and Methods

Insect. *L. entomphila* Badonnel was collected from simulative warehouse at Chongqing Key Laboratory of Entomology & Insect Control Engineering, Southwest University, Chongqing, China. The insects were reared on the mixture of whole wheat flour, brewer's yeast and milk powder(10∶1∶1) in 1 – liter glass jars at 27℃ ±0.5℃、75% – 80% RH、and a photoperiod of 0∶24(L∶D) in the laboratory.

Ethyl Formate

Ethyl formate (AI > 98.00%) was produced by Shanghai Chemical Reagent Group of China.

Effect of Temperature and EtF Concentration on Toxicity

1L glass jars were used in the fumigation. At 16, 19, 22, 25, 28, 31 and 34℃, booklice adults were fumigated 24 hours with EtF concentrations of 7, 9, 11 and13 μL/L. 30 adults of 24h old age were placed in a plastic box(d = 2 cm, h = 1 cm) for each treatment and then the plastic box wrapped with nylon gauze was placed at the bottom of the 1 000mL jars. The filter paper with quantitative EtF was placed in the glass jar and the Plastic film was used to seal the glass jars. At last the jars were put intoan incubator which was set at certain temperature and all dark. Each treatment has 3 replications. Controls were set up without EtF fumigation. Mortality was checked 24h after fumigation.

Effect of Fumigation Time and EtF Concentration on the EtF Efficacy

Same fumigation method as abovewas adopted. At 30℃, the EtF concentrations were 7, 9, 11, 13μL/L and fumigation times were 12, 24, 36, 48 and 60 h, respectively. Each treatment has 30 adults, 3 replications. Mortality was checked after fumigation.

LC_{50} Values of EtF on Liposcelis entomophila

The same fumigation method as the above in 1.2.1 was adopted. Fumigation time was 24 h and fumigation temperatures were 20, 25, and 30℃, respectively. At each temperature, 5 to 7 concentration levels were set up as follows. At 20℃, the concentration levels were 7, 7.5, 8, 8.5, 9, 9.5, 10μL/L, respectively. At 25℃, the concentrations were 8, 9, 10, 11, 12, 13μL/L, respectively. At 30℃, the concentrations were 7, 8, 9, 10, 11, 12 μL/L, respectively. These concentrations related temperatures were chosen to make the mortality from 16% to 84%. Each treatment had 30 adults, 3 replications.

Data Analysis

All the data concerning mortality were corrected by using Abbott's formula (Abbott, 1925). Mortality data were transformed using arcsine($x^{0.5}$) and ANOVA was carried out using SPSS software. Duncan's multiple range tests was used to test the difference significance and IRM software (developed by Southwest University) was used to obtain LC_{50} values and regression equations.

Results

Effect of Temperature and EtF Concentration on the Activities of EtF

The fumigation activities of EtF against L. entomphila under different temperatures and EtF concentrations after 24 h fumigation were listed in table1. Table 1 showed that EtF demonstrated relatively good activities on the adults of L. entomphila. At 19℃, the best effectiveness of EtF against the adults of L. entomphila. was obtained. Betweent16 and 22℃, the corrected mortalities reached up to 90% at 11 μL/L and 100% mortality was obtained when the EtF concentration was 13 μL/L. The corrected mortality decreased as temperature increased when the temperature ranged from 19 to 34℃, which indicated that the EtF efficacy was better at relatively low temperature than at relatively high temperature. Two-way ANOVA showed the effects of temperature, EtF concentration, and temperature EtF concentration interaction on the corrected mortalities were significant (for temperature: $F = 55.338$, $df = 6, 56$, $P = 0.000$; for concentration: $F = 265.059$, $df = 3, 56$, $P = 0.000$; for temperature EtF concentration interaction: $F = 3.726$, $df = 18, 56$, $P = 0.000$).

Table 1. The fumigation activities of EtF against *L. entomphila* under different temperatures(24 h)

Temperatures(℃)	Corrected mortality(%)			
	7μL/L	9μL/L	11μL/L	13μL/L
16	34.33 ±8.69 c	77.00 ±1.53 de	98.33 ±1.67 e	100.00 c
19	31.00 ±5.86 bc	86.77 ±1.94 e	99.00 ±1.00 e	100.00 c
22	27.67 ±2.67 bc	80.00 ±5.00 e	93.33 ±3.33 de	100.00 c

Temperatures(℃)	Corrected mortality(%)			
	7μL/L	9μL/L	11μL/L	13μL/L
25	22.57 ±2.57 abc	62.33 ±6.23 cd	84.33 ±1.33 cd	99.00 ±1.00 c
28	16.67 ±1.93 ab	54.43 ±2.94 bc	70.00 ±5.13 bc	84.33 ±4.67 b
31	13.33 ±1.93 a	40.67 ±1.86 b	66.67 ±5.24 b	76.67 ±10.53 b
34	23.00 ±4.16 abc	25.33 ±7.67a	45.00 ±2.89 a	50.00 ±5.77 a
F	3.055	18.817	24.435	22.120
Df	6,14	6,14	6,14	6,14
P	0.04	0.000	0.000	0.000

Note: The data shows the average of three duplicates. Data in the same row followed by different letters show significant difference at 0.05 level by Duncan's multiple range test.

Effect of Fumigation Time and EtF Concentration on the EtF Efficacy

Two-way ANOVA showed fumigation time and EtF concentration affected the corrected mortalities significantly (for fumigation time: $F = 12.975$; $df = 4, 40$; $P = 0.000$; for concentration: $F = 71.687$; $df = 3, 40$; $P = 0.000$), but the effect of the fumigation time × EtF concentration interaction on the corrected mortality was not significant ($F = 0.651$; $df = 12, 40$; $P = 0.785$) at 30℃. When the EtF concentrations were the same, the corrected mortality increased as the fumigation time increased (Table 2). Under the condition of 60 h fumigation time and 13 μL/L dose, the corrected mortality was 100%. Meanwhile, when the fumigation time was the same, the efficacy improved as the EtF concentration increased.

Table 2. The fumigation activities of EtF against *L. entomphila* under different time and concentration (30℃)

Treatment time(h)	Corrected mortality(%)			
	7μL/L	9μL/L	11μL/L	13μL/L
12	10.00 ±2.89 a	36.67 ±10.91 a	40.17 ±10.33 a	67.67 ±4.63 a
24	18.00 ±6.01a	40.67 ±2.03 ab	60.00 ±10.15 ab	85.00 ±7.57 b
36	13.33 ±3.33 a	41.67 ±1.67 ab	72.67 ±1.45 bc	89.33 ±2.33 b
48	15.67 ±2.96 a	67.67 ±9.02 ab	76.33 ±2.03 bc	95.67 ±2.96 bc
60	28.33 ±6.01a	70.67 ±10.35 ab	86.67 ±3.76 c	100 c
F	0.797	2.755	6.562	9.897
df	4,10	4,10	4,10	4,10
P	0.554	0.088	0.007	0.002

Note: The data shows the average of three duplicates. Data in the same row followed by different letters show significant difference at 0.05 level by Duncan's multiple range test.

LC_{50} Values of EtF against Adults of *L. entomphila*

LC_{50} values of EtF against adults of L. entomphila at 20, 25, 30℃ were listed in table 3. Table 3 showed LC_{50} value was smaller at 20℃ than that at 25 and 30℃, which demonstrated the efficacy of EtF at 20℃ against this booklice was better than that at 30℃. Based on the regression equations, the relatively big rates of slope proved the susceptibility of the adults of L. entomphila was consistent and the improvement of fumigation efficacy could be obtained by increasing EtF concentration.

Table 3. The LC_{50} Values of Ethyl Formate against Adults of *L. entomphila* at Different Temperatures

Temperatures (℃)	Regression equation (Y =)	R	X^2	LC_{50}/μL · L^{-1}	LC_{95}/μL · L^{-1}
30	−1.801 +6.8304x	0.962	6.636 *	9.900 ±0.20	17.237 ±1.18
25	−6.656 +12.139x	0.977	5.297 *	8.964 ±0.13	12.246 ±0.28

Discussion

Ethyl formate, as an old fumigant, has been used as a fumigant for dried fruits for many years (Ren, 2006). For the past few years, the phase out of methyl bromide and resistance of stored product insect pests to phosphine drove reevaluation of EtF efficacy. Muthu (1984), Hilton and Banks (1997) reported that EtF could control stored product insect pests effectively. The researchers in Australia used EtF to fumigate stored wheat and sorghum in unsealed conditions and their results show EtF killed insect pests within short time period. Damcevski and Annis (2000, 2006) studied the fumigation efficacies of EtF on *Sitophilus oryzae*, *Rhyzopertha dominica* and *Tribolium confusum* in the laboratories and the results also demonstrated that EtF had satisfactory fumigation activities in a short fumigation time. The toxicities varied with the stored product insect pest species. In China, Tang et al. (2006) researched the fumigation activities of EtF against *Sitophilus oryzae* and *Tribolium castaneum* in the laboratory and he proved that EtF killed insects in a short time and the toxicities of EtF were better at relative low temperature than at relative high temperature. Our research also showed that EtF killed most psocids in 24 hour fumigation time at 11μL/L and 13μL/L dosage, especially at 13μL/L dosage. Our results about EtF quick killing insect pests were in consistent with that of Tang and other researchers from Australia. Temperature is an important factor to affect the efficacy of EtF. We found that the efficacy of EtF was much better at 16, 19, and 22℃ than that at 31 and 34℃, which demonstrated that EtF functioned much more effectively at relatively low temperatures. This finding was also in agreement with that of Tang et al., who tested the fumigation activities of EtF on *Sitophilus oryzae* and *Triboium castaneum*. We speculated that EtF decomposed quicker at higher temperature than at lower temperature. Contrary to phosphine fumigation, in which its toxicity increased with temperature, we thought that EtF was more suitable for the fumigation at relatively low temperature. Our data about EtF fumigation activities was obtained under the condition of empty jar and could be used as reference data for empty warehouse fumigation to control *L. entomphila*. In the real warehouse filled with stored grains, many factors such as the grain species, bulk grain height, insect species and its developmental stage, and other environmental factors affects the fumigant efficacy. These factors that affect the ethyl formate efficacy need to be further researched in the future.

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0213

Carbon Dioxide——the Veteran and Versatile Fumigant

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Abstract: Reported ninety years ago to be the "most effective" fumigant compared to carbon disulphide (CS_2) and hydrogen cyanide (HCN), carbon dioxide still finds niche applications, e. g. "organic" grain protectant. In addition to its use in Controlled Atmospheres (>35%, 15days), carbon dioxide has been used to improve efficacy, pesticide distribution and the elimination of flammability in pesticide mixtures.

High pressure formulations in industrial gas cylinders use the unique solvent-propellant properties of liquid carbon dioxide to achieve mixtures of various fumigant chemicals. An early example of this formulation is the non-flammable mixture of 10% ethylene oxide in CO_2. More recent formulations include 2% phosphine [CYTEC product ECO_2 FUME] and 16.7% ethyl formate [LINDE Group-VAPORMATE].

In addition to its use in formulation of fumigant chemicals, the solvent-fpropellant property of carbon dioxide has been used to dispense aerosol mixture of contact insecticides. The "industrial aerosol formulation" marketed in a number of countries and typically using quick-knockdown and non-residue insecticides (e. g. natural pyrethrins, dichlorvos) are excellent for "fogging" applications.

Commercial carbon dioxide is recycled from by-product process streams (petroleum refineries, breweries, fertiliser manufacture). The carbon dioxide purified, liquefied and marketed by industrial gas companies is equivalent to less than 0.05% of the total carbon dioxide emissions to the atmosphere.

Key words: fumigation, fumigants, carbon dioxide, organic, aerosols, methyl bromide alternative, stored product pests

Introduction

The examples given below are examples of the variety of uses of carbon dioxide in pest control, using its solvent, propellant, toxic and synergistic properties.

Carbon Dioxide + Insecticides

An outbreak of encephalitis (inflammation of the brain transmitted by mosquitoes) initiated the development of an insecticide dispensing system to treat large areas. The development investigated liquid propellants that could be used in industrial gas cylinders (the high pressure rating of these cylinders extended the potential candidate propellants). The propellants included a range of liquefied gases that have the dual role of solvent and propellant (co-solvents can be added to assist in any solubility issues).

The pressure pack ("aerosol") can was invented by the Norwegian, Eric Rotheim, in 1924. He used dimethyl ether (DME) as the propellant of a ski wax aerosol. There was no wide acceptance of the aerosol can until World War II, when aerosol insecticides were used to help protect soldiers fighting in insect-infested jungles in the Pacific (40 000 000 aerosol units were distributed by the US Government). The potential for this unique packaging system was quickly recognised and, in 1947, the first consumer aerosol can appeared on the market. Chlorinated fluorocarbons (CFCs) were developed in 1930 by the Frigidaire Division of General Motors to replace toxic and corrosive ammonia and sulphur dioxide then in commercial use as refrigerants. After World War II, CFCs became the basic propellant for the new aerosol industry. Implications of ozone depletion by CFCs caused concern in the aerosol industry and led to developments of alternative propellants. Australian industry statistics show in 1970 more than 90% of all aerosols contained fluorocarbons but, by 1987, more than 80% of aerosols were CFC – free.

Fluorocarbons, hydrocarbons, nitrous oxide (NO_2) and carbon dioxide (CO_2) were among the candidate liquefied gases investigated as suitable solvent-propellants. Fluorocarbons had ozone depletion issues, hydrocarbons (propane, butane, isobutane) are flammable and nitrous oxide had the potential to decompose forming an

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Note: vol% and wt% are identical as EtO and CO_2 have similar molecular weights

oxidant which would react violently with combustible ingredients. Only low pressure gaseous nitrous oxide (used in whipped cream) and carbon dioxide (surface spray products) can be used as propellants, because their equilibrium vapour pressure is four times (4x) the pressure rating of pressure pack aerosol cans (pressure rating of cans is less than12 bar). The industrial gas cylinder chosen for the bulk aerosol dispenser have pressure rating >200 bar.

The solvent-propellant properties of liquid carbon dioxide were the basis for the internationally patented[1] BOC Australia ENVIROSOL product range. The ENVIROSOL system relied on liquid CO_2 at high pressure (50 bar) to act as a solvent and propellant to dispense liquid insecticide as an aerosol fog (non-flammable, ultra-fine particle sizes, sized for commercial and industrial needs and ease of automation). The active constituent dissolved in the liquid CO_2 is contained in an industrial high pressure gas cylinder fitted with a "dip" tube to enable the liquid mixture to be withdrawn and dispensed as an aerosol spray. The small droplets size range (2 – 20 microns-over 2 billion droplets are created from a gram of chemical) of the particles formed results in the insecticide being suspended for over two hours. A small quantity of insecticide applied as a liquid CO_2 aerosol can quickly fill a large space (spray travels >30m) and no insect can escape the treatment. The product can be dispensed manually using portable equipment or automatically using programmable timed release systems.

ENVIROSOL products have revolutionised pest control. They include PESTIGAS (0.4% synergised natural pyrethrum in liquid CO_2 a quick knockdown, non-residual and low mammalian toxicity insecticide) and INSECTIGAS (5% dichlorvos in liquid CO_2 a rapid action non-residual insect control agent used by professional pest controllers).

The older style treatments of wetting surfaces with large volumes of insecticide/water mixtures or fogging with oil-based carrier are inefficient, messy, hazardous and time consuming. The ENVIROSOL system treats the total space with low concentration (3ppm levels) of insecticide, minimises occupational health and safety hazards of handling chemicals. The process is clean, fast and biologically efficient.

This technology has been extended into animal health products (automatic spraying of sheep for lice control); the joint CSIRO project on ECO_2 FUME/SIROFLO treatment of bulk grain; the new, low toxic fumigant, VAPORMATE; the project with partner CSIRO on potential new fumigants-Ethane Dinitrile (EDN) and Carbonyl Sulphide (COS).

Carbon dioxide + Sterilant

The use of carbon dioxide + ethylene oxide [EtO] mixtures have been in use for over fifty years as both sterilant and fumigant. Detailed EtO flammability studies [2] showed that, for non-flammability in air, the initial mixture mustbe less than 12% EtO in CO_2. The international dangerous goods-approved non-flammable mixture is a conservative 9% EtO in CO_2.

While a range of mixtures have been marketed over the years, the major demarcation is between flammable and non-flammable EtO products. However carbon dioxide is usually the gas of choice for pre-purging and post-purging EtO vacuum chambers used for both medical device sterilisation and quarantine fumigation. The unique penetrative properties of EtO make it the ideal quarantine fumigant required to overcome barriers such as paints and plastic wrappings. A recent publication[3] describes EtO quarantine fumigation dosages, pre-purging and post-purging actions and *Ct*-product details for effectiveness.

The new fumigant, Ethane Dinitrile (EDN = cyanogen = C_2N_2) also has sterilant properties. In addition to the pure material, a non-flammable 20% EDN in carbon dioxide formulation has been developed [4].

Carbon Dioxide + Phosphine

The patented non-flammable mixture of 2% w/w phosphine in liquid carbon dioxide [PHOSFUME = ECO_2 FUME] usage became widespread because of its superior biological efficacy, accurately controllable dosage and operator safety. Supply of ECO_2 FUME also supported the CSIRO development of SIROFLO which revolutionised fumigation of grain stored-products in "leaky" storages.

Prior to SIROFLO development, the traditional liquid insecticide "grain protectants", applied as chemical sprays, were one of the very few insect control options for leaky storages. SIROFLO, the flow-through fumigation application, achieves insect-free and residue-free status in existing non-gastight storages. SIROFLO delivers a low continuous dosage of PH_3 over a period of 28 days-shorter exposure times are possible with increased PH_3 levels[5]. The phosphine dosage initially less than 50ppm has been

increased over time to triple this level as insect's phosphine tolerance has increased. The advantage of the SIROFLO treatment is the ability to treat any grain storage irrespective of air tightness as demonstrated in Cyprus[6].

Installation of relative simple PVC pipework allows recirculation of gases, converting SIROFLO to SIROCIRC, enabling fumigation of multiple grain storages of varying sizes with cylinders of ECO_2FUME [7]. For large storages, e. g. 1. 3 million tonnes grain storage at Dalian, China, the on-site mixing of ECO_2FUME using bulk carbon dioxide supply and VAPORPH_3OS (100% gaseous PH_3) is a more practical option[8].

A separate patent was also granted for the on - site mixing of VAPORPH_3 OS and air where it is critical to ensure that the initial dilution to 1% PH_3 in air was done externally i. e. prior to addition to the grain storage (important to avoid any possibility of flammable gas within the storage).

While cylinderised formulations are a relatively expensive form of PH_3 supply, it is possible to use ECO_2FUME in a SIROFLO installation to achieve the economical fumigation of "leaky" storage ina way not possible with solid formulations. Also ECO_2FUME can be used in fumigate sealed storage where time is critical or where "top-up" of PH_3 levels is needed using conventional solid PH_3 formulations. The instant delivery of PH_3 using ECO_2 FUME allows reduced exposure time which makes possible selective methyl bromide replacement applications.

Studies on the oxidation reaction of mixtures of PH_3 and CO_2[9] identified the formation of a polymeric solid, $(-CO_2-P_2H_4-)_n$. This solid, on prolonged exposure to air, forms a paste mixture which also contains phosphonic, phosphinic and phosphoric acids. This equipment-clogging material can be avoided by purging air remaining in delivery systems for ECO_2 FUME after use with carbon dioxide to less than 100 ppm O_2.

Carbon Dioxide + Ethyl Formate

The development of alternative fumigants is important because of threats to the widely used fumigants, methyl bromide and phosphine. Specifically, methyl bromide is an ozone depletor and there is increasing phosphine resistance in insects. Alternative fumigants should be efficacious against a wide range of insect pests, safe to consumers and workers, but not damage the product. Ethyl formate (EtF) is a fumigant that satisfies these requirements. Historically used as fumigant of dried fruit and packaged food, EtF is naturally occurring, found in many foods, e. g. green apples, cabbages.

VAPORMATE is a LINDE Group registered formulation of EtF in CO_2. When CO_2 is mixed with EtF, it not only eliminates flammability, but also acts synergistically to enhance the efficacy of the ethyl formate. The naturally occurring EtF has GRAS (Generally Regarded As Safe) status as a food additive. EtF is diluted six times (6x) in liquid CO_2 to formulate the non-flammable VAPORMATE (16. 7% w/w ethyl formate in liquid CO_2, equivalent to 11% v/v ethyl formate in gaseous CO_2). VAPORMATE is a post harvest fumigant that controls insects in stored grains[10], fresh produce and food processing equipment. VAPORMATE is dispensed as a "fog" (particle size -5microns) or warm (40℃) gas mixture to assist uniform distribution and optimise efficacy.

VAPORMATE ["No Withholding Period" status] applications include:

· Niche alternative for methyl bromide (e. g. grain, dried fruit, nuts);

· Rapid treatment system: e. g. 50 - tonne silo of grain: 12 minutes to apply, three hours to fumigate and two hours to air out, with no withholding period.

· Disinfestation of food processing equipment containing food residues.

· Modified Atmosphere Package [MAP] treatment for packaged food.

VAPORMATE dispensing innovations include:

· Product sprayed as a liquid via metering orifice nozzles to treat food processing equipment. The resultant "fog" permeates the space quickly, propelled by the high cylinder pressure [50 bar]. VAPORMATE installation can be piped to convenient locations throughout the food plant.

· Product vaporised as a warm gas using a hot water vaporiser and dispensed using aeration fans in grain storages (product is dispensed during one air change) or venturi devices to dilute the gaseous mixture with atmospheric air to optimise use.

VAPORMATE development had input from the (Australian) Grain Research and Development Corporation (financial support), CSIRO

Entomology (innovation from researchers) and BOC Limited (product development).

The approved dosage on the registered VAPORMATE label is 420 g/m^3 (i.e. equivalent to 2.3% v/v ethyl formate and 21% v/v carbon dioxide). CSIRO reports the VAPORMATE forced flow fumigation of stored grain is safe, efficacious and rapid (high level of mortality of tolerant insects was achieved in 3 hours).

Carbon Dioxide as the "Organic" Fumigant

There has always been the need to control insects in foodstuffs to prevent food losses and to satisfy marketing requirements. Global food customers are hardening in attitudes to pesticide residues in grain, following consumer demands for minimal or no chemical residues in food. An alternative to the traditional practice of spraying grain with liquid insecticide grain protectants is fumigation using gases. With increasing restrictions being placed on the chemical treatment of grain, CO_2 atmospheres, which have been approved by "organic" certifying associations, will be used more extensively.

Carbon dioxide controlled atmosphere fumigation has an important role in the protection of grain where residue-free, in situ treatment is needed.

CO_2 fumigations have been used worldwide for many years, but, to be effective, must be applied in gastight storages. There is a long history of CO_2 fumigation in Australia with a reccommended dosage in 1917 of 0.72kg CO_2 per tonne of grain in an airtight silo[11] being themost effective' fumigant then available, when compared with carbon disulfide (CS_2) and hydrogen cyanide (HCN). Another publication, in 1921[12], recommended a dose of 1.4 kg/tonne for maize in galvanised iron tanks. Oosthuizen et al, in 1942[13], in South Africa, tested the use of CO_2 against *Callosobruchus chinensis* (cowpea weevil). In France, CO_2 as soliddry ice' has been used[14] to treat wheat and gaseous CO_2[15] was used to treat wheat stored for two years. In USA, systems were developed for CO_2 fumigation of peanuts[16] and maize[17]. In the decades following the late 1980s, CSIRO Division of Entomology, Australia published extensively on the use of CO_2 as a fumigant and Controlled Atmospheres treatment in general.

Recirculation is known to be beneficial in the fumigation of bulk grain and its treatment with CO_2. CSIRO[18] recommended one air change/day in tall structures, 15m or higher, to prevent low concentrations developing in the upper part of the structure. A review[19] of the extensive laboratory studies of the lethal effects of controlled atmosphere data resulted in the following schedules for commercial controlled atmosphere treatments:

* **Oxygen deficient atmosphere**

0% – 1% oxygen for longer than 20 days

* **Constant CO_2 composition**

80% 16 days if *Trogoderma granarium* is present

8.5 days for all other species

60% 11 days for all species except *Trogoderma granarium*

40% 17 days for all species except *Trogoderma granarium*

* **Declining CO_2 concentrations**

Initial concentration above 70% CO_2 in air, declining to 35% in 15 days or longer.

General details of how to convert existing storage to a gas tightness level suitable for modified atmospheres have been documented[20]. The acceptable level of sealing is that which gives a pressure half-life decay of greater than 5 minutes (often relaxed to 3 minutes in less than 300 tonne storage) when the structure is full. The application of fumigation using carbon dioxide in concrete storage can result in carbonation reaction with alkaline constituents[21]. However no corrosion was observed in 50 year old stores, suggesting there is no risk associated with carbonation as long as storage atmosphere maintains a dry environment around the steel reinforcement.

Conclusions

The unique properties of carbon dioxide allow it to be used to dispense insecticide chemicals ("giant" industrial aerosols) in addition to its use as an approved "organic" fumigant. Current developments continue to find applications for carbon dioxide in fumigant gas mixtures with benefits of improved efficacy, uniform distribution and the elimination of flammability.

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0214

Fumigant Activities of 2 Essential Oils Extracted from Dried Fruits of *Zanthoxylum bungeanum*a gainst the Adults of *Sitophilus zeamais*

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Abstract: Prickly ash tree, *Zanthoxylum bungeanum* (Maxim), which is a deciduous shrub, is widely grown in southwest China, especially in Sichuan Province and Chongqing municipality. The dried fruits of *Zanthoxylum bungeanum* are important condiment and traditional Chinese medicine. Chinese farmers have a long history to use the dried fruits and other parts of *Zanthoxylum bungeanum* to control stored grain insect pests. The essential oils extracted from the dried fruits of *Zanthoxylum bungeanum* are the promising resources of insecticide and fungicide. In this paper, the fumigation activities of green prickly ash essential oil and red prickly ash essential oil against the adults of *Sitophilus zeamais* Motschulsky were researched at 20, 23, 26, 29, 32, and 35℃. The results showed that essential oil, temperature and essential oil concentration influenced the mortality significantly. At 29, 32, and 35℃, 12μL/L and 15μL/L doses of green prickly ash essential oil led to 100% mortality after 24 h fumigation. Meanwhile, red prickly ash essential oil caused 100% mortality at 16 and 19μL/L essential oil dosage under the same temperatures. In addition, LC_{50} values at 30℃ after 24, 36, 48, and60 h fumigation were also studied and the results indicated that LC_{50} value of green prickly ash essential oil was smaller than that of red prickly ash essential oil after the same fumigation time. The objective of this research was to provide reference data for using essential oils of *Zanthoxylum bungeanum* to control stored grain insect pests.

Key words: essential oil of *Zanthoxylum bungeanum*, *Sitophilus zeamais*, fumigant activities

With the increasing concerns to ecological environment, food safety and human heath, the stored product insect pests control approaches must be safe, high efficient and environmentally friendly. Because botanic insecticides possess the advantages of low mammalian toxicity, easy degradation and less possibility to develop resistance for pests, the researchers in the world are trying their best to develop insecticides from the botanic resources (Shaaya et al., 1997; Zhang et al., 2004). Prickly ash tree, *Zanthoxylum bungeanum*, which belongs to *Zanthoxylum* Lin., Rutaceae, is widely grown in southwest China, especially in Sichuan Province and Chongqing municipality. The dried fruits of *Zanthoxylum bungeanum* are important condiment and traditional Chinese medicine (Sun and Duan, 1996). Volatility of *Zanthoxylum bungeanum* enssential oil and its monomers is strong and its scent is easily emitted. After the grains are treated, the scent residue can be gradually emitted. Hence, it is a promising resource of insecticide and fungicide (Jiang et al., 1992; Liu et al., 1994; Lu et al., 1995). In recent years, some researchers have been conducting experiments to use *Zanthoxylum bungeanum* to develop new botanic insecticide.

Maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), is a cosmopolitan pest of stored products and it was reported as the most important pest on stored maize in many countries including China (Yang et al., 1993; Zhang et al., 1998). The control of *S. zeamais* mainly depends on chemical approaches. The main chemicals used at present are phosphine and methyl bromide. However, *S. zeamais* has developed high resistance to phosphine and methyl bromide will be phased out in China in 2015 due to its ozone depleting (Wang et al., 1998). In order to solve the problems of chemicals, people are more and more interested in developing botanical insecticides, which have become research focuses in many countries (Zhang et al. 2004). The fumigation activities of green prickly ash essential oil and red prickly ash essential oil against the adults of *Sitophilus zeamais* were reported in this paper and the objective of this research was to provide reference data for using essential oils of *Zanthoxylum bungeanum* to control stored grain insect pests.

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Material and Methods

Insects. *Sitophilus zeamais* was maintained at Chongqing Key Laboratory of Entomology & Insect Control Engineering, Southwest University, Chongqing, China. The insects were reared on wheat in 1 – liter glass jars at 30℃ ± 0.5℃、70% – 80% RH、and a photoperiod of 0:24 (L:D) in the laboratory.

Essential Oils

Green prickly ash essential oil (85% AI) and red prickly ash essential oil (85% AI) were provided by Mountain Simian Prickly Ash Company of Jiangjing, Chongqing.

Effect of Temperature and Essential Oil Concentration on Toxicities

The 1L glass jars were used in the fumigation. At 20, 23, 26, 29, 32 and 35℃, adults were fumigated 24 hours under the green prickly ash essential oil concentrations of 6, 9, 12, and 15μL/L and red prickly ash essential oil concentrations of 10, 13, 16, 19μL/L. 30 adults of mixed age were used for each treatment. Adults were placed in glass jar. Filter paper slip (1cm × 15cm) was glued to jar lid at one side. The filter paper of the other side was applied with quantitative essential oils with micropipette and was quickly placed in the glass jar and the plastic film was used to wrap the glass jars to make them sealed. Put jars in the incubator which was set at certain temperature and 24 h dark. Each treatment has 3 replications. Contrasts were set up without essential oil. Mortality was checked after 24 h fumigation.

LC_{50} Values of 2 Prickly Ash Essential Oils on S. zeamais under Different Fumigation Time

The same fumigation method as above was adopted. Fumigation time were 24, 36, 48, 60, 72 h, respectively, and fumigation temperature was 30℃. At 24h fumigation time, the concentrations of green prickly ash essential oil were 6.5, 7.5, 8.5, 9.5, 10.5, 11.5 μL/L and the concentrations of red prickly ash essential oil were 12.5, 13.5, 14.5, 15.5, 16.5, 17.5 μL/L, respectively. At 36 h fumigation time, the concentrations of green prickly ash essential oil were 6, 7, 8, 9, 10, 11 μL/L and the concentrations of red prickly ash essential oil were 9, 10, 11, 12, 13, and 14 μL/L, respectively. At 48 h fumigation time, the concentrations of green prickly ash essential oil were5, 6, 7, 8, 9, and 10 μL/L and the concentrations of red prickly ash essential oil were 9, 10, 11, 12, 13, 14μL/L, respectively. At 60 h fumigation time, the concentrations of green prickly ash essential oil were5, 6, 7, 8, 9, 10 μL/L and the concentrations of red prickly ash essential oil were 8, 9, 10, 11, 12, 13 μL/L, respectively. At 72 h fumigation time, the concentrations of green prickly ash essential oil were 2, 3, 4, 5, 6, 7 μL/L and the concentrations of red prickly ash essential oil were 4, 5, 6, 7, 8, and 9 μL/L, respectively. These concentrations were chosen to make the mortality from 16% to 84%. Each treatment had 30 adults, 3 replications.

Data Analysis

Insect mortality were corrected by using Abbott's formula (Abbott, 1925). Mortality data were transformed using arcsine ($x^{0.5}$) and ANOVA was carried out using SPSS software. Duncan's multiple range tests was used to test the difference significance and IRM software (developed by Southwest University) was used to obtain LC_{50} values and regression equations.

Results

Effect of Temperature and Prickly Ash Essential Oil Concentration on the Activities

Under the condition of 24 h fumigation time and different temperature, the activities of green prickly ash essential oil and red prickly ash essential oil against adults of *S. zeamais* were listed in Table 1 and Table 2. Table 1 and Table 2 showed the corrected mortality increased significantly as the temperature and essential oil concentration increased. At 29, 32, and 35℃, with the green ash essential oil dose of 12 and 15μL/L, the corrected mortalities reached up to 100% after 24 h fumigation. Meanwhile, for red prickly ash essential oil, when the essential oil concentrations were 16 and 19μL/L and the temperature was higher than 26℃, the corrected mortalities were also 100%. ANOVA showed essential oil, temperature and essential oil concentration affected the corrected mortality significantly (for green prickly ash essential oil, its temperature factor: $F = 34.287$; $df = 5, 48$; $P = 0.000$; concentration factor: $F = 129.004$; $df = 3, 48$; $P = 0.000$; temperature × concentration interaction: $F = 5.600$; $df = 15, 48$; $P = 0.000$; for red prickly ash essential oil, its temperature factor: $F = 65.260$; $df = 5, 48$; $P = 0.000$; concentration factor: $F = 162.712$; $df = 3, 48$; $P = 0.000$; temperature × concentration interaction: $F = 4.345$; $df = 15, 48$; $P = 0.000$).

Table 1. The activities of green prickly ash essential oil against adults of *S. zeamais* Under different temperatures (24 h)

Temperature(℃)	Corrected mortality (%) (mean ± SE)			
	6μL/L	9μL/L	12μL/L	15μL/L
20	13.20 ± 5.00 a	48.33 ± 10.68 a	91.67 ± 3.53 a	99.00 ± 1.00 ab
23	25.67 ± 4.67 ab	77.67 ± 12.45 b	90.00 ± 0.00 a	94.67 ± 2.33 a
26	31.67 ± 1.33 b	73.20 ± 7.91 ab	91.27 ± 2.98 a	95.67 ± 4.33 ab
29	46.67 ± 4.98 bc	93.23 ± 2.11 bc	100.00 ± 0.00 b	100.00 ± 0.00 b
32	68.33 ± 5.21 c	96.67 ± 1.33 c	100.00 ± 0.00 b	100.00 ± 0.00 b
35	91.33 ± 8.67 d	100.00 ± 0.00 c	100.00 ± 0.00 b	100.00 ± 0.00 b
F	20.484	8.649	18.733	2.271
Df	5,12	5,12	5,12	5,12
P	0	0.001	0	0.114

Note: The data shows the average of three duplicates. Data in the same column followed by different letters show significant difference at 0.05 level by Duncan's multiple range test.

Table 2. The activities of red prickly ash essential oil against adults of *S. zeamais* under different temperatures (24 h)

Temperature(℃)	Corrected mortality (%) (mean ± *SE*)			
	10μL/L	13μL/L	16μL/L	19μL/L
20	23.33 ± 6.89 a	51.67 ± 5.55 a	66.33 ± 6.67 a	82.33 ± 5.33 a
23	38.33 ± 2.73 ab	75.00 ± 6.56 b	95.67 ± 1.33 b	99.00 ± 1.00 b
26	47.33 ± 7.84 c	78.33 ± 6.77 bc	100.00 ± 0.00 c	100.00 ± 0.00 b
29	62.33 ± 5.24 cd	91.00 ± 1.00 c	100.00 ± 0.00 c	99.00 ± 1.00 b
32	69.33 ± 8.41 d	88.67 ± 1.33 c	100.00 ± 0.00 c	100.00 ± 0.00 b
35	93.00 ± 2.00 e	100.00 ± 0.00 d	100.00 ± 0.00 c	100.00 ± 0.00 b
F	15.365	21.425	49.989	12.871
df	5,12	5,12	5,12	5,12
P	0	0	0	0

Note: The data shows the average of three duplicates. Data in the same column followed by different letters show significant difference at 0.05 level by Duncan's multiple range test.

LC_{50} values of 2 prickly ash essential oils on *S. zeamais* under different fumigation times

The LC_{50} values of essential oils from both green prickly ash and red green prickly ash decreased as the fumigation time increased (Table 3 and 4). For green prickly ash essential oil, LC_{50} values after 24 and 72 h fumigation were9.88 μL/L and 2.24 μL/L, respectively. The LC_{50} value was 4 times shorter at 72 fumigation time than that at 24 h fumigation time, which demonstrated green prickly ash essential oil possessed a longer residue period. Similarly, for red prickly ash essential oil, the longer the fumigation time, the better the fumigation efficacy. Hence, the essential oil amount could be relatively reduced as the fumigation time was increased. In addition, based on the Table 3 and Table 4, we found that LC_{50} of green prickly essential oil was smaller than that of red prickly ash essential oil after the same fumigation time, which proved that the efficacy of green prickly ash essential oil was better than that of red prickly ash essential oil.

Table 3. The fumigation activities of green prickly ash essential oil against adults of *S. zeamais* under different treatment time(30℃)

Treatment Time (h)	Regression Equation(Y =)	R	X^2	LC_{50} (μL/L)	LC_{95} (μL/L)
24	−3.25 +8.29X	0.994	1.258 *	9.88 ± 0.18	15.61 ± 0.82
36	0.17 +5.52X	0.993	1.373 *	7.50 ± 0.20	14.91 ± 0.90

Treatment Time (h)	Regression Equation (Y =)	R	X^2	LC_{50} (μL/L)	LC_{95} (μL/L)
48	−0.71 +6.59X	0.986	3.258 *	7.34 ±0.15	13.05 ±0.74
60	−1.76 +8.55X	0.996	1.029 *	6.17 ±0.12	9.61 ±0.32
72	3.88 +3.19X	0.989	1.695 *	2.24 ±0.16	7.35 ±0.66

Table 4. The fumigation activities of red prickly ash essential oil against adults of *S. zeamais* under different treatment time (30℃)

Treatment time (h)	Regression Equation (Y =)	R	X^2	LC_{50} (μL/L)	LC_{95} (μL/L)
24	−9.20 +12.41X	0.982	3.652 *	13.94 ±0.17	18.92 ±0.52
36	−3.92 +8.16X	0.979	4.630 *	12.41 ±0.19	18.73 ±0.97
48	−3.29 +7.91X	0.966	5.526 *	11.15 ±0.19	18.00 ±0.99
60	−2.41 +7.46X	0.971	4.960 *	9.86 ±0.18	16.38 ±0.89
72	2.71 +3.79X	0.987	1.451 *	4.02 ±0.26	10.93 ±1.00

Discussion

China is the largest country to grow *Zanthoxylum L.* in the world. Except for northeast and inner Mongolia, it is widely cultivated in many provinces such as Henan, Shangxi, Gansu, Hunan, Hubei, Yunan, Guizhou, Sichuan, Chogqing etc. (Zheng, 2000; Bi et al., 2002, 2003). Among the prickly ash growing provinces, Chongqing and Sichuan are the most famous places for prickly ash production, because people in these two places like hot and spicy food, in which the dry fruits of prickly ash are the most important condiments. *Zanthoxylum* L. as a traditional Chinese medicine has a long history. People used the dry fruits and other part of prickly ash tree to cure different diseases (Shi et al., 2003). As to insecticidal and fungicidal activities, Chinese people like to place the dry fruits and leaves of prickly ash in their stored grains to control insect and mold pests. Therefore, the research on insecticidal activity of prickly ash is focused on stored insect pests. Ge et al. (1995) proved that prickly ash essential oil caused *S. cerealella* to refuse to feed and inhibited oviposition. Dube et al. (1990) found that essential oil of prickly ash possessed fungicidal and repellent activities. He et al. (1980) reported that the bags dipped in water containing prickly ash dry fruits could make the paddy rice over summer without any insect and mold problems. Lu et al. (1995) separated and identify the components of volatile oil and found β − phellandrene and linalool could kill adults of *Tribolium confusum*. Jiang et al. (1992) demonstrated that essential oil of prickly ash could be used as a fumigant to control *Sitophilus zeamais*. Our research indicated that at 29, 32, and 35℃, 12μL/L and 15μL/L doses of green prickly ash essential oil led to 100% corrected mortality after 24 h fumigation. Meanwhile, red prickly ash essential oil caused 100% corrected mortality at 16 and 19μL/L essential oil dosage under the same temperatures. Our result is in agreement with that of Jiang et al. (1992). We couldn't make sure the components to be acted as insecticide in prickly ash essential oils. Whether β − phellandrene or linalool function as insecticidal components in prickly ash essential oils need to be further studied.

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0215

Alternatives to Methyl Bromide in Italian Food Industries Results of Two-year Practical Applications

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Abstract: In 2005 – 2006, the Italian Ministry of Environment gave a grant to the Institute of Entomology of the University of Milan for a research project. The goal was to find out several alternatives to the use of methyl bromide for the control of pests in food industries. The MB alternatives tested in Italy were fumigation with sulfuryl fluoride, heat treatments, automatic systems for the distribution of pyrethrins with the ULV method and integrated pest management.

Different food industries participated in the project and the results were reported in a special meeting in Italy. The general conclusion is that it is completely possible to replace methyl bromide to control pests. Every industry can choose the most appropriate alternative to methyl bromide.

Key words: sulfuryl fluoride, heat treatments, fogging, IPM

Introduction

Methyl bromide (MB) is widely spread all over the world, thanks to its broad spectrum of application, the possibility of doing treatments effectively, even at low temperatures, its easy-to-use versatility and its relatively low cost.

But as already well known, in 1992, MB was included in the Montreal Protocol on substances that deplete the ozone layer because of its ozone depletion potential. The reduction calendars were defined three years after its inclusion in the Protocol. This led to the elimination of MB as fumigant agent in food industries. Many alternatives have now been tested, and continue to be tested, as replacement for methyl bromide in the control of stored-product insect infestations.

In 2005 – 2006, the Italian Ministry of Environment gave a grant to the Institute of Entomology of the University of Milan for a research project. The goal was to test possible alternatives to methyl bromide in Italian food industries, particularly fumigation with sulfuryl fluoride (SF), heat treatments, the use of an automatic system for the distribution of insecticides with the Ultra-Low-Volume (ULV) method and the application of IPM. Different food industries participated in the project, allowing tests of alternative technologies to MB.

Material and Methods

Tests were carried out with different methods, according to the different needs. In particular:

Test Insects

Table 1. Test insects

Species	Life stage	Treatment
Tribolium confusum J. du val	Eggs, larvae, adults	ULV, heat treatment, sulfuryl fluoride
Tribolium castaneum (Herbst)	Eggs, larvae, adults	ULV, heat treatments, sulfuryl fluoride
Sitophilus oryzae (Linnaeus)	Eggs, larvae, pupae, adults	sulfuryl fluoride, heat treatment
Rhyzopertha dominica (Fabricius)	Eggs, larvae, pupae, adults	sulfuryl fluoride
Stegobium paniceum (L.)	Eggs, larvae, pupae, adults	sulfuryl fluoride
Plodia interpunctella (Hubner)	Eggs, larvae, pupae	sulfuryl fluoride, ULV, heat treatment
Ephestia kuehniella Zeller	Eggs, larvae, pupae	sulfuryl fluoride, ULV, heat treatment
Musca domestica Linnaeus	Adults	ULV

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Test Environments

Table 2. Test enviroments

Industry	Treatment
Confectionery industry	ULV
Mill	sulfuryl fluoride, heat treatment
Industrial bakery	heat treatment
Rice mill	heat treatment

Monitoring

· pheromone traps for moths

· traps baited with aggregation pheromone lure and an oil-based food attractant for beetles

· Oil-and water-filled traps for moths

The number of insects used for each test was different, according to the kind of test, the size of the area to be treated and the specific needs. Some details are given in the discussion of the results.

Results and Discussion

Fumigations with Sulfuryl Fluoride

The Institute of Agricultural Entomology onitored several applications directly, both in the mill industry and in pasta factories, through the installation of an adequate number of monitoring points in the areas to be treated. The bioassays were carried out with different life stages. Great attention was devoted to the gas action on insect eggs, particularly on *Tribolium* ones, since they represent the most resistant stage to treatments.

The following bioassays, shown in table 1, were used in the first test, carried out in a semolina mill, with an estimated volume of 16 250 m^3.

Table 3. Details of test insects introduced into the mill

Species	Life stage	Number of replicates	Number per replicate
Sitophilus oryzae	mixed population	1 single test for each location	5 adults + mixed population
Rhyzopertha dominica	mixed population	1 single test for each location	5 adults + mixed population
Plodia interpunctella	eggs pupae	1 single test for each location	50 20
Ephestia kuehniella	eggs pupae	1 single test for each location	50 20
Tribolium confusum	eggs adults	1 single test for each location	50 20
Tribolium castaneum	eggs adults	1 single test for each location	50 20
Stegobium paniceum	mixed population	1 single test for each location	5 adults + mixed population

During fumigation, the temperature ranged from approximately 28℃ to 31℃. The CT product with SF fumigation ranged from 1 101 g. h/m^3 to 1 501 g. h/m^3, with a mean of 1 353 g. h/m^3. At this CT product, 100% mortality was confirmed for all the insect species included in the bioassays: *S. oryzae*, *R. dominica*, *S. paniceum* (mixed life stage culture), *P. interpunctella*, *E. kuehniella* (eggs, pupae), *T. confusum* and *T. castaneum* (eggs, adults). Many of the test insects had been placed in protected and difficult-to-reach locations in the mill, providing an exacting test of efficacy for the fumigant[1].

Further tests, carried out in different mills, showed that sulfuryl fluoride could be considered as an effective disinfestation fumigant for food industries, provided that its use is assigned to qualified staff. In several cases, pests reappeared afterwards and quickly in the treated areas. This is due to the presence of insects in the industry yards which are not taken into account with adequate cleaning and disinfestation treatments. This situation is hence not due to ineffectiveness of the application but to a bad global management of the pest control. This occurs obviously with every kind of toxic gases[2,3,4].

Heat Treatments

The use of warm air was particularly interesting. Also for this method, there was the opportunity to follow and organize some applications in mills, industrial bakeries and in some departments of pasta factories and rice mills. Temperatures around 42℃ cause a reduction of oviposition; when 50℃ are reached, insects die in short time. The data at disposal show indeed

that most species do not survive for more than 24 hours at 40℃, 12 hours at 45℃, 5 minutes at 50℃ and for more than a single minute at 55℃[5]. In practical applications, several parameters are to be taken into consideration; in fact, it is important not only to identify pests but it is absolutely necessary to consider the materials used to build the environments and the plants. The thermal conductivity of a concrete or plate wall, of a tiled or wooden floor, of metal removable coversor conveyor belts made of plastic materials is indeed very different. Furthermore, since the movement of warm air is upward, temperatures, even higher than those needed, can be reached quickly on the premises ceilings, whereas walls and floors still remain half – cold. In these cases, it is necessary to improve air circulation with fans, in order to uniform the environmental temperature as quickly as possible. Before dying, insects flee from overheated nesting points and they move where temperature is more tolerable.

In an industrial mill, tests were made in order to obtain a gradual warming of premises and plants up to 55℃, for 48 hours. Ten bioassays were arranged, each containing *P. interpunctella* (eggs, larvae, pupae), *E. kuehniella* (eggs, larvae, pupae), *Tribolium* spp. (eggs, larvae, adults), *R. dominica* and *S. oryzae* (mixed population).

The mortality rate of all the considered species, in all the life stages, turned out to be 100%. The production shutdown lasted as long as that necessary for a MB disinfestation.

In the case of an industrial bakery, the intervention lasted only 48 hours because of production needs. As table 4 shows, the mortality rate noted in the bioassays did not reach 100%.

Table 4. Details and mortality rate of insect bioassays introduced into the industrial bakery.

Species	Life stage	Total number	Alive after treatment	Mortality rate (%)
T. castaneum	eggs	40	2	95
	larvae	40	1	97.5
	adults	40	0	100
T. confusum	eggs	40	19	52.5
	larvae	40	10	75
	adults	40	16	60
E. kuehniella	eggs	200	0	100
	larvae	40	0	100

The present method, which is very interesting and also gradually spreading in Italy, requires PCOs with specific experience, sometimes flanked by conditioning technicians of industrial environments. In any case, the required temperatures, needed to kill insects, must be reached and maintained for the necessary time, in every point of each department and plant to be disinfested.

Space Spraying

Automatic systems to disinfest wide volume areas, employing only active ingredients with knock-down effect, have been used in different places. Space spraying devices are different and they are based on the micronization of the product which is distributed nearly as if it were fog. It is evident that the more the insecticide is distributed uniformly with an ultra-low volume treatment, the more the intervention is effective. The limit of these applications derives from the characteristics of the active ingredients at disposal, as natural pyrethrum or non-persistent synthetic pyrethroids. Since they are contact insecticides, they work essentially on adults but they are not effective on nested insects and on life stages characterized by reduced or no motion.

A similar system for insecticide distribution was already tested in Italy[6]; the obtained results were interesting but the method, called Turbocide GOLD (Trademark AgrEvo) was not further developed.

The present research was made with the use of a space spraying device called Nebbia Secca (Trademark GEA, Italy). Thanks to its very high insecticide micronization (droplets of about 12 μm, produced by collision), this method gives the advantage of determining an optimal distribution of the a. i., without smearing plants, as often occurs with thermofog. Each spray nozzle of Nebbia Seccacovers about 1000 m^3. Treatment times depend on premises volume and on the a. i. which is used.

In a test, carried out in a confectionery industry, a department of about 3 000 m^3 was treated in 8 – 10 minutes. In this industry, several applications were made using 200 g of AquaPy (Bayer) for each test(*). Immediately before each of the three tests, 36 containers had been placed in the environment to be treated (18 containing each 10 Indian Meal Moth adults and 18 containing each 10 *Musca domestica* adults). After space spraying, insect containers remained in the department for 14 hours before their collection and control. The mortality ratewas 100% of the bioassays.

This centralized insecticide spraying system can be a valid instrument for knocking

down fly and moths adults. However, its use requires repeated and frequent applications to give completely positive results. This system is particularly effective in areas with low levels of infestation, where an accurate monitoring of pest flight can be done. Moreover, it is effective also in big goods stocking or distribution warehouses, where rational monitoring is extremely difficult or inapplicable. In this case, it is necessary to make frequent treatments.

Integrated Pest Management

Localized insecticide treatments, use of pheromones for monitoring, mass trapping and mating disruption are a good help for the IPM. If this method is used in food industries, it is necessary to consider that, at least in Italy, there is no tolerance threshold regarding the presence of pests. Integrated pest management is essentially based on prevention and on careful monitoring; these two aspects aim at reducing traditional pest treatments, since the general situation of the environments tends to be always under strict control. By implementing such practices it is also possible not to use toxic gases. Integrated pest management requires well technical trained users and a regular application. Implementing a pest management programme with this method determines for sure remarkable technical progress for the industries that want to adopt it. The results obtained in Italy in an IPM annual test were referred[7].

Conclusions

The general conclusion is that it is completely possible to replace methyl bromide in the control of pests and that every industry can choose the most appropriate alternative.

In fact, none of the considered IPM methods can totally solve each problem. Nevertheless, as already known, also the use of methyl bromide was not able to eradicate pest attacks in a definitive way. The transition towards new methods requires good willingness to come to alternative techniques; furthermore, it requires higher technical skills from both the people in charge of the food industries concerned and from PCOs. It can also require much time to be devoted to the pest problem but in this way, insects can be always kept under control. The appearance of serious infestations, which may cause surprise and worries to food safety supervisors, is thus prevented.

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* *AquaPy* ®: *natural pyrethrins* 3%; *ppb* 13.5 %.

0216

Preliminary Study on Adsorption and Degradation of Methyl Bromide by Activated Carbon

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Abstract: Preventing emissions of methyl bromide is important for the recovery of the ozone layer. Methyl bromide is routinely emitted from fumigation facilities which could be prevented with equipment to capture the emissions. We examined the ability of activated carbon from coconut fibre to absorb methyl bromide. We showed that about 94 to 98% of the methyl bromide held in a tank was absorbed by activated carbon and not vented. About 95% of the methyl bromide was decomposed from the carbon using water at 80℃ for 16h. We showed regenerated carbon could be re-used for recovering more methyl bromide and it seemed to be little affected by a previous absorptive use. The properties of water and carbon together at 80℃ may be more effective at decomposing methyl bromide, rather than dry heat which requires temperatures as high as 900℃.

Introduction

Methyl bromide is widely used as fumigant for the treatment of quarantine pests worldwide. But methyl bromide is highly reactive to ozone and is classified as a potent stratospheric ozone depleter. About 37% of the global production of methyl bromide is now used for quarantine and pre – shipment treatments (Batchelor and Miller 2008). A range of alternatives to methyl bromide have been implemented for soil uses which has reduced the overall amount of methyl bromide.

However, although alternatives exist for many uses (TEAP 2006), they have generally not been implemented because methyl bromide for quarantine uses is not limited by the Montreal Protocol and therefore there has been little incentive to adopt alternatives (TEAP 2007).

methyl bromide used for quarantine treatments is used in a closed space or fumigation facility, and then released to the atmosphere after the treatment where it damages the ozone layer. If methyl bromide can be captured, absorbed and degraded, the amount emitted to the atmosphere from quarantine treatments could be reduced significantly. The use of recapture techniques could be used in the short term until methyl bromide-free alternatives become available as a more permanent solution.

Gan *et al.* (2000) studied 5 types of activated carbon that adsorbed methyl bromide and methyl iodide, and reported that granules of activated carbon made of coconut can absorb both chemicals. Leesch and Gerhard (1998) indicated that activated carbon can recover 95% of the methyl bromide which is vented from a closed fumigation chamber.

With this in mind, we examined the ability of activated carbon to absorb methyl bromide, the ability of hot water to remove methyl bromide from the activated carbon, and the prospects for re – using the activated carbon once the methyl bromide has been removed.

Materials and Methods

Materials

We used granular activated carbon 40 mesh produced from coconut shell. The samples of methyl bromide were obtained from the Lianyungang (Jiangsu) Seawater Chemical No. 1 Plant. We measured methyl bromide concentrations relative to a methyl bromide Standard of 200μg/ml which was obtained from SUPLECO USA. We used a portable photo-ionization gas chromatograph ("portable GC") to measure the methyl bromide concentrations (PHOTOVAC – 10S50, HAMILTON Co. Ltd. UK). We used an oxygen bag YD – 50 Type obtained from the Shanghai Huifeng Medical Instrument Co. Ltd to mix the pure fumigant to obtain the desired concentration.

Experiment Equipment

Figure 1 describes the fumigation chamber which has a capacity of $1m^3$.

Determine of Methyl Bromide Adsorbing rate by Activated Carbon

The oxygen bag was used to generate dif-

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ferent concentrations of methyl bromide (24, 48, 56g/m^3) at 25℃ and 30℃ in the fumigation chamber (Fig. 1). The concentration of methyl bromide in the chamber was recorded by the portable GC. A known concentration of methyl bromide was vented out of the chamber at a rate 500mL/m through the column which is loaded with 1 kilogram of activated carbon. The concentration of methyl bromide at the end of column was measured on the portable GC at1, 5, 10, 15, 20m intervals.

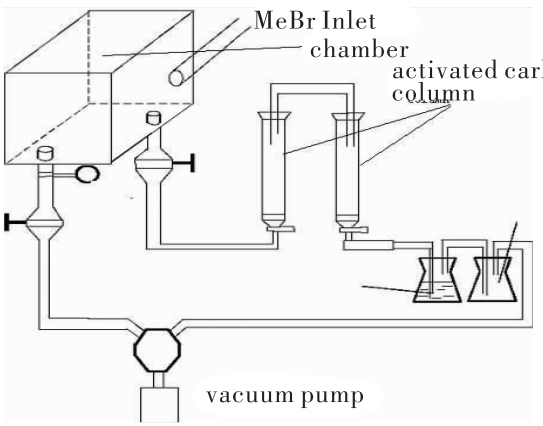

Fig. 1 Schematic of activated carbon test equipment

We repeated the test 4 times at each of the two temperatures to measure the difference between the initial methyl bromide concentration in the chamber and the average methyl bromide concentration being vented at the end of column. This enabled a calculation of the methyl bromide adsorption rates by the activated carbon at different temperatures. We repeated the test three times to get an average absorption rate.

Determination of Methyl Bromide Degradation

The activated carbon which adsorbed the methyl bromide vented from the fumigation chamber was taken out of the column and macerated. The macerated activated carbon was divided into 4 equal-sized parts. One part was weighed to 20g in 50mL conical flask. Fifty millilitres of methanol were added to the flask and after 10 minutes of stirring, the liquid was filtered by vacuum in Bush filter. The filtrate was collected in volumetric flask and is added to 50mL methanol.

The methyl bromide concentration was determined by gas chromatography equipped with an FED detector. Each of the remaining three parts was weighed to produce four 20g samples which were added to separate 50mL conical flasks containing 20mL of water. The flasks were placed at 60℃, 70℃ and 80℃ in a constant temperature bath.

At intervals of 8 h, 16 h, 24 h and 36h, a sample of the macerated activated carbon was removed from the bath and filtered. Fifty millilitres of methanol were added to the flask and after 10 minutes of stirring, the liquid was filtered by vacuum in Bush filter. The filtrate was collected in a volumetric flask and added to 50mL methanol.

The methyl bromide concentration was determined by gas chromatography. In addition, the filtrates from the 24 h and 36h macerated activated carbon were concentrated to 3mL by low-pressure, after which 5 mL of methanol was added and the concentration of methyl bromide was determined by gas chromatography. All treatments were repeated three times.

The gas chromatographic conditions for determination of the methyl bromide concentrations were: ANGILENT 6890 gas chromatograph with electron capture detection (ECD); Column: HP-50, 30m × 0.32mm × 0.25μm; Inlet temperature: 220℃; Column temperature: 70℃; Detector temperature: 320℃; Sample volume: 1μL. The quantity of methyl bromide was calculated by reference to the external standard methyl bromide concentration.

Regeneration of Activated Carbon

The activated carbon was regenerated by adding water at 80℃ followed by heating in an oven for 2h at 300℃. The regenerated activated carbon was then re-loaded into the absorption column and the trials are ready to begin again.

Results and Analysis

Absorption of Methyl Bromide by Activated Carbon

Most of the methyl bromide passed through the activated carbon column is adsorbed onto activated carbon (Table 1).

Table 1. Average concentration change of methyl bromide at venting after being absorbed by activated carbon (N = 3).

Temperature℃	methyl bromide applied rate (g/m^3)					
	24	48	56	24	48	56
	methyl bromide in tank (ppm)			methyl bromide at venting (ppm)		
25	6117.7	12331	13083	155.5	548.6	719
30	6249.7	12523	13530	168.9	621.5	733

The absorption rate of methyl bromide by activated carbon is shown in Table 2.

Table 2. Average percentage absorption rate of methyl bromide by activated carbon at 25 and 30℃ (N = 3).

Temperature℃	methyl bromide applied rate (g/m³)		
	24	48	56
	methyl bromide in tank (ppm)		
25	97.64 ± 0.07	95.50 ± 0.11	94.49 ± 0.13
30	97.49 ± 0.12	95.04 ± 0.09	94.62 ± 0.14

Activated carbon has a strong affinity for methyl bromide. Methyl bromide recovery rate by activated carbon is about 95%. Temperature makes little difference to recovery rate.

Decomposition of Methyl Bromide from Carbon Using Hot Water

Figures 2, 3 & 4 show the methyl bromide decomposition at 40, 60 and 80℃, respectively.

After 36h, 83.88% – 92.28% of the methyl bromide was decomposed at 40℃; 96.45% – 98.60% at 60℃; and 99.84% – 99.98% at 80℃. At 80℃, methyl bromide was decomposed to 0.08 – 1.5g/kg in hot water after 36 hour. About 64% of the methyl bromide was decomposed at 80℃ after 8h, and about 95% after 16h. We conclude that methyl bromide is decomposed rapidly at 80℃ in hot water.

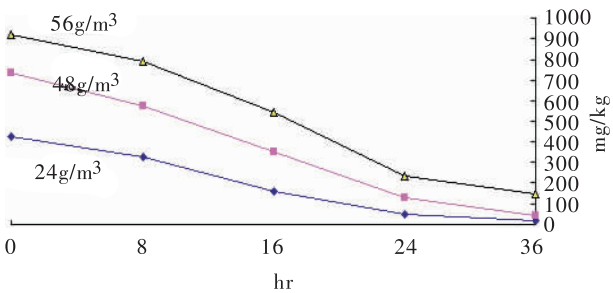

Fig. 2 Decomposition of methyl bromide in water at 40℃

Methyl Bromide Absorption by Regenerated Activated Carbon

Absorption of methyl bromide by regenerated activated carbon is not markedly different to the absorption of methyl bromide on activated carbon for the first time. Regenerated activated carbon could therefore be re-used to absorb methyl bromide vented from fumigation facilities.

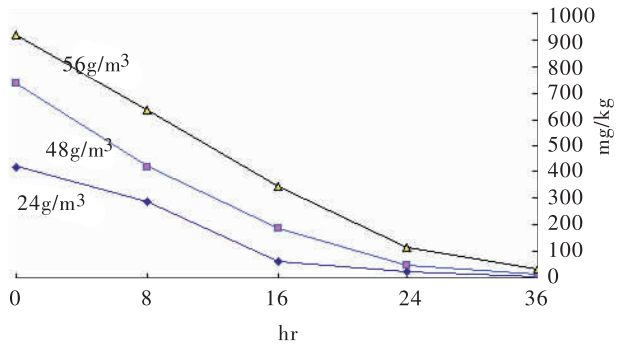

Fig. 3 Decomposition of methyl bromide in water at 60℃

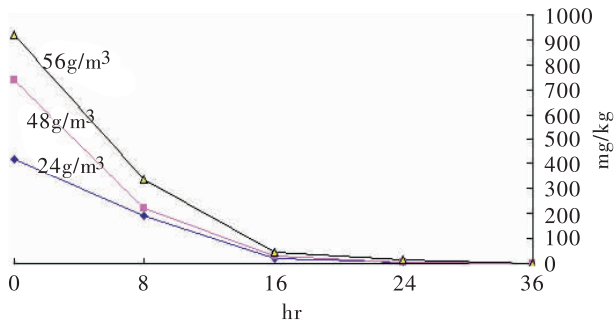

Fig. 4 Decomposition of methyl bromide in water at 80℃

Table 3. Percentage rate of methyl bromide being absorption by regenerated activated carbon at 25 and 30℃ (N = 3)

Temperature(℃)	methyl bromide applied rate (g/m³)		
	24	48	56
	methyl bromide in tank (ppm)		
25	95.64 ± 0.17	94.50 ± 0.21	93.49 ± 0.23
30	93.37 ± 0.14	92.14 ± 0.19	91.62 ± 0.18

Discussion

The results of experiments indicate that methyl bromide emitted after a fumigation treatment could be adsorbed by activated carbon which is made of coconut. Activated carbon has a strong affinity for methyl bromide. The results are similar to those reported by Gan *et al.*

(2001) and Leesch & Gerhard (1998). Although the absorption was similar at 25℃ and 30℃ in our work, the rate of methyl bromide absorption is higher because absorption is inversely proportional to the temperature (based on the principle of Langmuir). Leesch and Gerhard (1998) considered zeolite to absorb methyl bromide similarly to activated carbon.

Methyl bromide may be decomposed from the activated carbon by hot water. The higher the temperature, the more rapidly methyl bromide is decomposed. At 80℃, methyl bromide took the least time to decompose. Gan *et al.* (2001) and Kitagawa (1998) thought that water and activated carbon provide a catalyzing active centre. After methyl bromide is adsorbed onto the surface of activated carbon, the CH_3-Br covalent bond could be ruptured by the molecular effect of water and, moreover, the rate of covalent bond rupture increased with increasing temperature. Without water, methyl bromide could be regenerated from activated carbon at an incineration temperature of 900℃.

Acknowledgements

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0217

Integrated Pest Management in the Italian Mill Industry

Sara Savoldelli * and Elena Panzeri

Abstract: The efficacy of integrated pest management (IPM) in an Italian mill was evaluated as a substitute for annual fumigation with methyl bromide, an ozone-depleting pesticide which is now banned in developed countries for most of its uses.

Traps baited with aggregation pheromone lure and an oil-based food attractant were used to monitor populations of stored-product beetles. sticky traps baited with a pheromone lure were used to monitor the Mediterranean Flour Moth (MFM) and the Indian Meal Moth (IMM). In addition, several bins, oil-or water-filled and baited with a pheromone lure, were used to catch adults of the MFM and of the IMM in the most critical areas.

Monitoring and inspection were carried out weekly; cleaning was carried out more often than in the past; local treatments were made if necessary; and sometimes there were changes to the buildings to reduce insect hiding places. Several sources of infestation were detected and critical areas were more often monitored. The intent was to avoid fumigation in the following years.

Due to the IPM activities, it was possible to reduce the population peak to a lower level than recorded in the previous years. The frequent inspections led to the identification of foci of infestations and occasional pests, such as *Tineola bisselliella* Hummel, *Tetramorium caespitum* (Linnaeus), and *Blatta orientalis* Linnaeus.

IPM is a useful alternative to methyl bromide, but its application requires specialized knowledge by technicians who know the biology and the behaviour of stored-product pests, and are able to manage the monitoring program and to organize the most appropriate control strategies.

Key words: methyl bromide, alternative, IPM, trapping, monitoring, mill industry, stored-product pests.

Introduction

Pest management has been an established technique in agriculture for more than twenty years, whereas attempts to extend it also in the food industries are more recent. The acquisition of more knowledge about pest bioethology and the availability of pheromone lures and monitoring food traps contributed to this trend.

Another contribution was recently given by the necessity of finding the most effective alternative methods to methyl bromide for disinfesting production areas[1]. Among these ones, a pest management program was carried on in a mill where fumigation with methyl bromide and phosphine had been done for years.

The aim of the present work was to implement, where necessary, the monitoring already done by the mill in the past years, to follow the trend of pest captures and to localize the presence of infestation traces every week. In this way, it was possible to intervene by intensifying cleaning, with localized treatments and by realizing possible structural improvements. Finally, the effectiveness of integrated pest management was considered a valid alternative to the annual fumigation.

Materials and Methods

The industry is a soft wheat mill in the north of Italy. It extends on about 10 000 m^2 and has a productive capacity of 200 t/h.

The production area is placed in a 5 – floor building and it is near four warehousing areas (for raw materials, loose manufactured goods, packaged manufactured goods and by-products).

Pest management occurred according to a specific practice, contained in the Management and Quality Control Handbook. In the mill there were 33 funnel traps for Lepidoptera, baited with a pheromone lure for moth pests. They were controlled every month.

Every year, in summer, a pest control treatment with methyl bromide was scheduled and carried out in the production areas, whereas phosphine was used in the areas where raw materials and manufactured goods are stored.

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After fumigation, the funnel traps, already present in the mill (Mastrap), were restored, together with 13 Anobiidi Trapto monitor *Lasioderma serricorne* (F.) and *Stegobium paniceum* (L.), 5 Pantry Patrol, baited with different pheromone lures and an oil-based food attractant to monitor simultaneously *Tribolium* spp., *Oryzaephilus* spp., *L. serricorne*, *Plodia interpunctella* (H bner), *Sitophilus* spp., *S. paniceum* and *Trogoderma* spp.

Besides these ones, after having at disposal the monitoring data, also other traps were used to mass trapping moths. They were composed of plastic rectangular boxes (18 × 24, 5 × 12 cm), filled with water or seed oil and baited with pheromone lure for moth pests[2]. These traps (one for each type) were placed in the two areas of the mill which were considered to be the most critical (basement "wheat silos" and by-products area) because of an increase in the captures of *P. interpunctella* in the funnel traps present there.

During the whole period of experiment (July 2005, August 2006), monitoring had a weekly frequency: the captured insects were collected and classified and thanks to the direct comparison with the data referring to the previous weeks, every time it was possible to detect the areas which had to be taken under control or where it was necessary to intervene promptly.

Results and Discussion

Lepidoptera

The 2005 fumigation with methyl bromide eliminated the presence of the moths in the mill but it did not prevent their recolonization, since captures of moths were already noted in the weeks following the treatment, particularly in the by-products area and in the basement "wheat silos" (Fig. 1、Fig. 2). In this area the reinfestation was due to the losses of the wheat transportation redler, from the unloading hole to the pre-winnowing, and to the fact that the wheat silos area is linked to the outside.

The problem concerning sudden room infestations, even from the week following the fumigation, and due in particular to the presence of infestation foci outside the building, was highlighted in different works[3,4,5].

After the increased captures in the basement "wheat silos", some targeted interventions were implemented, such as extraordinary cleaning and mass trapping with water-and oil-filled traps, baited with a pheromone lure. Thanks to these interventions, population diminished (Fig. 2). At the end of October, captures ceased with the arrival of cold weather.

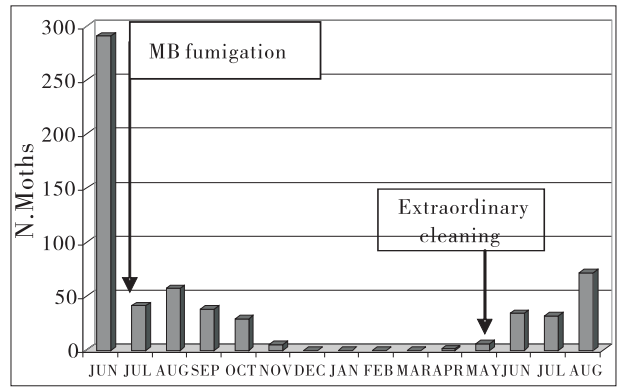

Fig. 1 Number of captured IMM and MFM in the mill, from June 2005 to August 2006.

Also the by-products area was involved in a targeted cleaning intervention (July, 25 2005) which made it possible to reduce captures for several weeks (Fig. 2).

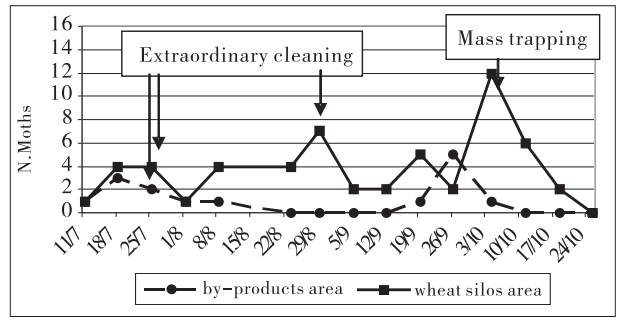

Fig. 2 Weekly captured moths in by – products area and "wheat silos" area and treatments to reduce moths population.

In the remaining mill departments, from July to November 2005, only some Lepidoptera captures were noted but they were not considered worrying. In winter, captures ceased and the presence of moths in the mill began again at the end of May 2006 (Fig. 1).

As a precautionary measure, during the second week of May, the winnowing area and the milling area of the entire building were completely cleaned because the monitoring data of the previous years showed a top of presence after the first spring captures. The extraordinary cleaning intervention aimed at eliminating infestation foci (in cracks and crevices, machinery, corners, overhead wires, etc.) from which new adults could flutter.

In fact, the weekly monitoring data showed that the population density in the mill remained at non-worrying levels for the rest of the warm season, thanks to the targeted cleaning and pest control interventions (Fig. 2).

Comparing the monthly captures of the

years 2004 – 2005 – 2006, the flight curve showed a peak in June 2004 and 2005 and then a fall due to the fumigation carried out in the first week of July (Fig. 3).

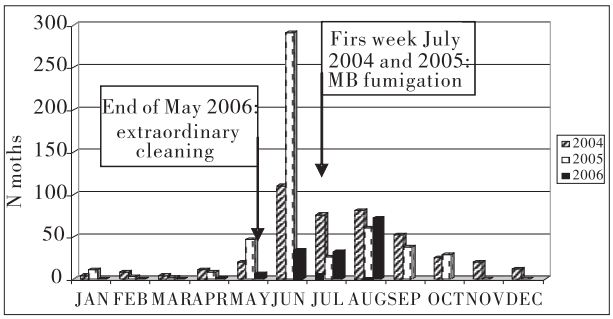

Fig. 3 Monthly captures of moths inside the mill in 2004, 2005, 2006.

In 2006, there was a foreseeable increase in the presence of Lepidoptera during the summer period. In June and in July, captures were strongly inferior compared with those of the same month, in the previous years, although fumigation was not done in 2006. In 2006, the peak of presence was noticed in August but it was inferior if compared with June 2004 and 2005. In these years, there was a boom in captures because of the absence of frequent monitoring and of targeted and prompt interventions. Captures decreased only with fumigation in July.

Beetles

Pantry Patroltraps, used to monitor beetles, captured also many *P. interpunctella* individuals, which turned out to be the main pest in the mill (Fig. 4). More than 20% of the noted captures was composed of insects coming from the outside and linked to the traps position, placed mostly on the ground floor and on transit areas with openings towards the outside. This data showed an inadequate structural prevention, linked to the presence of areas opened towards the outside, windows without the appropriate anti-insect net and because of the frequent habit of leaving the doors open in the transit areas. In the warehouse containing manufactured products in sacks, the introduction of pallets constituted another source of infestation from the outside.

In the mill, the sporadic captures of Anobidae showed that they are occasional pests, as proved also by the data collected with *Pantry Patrol* (Fig. 4).

However, *Anobiidi Trap*, especially those ones near doors and windows or placed in areas

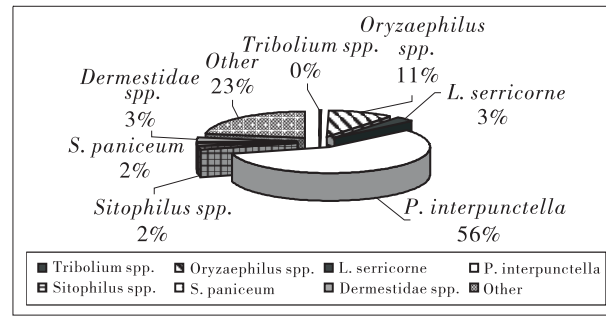

Fig. 4 Percentage of captured species with Pantry Patroltraps (July 2005 – August 2006).

opened towards the outside, captured a considerable number of occasional pests, coming from the outside, such as Hymenoptera and Diptera. This shows again the importance of prevention from the entrance of insects coming from the surrounding areas.

Water-and oil-filled traps

These traps, baited with a pheromone lure, were placed in some critical areas of the mill. The direct comparison among the captures data of the different traps (oil-filled, water-filled, funnel) showed that, especially in the by-products area, water-and oil-filled traps were more effective in capturing Lepidoptera than funnel traps (Fig. 5).

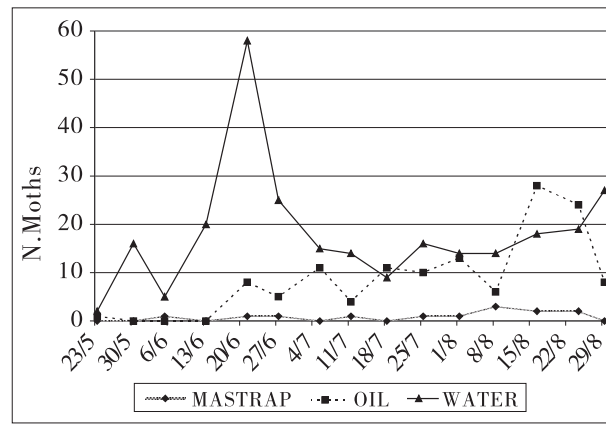

Fig. 5 Weekly captures of moths in by – products area with funnel traps (Mastrap), oil – and water – filled traps.

In general, it is known that there are different factors which can influence insect capture; these factors are linked to the trap, such as the structure and colour, the kind of lure, the capacity to detain insects after capture but they are also linked to the environment, such as placement, temperature, the kind of preserved goods and the way they are stored[6,7,8]. A study, in which four different traps to capture *P. interpunctella* were tested, showed that the funnel trap captures the lowest percentage of adults,

whereas "pagoda traps" were the most effective among sticky traps, followed by delta traps[9].

However, it is important to point out that in particular situations, such as in dusty environments like mills, funnel traps are the most appropriate. In fact, in these cases, sticky traps surfaces would be quickly inactivated by powders.

The strong attractiveness of water to Lepidoptera adults, observed in this study, had been already pointed out on *Ephestia cautella* Walker by Ryne *et al.* [2].

Trapped adults were sexed and the collected data showed that these traps were not only able to attract males, but also a small quantity of females (Fig. 6). Ryne *et al.* [2] analysed if water attractiveness varied between sexes of *E. kuehniella* Zeller and *E. cautella*. For the last one, they pointed out substantial differences in capturing individuals of both sexes with water-filled traps, either baited or not with a pheromone lure, compared to the same traps without water and to funnel traps. On the contrary, for *E. kuehniella*, females were not attracted, whereas males were captured only in the traps baited with a pheromone lure; once again, sack traps were less effective than water-filled traps, baited with pheromone lure. As for *P. interpunctella*, the percentage of captured females was small; more precisely, water-filled traps captured 3, 4% more females than oil-filled traps. (Fig. 6). Capture percentages of female individuals were not high but these traps proved to be more effective than funnel traps in mass trapping.

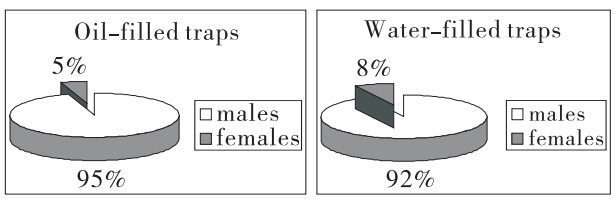

Fig. 6 Percentages of *P. interpunctella* males and females captured with oil – and water – filled traps.

Also some beetles showed their attractiveness to water-and oil-filled traps because a week after their placement, numerous captures of *Sitophilus oryzae*, *Oryzaephilus surinamensis*, *Cryptolestes* spp. were noticed in the basement area "wheat silos". In the following week, captures increased abnormally and this fact led to the identification of a considerable infestation in the raw materials stocking area above this room. A targeted intervention was hence scheduled with the use of phosphine in the stocking silos and deltamethrine on the walls of this area. After the interventions, captures diminished until they ceased after two weeks. These traps were therefore very useful to promptly identify a strong infestation existing in the stocking silos. A following and immediate intervention was hence possible.

Interventions against Accidental Insects

During this study, besides ordinary and extraordinary cleaning, made after real necessities, structural interventions and some treatments towards occasional pests, such as cockroaches, ants and tineids, were carried out.

The capture of some individuals of *Blatta orientalis* on the ground floor of the flour silos area (June, August 2006) was attributed to the presence of a manhole on the external yard of the mill which was linked to the urban sewerage system. The interventions regarded localized treatments in the interested area, with imidacloprid gel baits.

On the ground floor of the flour silos area, there was also the presence of *Tetramorium caespitum* coming from the outside. The interventions aimed at eliminating flour remains, at sealing crevices with concrete and at treating the area with a deltamethrine-and pyrethrum-based product.

In May 2006, an infestation of the clothing moth *Tineola bisselliella* was noticed on the cloth lid filters of the flour silos used for sacking. The filters were removed and cleaned. After this intervention, the infestation disappeared.

Intervention on the Flour Silos

An important structural intervention was sealing the flanges of the stocking silos containing flour to be packed. Product loss was in fact due to the lack of grip of the flanges because of the loading pressure of the flour silo; this problem determined not only an economic loss, but it constituted also an attractive source for insects (the presence of Dermestidae larvae was often noticed). All silos were therefore sealed with silicon: after this intervention, there were no more product losses and the presence of traces diminished considerably.

Conclusions

Thanks to the implementation of IPM program, with an increment in the control frequency and an increase in the number of traps, it was possible to detect infestation foci and to define critical areas in order to intervene promptly and in a specific area. In this way, infestations

can be eliminated and after that, the success of the intervention can be checked.

In 2006, the number of insects in the mill was inferior if compared to the previous years, thanks to all the interventions implemented. Fumigation with MB was hence unnecessary.

Using other kinds of traps (Pantry Patrol, Anobiidi Trap, water-and oil-filled traps), which in some cases proved to be more effective than the traps generally used in monitoring to attract and capture insects, it was possible to trap a greater number of pests. Infestations were consequently kept under control, particularly in the basement "wheat silos" and in the stocking area of milling by-products, which were considered to be the two most critical areas in the mill. In particular, although water-and oil-filled traps captured few female adults of *P. interpunctella*, the mass trapping technique was developed with the aim of capturing as many adults as possible. Female capture, besides lowering the mating possibilities between individuals, diminishes also the possibility of oviposition.

Thanks to the traps placed on the ground floor and near openings, the entrance of pests from the outside was monitored and the need to prevent it was stressed. To stop the entrance of pests, nets were installed where they lacked and doors were kept closed when not used.

Infestation foci, which were promptly removed, and occasional pests, such as Tineidae, ants and beetles, managed through targeted interventions, were detected during the weekly visual monitoring.

It is also important to monitor the presence of pests weekly and not monthly; to plan to clean more frequently and if necessary, extraordinarily and promptly; to invest money in structural improvements or new machinery; and to train the staff about stored-product pests and IPM techniques.

The obtained results show that infestation management in a mill industry can be realized through an integrated pest management program, leaving aside the annual fumigation.

However, it must be highlighted that this approach requires a more technically skilled staff able to organize and manage monitoring.

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0218

Vapormate® as a Quarantine Fumigant for Orange Treatment

Bo Kyung Sung[1], Min Goo Park[1], Robert Ryan[2], Yonglin Ren[3], ByungHo Lee[4*] and Tae Joon Kim[4]

Abstract: In Korea, 10% methyl bromide (MB) has been used to the imported oranges for QPS (Quarantine and Pre-Shipment) purpose, and amount of the consumption of MB was reached to 82t in 2007. Mandatory fumigation with MB was carried out for treatment of imported oranges (96.7% of total) in order to meet National Plant Quarantine Service (NPQS) requirement. Although MB use for soil fumigation has been decreased by year to year due to the ozone depletion issue, its use for QPS fumigation is increasing continuously because of increasing of global trades and protection of agro-ecosystem in imported counties against quarantine pest. Currently, as one of the potential alternatives, ethyl formate is considered to use for treatment of postharvest commodities. A cylinder formulation of 16.7% ethyl formate, named Vapormate, was developed by the Linde Group for fumigation of durable commodity. To extend the application purposes, the Linde Group and Dongbu HiTek have tested Vapormate for perishable commodity fumigation, especially for imported and exported fruits. This study was conducted to obtain the more systematic data to control different stages of two spotted mites (*Tetranychus urticae* Koch) and citrus mealybugs (*Planococcus citri* (Rossi)) in terms of estimated CT product. The L(CT)$_{99}$ values of ethyl formate were 96 and 21 g·h/m^3 against eggs of two spotted mites and adults of citrus mealybugs at 21 ±2℃, respectively. In a semi-field test with Vapormate on oranges, the completed control of mites and mealybugs were achieved at 210 g/m^3 for 4hr at both 5℃ and 17 ±2℃. In the post fumigation, the quality (loss of firmness, color change and total soluble solid) for 1, 6 and 15 days at 6℃ storage was not significantly different. The final residue of applied ethyl formate was detected at the level of 0.042 ppm that is under the Maximum Residue Limits (MRLs) for dried fruits in Australia. Vapormate could play an important role to reduce MB use and to protect ozone layer as a MB replacement for QPS treatment purposes.

Introduction

In Korea, 10% methyl bromide (MB) has been used to treat the imported oranges for Quarantine and Pre-Shipment (QPS) purpose, and amount of the consumption of MB was reached to 82t in 2007. Mandatory fumigation with MB was carried out for treatment of imported oranges (96.7% of total) in order to meet National Plant Quarantine Service (NPQS) requirement. Although MB use for soil fumigation has been decreased by year to year due to the ozone depletion issue, its use for QPS fumigation is increasing continuously because of increasing of global trades and protection of agro-ecosystem in imported counties against quarantine pest. Currently, as one of the potential alternatives, ethyl formate is considered to use for treatment of postharvest commodities. A cylinder formulation of 16.7% ethyl formate, named Vapormate®, was developed by the Linde Group for fumigation of durable commodity. To extend the application purposes, the Linde Group and Dongbu HiTek have tested Vapormate® for perishable commodity fumigation, especially for imported and exported fruits. In this paper, we report more systematic data regarding with use of Vapormate® to control different stages of two spotted mites (*Tetranychus urticae* Koch) and citrus mealybugs (*Planococcus citri* (Rossi)) in laboratory test and semi-field conditions. Also, assessment of the quality and residue were evaluated post fumigation.

Materials and Method

Fumigant Bioassays

A different stages of two spotted mites (*Tetranychus urticae* Koch) and citrus mealybugs (*Planococcus citri* (Rossi)) were used to predict L(CT99) values. The Ethyl formate fumigation was conducted for 4hr at 21 2℃ in 8L gas-tight glass desiccators. After 4 hr fumiga-

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tion, the desiccators were opened and aerated in the fume hood for 2hr. Mortality of adult and nymph was assessed under a microscope at 24hr post fumigation. In case of *T. urticae*, hatching rate of eggs was investigated after incubation for 4 – 5 days.

Fruit Quality Assessment

Navel oranges imported from Australia and California were used for quality assessment. Vapormate at 210 and 420 g/m^3 applied for 4hr at both 5 and 17℃ in 0.5m^3 stainless steel fumigation chambers filled with 25% of orange. After 4 hr exposure, the chambers were opened and aerated for 2hr using fan and then the commodity was stored at 6 2℃. The quality (loss of firmness, color change, total soluble solid and pitting or pericarp browning and fungal decay) were accessed for 1, 6 and 15 days at 6℃ storage

Residue Analysis

The oranges were fumigated with 50 g/m^3 of ethyl formate for 24hr at 21 2℃ in 6L gastight glass desiccators and then aerated for 3hr. For residue analysis, all samples were analyzed by a solvent extraction method as described by Vu and Ren (2004)[1].

Results and Discussions

The efficacy of ethyl formate for different stages of two spotted mites (*T. urticae*) and citrus mealybugs (*P. citri*) is shown Table1. The LC values are useful fundamental data for future practical trials because dose of ethyl formate in fumigation chamber is variable depending on sorption of target fresh fruits, loading factors and their application methods (formulated with CO_2) as well as fumigation conditions. The estimated $L(Ct)_{99}$ values of ethyl formate were 96 and 21g · h/m^3 against eggs of two spotted mites and adult citrus mealybugs, respectively.

Table 1. Toxicity of ethyl formate to two spotted mites and citrus mealybugs.

Species	Stage	LC_{50} (mg/L)	LC_{95} (mg/L)	LC_{99} (mg/L)	Slope (SE)[a]	DF	x^2
T. urticae	Adult	4.96	7.39	8.71	9.51 (±1.1)	23	2.56
	Egg	12.68	36.82	55.95	3.63 (±1.3)	23	15.43
P. citri	Adult	5.19	8.25	10.00	8.18 (±1.5)	23	2.84
	Nymph	2.89	5.58	7.34	5.77 (±1.4)	26	5.46

[a]Standard error

There were no found in significant quality differences between the fumigated orange (Vapormate 210 and 420 g/m^3) and untreated one in terms of firmness, pitting or pericarp browning, fungal decay, or total soluble solids for the 1st and 6th day of storage (Table 2 and Table 3). A slight pitting on surface of orange fumigated at 420 g/m^3 of Vapormate at 17℃ was investigated in 15th day of storage.

Table 2. Phytotoxic effect of Vapormate to orange fumigated for 4hr at 17℃ (15th day storage at 6 ± 1℃)

Vapormate concentration (g/m^3)	Pitting ± SD a	Firmness ± SD (kg or N)	Fungal decay ± SD b	Soluble solids ± SD (%)
0	0.40 ± 0.50 abc	0.83 ± 0.01	1.00 ± 0.00	10.67 ± 0.74
210	0.20 ± 0.41 a	0.82 ± 0.03	1.00 ± 0.00	10.67 ± 0.55
420	1.25 ± 0.55 c	0.83 ± 0.05	1.00 ± 0.00	10.93 ± 0.15
Significance (*P*)	0.000	0.963	1.000	0.790

Table 3. Phytotoxic effect of Vapormate to orange fumigated for 4hr at 5℃ (15th day storage at 6 ± 1℃)

Vapormate concentration (g/m^3)	Pitting ± SD a	Firmness ± SD (kg or N)	Fungal decay ± SD b	Soluble solids ± SD (%)
0	1.00 ± 0.72 ac	0.79 ± 0.02	1.10 ± 0.32	12.07 ± 2.01
210	1.25 ± 0.67 a, b	0.79 ± 0.03	1.00 ± 0.00	12.55 ± 1.50
420	1.40 ± 0.55 b	0.80 ± 0.02	1.00 ± 0.00	12.63 ± 1.59
Significance (*P*)	0.023	0.197	0.381	0.734

[a] Damage score: 0 (none), 1 (slight), 2 (moderate), 3 (severe).
[b] Decay score: 1 (none), 2 (25%), 3 (50%), 4 (75%), 5 (entire fruit)
[c] Means in a column followed by the same letter are not significantly different at the 5% level.

The partitioning of ethyl formate between air and the salts in spiking sample system is shown in Figure 1. Equilibrium partition between air and each salt was examined within 20min. Ethyl formate was stable in the headspace over either sodium sulfate plus orange or sodium sulfite plus orange. Ethyl formate in the headspace of ammonium nitrate and zinc sulfate wasn't even detected at 1 min. The final residue of ethyl formate exposed to 50 g/m^3 for 24h at 21 ± 2℃ and aerated for 3hr was detected at the level of 0.042 ppm.

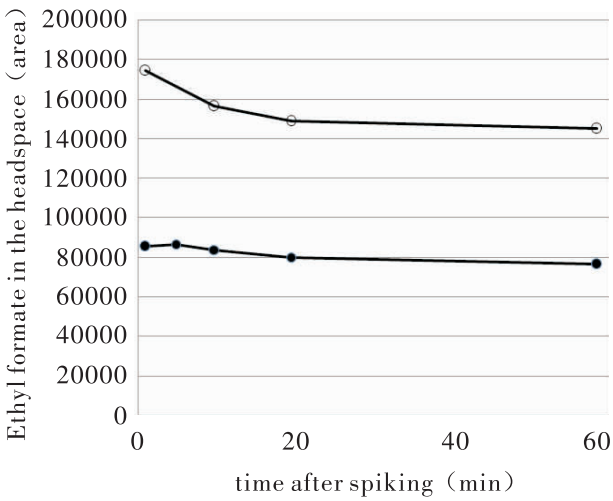

Fig. 1 Amount of ethyl formate in the headspace above spiked standards of orange in – ○ – sodium sulfate, – ● – sodium sulfite.

The L(CT)$_{99}$ values of ethyl formate were 96 and 21 g · h/m^3 against eggs of two spotted mites and adults of citrus mealybugs at 21 ± 2℃, respectively. In a semi – field test with Vapormate on oranges, the completed control of mites and mealybugs were achieved at 210 g/m^3 for 4hr at both 5℃ and 17 ± 2℃. In comparison with untreated oranges, the quality (loss of firmness, color change and total soluble solid) of treated oranges have not significan different or changes for 1, 6 and 15 days storage at 6℃. The final residue of ethyl formate was detected at the level of 0.042 ppm which is below the Maximum Residue Limits (MRLs) for dried fruits in Australia.

Vapormate® could play an important role to reduce MB use and to protect ozone layer as a MB replacement for QPS treatment purposes.

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Fumigation Efficacy of Ethyl Formate on Wheat, Corn and Rice in Sealed Desiccators

He Yanping, Xu Li, Chen Sisi, Xu Guangwen, Xie Lingde

Abstract: Ethyl formate (EF) is a potential alternative fumigant to methyl bromide and phosphine for the control of insect pests in stored commodities. Effects of temperature, relative humidity and grain m. c. on toxicity of EF were studied using sealed desiccators containing maize, wheat or rice. Lower temperatures decreased efficacy of EF. A concentration of 80 mg/L EF killed >98% of *Sitophilus zeamais* Motschulsky placed on top of the grain exposed 72h at 16, 24 or 32℃. However, very low mortalities of *S. zeamais* placed under rice at all three temperatures and under wheat at 16℃. A concentration of 80 mg/L EF could not control *Rhyzopertha dominica* (F.) on top of wheat or rice and below all three grains at 16℃, 72h exposure. Increased r. h. increased efficacy of EF. At 35 and 65% r. h., very low mortalities of *S. zeamais* and *R. dominica* were found below maize after an applied concentration of 80 mg/L EF after 72h at 25℃. However, when r. h. was increased to 95%, it killed >90% of both pests. The efficacy of EF was reduced at higher grain moisture contents. At >12% m. c., mortality of *S. zeamais* and *R. dominica* under maize was below 20% even though higher r. h. of 60 and 90% were used.

Key words: ethyl formate, temperature, r. h., moisture content, mortality, fumigation

Introduction

Ethyl formate (EF) is an easily vaporised organic liquid. It has been investigated as a rapid disinfestant for many stored product pests and other pests[1,2,3]. EF has been used for disinfestation of pests in stored dried fruit since 1927[4]. However, it as a grain fumigant has waned following development of methyl bromide and phosphine[5]. Methyl bromide is now being phased out internationally under the Montreal Protocol because of its damaging effect on the ozone layer. Use of phosphine is under increasing pressure because of the frequency of resistance to it[6]. Ethyl formate has been considered as a candidate for development as a fumigant of stored grain.

The rapid toxic action of EF has been observed in many insects. Damcevski and Annis showed that EF could give >99% mortality in adult insects and >95% mortality in other stages with a 24 h exposure period[1,7,8]. EF also is an environment friendly fumigant. EF naturally occurs in many grains, vegetables, fruit, and animal products such as milk and cheese[5-9]. Its residues break down to the naturally occurring products, formic acid and ethanol[1]. However, EF is easily sorbed by all commodities and has poor penetration through bulk grain. Its fumigant toxicity is affected by many complex factors. Carbon dioxide (CO_2) can enhance fumigant toxicity of EF, and many other fumigants, to insects[10,11]. CO_2 mixed with EF can substantially reduce its ammability and has the effect of raising the lower ammability limit[12]. Furthermore, CO_2 has ability to improve the movement of fumigant through the grain[13]. Efficacy of EF against pests was markedly increased at increased r. h. in the absence of grain[14]. However, the effect was less in presence of grain, possibly due to the high solubility of EF in water (105 g/L at 20℃)[14,15]. Ren and Mahon[5] showed that using two dosages of 85 g/t, EF gave a high level of control of all stages of most of the test insects in the wheat, split faba beans (*Vicia faba*) and sorghum. Split faba beans and sorghum took up EF more strongly than wheat. EF residues in wheat were reduced to natural levels within 2 weeks from application, but in split faba beans and sorghum it took more than 4 weeks for residues to return to near background levels.

Maize weevil, *Sitophilus zeamais*, and lesser grain borer *Rhyzopertha dominica* are two serious pests of stored grain. High mortality of *R. dominica* by EF has been reported[8], but there is little reported data on EF against *S. zeamais*. Two pests were chosen for comparative studies.

In this paper, we report the influence of temperature, kind of grain, relative humidity and moisture content (m. c.) of grain on EF effects on these two species.

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Materials and Methods

Insects

All test insects were reared at Wuhan Polytechnic University in electronically controlled incubators 27 ± 1℃ and 75% 5% r. h.. *Sitophilus zeamais* was reared on whole wheat, *Rhyzopertha dominica* on broken wheat, *Tribolium castaneum* on whole wheat flour with 5% yeast. The wheat to be used was sterilized at 80℃ for 2h. The moisture content was then adjusted to 13% ±1% w. b. Adults (7 – 14 days old) were used in this study.

Chemicals

The EF used was analytical grade (≥97% purity), purchased from Tianjin Basifu Chemical Ltd.

EF Treatment at Different Temperatures

The insects were treated with EF in gastight 15 L glass desiccators, sealed with glass stoppers fitted with a septum for gas injection. Four fifths of the volume of the desiccators were filled by 12% m. c. grain (maize, wheat or rice). Each test replicate used 30 adult maize weevils and 30 adult lesser grain borers. Three replicates of each kind of insect were put below the grain and also on the top of the grain. 1mL liquid EF was added onto a filter paper in a dish on the grain surface. The final calculated concentration of EF in the desiccators was 80 mg/L. EF treatments were carried out at 16, 24 and 32℃, each at 70% r. h. in electronically controlled incubators. After the exposure of 72h, the desiccators were opened and mortalities were assessed for each replicate.

EF Treatment at Different Humidities

Test insects were treated in desiccators, as above. The desiccators were filled to two-thirds capacity with 6 kg maize. Prior to being sealed, the desiccators, with grain were held at 25℃ at 35%, 65% or 95% r. h for 30 minutes. The containers were then sealed and 1mL EF was applied introduced through the septum using a gas-tight syringe. After 72h, the desiccators were opened and mortalities were assessed for every replicate. Maize at 10% m. c. was used in r. h. 35% r. h. tests, and 12% m. c. maize was used in 65% and 95% r. h. tests.

EF Fumigation at Different r. h. and m. c. of Grain

Fumigations were carried out as above at 25℃ using maize of different moisture contents. The moisture contents and resulting r. h. is given in Table 1.

Table 1. Fumigation condition of EF for corn at 25℃

Weight of grain (g)	Grain m. c. (%)	r. h. (%)	Concentration of EF (mg/L)
6	9.2	30	80
6	12.7	60	80
6	16.25	80	80

Results

EF Treatment at Different Temperatures

Table 2 shows that a concentration of 80 mg/L EF killed >98% of *S. zeamais* placed on the top of the maize, wheat or rice after exposure of 72h at 16, 24 and 32℃ (Table 2). Very low mortalities were observed for insects placed below under rice at all three test temperatures and under the wheat at 16℃. A initial concentration of 80 mg/L EF could not control *R. dominica* after 72h at 16℃ (Table 3) did not kill all of the test insects either on top of or below the grain. It killed more than 90% lesser grain borers both on the top of the maize and wheat and underneath the two grains at 24℃ and 32℃. Lower mortalities of lesser grain borer were found in the rice at the three temperatures. These results showed that higher temperature increased the efficacy of EF. EF fumigant efficacy varied with the kind of grain used (Tables 2, 3).

Table 2. Mortality (%) of maize weevils with EF applied at 80 mg/L after 72h at different temperatures [a]

Grain		16℃	24℃	32℃
Maize	top	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
	bottom	97.0 ± 2.6a	100.0 ± 0.0a	98.8 ± 2.1a
Wheat	top	92.4 ± 10.3a	100.0 ± 0.0a	100.0 ± 0.0a
	bottom	29.7 ± 13.6a*	98.9 ± 1.9b	100.0 ± 0.0b
Rice	top	98.9 ± 1.9a	100.0 ± 0.0a	98.9 ± 1.9a
	bottom	0.0 ± 0.0a*	5.7 ± 2.1b*	6.9 ± 1.7b*

[a] Results are the means SD ($n = 3$). Means within columns followed by the same letter are not significantly different ($p > 0.05$,

LSD Fisher's multiple range test).

* Mortalities of pests is significantly different between top and down of the same grain ($p < 0.05$, Student's t test).

Table 3. Mortalities of lesser grain borers with EF applied at 80 mg L^{-1} for 72h at different temperatures (%) [a]

Grain		16℃	24℃	32℃
Maize	top	94.6 ± 5.0a	97.7 ± 4.0a	98.9 ± 2.0a
	bottom	74.1 ± 0.8a *	92.0 ± 4.9b	93.4 ± 5.8b
Wheat	top	51.9 ± 10.2a	98.9 ± 1.9b	100.0 ± 0.0b
	bottom	5.6 ± 3.8a *	94.4 ± 6.9b	92.2 ± 10.7b
Rice	top	68.6 ± 6.5a	63.3 ± 3.3a	61.2 ± 6.2a
	bottom	3.4 ± 0.1a *	4.4 ± 1.9a *	11.5 ± 3.2b *

[a] Results are the means SD ($n = 3$). Means within columns followed by the same letter are not significantly different ($p > 0.05$, LSD Fisher's multiple range test).

* Mortalities of pests is significantly different between top and down of the same grain ($p < 0.05$, Student's t test).

Efficacy of EF Fumigation at Different r. h.

Increased r. h. increased the efficacy of EF. At 35 and 65% r. h., very low mortalities of *S. zeamais* and *R. dominica* were found under the maize with an initial concentration of 80 mg/L EF after 72h at 25℃. However, when r. h. was increased 95%, it killed more than 85% of both pests (Table 4).

Table 4. Mortalities of the two pests with EF applied at 80 mg/L after 72h at 25℃ for maize at different r. h. (%) [a]

Pest		16℃	24℃	32℃
S. zeamais	top	92.3 ± 1.7a	100.0 ± 0.0b	97.8 ± 1.9b
	bottom	22.9 ± 5.0a *	71.9 ± 0.8b *	96.8 ± 5.6c
R. dominica	top	96.3 ± 6.4a	89.4 ± 9.8a	93.0 ± 3.2a
	bottom	40.9 ± 3.2a *	64.7 ± 3.3b *	86.3 ± 13.9c

[a] Results are the means SD ($n = 3$). Means within columns followed by the same letter are not significantly different ($P > 0.05$, LSD Fisher's multiple range test).

* Mortalities of pests is significantly different between top and bottom of the same grain ($P < 0.05$, Student's t test).

Effect of r. h. and m. c. of Maize to EF Fumigation

Both r. h. and m. c. of maize affect the efficacy of EF. An initial concentration of 80 mg/L, EF gave lower mortalities of both *S. zeamais* and *R. dominica* with the lowest r. h. (30%) (Table 5). At > 12% m. c., mortalities of *S. zeamais* and *R. dominica* below the maize were < 20%, even with higher r. h. (60% and 80%) (Table 5). These results were far below the mortalities observed for *S. zeamais* (71.9%) and *R. dominica* (64.7%) below maize at 65% r. h., 12% m. c. and 25℃ (Table 4). This shows that m. c. of grain affected efficacy of EF much more than r. h. (Tables 4, 5).

Table 5. Mortalities of two pests with EF applied at 80 mg/L after 72h at 25℃ for the different m. c. of maize (%) [a]

Pest		r. h. 30% m. c. 9.2%	r. h. 60% m. c. 12.7%	r. h. 80% m. c. 16.3%
S. zeamais	top	53.5 ± 3.2a	98.5 ± 2.6b	97.5 ± 4.3b
	bottom	5.1 ± 0.8a *	16.9 ± 3.6b *	13.3 ± 6.7b *
R. dominica	top	29.8 ± 3.1a	60.4 ± 17.0b	54.9 ± 11.7b
	bottom	5.3 ± 1.8a *	16.7 ± 5.8b *	7.5 ± 2.9a *

[a] Results are the means SD ($n = 3$). Means within columns followed by the same letter are not significantly different ($P > 0.05$, LSD Fisher's multiple range test).

* Mortalities of pests is significantly different between top and down of the same grain ($P < 0.05$, Student's t test).

Discussion

In general, fumigants show high toxicities at higher temperatures[16], but there is little data published on effects of temperature to EF. Damcevski et al. proposed that this should be studied[14]. Our results showed that EF gave higher mortality of pests at high temperatures, especially below a layer of grain (Tables 2,3). However, higher mortalities of *S. zeamais*, *R. dominica* and *Tribolium castaneum* were found at lower temperatures in the absence of grain (these Proceedings). It is suggested that higher temperatures increase uptake of EF, through increased respiration, and also increase fumigant movement thus increasing its effectiveness in presence of grain.

The presence or absence of grain and its amount directly affects the efficacy of EF. There is little loss of EF without wheat[14]. The loss of EF from the headspace above wheat has been studied observed. The loss is attributed to sorption and subsequent breakdown by the wheat[1,14,17,18]. Also, the influence of a commodity on the ef cacy of fumigation is due both to its sorption behaviour and also its effect on the metabolism of the insects[5]. Damcevski et al. reported the effect of wheat on the metabolism of adult stored-product Coleoptera[19]. Changes in the respiration and metabolism of *S. oryzae* were observed when in the presence of wheat[14]. Results in this paper showed effectiveness of a set dosage of EF was influenced by the type of grain treated (Tables 2,3). This implies that the kind of grain influences the sorption abilities to EF. Also, the different grains may affect metabolism of pests differently. This idea merits further study.

Results in this paper are consistent with previous observations that EF showed higher toxicity to pests in higher r. h. (Table 4). EF is sorbed by most commodities[15,20,21], especially where they have high moisture content or are warm[14]. Tables 4 and 5 show that higher m. c. of grain inhibited efficacy of EF more strongly than higher r. h. However, higher r. h. can increase the m. c. of grain, so the effect of r. h. on EF action is complex.

The m. c. of grain should be considered when EF is to be used to fumigate stored grain. In order to get best effectiveness of EF, it is necessary to choose the moost favorable conditions of temperature, kind of grain, m. c. of grain and r. h. .

This research will maximize the ability of the "green" fumigant, ethyl formate, as a fumigant for stored grain.

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0220

Semi-Continuous Ozonation System for Pest Control

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Abstract: Previous trials of ozone treatment for post-harvest food grains have proved ozonation as a potential non-chemical, non-residual and environmentally-friendly alternative for methyl bromide fumigation for treatment of stored food grains. Ozone is a strong oxidizing agent, which is used to control harmful microbes and volatiles. Stored grain products can harbor multiple microorganisms including molds that produce harmful toxins. To examine the effect of ozone on surface microbes, samples of stored maize were treated for a maximum of 60 minutes at a maximum ozone concentration of 1900 ppm. The data showed that the amount of microorganisms significantly decreased or were completely absent after the maize samples were treated with ozone. In order to improve the treatment effect, a counter-flow semi-continuous ozonation treatment system was designed in order to ozonate grain at a faster rate, based on the concentration-time product (CTP) of ozone required to eliminate microbial growth on grain kernels and achieve insect mortality. The ozonation treatment system can be installed in typical grain silos. Ozone is generated at a constant rate and introduced into the plenum of the silo with a variable speed fan to control treatment effect. The bottom grain layer is removed with a specially designed unloading conveyor after it has reached the desired ozone CTP. Air velocity was quantified for each grain layer to determine the theoretical ozone concentration. Ozone concentration was allowed to build up for 60 to 120 minutes in the bottom grain layer. As the layers of grain were removed, the ozone concentration in each layer increased until the maximum value was reached. A counter-flow semi-continuous ozonation system was successfully tested and proved to be a technically feasible non-chemical tool for pest control.

Introduction

Ozone (O_3) is a gas made up of three bonded oxygen molecules; it is highly unstable and reacts with many elements. O_3 reacts with microorganisms quickly by degrading them. Theories suggest that O_3 attacks the lipid double bonds in the cell membrane and results in a change in cell permeability to lyses[1]. Ozone has the ability to oxidize (i. e., cause the loss of electrons) the chains and break down the enteroviruses. Such strong oxidative abilities make O_3 a very reactive element.

Insects and molds cause a significant amount of damage to grain each year producing economic losses that affect farmers, elevators managers and processors throughout the world[2]. Stored grain protection relies heavily on the use of chemicals like phosphine and methyl bromide to control pests. Due to the increased concern over the use of post-harvest chemicals worldwide, there is much interest in the development and use of non-chemical treatments such as temperature, moisture management, modified atmospheres, heat treatment of empty structures, physical exclusion, non-chemical protectants, biological controls and ozonation to control stored product pests. Ozonation is a powerful oxidant that reduces or inhibits mold spore development and kills stored product insects, therefore serving as a non-chemical alternative for stored grain protection[3,4].

Fungi and bacteria contribute to quality deterioration of stored grains; they can cause nutrient loss (changes in vitamins, lipids, proteins and carbohydrates), functional property losses (germination), and aesthetic changes (discoloration, caking and odors). Given that ozone inhibits or eliminates fungal spores, it reduces the potential production of mycotoxins that can be toxic to humans and mammals when ingested. Ozone has the tendency to transform or decay into two molecules of oxygen within 20 to 50 minutes[5]. Therefore, a properly designed ozonated airflow system is important for the effective movement and distribution of ozone through the grain mass in a storage structure. A

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recirculation or exhausting system is needed so ozone is replenished once the leading front exits the grain mass and is exhausted to the air.

The ozone generator needs to be capable of producing ozone at a constant rate and at the capacity needed for the grain mass to be treated. Among the advantages of ozonation compared to traditional phosphine fumigation for pest control are that it can be generated on the treatment site and no residue remains on the treated product[4]. The initial ozonation treatment of a grain mass takes more time to complete than subsequent treatments on the same grain mass (as long as it is not moved and recontaminated with dust, etc.) because ozone reacts with the cell structures of mold spores, insects, bacteria and other biological matter attached to the grain kernel surface and contained within the grain mass. Completion of the sterilizing ozonation effect depends on the quantity of biological matter to be reacted with, the quantity of ozone available to react, and the supply of ozone to complete the reaction process throughout the grain mass within a timely manner. After this reaction process, ozone will move faster and more freely in the grain mass increasing in concentration.

Given ozone's tendency to decay into oxygen within a short time period, and to fully understand its behavior in any environment, it is necessary to determine its half-life (HLT). HLT is the time when the initial concentration of a chemical is reduced by half; it is most commonly denoted by $t_{1/2}$. The primary objective of this study was to determine the engineering parameters and half-life of ozone to design a counter-flow, semi-continuous ozonation treatment system in a grain bin in order to ozonate the grain mass at a faster rate based on the known concentration-time product (CTP) of ozone required to eliminate microbial growth on grain kernels and to achieve insect mortality.

Materials and Methods

The counter-flow, semi-continuous ozonation system was set up in metal silo of 9.1m diameter with a sidewall height of 4.9m and a capacity of 178t. The silo was equipped with grain drying system consisting of a 0.71m diameter axial-flow fan powered by a 9.7kW motor, a propane burner, and a sweep unloading auger system (Shivvers Blue Fame Corydon, IA) to remove a layer of maize from inside the silo after it was dried to the appropriate moisture content. The sweep auger consisted of a 4.5m radius tapered screw conveyor, which rotated around the silo diameter removing 0.10 to 0.15m of grain for each full rotation. The removed grain was transferred by a vertical screw conveyor to the top of the bin where it was transported by a roof-mounted screw conveyor into a nearby bin.

The ozone was produced by a four quad generator manufactured by O_3Co. (Aberdeen, ID) that has a capacity of producing 250g/h. It was powered by a 40kW diesel generator. The four ozone supply lines were positioned to empty into the plenum of the silo through to a fan plenum in order to achieve a uniform distribution of ozone below the perforated drying floor.

Ozone was quantified and controlled with a monitoring system that measured the ozone concentration in different layers of the grain mass using multiple monitoring lines. The ozone concentration was quantified using an ozone analyzer model IN-2000 made by INUSA (Boston, MA) and recorded using a data acquisition system. Ozone monitoring lines were placed in the silo at the following depths: 0.076, 0.17, 0.23, 0.30, 0.61 and 0.89m above the floor and in the headspace and at the grain surface. Each monitoring line was connected to a valve control manifold so only one monitoring line was sampled at a time.

The air velocity was measured using the procedure described by Bartosik[6] that uses a custom-built funnel and a vane-wheel anemometer (Omega HHF91, Omega Engineering Inc., Stanford, Connecticut). The funnel is placed with its larger diameter end on the grain surface and the anemometer is placed on the smaller diameter end to measure air velocity, which then is converted into volumetric airflow through the grain mass.

The ozone half-life was measured using a 40L cylinder which was filled with ozone and sealed off. Ozone was produced from a lab-scale ozone generator manufactured by O_3Co. (Aberdeen, ID) and pumped into the cylinder until it was completely filled with O_3. Once this occurred the chamber was sealed and measurements of O_3 concentration were taken using gas detection tubes. Concentration measurements were taken through a valve at the top of the cylinder using a Kitagawa Gas Sampling Tube.

Results and Discussion

The procedure for the counter-flow, semi-continuous ozonation treatment system involved removing the bottom grain layer with the taper-

ed unloading auger after the layer reached the desired ozone concentration to achieve insect mortality, mold reduction, and/or odor removal. The treated grain was subsequently transported to a storage or shipping silo. Mendez *et al.* [4] reported that in order to have optimal ozone concentration in the plenum, a minimum air velocity of 0.03 m/s is needed. Therefore, air velocity was quantified for each grain layer to determine the theoretical ozone concentration. Air velocity was measured at several locations along the grain surface after the grain surface was levelled. For each airflow measurement at the surface, the airflow was calculated for each grain depth, which was then used to determine the theoretical ozone concentrations using the following formula that relates ozone production of the 250g/hr generator with airflow (Table 1).

$$O_3 = \frac{O_3\text{Flow} \times R \times T}{P \times Q \times 1000 \times 60} \quad (1)$$

Where, O_3 = ozone concentration (ppm); O_3Flow = ozone flow (250g/h); R = 8314.4 J/kmol. K; T = Air temperature (K); P = Pressure (Pa)

Table 1. Calculated ozone concentration and airflow at various grain depths.

Grain Depth (m)	Airflow (m^3/s)	Ozone Concentration (ppm)
1.55	0.525	67
1.40	0.580	60
1.32	0.600	58
1.25	0.620	56
1.17	0.640	55
1.02	0.706	50
0.94	0.701	50
0.86	0.721	49
0.79	0.741	47
0.64	0.782	45
0.56	0.801	44
0.48	0.822	43
0.41	0.841	42
0.23	0.889	39
0.15	0.909	39
0.076	0.929	38
0	0.949	37

During the treatment process, the ozone concentration was allowed to build up for 60 to 120 minutes in the bottom grain layer. As the layers of grain were removed, no ozone concentration in the headspace was measured until only 0.90m of grain was left in the silo. After additional layers were then removed, ozone concentration was detected in the headspace and increased with time. This occurred because ozone moved through the shallower grain too quickly for it all to react with the grain kernels before reaching the surface and exiting into the headspace where it could be detected.

The comparison of the theoretical and measured values for ozone concentration showed a larger than expected difference at each grain depth. Two possible causes were identified. Firstly, the actual ozone producing capacity of the generator was likely lower than the rated capacity, and secondly there was likely ozone measurement error in the monitoring system as some monitoring lines were longer than they should have been.

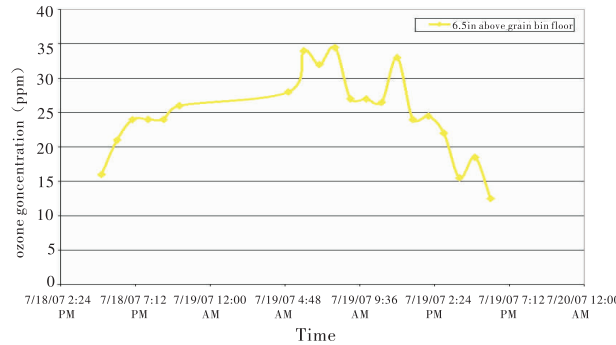

Fig. 1 Ozone concentration at the 0.165 m (6.5 inch) grain depth.

Design Parameters

In order to effectively control and design the semi – continuous counterflow system, the variables of airflow, ozone mass flow and exposure time were controlled. The airflow was controlled by sizing up the appropriate fan that can deliver at least the minimum air velocity of 0.03 m/s and the maximum airflow that will not cause a dilution effect with the initial amount of grain that will be ozonated.

The exposure time of the grain to ozone was controlled and determined by the amount of ozone concentration that was allowed to build up in each grain layer. Once the desired ozone concentration was achieved, the unloading sweep auger removed the bottom grain layer, allowing the ozone concentration to build up again. Based on the previous trial, the exposure time for each layer was between 60 to 120 minutes. Due to the lack of ozone producing capacity by the ozone generator, the CTP was not properly calculated. Therefore, a maximum ozone concentration of 25 ppm for 60 to 120 mi-

nutes was used.

The results of the half-life ozone experiments gave insight into how long the ozone was staying active under varying air conditions. Each of the experiments calculated the half-life of ozone, or the time at which half of the initial concentration was reached, under that particular condition. To examine the effect of ozone on surface microbes, samples of freshly-harvested and stored corn were treated with ozone for 60 minutes and 180 minutes at ozone concentrations of 1 600 – 1 700 ppm and 800 ppm. To determine the effect of ozone treatment on resistance to ozone flow, grain from each ozone concentration/time treatment was placed in a one-meter column and ozone was applied to the inlet of the treatment system. The time it took for the ozone concentration to exit the column and equal the intake concentration was measured. Although these values were not always an exact match, the output concentration generally approached the inlet concentration over time.

Concentration values from the ozone analyzer were continually recorded. The concentration value is important in understanding what concentration it will take to destroy all microbial growth over the shortest period of time. Data retrieved from the ozone analyzer were graphed and used as the basis of the research results.

Half – Life Time Calculation

The first tests performed were to determine the HLT of ozone. In this experiment, ozone was produced in the lab through dielectric excitation of oxygen. This excitation transforms the oxygen (O_2) molecules found in air to ozone (O_3). As the airflow increased within the cylinder, the HLT for O_3 decreased (Table 2). The first two tests were all with zero airflow for which the average HLT was approximately 26 hours. This value was much greater than the previously assumed 37 minutes for O_3 HLT. Weilandics *et al.* [7] determined that the chemical decay constant of ozone was 3.1e – 4 (s^{-1}). This value yields $t^{1/2}$ = 2235.97 sec = 37.26 min using Wolberg's equation of HLT = 0.69315/(Decay Constant).

Airflow Rate HLT Tests

In the next tests, mixing fans were incorporated to compare ozone HLT as a function of airflow rate. As the fan speed increased, the HLT decreased (Table 2). Incorporation of mixing fans showed marked reduction of HLTs. The greatest difference in HLT was noted with the 0.0283 m^3/s fan. Table 3 shows HLT results from tests when two different initial ozone concentrations were used. The HLTs for the two initial concentrations were consistent; there was no significant change in HLT when the initial concentration was reduced by half.

Table 2. Summary of ozone HLT values as a function of four airflow rates.

Exp. #	Fan Airflow (m^3/s)	HLT(min)
1	0	1590
7	0.0283	112
4	0.0519	57.7
5	0.104	47.5

Table 3. Summary of ozone HLT values as a function of two different initial concentrations at zero airflow.

Exp. #	Initial O_3 Conc.	HLT(min)
1	1300	1590
2	700	1553

Conclusions

A counterflow semi – continuous ozonation system was successfully tested and proved to be a technically feasible tool for properly ozonating grain at a faster rate than with a batch ozonation system. The half-life time of ozone was significantly decreased upon the incorporation of a mixing fan. This reduction can be explained by the fact that when ozone molecules are agitated they break down to oxygen more quickly.

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0221

Toxicity of Ethyl Formate to Three Stored Grain Insects in Absence of Grain

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Abstract: The effects of treatment time, temperature and concentration of ethyl formate (EF) against *Tribolium castaneum*, *Rhyzopertha dominica* and *Sitophilus zeamais* were studied in absence of grain. Some dosages of EF achieved complete kill within 24h in a sealed system. Mortality of each of the three pests decreased with increasing temperature at a fixed dosage. Mortality of *T. castaneum* and *R. dominica* at 16℃ was significantly different to that at 24 and 32℃ ($p < 0.05$). Mortality of *S. zeamais* at 16, 24 and 32℃ were significantly different to each other ($P < 0.05$). The LC_{50} values of *T. castaneum*, *R. dominica* and *S. zeamais* were 25.97, 12.50 and 7.45μL/L respectively with EF over 24h at 24℃.

Key words: Ethyl formate, fumigation, *Tribolium castaneum*, *Rhyzopertha dominica*, *Sitophilus zeamais*

Introduction

Currently, worldwide, methyl bromide and phosphine are the mainly used fumigants. However, methyl bromide has been classified as an ozone-depleting substance under the Montreal Protocol and its use as a fumigant will be phased out before 2015 worldwide [1]. Furthermore, resistance of stored grain pests to phosphine is growing and spreading, the dosage of phosphine and its frequency of use are increasing in response to this resistance, with decreasing quality of the fumigated products and increasing cost of pest control[2]. Developing a new type of fumigation to replace methyl bromide and reducing the dosage of phosphine is a priority in the current plan[3]. Ethyl formate is one of the closely studied candidate replacements in the process [4].

There are many reports on EF as fumigant for dry fruit and stored grain. It was reregistered as a fumigant for dried fruit and nuts in Australia in 2002[5]. Damecevski et al[6]. researched the effect of humidity on the fumigation activity of EF in empty containers. The results showed that r. h. influenced the observed mortality. The higher the r. h., the lower dosage of EF was required to achieve 99% mortality [6]. Tang Peian et al. researched various aspects of the fumigation activity of EF to *Rhyzopertha dominica*, *Sitophilus oryzae*, *Tribolium castaneum* and *Liposcelis bostrychophila* in empty containers[7-10]. They showed that the sensitivity of different species and different stages of insects to EF were different. The better efficacy of EF was showed at lower temperatures. This was not consistent with previous reports that higher temperatures are better for EF fumigation [2].

T. castaneum, *R. dominica* and *S. zeamais* are the main stored grain pests in China. There are many reports about the efficacy of EF against *Sitophilus oryzae* and *T. castaneum*, but there is little information on the efficacy of EF against *S. zeamais* and *T. castaneum*. Therefore, we chose *T. castaneum*, *R. dominica* and *S. zeamais* as test insects so that our studies could be compared with the previous ones and new data on the efficacy of EF against *S. zeamais* and *T. castaneum* was obtained.

In this paper, we determined the LC_{50} values of EF to *T. castaneum*, *R. dominica* and *S. zeamais* adults at 16℃, 24℃ and 32℃.

Materials and Methods

Insects

All test insects were reared at Wuhan Polytechnic University in electronically controlled incubators 27 ± 1℃ and r. h. 75% ± 5%. *Sitophilus zeamais* was reared on whole wheat, *Rhyzopertha dominicad* on broken wheat, *Tribolium castaneum* on whole wheat flour with 5% yeast. The wheat to be used was sterilised at

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Editor's note: No concentration measurements were taken for EF during the fumigation. It may be that no EF was present after 24h.

80℃ for 2h. The moisture content was then adjusted to 13% ±1% w. b. Adults (7 – 14days old) were used in this study.

Chemicals

The EF used was analytical grade (≥97% purity), purchased from Tianjin Basifu Chemical Ltd.

Toxicity Studies

Fumigation was carried out in 1L airtight jars. Firstly, 15 test insects were placed in the jar. Secondly, measured quantities of EF were added to a filter paper (311cm) which was glued vertically inside the jar. Thirdly, the jar was closed as soon as possible, and the lid was sealed on with parafilm. Finally, the jar was placed in the incubators at constant temperature and humidity. Each bioassay was carried out in duplicate with five EF dosages and an undosed control.

Fumigations were carried out at 16, 24 and 32℃, with exposure times of time was 24, 48, 60 and 72h.

Statistical Analysis of Data

The mortality results were analysed statistically using SPSS data processing software[11].

Results Mortality of adult insects to EFTables 1, 2 and 3 show the LC_{50} values for EF on *T. castaneum*, *R. dominica* and *S. zeamais*. At the same temperature, LC_{50} values of for each of the three species of insect were not significantly different ($P > 0.05$) at specific treatment times (Tables 1, 2 and 3), showing that EF had a rapid insecticidal ability and suggesting that all the ethyl formate had been lost (destroyed) within 24h.

The LC_{50} values of EF for *T. castaneum*, *R. dominica* and *S. zeamais* were 25.97, 12.50 and 7.45μL/L respectively, for 24h at 24℃, showing that *T. castaneum* was the most tolerant of the three species to EF.

The effect of Temperature to Fumigation activity of EF

Table 4 showed the observed mortalities with selected initial concentrations of EF of the three insects at different temperatures for 24h exposure. Lower mortalities to the selected dosage were observed at higher temperatures. This trend was statistically significant for each of the species tested ($P < 0.05$).

Discussion

Rapid Toxicity of EF

EF has a rapid impact on the test insects, giving high mortality within 24h in the absence of grain. No increase in effectiveness was observed when the exposure was extended to 72 h (Tables 1, 2 and 3). Damcevski et al.[5] studied that the potential of EF for use as a rapid disinfectant giving very high mortality (99%) in adult insects and high mortality (95%) in other stages with a 24h exposure period; and Aharoni et al.[6] found that a 3 h fumigation of grapefruit infested with the California red scale, *Aonidiella aurantii* (Maskell), at a concentration of 1.5% EF resulted in 100% mortality of all stages of the insect. In comparison, fumigation with phosphine needs 7 – 10 days. EF has great potential for grain fumigation[13].

The Effect of Temperature to EF

Generally, fumigants are more effective at higher temperatures. The volatility of the chemical is greater and the respiration of insects is higher. Consequently, it is easier to kill the insects. The experimental results, given here, showed that the efficacy of EF was better at relatively lower temperature in empty containers, which is consistent with the results of Tang et al.[13]. We suggest that the physiological state of insects is poor in the relatively low temperature. Despite the lower volatility of EF, a smaller quantity of the toxicant is effective against the pests. Alternatively, the toxicant may be able to penetrate more easily into the body of the insects through the intersegmental membrane and other parts. The presence of grain may affect the fumigation efficiency of dosages of EF in other ways, such as penetration of EF through the grain bulk, the sorption of EF on the grain and so on. Temperature can directly affect these factors. The effect of temperature on EF toxicity that we observe in an empty container may thus be different from the results observed in the presence of grain by others, where effectiveness increases with temperature. Further research is required.

Table 1. LC_{50} values of EF to *T. castaneum*.

Temperature (℃)	Exposure time (hours)	LC_{50} (μL/L) (95% ConfidenceIntervals)	Slope	P
16	24	17.94 (17.57, 18.11)	0.60	0.54
	48	17.70 (17.31, 18.11)	0.55	0.80

Temperature(℃)	Exposure time(hours)	LC_{50}(μL/L) (95% ConfidenceIntervals)	Slope	P
	60	17.70(17.31,18.11)	0.55	0.80
	72	17.44(16.95,17.93)	0.43	0.54
24	24	25.97(24.90,27.44)	0.19	0.91
	48	25.18(24.28,26.29)	0.21	0.97
	60	24.51(23.63,25.51)	0.22	0.96
	72	24.36(23.48,25.33)	0.22	0.98
32	24	26.16(24.57,29.13)	0.15	0.14
	48	25.59(24.21,27.85)	0.18	0.11
	60	25.64(24.12,28.27)	0.17	0.08
	72	25.53(24.01,28.13)	0.18	0.06

Table 2. LC_{50} values of EF to *R. dominica*.

Temperature(℃)	Exposure time(hours)	LC_{50}(μL/L) (95% ConfidenceIntervals)	Slope	P
16	24	10.45(9.87,11.12)	0.37	0.64
	48	10.11(9.56,10.72)	0.40	0.85
	60	9.83(9.33,10.38)	0.45	0.93
	72	9.58(9.02,10.21)	0.36	0.57
24	24	12.50(11.90,13.22)	0.38	0.42
	48	11.81(11.22,12.51)	0.41	0.53
	60	11.81(11.22,12.51)	0.41	0.53
	72	11.70(11.12,12.37)	0.42	0.63
32	24	16.61(15.29,18.11)	0.20	0.12
	48	16.44(15.11,17.72)	0.20	0.11
	60	16.23(14.66,18.05)	0.16	0.08
	72	15.46(14.25,16.72)	0.15	0.51

Table 3. The LC_{50} values of EF to *S. zeamais*.

Temper – ature(℃)	Exposure time(hours)	LC_{50}(μL/L) (95% ConfidenceIntervals)	Slope	P
16	24	6.00(5.60,6.41)	0.60	0.21
	48	5.78(5.38,6.18)	0.61	0.18
	60	5.70(5.14,6.26)	0.60	0.12
	72	5.66(5.25,6.06)	0.61	0.27
24	24	7.45(6.84,8.03)	0.33	0.29
	48	7.16(6.54,7.73)	0.34	0.28
	60	7.01(6.36,7.59)	0.33	0.16
	72	6.53(5.49,7.35)	0.34	0.06
32	24	9.52(8.94,10.19)	0.33	0.60
	48	8.62(7.74,9.62)	0.20	0.71
	60	7.65(5.94,9.19)	0.17	0.07
	72	6.36(4.54,7.56)	0.20	0.10

Table 4. Mortalities to EF of three insects at different temperatures for 24h exposure[a]

Insect species	Fumigant concentration(μL/L)	Mortality		
		16℃	24℃	32℃
Tribolium castaneum	25	100 ± 0.00a	60.00 ± 6.67b	53.33 ± 11.55b
Rhyzopertha dominica	11	55.56 ± 7.70a	17.78 ± 7.70b	6.67 ± 6.67b
Sitophilus zeamais	8	97.78 ± 3.85a	64.44 ± 10.18b	28.89 ± 13.88c

[a] Results are the means ± SD(n = 3). Means within rows followed by the same letter are not significantly different(p > 0.05, LSD Fisher's multiple range test).

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0222

Alternatives to Methyl Bromide in Grain Management in Kenya

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Abstract: Methyl bromide is one of the most commonly used fumigants in the Kenya for disinfesting commodities in grain silos and warehouses. The grain industry needs to act quickly to find alternatives to methyl bromide for use on grain because it will soon be banned in all developing countries due to its ozone-depleting properties. Kenya has identified carbon dioxide as an effective substitute for methyl bromide. Fumigation with carbon dioxide in well-sealed grain silos has been found to be technically feasible. A minimum carbon dioxide concentration of 35% to 40% in air for 10 days is required for complete insect mortality. Phosphine is also used as a fumigant but its use requires careful practice to avoid pest resistance. Three insecticidal sprays consisting of organophosphates and pyrethroids also proved effective for short term grain protection against stored product pests. A recent workshop in different parts of Kenya discussed the options for storage of grain. In the future, farmers may receive a premium price for grain supplied from protected, farm-based storage facilities as this action will reduce the need for methyl bromide fumigation in centralised facilities.

Introduction

Losses in storage as a result of infestation by insects can be significant. For example, maize is one of the most important food crops for the majority of Kenyans. Losses in storage on the farms have been estimated to range from 6% to 30% (De Lima 1979; Anon 1980; Mutambuki and Ngatia 2003).

To prevent such losses and to maximise the harvest, Kenya has begun to implement an integrated pest control programme for grain handling and storage. There are a range of pest control methods available including traditional, chemical and fumigation methods.

Adequate storage facilities and regular inspection of stores help to reduce the need for frequent use of chemicals, which also reduces cost and chemical residues. However, when chemical intervention becomes necessary, it must be used effectively to maximise the prospects for pest control, as well as safely and responsibly to avoid hazards to humans. Pest control must also be economic which can be achieved through proper training and the implementation of efficient management procedures.

In this paper, we report on a range of options for grain storage in Kenya that can be replacements for methyl bromide, and the actions that are underway to implement them.

Grain Protection in Kenya

Methyl Bromide

Methyl bromide is a major fumigant used to control pests in agricultural commodities. It is both effective and efficient in the control of all known storage insect pests in Kenya. It is used to disinfest commodities in stacks under sheet and in sealed silos, warehouses, rail wagons, metal containers and ships.

However, in the early 1990's, methyl bromide was identified as an ozone-depleting gas by the Parties to the Montreal Protocol. As a result, it will be removed from the list of the few remaining products capable of preventing pest damage to food and other valuable commodities. With global pressure to reduce usage of methyl bromide, the major strategies were employed by NCPB.

When methyl bromide is used, adequate distribution of methyl bromide within large silos is only possible where a forced circulatory system is incorporated in the facility. The National Cereal and Produce Board (NCPB) is responsible for storage of maize and has used methyl bromide for many years with such a system. However, methyl bromide can leak through the concrete walls, piping and manholes as sealing the storage facility is difficult.

Our initial work has been to reduce any imports of methyl bromide for grain protection by reducing the dose required for disinfestation. For example, we showed that we could re-

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duce the dose by 38% from the current recommended application rate of 16.1 g/m^3 if the treatment was carried out at 30℃, rather than a lower temperature which was the practice.

The amount of grain imported as also been significantly reduced, which has reduced the quantity of methyl bromide needed for fumigation. Before grain liberalization in 1993, NCPB was a monopoly handling all cereals and scheduled agricultural produce throughout Kenya. This led to the importation of large quantities of methyl bromide to fumigate 12 million 90kg bags of commodities annually. The quantities of commodities handled by NCPB have gradually declined to about 4 million bags annually. The reduction in imports by almost 70% has reduced the need for methyl bromide correspondingly.

Phosphine

Phosphine is also used in Kenya for the disinfestation of commodities. Application techniques for phosphine are relatively easy and this makes it a popular product.

However, unlike methyl bromide, phosphine requires not less than 5 days to be effective in our climate. The need for long exposure period to lethal concentrations means gas tightness is essential. Failure to create gas tight facilities can eventually lead to persistent survival of insects during fumigation and hence the development of resistance in certain species. This is the biggest challenge that fumigant operators are facing as effective phosphine fumigation practices have not been implemented consistently.

Phosphine is also being used to fumigate small quantities in drums which are gas tight. While fumigating bag stacks under gas proof sheets, care must be taken to avoid gas leaks. Fumigation must thus be carried out properly in terms of correct dosage to preserve this single fumigant available through reduction of insect resistance.

Carbon Dioxide

Kenya has its own natural supply of carbon dioxide which is tapped from the ground by a Kenyan company known as Carbacid Ltd. The trials carried out in concrete silos in Kenya showed that fumigation with CO_2 is technically feasible and more cost-effective than phosphine. However, the silos must be well-sealed and the study also recommended pressure testing be part of silo maintenance.

The disadvantages in CO_2 fumigation is the large quantities required and high dosages during initial fumigation since gas must be absorbed by the concrete by a process termed carbonation. For successful fumigation, gas concentrations must be maintained at a minimum of 35% for at least 10 days.

Insecticidal Sprays

We also tested the ability of insecticidal sprays such as pirimiphos methyl, fenitrothion and permethrin to control insects in silo – stored grain. These treatments proved effective for short term protection.

Training on Alternatives to Methyl Bromide

The impending ban on the use of methyl bromide in the grain sector was the subject of discussion during a two day workshop entitled "*Alternative to methyl bromide in post harvest grain management at farm level in Kenya*".

The workshops were held in Western and Eastern parts of Kenya covering most of the adjoining districts. The aim of the workshop was to raise awareness of the impending ban of methyl bromide and to explore options to replace it.

The workshop focused on the available alternatives such as phosphine and CO_2. It was emphasized that fumigation with CO_2 is technically feasible but required high doses and extended exposure period. For silo fumigation, pressure testing is a prerequisite to the use of CO_2.

The workshop further focused on appropriate treatment at farm level. If all the grain is protected, the need for immediate fumigation after reaching the central storage system would not be warranted. The result would be a reduction in the number of fumigations carried out in Kenya thereby reducing methyl bromide use in Kenya. The treated grain could attract a price premium which would be an incentive to farmers to protect stored grain.

Conclusions

The implementation of alternatives to methyl bromide such as CO_2, with procedural modifications where required and increased training of stakeholders, will reduce methyl bromide use.

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SESSION 3

SAFETY OF PRODUCTS UNDER CA AND FUMIGATION (EFFECTS ON QUALITY OF STORED PRODUCTS), PROTECTION OF THE ENVIRONMENT, HUMAN HEALTH ISSUES

Chairpersons:
An Yulin, China
Dagmar klementz, Germany

0301

Integrated Storage Pest Management System by Application of Warehouse Neem(Brand :Wellsto)(Azadirachtin 1 500 ppm min.)

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Introduction

Food grains constitute the bulk of the diet of human beings. The stored foodgrains require to be protected from the ravages of insect pests through application of various insecticides. The insecticide resistance combined with need for residue free food has prompted development of alternative methods of insect control. The development of resistance in eight insect pests of stored grain to Malathion 50% E. C. and other commonly used insecticide has been noticed (Madhumathi,1997). This calls for the need to consider the use of biorational or low risk insecticides such as neem formulations as a component in stored grain pest management. The effectiveness of plant derivatives against stored product insect has already been demonstrated, among which the extracts of Neem(*Azadirachta indica* A. Juss) have received more attention. The pesticidal and medicinal properties from the neem tree have been exploited for atleast the last 2 500 years. Neem oil is also known to be active against 400 insect pests.

Ali et al. (1983) reported that neem oil at 0.5 ml. Per 100 grams caused more than 50% mortality and at higher dosages of 1 mL. per 100 gram,cent percent mortality in *Callasobruchus chinensis* . In a study by Devi etal(2004) on *Callasobruchus chinesis* . ,it was noticed that 0.03% Azadirachtin affected the fecundity,fertility of eggs and development of Larvae. Mukherjee and Rama Chandran(1989) reported that Azadirachtin incorporated in the wheat flour at upto 10 ppm reduced growth and survival of the larvae in case of *Tribolium castaneum* at 1 ppm and above. Study carried out by Lakwah and Kashlan 1999 observed 100% mortality of *Sitophilous oryzae* at all tested concentration between 20 – 1 000 ppm 14 days post treatment. Mortality of *C. . maculatus* adults reached 38.4%,92.2% and 97.8% at concentrations of 50ppm,500ppm and 1 000 ppm one day after the treatment and from 97.8 to 100% at 50 – 1 000 ppm after 7days. Cent percent mortality of *Tribolium castaneum* at concentration of 1 000 ppm after 14 days treatment was noticed.

The Azadirachtin($C_{35}H_{44}O_{16}$) is a chemical extracted from the neem seeds. This has got a broad spectrum activity. . Neem pesticide being safe and harmless to non target and beneficial organisms like pollinators. Honey bee etc. was evaluated against Malathion,in a collaborative project between Central warehousing corporation, ITC Limited and Indian Grain Storage Management & Research institute ,to determine its suitability under Integrated Pest Management System.

The Project was Divided Intofollowing Stages

(Ⅰ)**Bio efficacy studies** carried out at Acharya N. G. Ranga Agricultural University (ANGRAU), Baptala(A. P) in a joint project with ITC Limited,GUNTUR

(Ⅱ)**Bio efficacy studies** at Indian Grain Storage Management & Research Institute(IGMRI) Hapur in association with Central Warehousing Corporation and ITC Limited,GUNTUR

(Ⅲ)**Field trials** at Central Warehouse Anakapally(A. P.). jointly by central warehousing corporation,I. T. C. GUNTUR and Indian Grain Storage Management & Research Institute (IGMRI) Hapur/hyderabad

(Ⅳ)**Residue analysis** of wheat stocks treated with Malathion and Warehouse Neem (Wellsto) and

(Ⅴ)**Baking test** of Chapathi (Indian Bread) made from wheat samples treated with Malathion and Warehouse Neem (Wellsto) through reputed laboratories.

(Ⅰ)**STAGE – 1:Study on Bioassay of Neem at Acharya N. G. Ranga Agricultural University(Angrau):**

A collaborative project of(1)*Central Warehousing Corporation*,(*A Government of India undertaking*),*New Delhi*(2)*Indian Grain Storage Management and Research Institute*(*IGMRI*),*Ministry of Consumer affairs*,*Food & Public distribution*,*Government of India*,*New Delhi &*(3)*ITC Limited ILTD Division*,*Guntur.*

Methods

The test insects included *Tribolium castaneum* (*Herbst*), *Callasobruchus chinensis* Linn., *Rhizopertha dominica* (Fabricius), *Corcyra cephalonica* (Stainton), *Cryptolestes ferrugineus* (Stephens), *Oryzaephilus surinamensis* and *Lassioderma serricorne*. The population of these insects were collected from various locations of CWC in the State of A. P. and reared under laboratory conditions. Series of concentration was prepared by using sereal dilution techniques from the commercial preparationof warehouse neem (Wellsto) provided by ITC Limited. The wide range of concentrations were tested followed by a narrow range of concentrations to get mortality in the range of 10 to 90%.

In the present study the LC_{50} values of Warehouse Neem (Wellsto) ranged from 0.00017 to 0.0004% for the above mentioned stored grain insect pests. The LC_{80} values of Warehouse Neem (Wellsto) ranged from 0.00158 to 0.0082% for the above mentioned stored grain insect pests. The LC_{90} values of Warehouse Neem (Wellsto) ranged from 0.0012 to 0.00757% for the above mentioned stored grain insect pests. Warehouse Neem (Wellsto) found to be 39.88, 31.89 and 27.26 times more toxic than malathion against *T. castaneum*. Since Warehouse Neem (Wellsto) has showed better toxicity than Malathion to which many of the stored grain pests have developed resistance, it can be better used as a tool in the management of stored grain insect pests.

(Ⅱ) STAGE – 2: Bio Efficacy Studies at Indian Grain Storage Management & Research Institute (Igmri) Hapur.

Methods

Malathion 50% EC at a normal dosage of 1:100dilution with water @ 3Lit./100 Sq. m was compared with Warehouse Neem (Wellsto) Azadirachtin 1 500ppm using 3 dosages (40, 50 and 60mL./litre of water @ 3Lit./100Sq. m. The statement of corrected mortality percentages on an average of 3 replications of ITC's Warehouse Neem azadirachtin 1 500 ppm (min.)) (Brand Wellsto) and Malathion 50% E. C. formulations are presented in Tables 1 and 2 below respectively.

Table 1. Warehouse Neem (Wellsto) (Azadirachtin 1 500 ppm (min.))

S. No.	Concentration	Period afterSpray	*Sitophilus oryzae*	*Rhizopertha dominica*
1	40 mL/Litre (1.8 mg a. i./Sq. m)	60 minutes	1.6%	51.7%
2	50 ml/Litre (2.25 mg a. i./Sq. m)	60 minutes	70%	100%
3	60 ml (2.7 mg a. i/Sq. m)	60 minutes	80%	100%

Table 2. Malathion 50% E. C.

S. No.	Concentration	Period afterSpray	*Sitophilus oryzae*	*Rhizopertha dominica*
1.	Malathion 150 mg a. i./Sq. m (10 ml/litre).	60 minutes	68.3%	60.9%

From the above tables, it is observed that at a concentration of 1.8 mg a. i./Sq. m (40 ml/litre) of Warehouse Neem (Azadirachtin 1 500 ppm (min.)) (Brand Wellsto), the corrected mortality of *Rhizopertha dominica* was at 51.7% comparable with 60.9% mortality of the same insect with Malathion 50% E. C. at 150 mg a. i./Sq. m (10 mL/Litre). At higher concentration of Warehouse Neem i. e., 2.25 mg and 2.75 mg of a. i/Sq. m., the corrected mortality rates of *Rhizopertha dominica* and *Sitophilus spp.* were higher than 150 mg a. i./Sq. m of Malathion 50%. Especially in case of *Rhizopertha dominica*, the corrected mortality was 100% in both the higher concentrations. Warehouse Neem (Azadirachtin 1 500 ppm (min.)) (Brand Wellsto) had considerable impact over *Rhizopertha dominica* at lower concentrations i. e. 1.8 mg a. i./Sq. m (40 ml/Litre). However, higher concentrations of the same formulation showed better mortality rates in case of *Sitophilus oryzae*.

(Ⅲ) STAGE – 3: Field Trials at Central Warehouse Anakapally (East Coast of Andhra Pradesh State) Jointly by Central Warehousing Corporation, ITC Limited, Guntur and Indian Grain Storage Management & Research Institute (IGMRI) HA-

PUR/HYDERABAD

The field studies(Surface & space spray) were carried out at Central warehouse – Anakapally(AP). Two compartments of the godown belonging to CWC comprising of minimum of 6 stacks(150 – 200 metric tones) were selected for the studies. Freshly procured wheat, packed in jute bags, untreated with any of insecticide were selected for trials. 6 stacks from one of the compartment were treated with Warehouse Neem (Azadirachtin 1 500 ppm (min.)) (Brand Wellsto) and 6 stacks in the second compartment were treated with Malathion 50% EC(500 000 ppm).

The stacks were left without any prophylactic/fumigation operations so that crawling infestation appear prior to initiation of the study. The stocks were fumigated as per normal schedule(i. e. up to 3 fumigations in a year) as per requirement.

Dosages

The dosage of Warehouse Neem (Azadirachtin 1 500 ppm(min.))(Brand Wellsto) formulation was recommended by ITC Limited @ 40 ml./litre of water, the solution of 3 litre sprayed per 100 m^2 of surface area. The prescribed dosage for Malathion 50% E. C. @. 10 ml/litre of water, the solution of 3 litre sprayed per 100 m^2 surface area used in the compartment under control.

Record of Observations

6 stacks were selected for the purpose of making observations to count insect population before and after the treatment. Each stack was marked with 5 spots of an area of 0. 25 sq. mtr., (50 cm × 50 cm) @ one each on all four sides and one at the top of the stack. Thus, 6 stacks selected for study had 30 spots for recording observations, which were labeled as S – 1 to S – 30. Similarly, 10 spots were selected on the floor, which were marked as F – 1 to F – 10. 10 spots were also marked on the walls, which were marked as W – 1 to W – 10. The data sheets for all these 50 spots each for the control compartment (Malathion 50% EC) and the experimental compartment (Warehouse Neem (Wellsto) Azadirachtin 1 500 ppm. (min.) were prepared with details of insect population before and after the treatment. In order to ensure representative sampling, these spots were marked at different lengths.

Frequency of Treatment

The neem preparation Warehouse Neem Azadirachtin 1 500 ppm (Brand : Wellsto) was sprayed in the experimental compartment at fortnightly cycle as in the case of malathion 50% E. C. The experiment was carried out for approximate 10 – 12 months period.

Data Recording

Mortality of insects on the marked spots were recorded at an interval of 1, 3, 7 and 15 days from the date of spraying. The scientist from IGMRI – Hapur/Hyderabad recorded data in association with CWC & ITC on 1st, 3rd, 7th and 15th days of initial spray. The data for the study period was recorded by all officials involved in the trials.

Statistical Analysis and Interpretation

Relavent statistical tools are used for compiling and analysis of data as given below.

Table 3. Field trials Anakapalli: Test on average population(statistical t – test' at 95% level of confidence) compared to Malathion:

PARAMTERS CONSIDERED	HIGHER/EQUAL/LOWER
Live population at 0th day	Equal
Overall live population on Stacks (18 cycles)	Equal
Overall dead population Stacks (18 cycles)	Lower
Overall live population on Walls (18 Cycles)	Equal
Overall dead population on Walls (18 Cycles)	Equal
Overall live population on Floor (18 Cycles)	Equal
Overall dead population on Floor (18 Cycles)	Lower
Tribolium – Stacks + Walls + Floor – 18 Cycles – Live	Equal
Tribolium – Stacks + Walls + Floor – 18 Cycles – Dead	Equal
Rhizopertha – Stacks + Walls + Floor – 18 Cycles – Live	Equal
Rhizopertha – Stacks + Walls + Floor – 18 Cycles – Dead	Lower
Ephestia – Stacks + Walls + Floor – 18 Cycles – Live	Equal
Ephestia – Stacks + Walls + Floor – 18 Cycles – Dead	Equal

Table 4. Field trials Anakapalli: Test on variation ('ANOVAF test' at 95% level of confidence) compared to Malathion:

PARAMTERS CONSIDERED	HIGHER/EQUAL/LOWER
On Stacks (Live population) Between Treatments	Equal
On Floor (Live population) Between Treatments	Equal
On Walls (Live population) Between Treatments	Equal
On Stacks (Dead population) Between Treatments	Equal
On Floor (Dead population) Between Treatments	Equal
On Walls (Dead population) Between Treatments	Equal

Table 5. Field trials Anakapalli: Analysis of Pest infestation in Wheat Samples (Chi – Square test at 95% level of confidence) compared to Malathion:

PARAMTERS CONSIDERED	HIGHER/EQUAL/LOWER
Clear Infestation	Equal
Few Infestation (up to 2live insects/kg)	Equal
Heavy Infestation (> 2live insects/kg)	Equal

The above statistical analysis with several parameters showing equality both in mean and variance analyses in case of Warehouse Neem (Wellsto) (Azadirachtin 1 500 ppm) and Malathion, converge to the point that Warehouse Neem (Azadirachtin 1 500 ppm) efficiency is not less than that of Malathion amongst several key parameters and also in some cases, superior in terms of lower variances. This leads to the statistical conclusion that Warehouse Neem (Azadirachtin 1 500 ppm (min.)) (Brand Wellsto) can be included in Integrated Storage Pest Management. This statistical statement is validated by the above tests at 95% level of confidence.

(Ⅳ) STAGE – 4: Residue Analysis of Wheat Stocks Treated With Malathion and Warehouse Neem (Wellsto).

The Wheat samples from both the compartment of CWC Anakapalli Warehouse where field trials with Warehouse Neem (Azadirachtin 1 500 ppm (min.)) (Brand Wellsto) and Malathion 50% E. C. were subjected for evaluation of residues at Indian Institute of Chemical Technology, Hyderabad and CFTRI Mysore The results are presented in the table below.

Table 6. Chemical Residue analysis in Wheat samples.

CHEMICAL	ANAKAPALLI	TEST CARRIEDOUT BY IICT, CFTRI
Malathion	1.98 ppm	CFTRI, Mysore
Azadirachtin	NIL	IICT, Hyd

Perusal of the above residue analysis table clearly shows that there are absolutely no traces of Azadirachtin, active ingredient of Warehouse Neem (Azadirachtin 1 500 ppm (min.)) (Brand Wellsto) vis – a – vis Malathion 50% E. C. where the residues were observed in the Wheat samples collected from the control compartment.

(Ⅴ) STAGE – 5: Baking Test of Chapathi (Indian Bread) Made from Wheat Samples Treated with Malathion and Warehouse Neem (Wellsto)

The flour of Wheat samples taken from both the compartments of CWC, Anakapalli where field trials were conducted with Warehouse Neem (Azadirachtin 1 500 ppm (min.)) (Brand Wellsto) and Malathion 50% E. C., were analysed for any impact of bitter compounds on the baking quality and other quality parameters as per the Prevention of Food Adulteration Act (PFA, 1954).

Table 7. Baking test of chapathi (Indian bread)

S. No	Parameters	Scale/Unit	Warehouse Neem (Azadirachtin 1 500 ppm (min.)) (Brand Wellsto) (B2 Compartment in CWC Anakapalli)	Malathion 50% E. C (B3 Compartment in CWC, Anakapalli)
1	Appearance	10	8.5	8.0
2	Tearing strength	10	9.0	8.5
3	Pliability	10	8.5	8.5
4	Aroma	10	9.0	8.5

S. No	Parameters	Scale/Unit	Warehouse Neem (Azadirachtin 1 500 ppm (min.)) (Brand Wellsto) (B2 Compartment in CWC Anakapalli)	Malathion 50% E. C (B3 Compartment in CWC, Anakapalli)
5	Eating quality	10	9.0	8.5
6	Overall quality	10	44.0	42.0
7	Shear force	g	770	650

Table 8. Evaluation of Wheat Flour Based on Prevention of Food Adulteration Act(PFA,1954)

S. No	Parameters	Warehouse Neem) (Azadirachtin 1 500 ppm (min.)) (Brand Wellsto) (B2 Compartment in CWC Anakapalli)	Malathion 50% E. C(B3 Compartment in CWC, Anakapalli)	PFA Specification-sA. 18.01	Test Method
1	Moisture,%	9.6	10.0	Max:14.0	AOAC 17th Edn. 2000,925.10
2	Total ash,% (on dry weight basis)	1.7	1.8	Max:2.0	AOAC 17th Edn. 2000,923.03
3	Acid insoluble ash in dil HCl,% (on dry weight basis)	Not detected	Not detected	Max:0.15	AOAC 17th Edn. 2000,941.12
4	Gluten,% (on dry weight basis)	9.1	9.2	Min:6.0	IS:1155 – 1968
5	Alcoholic Acidity expressed as sulphuric acid,% (on dry weight basis)	0.13	0.13	Max:0.18	IS:1155 – 1968
6	Rodent hair and excreta	Not detected	Not detected	Should be free from Rodent hair and excreta	AOAC 17th Edn. 2000, 993. 26 & 943.06

On perusal of results of both the tests (baking quality of Wheat flour and other quality parameters as per PFA,1954), it is observed that samples from Warehouse Neem(Azadirachtin 1 500 ppm(min.))(Brand Wellsto) is on par with Malathion 50% E. C. treated samples.

Conclusion

In light of concerns over pesticide residues in agricultural produce coupled with increasing pest resistance to chemical pesticides, there is an immediate need for developing or adopting effective alternative methods or natural pesticides which do not pose such serious negative impact. The concept of Integrated Pest Management(IPM), addressing the problems of insect resistance and pesticide residues, needs to be applied in Storage pest management also.

The results of the bio-efficacy trials conducted at ANGRAU, Bapatla show the target species *T. castaneum* has developed resistance to Malathion 50% E. C. and Warehouse Neem (Wellsto) is 40 times more effective than Malathion 50% E. C. in terms of efficacy. The trials conducted at IGMRI, Hapur show that Warehouse Neem(Azadirachtin 1 500 ppm(min.))(Brand Wellsto) is more effective against *Rhizopertha dominica* at 40 ml/litre and at higher concentrations 50ml/litre and 60 mL/litre it is effective against *Sitophilus oryzae*.

The field trials conducted at. CWC, Anakapalli, Andhra Pradesh, for a year clearly show that Warehouse Neem (Wellsto) (Azadirachtin 1 500 ppm) is on par with Malathion 50% E. C. in terms of pest management. The pest infestation(as per the standard guidelines) recorded in the stored product(Wheat grains) clearly show that the grain is well protected by Warehouse Neem (Wellsto) (Azadirachtin 1 500 ppm) during the entire one year study which was the objective of the study.

The overall quality of the grain, in terms of pest infestation, chemical residues, baking quality and other quality attributes as per the PFA, 1954, was maintained in good order even after one complete year of trial. This shows that the

product has no deleterious impact on the quality of the food grain and is a best fit in Integrated Pest Management System.

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0302

Mortality of Three Stored Product Pests Exposed to Sulfuryl Fluoride in Laboratory and Field Tests

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Abstract: Effectiveness of sulfuryl fluoride(SF) for the control of three stored pests was investigated as alternatives to methyl bromide(MB) and resistance to phosphine(PH_3). The toxicity test indicated that SF at low concentrations was effective to control *Sitophilus oryzae*(L.), *Rhyzopertha dominica* Fabricius and *Tribolium castaneum*(Herbst). In addition, 5.94g/m^3 SF mixed with 14.27g/m^3 CO_2 were used for fumigation of grain for 30d. The gas mixture was effective in the control of the adult pests and F_1 progeny without negative effects concerning the quality of treated wheat, long – grain non – glutinous rice and medium to short – grain non – glutinous rice.

Key words: Sulfuryl fluoride(SF), *Sitophilus oryzae*, *Rhyzopertha dominica*, *Tribolium castaneum*, gas mixture, CO_2

Introduction

Since about 1950, China has started to fumigate against stored grain pest insects to prevent losses. The fumigants included mainly chloropicrin, methyl bromide and hydrocyanic acid. Phosphine(PH_3) is in use as fumigant in China since about 1960. Methyl bromide(MB) and PH_3 have been developed to bethe main fumigants in the grain storage industry.

For a long time, PH_3 was widely used in grain storage because of special advantages in cost, efficacy, application method, low residual toxicity and worker safety; whereas, after long and exclusive use of PH_3, stored grain insects have increasingly developed resistance to this gas. Therefore, in some places many failures of fumigation occurred after using PH_3 to kill insects. PH_3 fumigation requires improved gas tightness of the fumigated enclosure, temperature higher than 15℃, and longer treatment time. Therefore, many grain stores, especially, those with old structures and lower gas tightness preferred to use methyl bromide fumigation instead. However, with greater awareness of environmental protection, methyl bromide was eliminated because of its ozone depleting property to the atmosphere. According to the International Montreal Protocol and related conventions, developing countries were demanded to accept the phase out of methyl bromide in 2015. The Chinese government decided that the grain storage industry of the whole country must not use methyl bromide from January 1, 2007 onwards. At present, the national stored grain insect prevention fumigation mainly relies on PH_3. Using PH_3 for a long time resulted in stored grain insects with fairly high resistance to PH_3. Therefore, it is necessary to study and develop a new chemical, which is efficient, has low mammalian toxicity, does not lead to pollution and will preferably not cause resistance.

Sulfuryl fluoride(SF) is a toxic gas and has been used since 1960 for controlling various insects in wooden buildings especially against termites. It is a universal insecticide and rodenticide. Its mode of action consists in the release of fluoride ions to restrain the circulation of glycolysis and fatty acids, which deprive the necessary energy for the survival of insects. In July 2005, SF was registered in the United States of America, being used as fumigant for the control of stored grain insects. In the beginning of 2008, it was approved for use as fumigant against stored grain insects in China. The fumigant is targeted for the wide use of grain fumigation in China. This paper reports on results of the experiments in providing additional theoretical and practical knowledge for the wide use

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of this new gas for insect pest control.

Materials and Methods

Test place

Toxicity test: Tests were carried out in the Chengdu Grain Storage Research Institute.

Field test: Storehouse No. 29 at Gaomi Grain Depot, which has horizontal warehouse, with brick structure, door and window air-proofed by sheet. The warehouse has a volume of 5 000 m^3, with 3 280 m^3 storage capacity of grain. The gastightness was tested to hold for 40s the pressure decay from 500Pa to 250Pa, which is the national standard for fumigation. The warehouse is equipped with aeration ducts and it has computerized temperature monitoring systems.

Tested Grain

Tests were carried out on 2 500 tonnes of wheat produced at Shandong province, which was stored for two years, with impurities of 5%, moisture content 11.3%. The temperature of the warehouse was 31.6℃, relative humidity 66%, average temperature of grain was 19.3℃, the lowest grain temperature was 12℃, long-grain nonglutinous rice and medium to short-grain nonglutinous rice produced at Sichun province was placed on the surface of the grain in the warehouse.

Tested Insects

ⅰ) Toxicity test

Two weeks old adults of susceptible and phosphine resistant strains of *Sitophilus oryzae*, *Rhyzopertha dominica and Tribolium castaneum* were used for toxicity tests.

ⅱ) Field test

Tested insects were eggs, larvae, pupae and adults of susceptible and phosphine resistant strains of *Sitophilus oryzae*, *Rhyzopertha dominica* and *Tribolium castaneum*. Every cage contained the four developmental stages of the tested insects. The cages were made of nylon meshwork about 15cm(5cm. There were a total of 54 cages used in these tests. Each cage contained 5 g food and 50 adult insects. Each test was carried out on 9 cages containing susceptible and resistant strains of insects.

Fumigant

The SF concentration in the cylinder was 99.5%, that of CO_2 in the cylinder was 99.5%. The ratio of SF: CO_2 used was 1:2.5, the dose of SF was 5.94 g/m^3 and the dose of CO_2 14.27g/m^3 was used in the mixture.

Equipment

For field tests, caged insects of 15 cm × 5 cm made of nylon meshwork that enabled penetration of the gas were used. For toxicity tests in the laboratory, phosphine was generated on – site and desiccators equipped with recirculation units were used.

Experimantal Methods

Toxicity Test

Laboratory tests to determine the LC_{50} and $LC_{99.9}$ of SF values exposed for 20h and 48h were carried out for *Sitophilus oryzae*, *Rhyzopertha dominica* and *Tribolium castaneum* using the FAO method [1].

Field Test

The field test was carried together with the Chinese Academy of Inspection and Quarantine, Gaomi Grain Depot of Shandong Province and Longkou City Chemical Plant.

ⅰ) Placement of cages with test insects

There were a total of 54 cages that consisted of 3 insect species, 2 strains, 3 test points in the warehouse, and 3 depths in the grain bulk. The three points selected to place the tested insects were in the middle, northeast corner and southwest corner, and every point was tested at three depths of 0.2m, 2.5m and 3.5m deep below the surface.

ⅱ) Fumigation

The fumigation was carried out with a mixture of SF with CO_2. The ratio SF: CO_2 was 1: 2.5, the dose of SF was 5.94 g/m^3 and the dose of CO_2 14.27 g/m^3. At the beginning, the fumigants were supplied and recirculated for 4h with the recirculation unit. Subsequently, each third day the gas was recirculated for 2h. The fumigation lasted 30d.

ⅲ) Quality of grain

Germination and other quality parameters were determined after the fumigation of wheat and paddy.

Results

Toxicity Test

The LC_{50} and $LC_{99.9}$ values of SF to control *Sitophilus oryzae*, *Rhyzopertha dominica*, *Tribolium castaneum* using the FAO method[1] for 20h and 48h are shown in Table 1.

Table 1. Response of adult *Sitophilus oryzae*, *Rhyzopertha dominica*, *Tribolium castaneum* towards exposure to SF (25℃ and R. H. 70%).

Strain		Time (h)	LC_{50} (g/m^3)	$LC_{99.9}$ (g/m^3)	Ct (gh/m^3)	dF(degree of freedom)	CHI SQ(95% confidence index)	Reference values
Sitophilus oryzae	S*	20	1.39	3.48	69.60	8	6.17	15.507
		48	0.73	2.06	98.88	6	10.06	12.592
	R*	20	1.27	4.68	93.60	8	2.53	15.507
		48	0.63	1.23	59.04	2	5.47	5.991
Rhyzopertha dominica	S	20	1.06	1.72	34.40	4	2.66	9.488
		48	0.57	0.89	42.72	3	3.75	7.815
	R	20	1.05	1.57	31.40	3	6.28	7.815
		48	0.56	0.94	45.12	5	3.96	11.070
Tribolium castaneum	S	20	2.04	3.06	61.20	6	9.71	12.592
		48	0.88	1.29	61.92	7	3.25	14.067
	R	20	1.97	3.14	62.80	6	7.17	12.592
		48	0.80	1.05	50.40	5	9.75	11.070

*: S: susceptible strains, R: phosphine resistant strains

Field Test

SF concentration in field fumigation

Gas concentrations were not measured in the field tests because of lack of portable gas analyser. But gas samples were taken to the laboratory and the concentrations were measured using gas chromatograph equipped by electron – capture detector. The gas concentration after 11 days of fumigation was 77.078 mg/m^3, and after 30 days it was 0.0367 mg/m^3. It showed the SF concentration is very low after fumigating 11 days.

Effect of field fumigation on insects

Mortality of insects was tested 30d after fumigation, and the data are shown in Table 2.

Table 2. Response of SF fumigating in field.

Tested insect			Mortality(%)		
			southwest corner	Middle	northeast corner
adult	*Sitophilus oryzae*	S	100	100	100
		R	100	100	100
	Rhyzopertha dominica	S	100	100	100
		R	100	100	100
	Tribolium castaneum	S	100	100	100
		R	100	100	100
Count the number of adults hatched after 42d					
Eggs, larvae, pupae	*Sitophilus oryzae*	S	0	0	0
		R	0	0	0
	Rhyzopertha dominica	S	0	0	0
		R	0	0	0
	Tribolium castaneum	S	0	0	0
		R	0	0	0

*: S: susceptible strains, R: phosphine resistant strains.

Table 3. Influence of SF on the germinating capacity of the treated grain

grain		germinating capacity(%)			Average germination capacity(%)
		Replicate 1	Replicate 2	Replicate 3	
wheat	Prior to fumigation	96	96	94	95.7
	After fumigation	96	92	96	95
long – grain nonglutinous rice	Before fumigating	86	86	88	86.6
	After fumigating	86	86	86	86
medium to short-grain nonglutinous rice	Before fumigating	94	96	94	95
	After fumigating	94	94	96	95

Table 4. Influence of SF fumigation on quality of paddy

Item	medium to short-grain nonglutinous rice		long-grain nonglutinous rice	
	Prior to fumigation	After fumigation	Prior to fumigation	After fumigation
Brown rice yield(%)	86.1	85.3	78.8	78.7
Crude protein(% dry weight basis)	9.6	9.7	10.0	9.8
Paddy fatty acid value(KOH/dry basis)/(mg/100g)	20.3	19.0	17.7	19.0
Sensory evaluation(%)	87	87	86	85
Yellow-colored rice(%)	0	0	0	0
Colour and luster, odor	normal	normal	normal	normal

Table 5. Influence of SF fumigation on wheat quality

Item	Before fumigating	After fumigating
Gluten water absorption(%)	215	216
Crude protein(% dry basis)	15.0	13.7
sensory evaluation of steamed bread(cent/100)	84.0	82
sensory evaluation of bread(cent/100)	79	77
Viscosity(mm^2/S)	5.6	6.7

Discussion

The test indicated that the mixture of SF (5.94g/m^3) and CO_2 (4.27g/m^3) to fumigate for 30 d was effective to control insects pest in grain. The fumigation did not deteriorate the quality of treated wheat and paddy.

Compared with methyl bromide, SF has some advantages, such as its physical and chemical properties. The gas does not cause any damage to the ozone layer with better dispersion and infiltration through the fumigated products than methyl bromide. The American DOW Company used *Tribolium confusum* imagoes and large larvae of the black larder beetle to test the insecticidal effect of SF and MB[2]. The results showed that the dosage of SF was less than that of MB under the same conditions. Other research showed that mixed SF and MB had a better effect to control pupae and eggs of the khapra beetle[3]. As for the toxicity on higher animals, SF has not teratogenic, carcinogenic, mutagenic effects[3,4,5].

Additional research using SF on wheat and paddy did not show deleterious influence on their germination rate or their quality, and it could cause complete mortality of adults, larvae and eggs of the tested insects.

Therefore, SF after the phase out of MB,

seems to be a suitable fumigant for the grain storage industry,. The implementation of SF would not only provide a new technology for grain storage,but also consolidate the achieved success after removal of MB from the market and prevent the grain storage industry use of MB again.

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0303

Phosphine Dosimeter Tubes A Alternate Approach for Fumigation Monitoring

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Abstract: To ascertain the adequacy of fumigation as well as to comply with the regulatory norms one has to monitor the phosphine concentration from the beginning to the end of fumigation which can last five to seven days. When aluminium phosphide tablets are used in an enclosure to generate phosphine the phosphine concentration starts building up from zero and reaches a maximum and starts declining from the peak value with time. To calculate the total phosphine dose (which is the most important parameter) received by the food grains, it is necessary to make several measurements during the entire period of fumigation. The phosphine dose is then determined by by calculating the area under the curve phosphine concentration v/s time.

As an alternative to making several measurements for measuring the total dose, we have developed a phosphine dosimeter tube, which does the same job with a single measurement. For this purpose, a calibrated freshly opened phosphine dosimeter tube is kept at a suitable place inside the enclosure at the beginning and the phosphine dosage in ppm h is read at the end of fumigation on the dosimeter scale.

The total phosphine dosage received by the food commodities is governed by the phosphine loss due to leaky enclosures, the amount of aluminium phosphide used, its quality, and the total time of exposure. Hence the total dosage measured on the dosimeter tells about the adequacy or other dosage related factors of fumigation and can throw light on the underlying cause for the eventual failure of a fumigation like substandard amount of aluminium phosphide tablets, leaky enclosure, or inadequate time of exposure etc.

The paper will describe the method of calibration of dosimeter tubes and their various uses in fumigation experiments.

Key words: phosphine dosimeters, fumigation monitoring

Introduction

Phosphine is one of the most widely used fumigant for disinfestation of cereals, tobacco, spices and other stored products. Fumigation is carried out generally by producing Phosphine (PH_3) in situ by dosing the fumigation enclosure with calculated amounts of aluminium phosphide. The aluminium phosphide reacts with the atmospheric and grain moisture to produce PH_3. When this method is followed the PH_3 concentration starts building from zero and attains a maximum in about 24 hours and then starts falling as the PH_3 generation stops while the leaks from the enclosure and the absorption of PH_3 in the grain and on other sources of losses continue to bring down slowly the PH_3 concentration. The concentration profile of PH_3 starting from zero and going through a peak and its subsequent continuous fall is governed by several factors like total aluminium phosphide used, the total volume of the enclosure, amount and nature of the commodity inside, the moisture content in the stored products, the leak rate from the enclosure, the absorption characteristics of the commodities etc.

Fumigation procedure requires a certain concentration profile to be maintained during the entire period of fumigation to ensure that the fumigation is adequate to effect total insect mortality. The insect mortality is governed by an entity called the fumigation dose' or PH_3 dose which is defined as the concentration time product expressed in ppm hrs or ppm days. It is not the PH_3 concentration only at any time or the time of exposure alone which determines the adequacy of the fumigation but it is the total dose expressed in ppm hrs which determines the insect mortality or the effectiveness of the fumigation. Hence in order to determine the adequacy of the fumigation, it is necessary to measure the total

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PH_3 dose received by the food products.

The concentration time product,by its very definition turns out to be the area under the curve obtained by plotting the concentration Vs time. If this quantity is to be measured when the concentration is not constant but varies over a wide range starting from zero,it is necessary to make several,on-the-spot measurement of concentration at regular intervals over the entire period of fumigation to generate the concentration Vs time curve to find out the area under that. This involves several measurements and it is time consuming. Hence an alternate method which can directly measure the total dose received by the food grains by a single measurement if available is ideal for this application.

The dosimeter tube described in this paper does that job and measures the total dose received by the stored products during the entire course of fumigation with a single measurement. This paper describes this dosimeter tube including its working principle, calibration method and its uses for fumigation.

Experimental Method

The dosimeter tube consists of a narrow glass tubing of about 4mm OD and 3mm ID with a length of about 15cm filled with PH_3 sensing chemical. Both ends of the tube are sealed with round tips. The sensing chemical occupies 8 to 10cm of the tube beginning from one end. At the other end of the sensing chemical,a plug is placed to hold it in position. There is a scratch mark immediately after the plug for facilitating the breaking of the tube before using.

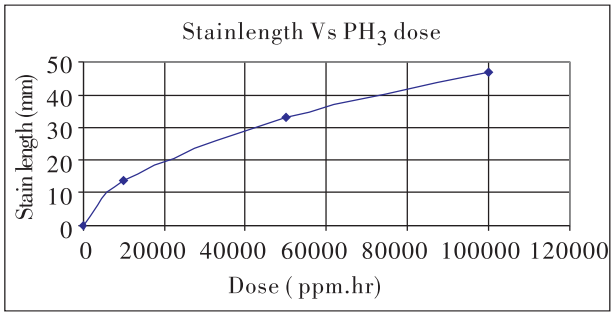
Working Principle

The dosimeter tube works on the diffusion of PH_3 gas into the sensing chemical in the tube and on the reaction with it producing a colored stain when the tube is placed in the environment containing PH_3. The tube has to be broken at the scratch mark before it is placed in the PH_3 environment for measurement. The diffusion through the tube follows the Fick's law of diffusion and the stain length produced depends on the concentration and time of the fumigation,which can be expressed as the concentration time product or the dosage.

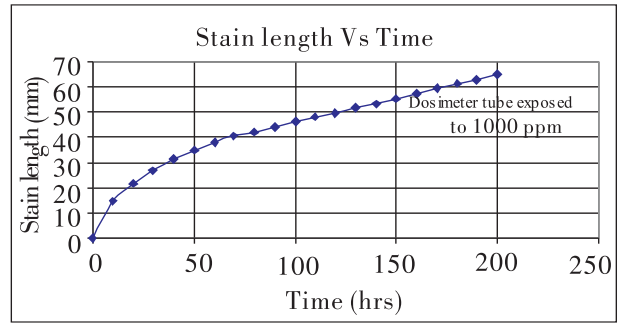
It should be noted,a PH_3 dosage of any magnitude can be produced by several combinations of concentration and time,and for all the combinations of time and concentrations for the same dosage,the same stain length is obtained within experimental errors. This is illustrated in Table 1 and Graph 1.

Table 1. Stain length Vs Phosphine Dose

Conc. [ppm]	Time [h]	Dosage [ppm h]	Stain length on dosimeter tube [mm]	Average stain length [mm]	Average standard deviation
50	200	10000	14.2		
100	100	10000	14.0	13.7	4.3%
200	50	10000	12.5		
1000	10	10000	14.0		
250	200	50000	33.0		
500	100	50000	34.5	32.9	2.7%
1000	50	50000	32.0		
5000	10	50000	32.0		
500	200	100000	42.0		
1000	100	100000	48.0	46.9	5.4%
2000	50	100000	49.0		
10000	10	100000	48.5		



Graph 1 **Stain length Vs PH_3 Dosage**



Graph 2. **Stain length Vs time**

Calibration

The dosimeter tube can be calibrated by using the stain length for different dosages and marking them on the tube for different doses. Refer Graph 1.

Alternatively, dosimeter tube can be exposed to a constant concentration of 1 000 ppm (or any other suitable concentration) and the stain length obtained ploted for different time intervals (refer graph 2). The dosimeter tube is marked with time t = 0 to t = 200 hours. Reading the dosimeter tube (i. e. time) and multiplied by 1 000 when it was exposed to this gas content gives the dosage. This method can be used for calibration.

Table 2. **Stain length vs time for** 1 000 **ppm exposure**

Time h	Stain length on dosimeter tube (mm)	Time h	Stain length on dosimeter tube (mm)
0	0	110	48
10	14.5	120	49.5
20	22	130	51.5
30	27	140	53.5
40	31.5	150	55.5
50	35	160	57.5
60	38	170	59.5
70	40.5	180	61.5
80	42	190	63
90	44	200	65
100	46		

Application

Determination of the Adequacy of Fumigation

The dosimeter tube can be used to determine the adequacy or other important factorts of the fumigation. If the fumigators know the PH_3 dosage to be applied for the fumigation experiment, they can measure the actual dosage received by the commodity and compare it with the expected dosage. This can be carried out by placing the dosimeter tube at the beginning of the fumigation at a suitable location within the fumigation cover and taking it out after the fumigation and aeration and read the total PH_3 dosage received. The tube-before placing it inside-should be broken at the scratch mark and placed either horizontally or vertically.

In a silo of a fixed volume and for a fixed volume of stored products and a fixed dosage, one can expect the same stain length for repeated fumigations. If in any experiment the stain length obtained is much smaller than the average stain length which one used to get, it is a sure indication that the fumigation had failed. The failure could have happened because of leaks or inadequate amount of fumigant or substandard fumigant material used. This becomes a handy method for fumigators to ascertain the adequacy of fumigation and provide a proof of fumigation to their clients.

PH_3 Concentration Distribution

Many a time though, the AlP dosing is adequate PH_3 concentration distribution is not uniform for various reasons. At many places, the PH_3 concentration can be very very low, which is not adequate to kill the insects. The dosimeter tube can be used to find this non-uniform distribution by keeping several dosimeter tubes at different locations and measure the PH_3 dosage on the tubes placed at different locations. Non-uniform distribution will lead to widely varying stain lengths (dosages) on the dosimeter tubes placed at different locations.

To Provide the Proof of the Fumigation

The dosimeter tube which measures the PH_3 dosage can be used as a proof of fumigation and also to ascertain that it is carried out properly. The stain length on the tube remains the

same after it is taken out of the silo after the fumigation and can be produced as a proof.

Conclusion

This novel device should prove to be a very useful device for the applications already described in the paper.

0304

The Quality of Candle Nut (*Aleurites moluccana* (L.) Willd.) Stored under Controlled Atmosphere

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Abstract: The effects of carbon dioxide on fungal populations, lipid and free fatty acid (FFA) contents of shelled broken candle nut (*Aleurites moluccana* (L.) Willd.) were investigated together with moisture contents. As much as 300 g of shelled broken candle nut with initial moisture content of 5.4% were placed in a three-liter jar. CO_2 contents used were 10, 30, 50 and 70% in air, respectively. As control, air was incorporated into the jar instead of CO_2. The candle nuts were stored for 30, 60 and 90 days, respectively, at 25 – 28℃ and at relative humidity of 60% – 80%. Three replicates were used for each treatment and the control. The moisture contents were relatively constant during storage. The lowest moisture content (5.3% w. w) occurred at 70% CO_2 content after 90 days of storage, while the highest moisture content (5.5% w. w) was in the control after 60 days of storage. Total fungal population decreased with the increase of CO_2 contents. After 90 days of storage, the lowest total fungal population (6.7×10 cfu/g w. w) occurred at 70% CO_2 content, but it was not significantly different with that of 50% CO_2 content (4.5×10^2 cfu/g w. w) and 30% CO_2 content (1.2×10^5 cfu/g w. w). Although the lowest lipid content (46.38% w. w) occurred at 70% CO_2 content after 90 days of storage, it was not significantly different with that of 50% CO_2 content (51.20% w. w). FFA contents increased with the increase of CO_2 content. The lowest FFA content (2.94% w. w) occurred at 70% CO_2 content after 90 days of storage and it was not significantly different with that of 50% CO_2 content (3.93% w. w). Storage of shelled broken candle nuts up to 90 days can be carried out using 50% CO_2 content.

Key words: candle nuts, controlled atmosphere, quality

Introduction

In Indonesia candle nut is used especially for seasoning. The oil of candle nut is servesed among others for preparing soap, cosmetics, and medicines. Candle nut is exported in the form of rolling seeds, sound shelled seeds, and broken shelled seeds. Shipping in the form of rolling seeds needs big transportation space and reduces the capacity of exported candle nut, consequently shipping in the form of shelled seeds will be a good solution to overcome this limitation. Nevertheless, shelling of rolling seeds has a constraint, because the percentage of sound shelled seeds is low. Besides, the quality of shelled seeds could be easily reduced, because its lipid content is high and it is easily infected by fungi during storage. Dominant fungi infecting 19 candle nut samples collected in Bogor (West Java) and Yogyakarta (Central Java) were *Aspergillus flavus*, *A. niger*, *A. tamarii*, *A. wentii*, *Eurotium chevalieri*, *E. rubrum*, and *Penicillium citrinum*[1]. Carbon dioxide is used for insect control, but no study has been conducted on the effect of CO_2 on the quality of candle nut. The objective of this study was to investigate the change of broken shelled seed candle nut quality (moisture contents, fungal population, lipid and free fatty acid contents) stored under controlled atmosphere using CO_2.

Materials and Method

Preparation of Broken Shelled Candle Nut

Broken shelled candle nut seeds were derived from candle nut fruits. The fruit were collected from Camba district, Maros regency, South Sulawesi Province. Broken shelled seeds 2 4 were prepared for the experiment. The seeds were fumigated using phosphine (2 g/ton) for 7 days for disinsectisation all insect stadia that may exist.

CO_2 Treatment and Storage of Candle Nut Seeds

Twenty four hours after phosphine fumigation, 300 g broken shelled seeds (moisture contents 5.0 0.5%) were packed in a sac made from plastic net. They were then placed in a

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3litre glass jar which could be vacuumed and filled with atmospere with different CO_2 contents in air. The sac containing the seeds was supported by a perforated plastic container. CO_2 contents used were 10, 30, 50 and 70%, respectively. As control, air was incorporated into the jar instead of CO_2. The seeds were stored for 30, 60 and 90 days, respectively, at 25, 28℃ and 60, 80% rh.. Three replicates were used for each treatment and the control.

Sampling and Obtaining Working Sample

After the treatment, the seeds from each jar were stored subsequently for 30, 60, 90 days, respectively. Samples were taken after the different storage periods, mixed thoroughly, divided twice to obtain working samples for determination of moisture content, fungal population, lipid and free fatty acid content, and as a reserve sample.

Determination of Nut Quality

Moisture content, lipid and free fatty acid contents were determined based on BSI [2] and AOAC[3], respectively. Fungal population was determined based on a serial dilution method followed by pour plate method on Dichloran 18% Glycerol Agar [4]. The data were analyzed using Completely Randomized Design with one factor. The factor consisted of five CO_2 contents (including the control).

Results and Discussion

The Effect of CO_2 on Moisture Content

At the beginning of storage, the moisture contents of candle nuts in the control were not significantly different from those treated with CO_2 at 10%, 30%, 50% and 70% contents in air, respectively. The same results were obtained with the moisture contents of candle nuts after 30, 60 and 90 days of storage (Table 1). The moisture contents were still higher than those determined by the Indonesian National Standar (*Standar Nasional Indonesia* or SNI) (BSN)[5], e. g 5% w. b.

Although based on the analysis of variance, CO_2 contents did not result in any significant differences of the moisture contents of treated candle nuts. The moisture contents were relatively similar and constant during storage. Based on their averages, moisture contents decreased after 30 days of storage, increased again slightly after 60 days and decreased again slightly after 90 days of storage. The moisture contents were always in equilibrium with the relative humidity of the storage.

The Effect of CO_2 on Fungal Population

After 90 days of storage, 13 fungal species and one unidentified isolate were isolated from from the candle nuts. The 13 fungal species were *Aspergillus candidus*, *A. flavus*, *A. niger*, *A. penicillioides*, *A. wentii*, *Cladosporium cladosporioides*, *Eurotium chevalieri*, *E. repens*, *Hyphopichia burtonii*, *Penicillium citrinum*, *P. rugulosum*, *Syncephalastrum racemosum* and *Wallemia sebi*. The dominant species were *Aspergillus*, *Eurotium*, *Penicillium*, and *Hyphopichia*.

In tropical regions, the most spoilage fungi in storage were *Aspergillus* and *Eurotium*, while the role of *Penicillium* was less than the two previous genera[6].

Fungal population decreased with the increase of CO_2 contents (Table 2). It was presumably due to the decrease of O_2 contents. In general, fungi are aerob obligate organisms[7]. Aerob fungi need O_2 for their growth. CO_2 at 20% content in air started to inhibit mycelia, conidiation (sporulation) and conidial germination of *A. flavus*[8]. The four parameters were inhibited with the increase of CO_2 content.

Total fungal populations were reduced significantly at different CO_2 contents after 30, 60 and 90 days of storage, respectively. Fungal populations started to become inhibited by CO_2 at 10% contents, but they were inhibited significantly at 30% CO_2. The highest increase of total fungal populations occurred in the control (1.8×10^2 cfu/g w. b.) before storage. They increased to 1.4×10^6 cfu/g w. b. after 90 days of storage. Total fungal populations in the control were very significantly different from those treated with CO_2 at 10, 30, 50 and 70% contents.

The Effect of CO_2 on Lipid Content

In general, lipid contents decreased with the increase of CO_2 contents after 90 days of storage (Table 3). The ranges of lipid contents before storage and 90 days after storage were 58.08% – 58.51% w. b. and 46.38% – 55.37% w. b., respectively. 70% CO_2 content caused the highest decrease of lipid contents, e. g. the contents before storage and 90 days after storage were 58.48% and 46.38% w. b., respectively. After 90 days of storage, lipid contents at 70% CO_2 content were significantly different from those at 10 and 30% CO_2 contents. But they were not significantly different from

those at 50% CO_2 content and the control.

Fungi will accelerate the degradation of lipid during storage[7]. *Aspergillus* and *Penicillium* are capable to degrade lipid into free fatty acid and glycerol by lipase.

The Effect of CO_2 on Free Fatty Acid Content

FFA content increased with the increase of storage duration (Table 4). After 90 days of storage, the highest and the lowest FFA contents occurred in the control (7.43% w. b.) and at 70% CO_2 content (2.94% w. b.), respectively. FFA in the control (7.43% w. b.) was not significantly different compared with FFAs on nuts stored at atmospheres with 10% (6.58% w. b.) and 30% (5.73% w. b.) content of CO_2 in air. The content of FFA at 70% CO_2 (2.94% w. b.) content was not significantly different from the values at 50% CO_2 (3.93% w. b.)

BSN determined the maximum FFA content of candle nuts to be 5%[5]. Candle nuts treated with CO_2 at 50 and 70% contents in air were accepted to be stored, because their FFA contents were 3.93 and 2.94% w. b., respectively.

Conclusions

CO_2 contents did not cause any significant effect on the moisture contents of candle nuts during storage.

Thirteen fungal species and one unidentified isolate have been isolated from candle nuts during storage. The dominant species were *Aspergillus*, *Eurotium*, *Penicillium* and *Hyphopichia*. After 90 days of storage, the lowest total fungal populations occurred at 70% CO_2 content, but they were not significantly different with those of 30 and 50% CO_2 contents.

The lowest lipid contents occurred at 70% CO_2 content after 90 days of storage, but they were not significantly different with those of 50% CO_2 content. FFA contents increased with the increase of CO_2 contents. After 90 days of storage, the lowest FFA content was found in samples which had been stored at 70% CO_2 content in air. It was not significantly different with that at 50% CO_2 content.

Storage of shelled broken candle nut up to 90 days can be carried out using 50% CO_2 content.

Acknowledgements

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Table 1. The effect of CO_2 contents on the moisture contents of candle nut during storage

CO_2 content (%)	Moisture content (% w. b.)			
	Duration of storage (day)			
	0	30	60	90
Control	5.44 a	5.43 b	5.54 c	5.36 d
10	5.43 a	5.41 b	5.52 c	5.34 d
30	5.40 a	5.38 b	5.51 c	5.33 d
50	5.43 a	5.39 b	5.50 c	5.32 d
70	5.41 a	5.37 b	5.47 c	5.31 d

Numbers followed by the same letter in the same column do not differ significantly according to Duncan's Multiple Range Test at 95% confidence level

Table 2. The effect of CO_2 contents on the total fungal population of candle nuts during storage

CO_2 content (%)	Total fungal population (cfu/g w. b.)			
	Duration of storage (day)			
	0	30	60	90
Control	180 a	1007 b	496 400 d	1 402 307 f
10	147 a	330 c	226 017 de	956 500 g
30	170 a	147 c	2 647 e	122 150 h
50	253 a	43 c	83 e	447 h
70	236 a	37 c	77 e	67 h

Numbers followed by the same letter in the same column do not differ significantly according to Duncan's Multiple Range Test at 95% confidence level

Table 3. The effect of CO_2 contents on the lipid content of candle nuts during storage

CO_2 content (%)	Lipid content (% w. b.)			
	Duration of storage (day)			
	0	30	60	90
Control	58.51 a	51.44 b	51.29 cd	49.43 ef
10	58.39 a	51.60 b	54.64 cd	54.17 e
30	58.08 a	50.65 b	55.79 c	55.37 e
50	58.27 a	51.42 b	53.63 cd	51.20 ef
70	58.48 a	51.23 b	49.53 d	46.38 f

Numbers followed by the same letter in the same column do not differ significantly according to Duncan's Multiple Range Test at 95% confidence level

Table 4. The effect of CO_2 contents on the free fatty acid content of candle nuts during storage

CO_2 content (%)	Free fatty acid content (% w. b.)			
	Duration of storage (day)			
	0	30	60	90
Control	1.78 a	2.85 b	7.30 c	7.43 h
10	1.84 a	2.86 b	6.26 d	6.58 h
30	1.83 a	2.22 b	4.78 e	5.73 hij
50	1.51 a	2.18 b	3.51 f	3.93 ij
70	1.52 a	2.02 b	2.89 g	2.94 j

Numbers followed by the same letter in the same column do not differ significantly according to Duncan's Multiple Range Test at 95% confidence level

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0305

Research on the Influence of Carbon Dioxide Content, Exposure and Temperature on the Quality of Stored Paddy Rice with Different Moisture Contents*

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Abstract: The determination of quality aspects of stored paddy rice was the subject of the presented study. After airing and drying in the sun and adjusting the moisture content of the rice at relative balance humidities of 60%, 70%, 80%, the rice was exposed into four different atmospheres at five different constant temperatures: 10℃, 15℃, 25℃, 35℃ and normal ambient temperature, which containing high contents of carbon dioxide. The atmospheres were applied by ventilation into the tested bulks of paddy after reduction of pressure and adjustment of the moisture. By periodic sampling, many performance indexes for quality were determined, such as viscosity, fatty acid value, germination rate, hydrogen peroxidase activity, taste panel scores, light transparency difference and alteration of microbes.

It was shown by experimental data that the quality of stored paddy was degraded as grain storage temperature and grain moisture content and storage period increased. The rate of degrading was correlating to the storage temperature and moisture content of the rice. At a storage temperature of 15℃, paddy with high moisture content kept excellent palatability. At 25℃, except for paddy with high moisture content, the quality of paddy with lower moisture contents did not change. At 35℃, except for paddy with low moisture content, the performance of paddy decreased significantly, and paddy with high moisture content loosing its dietary property completely. Based on the research on paddy performance, alteration rules were developed for different storage conditions, such as different temperature, humidity and content of CO_2 in the storage atmosphere, fatty acid value, taste panel scores and germination rate.

The CO_2 content influenced the quality performance of paddy, which had been stored for one year. However, it played obviously a role on grain storage pest control, and had a pronounced inhibition effect on the growth of grain microbes. When the volume content of CO_2 was beyond 15% and the temperature at 35℃, it had some inhibition effects on increase of the fatty acids for paddy with high moisture content above 15%. The further increase of the CO_2 content did not show a pronounced inhibition effect. When the moisture content was below 15% and the grain temperature below 15℃, the carbon dioxide inhibited excellently the growth of fungi, as well as with common paddy or paddy with lower performance. When the content of CO_2 increased above 35% and the grain storage temperature above 25℃, growth of fungi was inhibited in shortly stored paddy with high moisture content above 15% to some extent. Modified atmospheres (MA) with increased carbon dioxide content reduced significantly the mass growth of grain fungi but it could played no roles on the variety of fungi occurring in the stored rice. When the moisture content and the temperature of the stored paddy were high, it could not be stored for long time even under MA.

Key words: carbon dioxide, gas content, temperature, moisture, paddy, quality

Introduction

During the period of the seventh-five-year plan, the national emphasis science and technology brainstorm project of "research on application technologies in grain storage with MA" has been carried out in China. Mock-up tests have been carried out in granaries under different storage conditions[1]. The influence of different storage factors like temperature, moisture content of the stored paddy varieties and modification of the composition of the storage atmosphere by use of different amounts of carbon dioxide in air have been investigated with respect to pests control, mould inhibition and quality performance. Meanwhile, the limitations of use of such atmospheres especially under the aspect of safe storage period have been explored. It has been considered that modified atmospheres (MA) with increased amounts of carbon dioxide in air play a significant role to improve the storability of various grain varieties under otherwise bad conditions like high storage temperature and moisture.

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The presented research results belong to special research projects for social public welfare businesses by the research institute and the academy in 2001. Based on researches achievements in the "seventh-five-year plan", elaborate choices to detect storage indexes have been made, for instance for viscosity, fatty acid value, germination rate, hydrogen peroxidase activity, taste panel scores. The light transparency difference has also been investigated.

In comparing with normal ambient atmosphere, modified atmospheres with three different contents of carbon dioxide of 15%, 35% and 60%, respectively, with rest air, have been investigated as storage atmospheres in granaries and in the laboratory. To implement the moisture content of paddy and the temperature as experimental factors, paddy rice was stored in balance with relative humidity at 60%, 70%, 80%, respectively, at different temperatures. Five different temperatures were adjusted. Some sensorial indexes, which have describe the quality of paddy rice were implemented into the investigation of the stored paddy.

Materials and Methods

Samples

The investigated paddy was harvested in former years in Gucheng Town, Pi count, Sichuan province.

Temperature

Five temperatures, comprising 10℃, 15℃, 25℃, 35℃ and ordinary temperature, were investigated. The temperature of 10℃ was gained by freezing. The conditions of 15℃, 25℃ and 35℃ were adjusted by use of a constant temperature generator. Controlling of temperature was performed by installing a SWP - F803 - 01 digital temperature controller with relative accuracy of ±1℃. The ordinary temperature referred to was room temperature in the laboratory and varied from 6℃ to 34℃.

Moisture Content

According to the requirement of adjusting the grain moisture content at different temperatures, the grain was exposed in balance to air with different relative humidities of 60%, 70% and 80%, respectively, and the grain regarded accordingly as grain with low moisture content, safe moisture content and high moisture content, respectively (see table 1). After thorough mixing of the corresponding large samples, they were divided into samples of 10kg weight. The moisture content of the grain was adjusted by adding corresponding amounts of water or airing in the sun. Subsequently, the sub samples were introduced into glass bottles with twenty liter capacity. When the temperature reached about 15℃, they were transferred into the storage experiments at the respective temperature after balance time of 14 days.

Relative Humidity of the Atmosphere and Control of CO_2 Content

Carbon dioxide content and relative humidity of the atmosphere were controlled by the way of "gas split stream ways"[2]. After passing through oxygen gasometer, flow controller and flow meter in succession, the mixture of pressurized air from the cylinder and carbon dioxide from a liquefied source became a modified atmosphere with increased volume ratios of carbon dioxide, including 0%, 15%, 35%, 60%, respectively with the rest being air. The oxygen contents of the atmospheres were correspondingly about 20%, 17%, 13% and 8%, respectively. Subsequently, the atmospheres were adjusted to three different relative humidities of 60%, 70% and 80%, respectively. The gas was ventilated into rice samples with the corresponding moisture contents that were sealed by linking the sample outlet to a pot of water(gas washing vessel). From there the gas was ejected outside.

At the same temperature and gas content of the mixture, the flow rate of gas through each sample was 350mL/min. To keep the content of CO_2, it took one hour for ventilation every day, equal to about one volume exchange. By detection with CHX - 3010D infrared measuring device, the relative accuracy of CO_2 content was below 2%. The sampling for determination of the moisture content was carried out four times every year and showed that the adjustment of the relative humidity of the atmosphere fulfilled the requirement of the experimental design.

Table 1. Moisture content of paddy at different temperatures and humidities

Balance relative humidityin %	Moisture content	Temperature in℃		
		15	25	35
		Moisture content in %		
60	Low moisture	13.5	12.7	12.0
70	Safe moisture	15.0	14.2	13.5
80	High moisture	16.5	15.7	15.0

(Note: at 10℃, the paddy moisture content can be related to the result at 15℃ in the above table, the ordinary temperature was about℃)

Sampling and Quality Analysis

Sample

According to the storage condition and the paddy moisture content, paddy with safe or low moisture content was sampled every three month, four times in the whole year. Paddy with high moisture content stored at high and middle temperature was sampled already after the first month. At 10℃, samples were taken after half a year.

Samples for detection and determination of microbes: In the first two months, paddy with high moisture content was sampled one time every fifteen days and later on one time every month. Paddy with safe or low moisture content was sampled one time every three month.

Performance analysis method

Viscosity: refer to GB/T 5516 – 85

Fatty acid value: refer to GB/T 15684 – 1995

Germination ratio: refer to GB/T 5520 – 85

Hydrogen peroxidase activity: refer to GB/T 5522 – 85

Taste panel scores: refer to GB/T 15682 – 1995

Light transparency difference: There exists no recent national or professional criterion. Therefore, a method was used designed by the project group.

Detection for microbes:

Detection on outer mould of grain: refer to GB/T 4789.15 – 94

Detection on inner mould of grain: refer to GB/T 4789.16 – 94.

Results and Discussion

Rules on Performance Alteration Germination ratio

It was one of most important factors for the detection of fresh and good quality of rice. In the first one year depending on storage period, temperature, moisture content and storage period, germination ratio of paddy decreased slightly to different extents in the various samples (Fig. 1) (Please present the figure as it can be read with Latin letters in captions and explanations in English, otherwise it has to be omitted). At 35℃, germination ratio of paddy with safe and high moisture content decreased significantly. After storage for three months, germination ratio was above 90%. After six months of storage, the velocity of degradation was accelerating. Within a storage time of nine months, germination ratio decreased to zero. However, when the temperature was below 25℃, germination ratio after storage for one year remained above 80%. When the content of CO_2 was kept below 60%, the difference of carbon dioxide content did not play a significant role on germination ratio.

Viscosity

Because many factors play a role in judging quality, the viscosity change seemed not be a very clear factor (Fig. 2) (Fig. 2 can not be deciphered, may be omitted). With increasing storage time, viscosity began to rise to certain value and decreased again gradually. As paddy has a weak tolerance towards heat, the tendency of decreasing became more obvious at higher storage temperature.

Hydrogen peroxidase activity

H_2O_2 is produced during grain respiration. If hydrogen peroxidase activity decreases, H_2O_2 content is increased and the ageing of grain accelerated. With increasing storage time, hydrogen peroxidase activity dropped gradually. The speed of change was in accordance with temperature and time of grain storage (Fig. 3) (Fig. 3 can not de deciphered, present a readable version or omit it). Overall, it took about six month for the decrease from 63.5 mgH_2O_2/g to 32mgH_2O_2/g. If the grain would be stored for one year at ordinary temperature, hydrogen peroxidase activity would decrease to 80%.

Fatty acid value

Under all tested conditions in this study, all fatty acid values kept raising (In Fig. 4) (Fig. 4 can not be deciphered, present a readable version or omit it). With progressing storage time, the fatty acid value raised gradually. The speed of change correlated with storage temperature and moisture content of the rice in storage. At 25℃ and storage for one year, the fatty acid value was below 30 mgKOH/100g dry matter. At 35℃ and within nine month of storage, the fatty acid peak value of paddy with high moisture was obtained with 40 mgKOH/100g dry matter. When the content of carbon dioxide increased above 15% and the storage temperature was 35℃, the speed of change of the fatty acid value was reduced.

Light transparency difference

In recent years, light transparency has been investigated and brought forward as rice storage ageing index. Comparing with distilled water as reference, the light transparency ratio between rice and water and its iodine color has been determined by spectroscopy. The difference of the light transparency ratio between rice and water and its iodine color was regarded as the light transparency difference. As the starch

content of paddy is generally about 60% – 80%, an alteration of this paddy performance would reflect changes in the starch structure. This change would lead to changes of the light transparency. Following the increase of the grain storage period and the increase of temperature and moisture, the tendency of change of the light transparency difference decreased(Fig 5)(Figure five needs to be presented in a readable form otherwise omit!). At 35℃ and 120 days of storage, the light transparency difference of the paddy with high moisture content decreased to 50%, from 8.2 to 4.0. The light transparency difference of paddy with low moisture always remained at about 70%. When the storage temperature was below 25℃, the light transparency difference of paddy with high moisture decreased to 60%. However, paddy with safe or low moisture content did not change its light transparency difference.

Taste panel scores

A taste panel determined directly the sensorial factors color, smell and flavor with samples of the stored and treated paddy. This determination was considered as easy and comprehensive index for the evaluation of the grain performance. When grain was stored for one year, the taste panel observed some changes (Fig 6)(A readablefigure 6 is neded, otherwise omit). With samples from a storage temperature of 10℃, the taste panel scored a slight decrease with low velocities of change. At storage temperatures of 15℃ to 25℃, the taste quality of paddy with safe moisture content did not change; all tested samples obtained about 80 scores. When the moisture content of the stored paddy was below the safe margin, the scores remained stable. So, under general storage condition s at about 25℃, the safe paddy could be stored safely for at least one year without loss in taste quality. High temperature played a significant role on scores. At a storage temperature of 35℃, paddy with safe moisture content could not be stored for one year without loss in taste quality, paddy with high moisture content not even 6 – 9 months.

Sensorial Indexes on Quality Changes

Sensorial indexes on quality changes were determined by variance analysis in this study. Variance analysis was carried out on every data set. The significance of a factor would be determinated by determining the "F" value and then indicated as of significant influence by one fold underlining(fiducial probability 95%). Special significant influence was indicated by double underlining(fiducial probability 99%). No-obvious influence was indicated by non-single underlining(fiducial probability 95% or 99%). The results of variance analysis are presented in table 2. When the sensorial index of quality was determined, and if there were at least one special significant factor and one significant factor among four factors, from the mathematical analysis standpoint it would be determined as primary sensorial index.

It is shown with results of variance analysis(Table 2) that five kinds of indexes, including the germination ratio, taste panel scores, fatty acid value, hydrogen peroxidase activity and light transparency difference have been regarded as sensorial index for the quality of paddy.

Table 2. Variance analysis on influence of temperature, moisture, gas composition and storage period on paddy storage quality

Resource	Free degree	Germination ratio		Viscosity		Hydrogen peroxidase activity		Fatty acid value		Light transparency difference		Taste panel scores	
		Average variance analysis	F value	Average variance analysis	F value	Average variance analysis	F Value	Average variance analysis	F value	Average variance analysis	F value	Average variance analysis	F value
T(℃)(A)	2	29099.1	30.09	1354.36	78.24	1317.31	3.42	53.54	4.92	938.38	12.77	49.72	1.02
Moisture(%)(B)	2	6328.78	4.39	31.50	0.70	380.24	0.94	5.82	0.49	68.31	0.75	435.13	10.74
CO_2(%)(C)	3	13.43	0.01	59.20	1.36	43.50	0.11	2.04	0.17	31.87	0.35	2.25	0.05
T(h)(D)	3	5044.48	3.56	14.71	0.33	11216.9	181.74	116.36	13.92	1512.74	33.04	782.19	31.11

Note: Among five different levels of temperature in table 2, sample capability at 10℃ and ordinary temperature were 6 and 12 respectively, all others three levels of sample capabilities were 48. All sample capabilities in three different levels of moisture content were 48. Among five different levels of storage period, sample capability of one month was 12, others were 48. They remained the same as the explanation in "t" inspection table.

Influential Factors on Change of Quality and Analysis on its Significance

(Since Table 3 can not be deciphered is has to be presented again in a readable version or the whole has to be discarded.)

Table 4 "t" inspection on paddy Hydrogen peroxidase activity and Fatty acid value

From table 3 it can de deduced, that at 35℃ the average germination ratio of paddy equaled to 55%. It was obviously lower than the germination ratio at the other four temperatures. The storage of paddy with low moisture content showed, that the germination ratio of paddy remained higher than from samples that were stored with safe and high moisture content. During the first three months, the germination ratio of paddy showed generally no variance. In the period of 3 – 6 months, the tendency of decreasing became obvious, and till 6 – 12 months, the tendency was very stable.

It is shown in table 4 that hydrogen peroxidase activity at a storage temperature of 10℃ remained higher in paddy than at other temperatures. Hydrogen peroxidase activity at 35℃ was lower than at other temperatures. After storage for three months, hydrogen peroxidase activity decreased significantly, especially after 6 months. The tendency increased until after one year of storage the activity dropped to almost zero.

The fatty acid value at 35℃ remained obviously higher than the values of paddy storage at the other temperatures. At 25℃ it was higher than at 10℃, 15℃. During the first nine months of storage, it appeared to raise.

Table 5: Inspection on paddy light transparency difference and taste panel scores

It can be derived from table 5 (Table 5 need s new presentation in a readable version!! Or discard!) that light transparency differences of paddy decreased as storage period increased and temperature raised.

The scores of the taste panel for paddy with high moisture content level were lower than for those samples with safe and low moisture content. As the storage period expanded, the taste panel scores of paddy samples with longer storage period decreased within the first half of the year. Temperature played a significant role on taste panel scores of paddy. At storage temperature of 35℃, it was lower than at other temperatures; at 25℃ it was lower than at 10℃ and 15℃.

Insect Pests

It is one of the keys of grain storage technologies to identify appropriate storage conditions for pest control. For the grain storage experiment for one year, pest development in paddy is described in Table 6.

Table 6. Pests, mould growth and sensorial quality determination of stored paddy

Relative humidity (%)	Temperature In℃	CO_2 content in air in %			
		0	15	35	60
Low(60)	15			Normal quality	
	25	*		Normal quality	
	35	* *	*	Normal quality	
Middle(70)	15			Normal quality	
	25	* *	*	Normal quality	
	35	* *	*	Normal quality	
High(80)	15			Normal quality	
	25	* *	*	Normal quality	
	35	* * *	*	Normal quality	

*: Low pest density, * *: Middle pest density, * * *: High pest density, temperature at about at 25℃

It is shown in Table 6 that pest growth in grain within one year of storage occurred in close relation with grain moisture, storage temperature and CO_2 content of the storage atmosphere. When the temperature ranged between 25℃ and 35℃ and the amount of carbon dioxide was zero, paddy with high moisture content suffers from serious damage by growth of pests and mould and looses its value completely. Slight increase of carbon dioxide content to 15% results in reduced pest growth. When the CO_2 content is increased to 35% or more, pest growth is completely inhibited, color and luster of paddy are kept fresh. So, when the storage

temperature is kept at 15℃ or CO_2 content above 35%, all grain can safely be stored without the risk of pest growth.

Succession Rules on Microflora

Experimental data indicated as follows:

(1) In the combination of all kinds of storage temperatures with content of carbon dioxide in the storage atmosphere, growth of fungi in stored paddy can be inhibited in grain with low moisture content.

(2) There is much alteration of fungi quantities in stored paddy with high moisture content in different storage conditions. When the temperature is kept at 15℃ or below and the carbon dioxide content at 60%, paddy can be stored safely without fungal problems at least 140 days. When the carbon dioxide content is between 15% and 35%, fungi quantities begin to increase 20 – 50 times within storage for 80 days, CO_2 does not inhibit growth of fungi under these conditions. When the storage temperature is above 25℃ and the content of carbon dioxide higher than 59%, CO_2 inhibits the growth of fungi in stored paddy to certain extent, fungi quantities would remain stable within a storage period up to 140 days. However, the sensorial qualities of the grain would slightly decrease, such as color and luster of grain kernel would become dark and some harmful smell would appear. When the content of CO_2 is below 35%, it would cause weak inhibition effects on growth of fungi, fungi quantities would increase 20 – 100 times, there could develop mildew stains on grain kernels, color and luster of grain kernel could become dark and some smell after mould growth could appear.

(3) At all tested temperatures and CO_2 contents, development of different species of fungi in low moisture paddy were as follows: the occurrence of agricultural field fungi, mainly *Fusarium* and *Alternaria* decreased slowly. However, storage fungi, represented by *Aspergillus flavus*, *Aspergillus candidus*, *Aspergillus glaucus* and *Aspergillus niger* developed gradually as dominating fungi. CO_2 inhibited weakly the growth of storage fungi growth in paddy with low moisture content.

(4) In all kinds of temperature and CO_2 contents, development of fungi species in safe moisture paddy was as follows: the detection rate of agricultural field fungi, mainly of *Fusarium* and *Alternaria* was generally stable. However, storage fungi, represented by *Aspergillus flavus*, *Aspergillus candidus*, *Aspergillus glaucus* G. and *Aspergillus niger* increasing and developed gradually as dominating fungi. CO_2 caused weak inhibition effects on growth of storage fungi in paddy with safe moisture content.

(5) In all kinds of temperature and CO_2 contents, development of fungi species in high moisture paddy was as follows: In the first period of grain storage, occurrence of storage fungi, represented by *Aspergillus flavus*, *Aspergillus candidus*, *Aspergillus glaucus* and *Aspergillus niger*, increased rapidly, even reached 100% during the whole storage period with constantly high detection rate. Also, the occurrence of agricultural field fungi remained generally stable. At high temperature, carbon dioxide caused weak inhibition effects on growth of storage and field fungi in paddy with high moisture content.

Conclusion

Sensitive Indexes on Qualities

According to comprehensive analysis above, taste panel scores, fatty acid value and germination ratio (paddy not dried in oven) have been regarded as sensitive index of paddy qualities.

Influence of MA on Fungi in Grain Storage

Carbon dioxide inhibited very effectively the growth of grain fungi, which grew on bad qualities paddy or paddy with low moisture (below 15%) or at low storage temperature (below 15℃).

Contents of carbon dioxide in the storage atmosphere of more than 35% inhibited to a certain extent and for a short time growth of fungi, which grew on paddy with high moisture content of more than 15% or high storage temperature of more than 25℃. However, as storage time increased, damaging effects of fungi became worse, especially the storage fungi were not inhibited.

Modified atmospheres with increased content of carbon dioxide caused inhibition of mass growth of grain fungi not so much on the reduction of species of fungi. The carbon dioxide rich atmospheres did not ensure safe storage of paddy with high moisture content at high temperatures for a long period.

Conditions for Safe Grain Storage

According to comprehensive paddy quality analysis on various sensorial indexes as pests growth, color and luster, smell and mould grow, conditions for safe grain storage for one year can be determined as follows (Table 7):

Table 7. Conditions for safe grain storage for one year

Moisture content of stored paddy (relative humidity in balance in %)	temperature in℃	CO_2 content in air in the storage atmosphere in %			
		0	15	35	60
Low(60)	15		Safe		
	25			Safe	
	35				Safe
Middle(70)	15		Safe		
	25				Safe
	35				Safe
High(80)	15		Safe		
	25				Safe
	35				

Note: "/" means unsafe.

It can be derived from the results presented in Table 7 that several factors influenced the safety of grain storage, such as grain moisture content, storage temperature and content of carbon dioxide in the storage atmosphere. So, grain could be stored safely by decreasing the temperature, lowering the moisture content or relative humidity and increase the amount of carbon dioxide in the atmosphere of the store.

0306

Fumisense
An Instrument for the Measurement of Phosphine, Methyl Bromide and Sulfuryl Fluoride during Fumigation

R. C. Naik*, R. D. Shroff

Abstract: Phosphine, methyl Bromide and sulfuryl fluoride are the common fumigants used by the fumigators for different commodities and under different circumstances. At present, no single instrument is available, which can measure all the three gases using the same instrument in the concentration range of interest to the fumigators.

The present instrument developed by us, uses a thermal conductivity detector for the measurement of methyl bromide(0 – 200 mg/L) and sulfuryl fluoride(0 – 100 mg/L). For the measurement of phosphine(0 – 2000 ppm) it uses an electrochemical sensor. There are two sampling lines, one for methyl bromide or sulfuryl fluoride and the other one for the measurement of phosphine.

The instrument is fully microprocessor based and all the operations are menu driven, which makes the instrument extremely user friendly. The instrument is calibrated for phosphine, methyl bromide and sulfuryl fluoride with a choice to select the gas and the sampling line. Two inbuilt sample draw pumps are used on two sampling lines to draw the sample air to the monitor and put it back to the source. Two thousand measurement data can be stored in the microprocessor of the monitor with gas name, silo number and all other pertinent parameters. The data can later be downloaded on the computer. The instrument is ideally suited for fumigators using all the three fumigants.

The paper also discusses the pros and cons of different measurement techniques used for the detection of phosphine, methyl bromide and sulfuryl fluoride.

Key words: fumigation monitoring, gas detection

Introduction

Methyl bromide(MB) & Phosphine(PH_3) are some of the common fumigants used for fumigating a wide range of agricultural, horticultural and wood products. In recent years the use of Sulfuryl fluoride(SO_2F_2) is also being talked about. The choice of a particular fumigant is dictated by several factors including the type of products, the time available for the fumigation etc.

Fumigation is generally carried out by covering the commodities within a leak proof enclosure and applying the fumigant to generate the desired concentration. As a process control measure it is necessary to monitor the gas concentration at various stages of fumigation.

Fumigant gas concentration used with MB/SO_2F_2 fumigation ranges from 50 to 100 mg/L and hence the monitoring instrument should have the adequate range of measurement. Thermal conductivity detector based on the differing thermal conductivity of fumigant gas or an interferometric based monitor which uses the differing refractive index of the fumigant gas with respect to air are used for the measurement of MB/SO_2F_2.

However for the measurement of Phosphine both these techniques fail as they do not have the sensitivity to measure the PH_3 concentration used for PH_3 fumigation which ranges from 0 – 3 mg/L approximately or 0 to 2 000 ppm. Generally the most suitable sensor for this measurement is an electrochemical sensor which is extensively used on PH_3 monitors. At present there is no single instrument which can measure all these three gases using the same instrument.

The instrument described in this paper is developed for use by fumigators who provide fumigation service using all the above mentioned fumigants.

Description

Figure 1 gives the block diagram of the instrument including the sample air path. It uses a TCD(Thermal Conductivity Detector) for the detection of MB/SO_2F_2 in the range of 0 to 200 mg/L and an electro-chemical sensor for

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the detection of Phosphine in the range of 0 – 2 000 ppm. Two separate sample draw pumps and two separate gas lines, one for PH_3 and the other for MBr/SO_2F_2 are used. The sensor output from the electro-chemical sensor or the Thermal Conductivity Detector are processed by two separate signal conditioning circuits and are digitized by a common ADC. The ADC outputs are processed by a common micro-controller and a common LCD displays the gas concentration after measurements. The instrument is fully micro-processor based and all the operations are guided by a user friendly menu. At a time the instrument can be used for making measurement for one of the three fumigants.

Being a micro-controller based instrument there is provision for logging the measurement data on the micro-controller which can also be downloaded through RS232 port to a PC.

Three thousand such data can be stored in the instrument. The instrument is powered by 14.8 V rechargeable battery with a DC – DC converter to give the desired ±5 V.

When the instrument is ON, and the gas line is chosen on the instrument, the corresponding pump starts to draw the fumigant gas for a predetermined time ranging from 1 to 3 minutes, after which the pump stops and the measurement is made. The gas concentration is displayed on the LCD display.

Calibration

The instrument has got several modes of operation such as measurement mode', parameter setting mode', calibration mode' etc. It is possible to navigate from one mode of operation to the other by using four membrane key pads on the monitor.

Once in calibration mode, the instrument is first Zeroed' in clean air. Subsequently the target gas of known concentration is applied to the sensor and using the UP/DOWN keys the monitor is made to read the applied gas concentration. It is also possible to use a surrogate gas like CO for PH_3 and CO_2 for MB/SO_2F_2 for calibrating the instrument. The relation between the surrogate gas and the target gas is given in Table 1.

Table 1 – A Thermal conductivity data of fumigant gases

Sr. No.	Name of Gas	Thermal conductivity data mW/m K @ 27;	Ratio of thermal conductivity of CO_2 : Target gas	Remark
1	CO_2 (Surrogate Gas)	26.20	–	–
2	Methyl Bromide (Target gas)	16.80	1:0.33	100 mg/L CO_2 gas and 33 mg/L CH_3Br gas gives the same signal
3	Sulphuryl Fluoride (Target gas)	5.04	1:0.647	100 mg/L CO_2 gas and 64.7 mg/L SO_2F_2 gas gives the same signal

Table 1 – B Sensor output ratio of CO and PH_3 Electrochemical Sensors

Sr. No.	Name of Gas	μA/ppm generated in an electrochemical Sensor	Ratio of electrical signal CO: PH_3	Remark
1	CO (Surrogate Gas)	0.1	–	–
2	PH_3 (Target gas)	0.3	3:1	100 ppm CO and 33 ppm PH_3 generate the same signal

Discussion

This portable instrument weighing 2.8 kg, housed in a rugged box should prove to be ideal for those fumigators who provide fumigation service to different customers and use different fumigants like PH_3, MB and SO_2F_2. Being a three gas monitor, it is very cost effective and it is designed keeping in mind the fumigator's as well as customer's requirement. It makes measurement and the measured data can be stored in its memory with all the relevant information such as warehouse number, silo number, the sampling location (Top, middle, bottom) in the silo, date, time etc. A GUI (Graphic User Interface) software provided with the instrument can

later sort out all the measurement data from individual silos. From the stored data various, analysis reports can be generated. The reports include fumigation reports to be given to the customers or silo wise fumigation report for records etc.

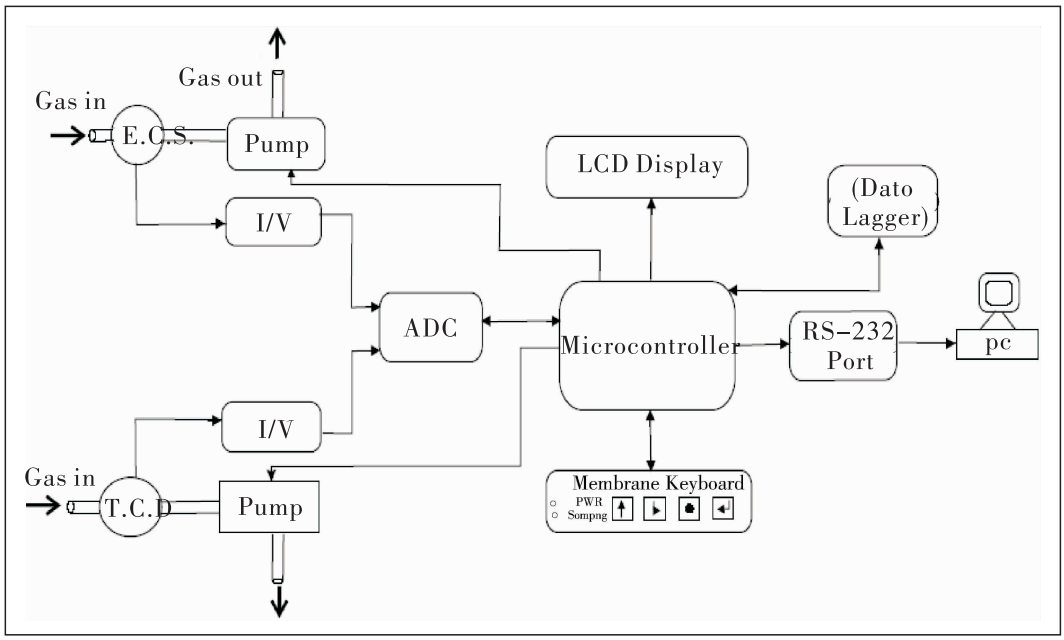

Fig. 1 **Block Dlagram of Fumisense**

0307

Effect of Ozone Gas on Brazil Nut (*Bertholletia excelsa* H. B. K.) Mycoflora and Aflatoxin Reduction

Giordano, B. N. E., Simão, V. and Scussel, V. M. *

Abstract: Raw Brazil nuts grow and are harvested in the wild of the Amazon forest. At post-harvest they are submitted to two storage stages prior to their drying process. The first storage is in the forest (on pallets) and the second in cities near the Amazon River or its tributaries to be subsequently send to the factories by boat. They are kept in wooden silos inside suspended stalls to keep them away from the environment. Despite of that, the relative forest humidity and temperature are high and suitable to fungi proliferation. The main biological factor that can affect in-shell nuts' quality during storage is fungi (deteriorating and aflatoxigenic strains) apart from forest termites. This work reports on an evaluation of ozone (O_3) gas influence on Brazil nut fungi load and its effect on aflatoxins (AFLs). Groups of *in – shell* Brazil nuts (14kg) from the year 2006 harvest, AFL contaminated with 5.62 (g/kg, collected in the Brazilian Amazon were submitted to O_3 treatment at different concentrations and conditions. After the gas exposure period, nuts were submitted to mycology tests, moisture and AFL analysis. Total fungi count was carried out utilizing malt extract agar and the aflatoxigenic fungi identification with *A. flavus* and *Parasiticus* agar. The nuts' moisture was determined by gravimetry and AFB_1 by high performance liquid chromatography with fluorescence-detection. As expected, the mycological tests showed that O_3 treatment affected mycoflora growth, lowering their cfu/g count and so the moisture content (from 8.2% to 5.6 %). The O_3 treatment applied within 5 hours at 31 mg/L was able to successfully destroy nuts' fungi contamination (initial cfu/g: 40×10^4). Fungi reduction just after harvesting by applying O_3 will certainly reduce the possibility of further fungi proliferation and so AFL formation. From a food quality and safety point of view, prevention is a better strategy than detoxification which is much more complicated and so are the implications towards human and animal health.

Key words: Brazil nut, ozone, post – harvest, mycoflora, aflatoxin

Introduction

Brazil nut (*Bertholletia excelsa* Humb. and Bonpl.) is native to the Amazon forests of South America and represents some of the oldest living tree species on earth. Many of these trees date back more than 1 100 years[12]. Harvesting of Brazil nuts, a major non-timber forest product, not only helps in preserving the Amazon rainforest but also creates an economy on which thousands of local people depend[3,25,26]. Brazil nut is widely recognized as the cornerstone species of the Amazonian extractive economy, and is the only internationally traded nut collected almost entirely from natural populations in mature forest[7,24]. The occurrence of aflatoxins (AFLs) produced by *Aspergillus flavus* Link, in Brazil nuts has been confirmed in several studies[6,36,15,5,25]. In many instances, the presence of the mycotoxins were detected on the surface of shelled nuts exhibiting visible mold growth and/or inside shriveled, cracked, or brown spotted nuts[15,8].

Several environmental factors are known to influence AFL production, but temperature and relative humidity (r. h.) are considered to be the most critical. Studies performed on hazelnuts and pistachios suggested that optimum temperature and r. h. for AFL production is 25℃ to 30℃ and 97% to 99%, respectively[9,10,34,22,35]. Additional factors such as water activity, moisture content, substrate composition[31], storage time, insect damage[18,33], and presence of a shell[4] also influence fungal growth and AFLs production. It is also important to recognize, however, that the interaction of all these factors may provide for varying results in regards to fungal growth and mycotoxin production even on identical substrates. The presence of AFLs is a serious concern for exporters of Brazil nuts especially since 1998, when the European Community decreased the maximum tolerance limit of total and B1 AFLs to 4 and 2 ng/g, respectively[11]. Moreover,

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since temperature and r. h. are important factors for AFLs production, it is of interest to evaluate the effect of these parameters on AFLs production during storage of Brazil nuts[24,26]. The main problems of Brazil nuts that reduces its quality and safety are fungi, AFB_1. and lipid oxidation. Since export companies must provide documentary evidence of laboratory analyses for AFB_1 and microorganisms, primary control for each nut lot is performed by sampling at the reception stage[23].

Many physical and chemical methods such as microwave heating, treatments with ozone (O_3) (ozonation) or ammonia have been recommended for detoxification of AFLs contaminated food[13,37,29]. Ozonation, an oxidation method, has recently been developed for the detoxification of AFLs in foods[32]. O_3 or triatomic oxygen, is a powerful disinfectant and oxidizing agent[20]. It reacts across the 8,9 double bond of the furan ring of AFLs through electrophilic attack, causing the formation of primary ozonides followed by rearrangement into monozonide derivatives such as aldehydes, ketones and organic acids[28]. The attractive aspect of O_3 is that it decomposes rapidly (half-life of 20 50 min) to molecular oxygen without leaving a residue[17]. As a disinfectant, O_3 is 1.5 times stronger than chlorine and is effective over a much wider spectrum of micro-organisms[37]. Several research studies have been undertaken to evaluate the effects of O_3 gas in reducing AFLs levels in contaminated agricultural products. Maeba et al. (1988)[19] have confirmed the destruction and detoxification of AFB_1 and AFG_1 with O_3. AFB_1 and AFG_1 were sensitive to O_3 and degraded with 1.1 mg/L of O_3 in 5 min in model experiments. O_3 is used to preserve the quality of fruit and vegetables after harvest. Frazier and Westhoff (1988)[14] reported that the storage period can be doubled when strawberry, raspberry, currant and apples are held in an environment including 2 – 3 mg/kg of O_3.

The objective of this research was to determine the influence of O_3 gas treatment on the mycoflora, moisture content and AFLs reduction in Brazil nuts.

Materials and Methods

Material

(*a*) ***Sample***: in-shell Brazil nuts (14 kg), 2005/2006 harvest, supplied by a Brazil nuts factory, located in Manaus city, Amazonas State (AM), Brazil. The AFL contamination was 5.62 g/kg.

(*b*) ***Storage***: (b. 1) seven vertical *silos*, made with vinyl polychloride (PVC) with 80 cm × 15 cm × 0.2 cm for height, diameter and width, respectively containing a lid and two apertures i. e. , top and lateral – inferior of the silos, for sample collection and O_3 application, respectively. (b. 2) ozoniser, Megazon (b. 3).

(*c*) ***Mycology tests***: (*c.* 1) glassware: Erlenmeyer (2 000mL), test tubes, Petri plates, microbiological pipettes (1, 10mL), automatic pipette 100, 1 000 L tips, microscope slides, Drigalski agar; (*c.* 2) culture media: malt extract agar (MEA), *A. flavus* and *A. parasiticus* agar (AFPA), peptone media, tween 80. (*c.* 3) equipment: autoclave, oven, microscope, incubator set at 20 – 25℃, scale, scissors, microscope stereoscope, colonies counter and tubes racks.

(*d*) ***Moisture content***: dissectors, microbiological oven, Fanen; analytical scale, Mettler; semi – analytical, CAB and industrial Brazil nuts cracker provided by CIEX, Manaus, AM.

(*e*) ***Aflatoxin analysis***: LC with isocratic pump and fluorescence detector, Gilson.

Methods

(*a*) ***Sample preparation for O_3 application***: in – shell Brazil nuts were weight and portions of 2 kg were aseptically added into the silos for O_3 treatment. Samples were collected from each silo for the following analysis: mycological, moisture content and AFLs.

(*b*) ***Preparation of the silos***: the silos (total = 7), after cleaned up with sulphite hypochloride, were filled with the 2 kg of nuts and had tightly closed the upper part with the lid. They were divided into 4 Groups for O_3 application at different concentrations: Group I (Control = no O_3 application), Group II (O_3 = 10mg/L), Group III (O_3 = 14 mg/L) and Group IV (O_3 = 31.5 mg/L), n = 2.

(*c*) ***Ozone application***: after closing the upper part of the silos, O_3 gas was applied through a lower lateral aperture by means pf a vacuum pump to get the following concentration in each silo: 10, 14 e 31.5 mg/L (n = 2) during 1, 3 and 5 hours and closed. The O_3 concentrations were measured utilizing the iodinemetrical method of APHA (1980).

(*d*) ***Storage***: after O_3 application, silos were placed in a room with temperature and UR monitored for up to 6 months. Brazil nuts were monitored for mycological tests, moisture con-

tent as well as R. H. and temperature.

(*e*) ***Sample collection for analysis***: samples were aseptically collected for mycology, moisture content and Afls from the top silo aperture, de-shelled and ground.

(*f*) ***Analysis***: (*f*. 1) *Mycology*: 225 mL of peptone media (0. 1% com Tween 80) were added to 25 g portions of ground Brazil nuts, shake and 0. 1 mL applied on the surface of MEA media. After their incubation at 25℃ for 7 days the fungi total colonies count was carried out. The fungi identification were carried out utilizing AFPA media and their strains toxigenicity checked utilizing the Machida & Saito (1999) method. (*f*. 2) *Moisture content*: by gravimetry. 5 g of each Group of Brazil nuts were taken to a drying oven with temperature of 105℃ up to constant weight (AOAC, 2005). (*f*. 3) *Relative humidity and temperature*: temperature and r. h. were monitored utilizing thermometer and hygrometer, respectively. (*f*. 4) *Aflatoxins*: by high performance liquid chromatography with fluorescence-detection HPLC/FD – (AOAC, 2005).

Results and Discussion

As expected, the mycological tests showed that O_3 treatment affected mycoflora growth, lowering their cfu/g count and so the moisture content (from 8. 2% to 5. 6 %). The O_3 treatment applied in 5 hours at 31 mg/mL was able to successfully destroy fungi contamination in the nuts (initial cfu/g: 40×10^4). As far as aflatoxigenicity is concerned, according to Saito & Machida (1999) [30], in order to identify the trains toxigenicity when utilizing the AFPA media, its (the media) reverse should present an orange colour. In our experiment the media turned orange-aflatoxigenic fungi genera *Aspergillus* detected-only in the Control Group nuts. From the nuts further O_3 gas treated i. e., Groups T2 to T4 no aflatoxigenicity was detected in any of the isolated strains thus, showing that the gas treatment was efficiently able to destroy them. O_3 gas produces a progressive oxidation of the cell vital components leading to apoptosis[16]. Table 1 shows the different strains of *Aspergillus* and *Penicilium* as well other genera isolated from the nuts. No AFLs were also detected in the nuts samples after O_3 gas treatment. To reduce yeast/mould activity, O_3 could be applied either for longer periods at low concentration, or conversely for short period with higher concentrations. Literature studies show that low concentrations and long exposure times were usually preferred for O_3 applications. In the study of Palou et al. (2002)[27] with the peaches cultivars Elegant Lady, they were treated for a four week period by O_3 at 0. 3 mg/L concentration in cold storage conditions at 5℃ temperature and 90% r. h. Fungi reduction just after harvesting by applying O_3 will certainly reduce the possibility of further mycelia proliferation and so AFL formation.

As far as moisture content is concerned, variations were observed between Groups: the Control and the three treated Groups with different concentrations and exposition time to O_3. The treated Brazil nuts (Groups T1, T2 e T3) presented lower moisture content than the Control Group (Table 2), either in – shell or shelled ones, with an average of moisture reduction of 18. 13 to 21. 63 % and 22. 76 to 28. 59 % of the initial moisture content, respectively. Considering the shelled Brazil nuts, where the moisture loss is more intense due to the lack of shell protection, it was observed that although Groups: T1 (exposition of 2 hs at 10 mg/L of O_3) and the T2 (exposure of 3 hs at 14mg/L of O_3) presented much lower moisture content (3. 97%, 3. 94 %, respectively), the difference was more intense in Group T3 of 5 hs of exposure to the gas and 31. 5 mg/L of O_3, a higher concentration applied, reaching 3. 67 %. Fig. 1 shows clearly that a reduction on the moisture content by the O_3 treatment was observed from the third treatment (Group T3) onwards.

This work is part of a Research Project on "Methodology Development for Reduction and Control of AFLs in Stored Brazil Nuts" that has been developed in the Food and Technology Department of the Federal University of Santa Catarina, Brazil.

Conclusion

It can be concluded that a minimum of five hours O_3 treatment at 31. 5mg/L could be successfully used for reducing the microbial count of Brazil nuts. O_3 reduced fungal growth and so AFLs in Brazil nut, consequently, that treatment could be an effective method for reduction of nut deterioration and so the AFLs contamination risk in the market. By destroying yeast and moulds just after harvesting will certainly reduce the possibility of AFLs formation before the next processing steps. On the other hand sensitivity of fungi to O_3 could be influenced by

other factors including location of fungi in the nut and interactions among the different parameters. O_3 could be used in packaged nuts, as long as proper method such as hermetic or vacuum resistant materials can be applied. From a food quality and safety point of view prevention is a better strategy than detoxification which is much more complicated and so the implications to human and animal health. Despite of the findings, there is a need of more studies, especially in pilot plants and application in larger amounts of nuts under the environment of Amazon forest in order to establish the optimal and practical O_3 gas concentration and the time of exposure for maximum reduction either of deteriorating or aflatoxigenic fungi growth and so moisture content and AFLs contamination.

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Table 1. Effect of different ozone gas concentrations and time of exposure on fungi development on in – shell and shelled Brazil nuts

Media	Fungi growth							
	Control In – shell	Treatment 1		Treatment 2			Treatment 3	
		Shelled	In – shell	Shelled	In – shell	Shelled	In – shell	Shelled
MEA								
10^{-1}	*A. flavus* (1) *A. parasiticus* (2) Yeasts (nbc)	*P. crustosum* (nbc) Yeasts (nbc)	*A. N ger* (1) Yeast (8)	Yeast (nbc)	Yeast (6) *P. nalgiovense* Laxa (1)	*Rhizopus stonifer* (Ehrenb) Lind. (1) *P. nalgiovense* Laxa (2)	Yeast (1)	Yeast (1)
10^{-2}	*Syncephalastrum recemosum* Cohn (1) *A. ochraceus* (1) Yeast (30)	*P. crustosum* (20) *A. versicolor* (25) Yeasts (15)	*A.. versicolor* (20) Yeasts (4)	Yeast (6)	Yeast (6)	*P. corylophilum* Dierckx (3)	*Byssochamy s nivea* Westling (1)	Yeast (1)
10^{-3}	Yeast (10)	Yeast (4)	Yeast (3)	Yeast (3)	Yeast (1)	*P. nalgiovense* Laxa (3)	NG	NG
10^{-4}	Yeast (4)	Yeast (2)	*Byssochamys nivea* Westling (1)	NG[a]	NG	NG	NG	NG
AFPA								
10^{-1}	*A. parasiticus* (4) *A. flavus* (2) *Cladosporium Spharospermum* Penzig (1)	*A. parasiticus*(1), *A. sydowii* (Bain. & Sart.) Thom & Church (2)	*A. parasiticus* (1)	*Cladosporium Spharospermum* Penzig (5)	*Cladosporium Spharospermum* Penzig (1)	NG	*Cladosporium Spharospermum* Penzig (1)	NG
10^{-2}	NG	*Cladosporium Spharospermum* Penzig (1)	NG	NG	*Cladosporium Spharospermu m* Penzig (1)	NG	NG	NG
10^{-3}	NG	NG	NG	NG	NG	NG	NG	NG
10^{-4}	NG	NG	NG	NG	NG	NG	NG	NG

[a] no microorganisms growth was observed

Table 2. Moisture content of Brazil nuts after ozone treatment

Group	O_3 treatment		Brazil nuts moiture content			
	Time (min.)	Concentration (mg/L)	In-shell (%)		Shelled (%)	
			Nuts	Reduction	Nuts	Reduction
C[a]	Zero	Zero	9.43	NA[b]	5.14	NA[b]
T1	120	10	7.72	21.63	3.97	22.76
T2	240	14	7.44	21.10	3.94	23.35
T3	300	31.5	7.39	18.13	3.67	28.60

[a] control [b] not applicable

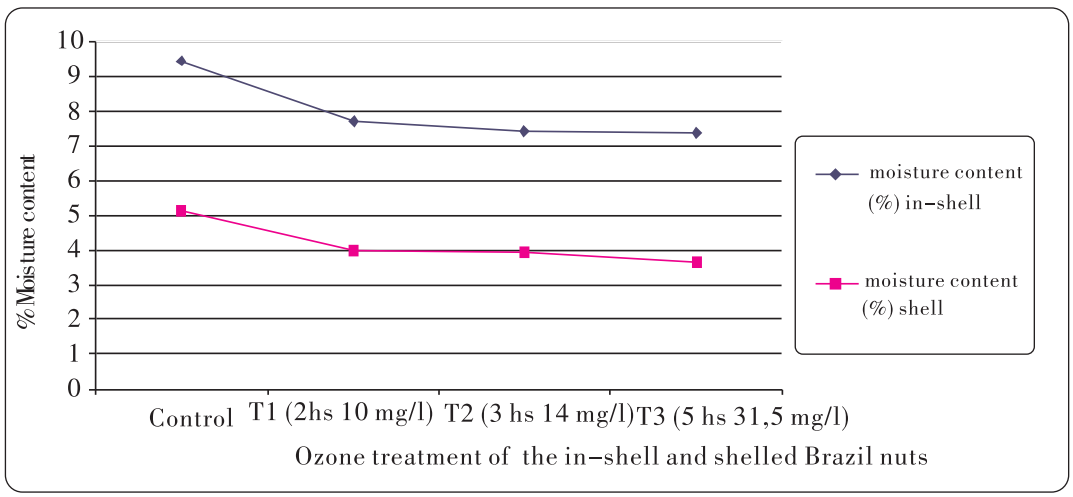

Fig. 1 Moisture content of in-shell and shelled Brazil nuts after treatment with different ozone concentrations

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Application Analysis on Alternative Technologies for Methyl Bromide Phase-out in Chinese Grain Storage Industries

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Abstract: According to the *Copenhagen Amendment* in the *Montreal Protocol on the ozone depleting substances*(*ODS*), putting the Chinese government's promise actually into practice and ensuring Chinese grain storage safety, a permanent guarantee mechanism for methyl bromide phase-out has been established. From a technical point of view, Chinese grain storage industry has taken *recirculation fumigation under film with phosphine* and *fumigation technologies by combination of phosphine with carbon dioxide* as alternative technologies for methyl bromide fumigation. Fumigation experiments to control pests in 32 demonstration depots of 9 provinces and cities in China have been carried out for more validation and later evaluation of application effects of alternative technologies. It has been shown that the lethal effects towards pest insects reached 100% in 31 cases and 90% in one case. Therefore, the application of the investigated alternative technologies for methyl bromide fumigation in grain storage seemed to be scientifically reasonable and sufficiently effective. Beside economical, social and environmental benefits, there was also technical support for the establishment of permanent mechanisms for phase-out of methyl bromide.

Key words: grain storage industries, methyl bromide alternatives, phosphine, application effect

Preface

It has been taken three years (2005 – 2007) to nearly phase out methyl bromide in Chinese grain storage industries. After making great efforts, lots of prominent achievements have been gained, and the project on phase-out of methyl bromide will be totally finished till the end of 2008. Meanwhile, as the described project was the first one of internationally appointed projects for Chinese grain storage industries, it was related to the international reputation, political attitude and social duties of Chinese grain storage industries. Beside of the significant influence, it was also an important Chinese contribution for protecting mankind's living environments. To consolidate the prophase achievement on methyl bromide phase-out, a permanent mechanism should be established with technical support to that methyl bromide will never be reused again. From May to October 2007, in elaborate organization and arrangement by the State Administration of Grain, the experiment on alternative technologies, such as *recirculation fumigation technologies with phosphine under film* and *fumigation technologies by combination of phosphine with carbon dioxide*, have been carried out for controlling pests in 32 demonstration depots. In another two depots that had been selected for demonstration as well, no pests were present and therefore no fumigation was applied. As a sub-project undertaker unit, Chengdu Grain Storage Research Institute sent professional engineers to the demonstration depots for trial inspection and to give guidance on fumigation technologies and on technical application. By inspection and later evaluation of the alternative technologies, the quick replacement of methyl bromide in China has been enhanced and technical support for the establishment of permanent alternatives for methyl bromide fumigation in grain storage provided.

Introduction of Alternative Technologies for Methyl Bromide

Main Technologies for Phasing out Methyl Bromide

According to the actual condition of grain storage industries and argumentation by professional experts, such technologies as *recirculation fumigation technologies with phosphine under film* and *fumigation technologies by combination of phosphine with carbon dioxide* have been chosen as the main alternative technologies for phasing out methyl bromide in grain storage industries.

Demonstration Depots that Applied alternative Technologies for Methyl Bromide

The specific demonstration depots are described in Table 1.

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Devices and Applied Chemicals

International assistance devices were taken for application as fumigation equipments. The supply with equipments was organized following public bidding. It comprised phosphine recirculation fans, phosphine generators, phosphine measuring devices, phosphine alarm devices, centrifugal fans, aluminum phosphide product, carbon dioxide in steel cylinders, equipment for worker safety, recirculation pipes, plastic liners, etc.

Main Survey on Fumigation

ⅰ) Fumigation modes

According to different types of granaries, different techniques of fumigation have been applied, such as recirculation fumigation under film in house granaries or large house granaries and recirculation fumigation in whole granaries in silos.

ⅱ) Actual fumigation situation

By making great efforts for several months, according strictly to *LS/T*1201 – 2002 (*Fumigation regulation of phosphine recirculation*, *LS/T*1201 – 2002)[1], fumigation experiments have been carried out under local guidance of technical engineers in 32 demonstration depots including tests on pest control. The total fumigated volume was 131 281m and the total amount of fumigated grain was 97 419t, including 67 642t of wheat, 17 489t of maize and 12 288t of paddy.

Out of 32 demonstration depots, 31 depots (96. 9%) passed the gas tightness test (LS/T1201, the tightness is at least 40s pressure from 250 Pa to 250 Pa in house granaries, and at least 40s in silos) as excellent. According to the test, a house granary should have a half life of pressure decay of not less than 40s after stored grains, and silos and squat silos not less than 60s. There was only one depot with worse gas tightness performance (grain supply center, HanDan grain administration). The specific fumigation situation is given in (Table 1).

Table 1. Description of the specifics of the 32 demonstration fumigation depots

Province	No	Name of depot	Gas tightness (pressure half life in s)	Grain	Amountoff grain (t)	Recirculation mode	Mortality (%)	Inhibition ratios of F1 generation (%)
Liaoning	1	Youyi State grain reserve and intermediate depot in Dalian	80	maize	8209	Fumigation in whole granary	100	100
	2	Jinzhou state grain reserve depot in Dalian	42	maize	3100	Fumigation under film	100	100
	3	Zhuanghe state grain reserve depot	40	wheat	2411	Fumigation-under film	100	100
Gansu	4	Grain depot in Wuwei city	42	maize	3500	Fumigation under film	100	100
Shanxi	5	802 Unit in Shanxi Province	40	wheat	4500	Fumigation under film	100	100
Hebei	6	Hanshan state grain reserve depot in Handan	42	wheat	4110	Fumigation under film	100	88
	7	ChengGuan grain supply center, Jize grain administration	40	wheat	5272	Fumigation under film	100	100
	8	State grain reserves depot in Ci county	40	wheat	1691	Fumigation under film	100	100
	9	purchase and sale reserve depot in Yongnian county	40	wheat	2202	Fumigation under film	100	100
	10	Daning state grain reserve depot	60	wheat	3311	Fumigation under film	100	100
	11	Hucun grain supply center, Handan grain administration	35	wheat	721	Fumigation under film	90	90

Province	No	Name of depot	Gas tightness (pressure half life in s)	Grain	Amountoff grain(t)	Recirculation mode	Mortality (%)	Inhibition ratios of F1 generation (%)
Hebei	12	Handan depot, China grain reserve Co.	45	wheat	6109	Fumigation under film	100	100
	13	grain depot, Feixiang county grain administration	40	wheat	620	Fumigation under film	100	100
	14	the second grain depot Guangping grain administration	40	wheat	840	Fumigation under film	100	100
Shandong	15	state grain reserve depot in Heze city	60	wheat	5814	Fumigation under film	100	100
	16	state grain reserve depot in Guang county,Liaocheng	52	maize	2680	Fumigation under film	100	100
	17	first depot of state grain reserve depot in Lingqing	50	wheat	1337	Fumigation under film	100	100
	18	state grain reserve depot in Zhengcheng	40	wheat	3008	Fumigation under film	100	100
	19	Dingtao state grain reserve depot	45	wheat	2562	Fumigation under film	100	100
	20	Juye state grain reserve depot	60	wheat	3447	Fumigation under film	100	100
	21	Dongjiao state grain reserve depot in Zibo city	45	wheat	3318	Fumigation under film	100	100
	22	Bingzhou state grain reserve depot	42	wheat	3500	Fumigation under film	100	100
	23	Donga state grain reserve depot	45	wheat	1907	Fumigation under film	100	100
Tianjing	24	Chengdong state grain reserve depot in Jinghai	43	wheat	2514	Fumigation under film	100	100
	25	JunLiangcheng state grain reserve depot	60	wheat	830	Fumigation in whole granary	100	100
	26	PuJidao state grain reserve depot	60	wheat	853	Fumigation in whole granary	100	100
	27	Binghai state grain reserve depot	60	wheat	1379	Fumigation in whole granary	100	100
Anhui	28	mechanization grain depot in Anhui	49	paddy	4065	Fumigation under film	100	100
	29	state grain reserve depot in Mengcheng	60	wheat	1000	Fumigation under film	100	100
	30	Anhui state grain reserve depot, China Grains&OilsGroup Science&Technology Corp.	56	paddy	8223	Fumigation under film	100	91
Guang dong	31	Haizhu grain depot in Guangzhou city	90	wheat	2463	Fumigation in whole granary	100	100

Province	No	Name of depot	Gas tightness (pressure half life in s)	Grain	Amountoff grain(t)	Recirculation mode	Mortality (%)	Inhibition ratios of F1 generation (%)
Guang dong	32	Maozhou state grain reserve depot in Guangxi	60	wheat	1923	Fumigation under film	100	100

Evaluation of Alternative Application Technologies for Phasing out Methyl bromide

Efficacy of the Alternative Technologies

According to the lethal effects against the pest insects, the fumigations in 31 depots showed excellent results (100% mortality of adult test insects). Due to 10% surviving insects in depot No. 11, where the pressure test had failed, total mortality rate of adults amounted to 96.9% of all tested depots. In depot No. 6, No. 11 and No. 30 some progeny (12%, 10% and 9%, respectively) developed.

Implementation of Alternative Technologies

During construction, each demonstration depot in the project of methyl bromide phase out mastered training of people, field guidance. Experts answered questions of storemen and carried out practical operations. However, as it was the first time to encounter and apply this technology in some depots, application of the technique was still weak and more effort will be needed.

Permanent Application Mechanism of Alternative Technologies

In the fumigation experiments, it was the first time for many demonstration depots to apply recirculation fumigation technologies. The local training and guidance by professional experts resulted in effective technology transfer at every depot. Meanwhile, comparing alternative technologies with old techniques, the alternative technologies succeeded in better pest control and revealed economical benefits towards conventional fumigation and fumigation with methyl bromide. It was also aim of the project to enable to lead these technologies into application in other granaries by self-financing investment. It is worth to mention that most depots with good facilities indicated that they would play a leading role in spreading the new techniques and also improve their own grain storage technologies. Therefore, from the point of development, alternative technologies will be more and more accepted and welcomed which is very useful for the quick phase out of methyl bromide.

Technical Support and the Union of Research institutes and Grain Storage

By practicing the sub-project on technical support for phasing out methyl bromide, some difficulties of applying alternative technologies have been solved. Especially for the handling of pesticide resistance, a system for inspection of resistance has been built up by collecting grain storage pests and by carrying out resistance inspection in regular depots and in those for demonstration. Meanwhile, by these experiments for validation and inspection of resistance, the union of the research institute with grain storage enterprises was formed. The union met and solved some difficulties to apply the alternative technologies for methyl bromide fumigation: the phenomenon of pests that could not be controlled with regular treatments and how to control them when they showed to be resistant. The union provided most grain storage enterprises with technical support.

Analyses on Benefit

Economical Benefit

Costs for recirculation fumigation with phosphine under film and fumigation by combination of phosphine with carbon dioxide

The main costs involved with the application of alternative technologies include costs for chemicals, fees for carbon dioxide steel cylinders, equipment depreciation charge, power fees and costs for plastic films. According to the specific condition and the pest infestation in every depot. carbon dioxide

a) Chemicals: applied at the dosage of 1.5 – 3g/m^3. The actual aluminium phosphide consumption was 414.7kg, which was equivalent to about 10 000 Yuan RMB (aluminium phosphide at a recommended price of 26 000 Yuan for each ton).

b) carbon dioxide: 276 bottles of cylinderised were used, which was equivalent to about 8 000 Yuan RMB (carbon dioxide at a recommended price of 30 Yuan for each bottle and 25kg of each bottle).

c) Equipment depreciation charge: including recirculation devices, generators, pipes and others ancillary equipments. The total assistance costs were about 4 millions Yuan RMB in 32

demonstration depots. According to the content of ten granaries in every depot and depreciation for 10 years, and every depot have 10 warehouses, the depreciation charge for each granary was about 40 000 Yuan RMB.

d) Supply with plastic film: according to 100 Yuan RMB for each granary (exception: five silos or squat silos), the total costs were 2 700 Yuan RMB.

e) Power fees were the last factor. The total power consumption during fumigation could reach 3456 degrees (two 750W power of each recirculation fan, intermittent recirculation for 3 days in 32 depots). At 0.8 Yuan RMB for each degree with industrial consumption, the total charges were about 2 700 Yuan RMB.

Therefore, during fumigation with alternative technologies in 32 demonstration depots, the total costs summed up to 63 400 Yuan RMB every year, of which the main part was used for equipments. Basically, all fumigations were fully effective and achieved mostly 100% control. Therefore, only one fumigation was necessary per year.

Costs for application of conventional fumigation with phosphine

The main costs involved with application of conventional phosphine fumigation include the cost for the aluminium phosphide product and labor costs. If conventional fumigation with phosphine was taken in 32 demonstration depots, the total fumigation volume would reach 206 629m^3. According to conventional fumigations two times every year at least with dosage of 6g/m^3 as the least, 2 279kg of aluminium phosphide would be required, equivalent to 59 000 Yuan RMB (recommended price of 26 000 Yuan per ton). Corresponding to 50 Yuan per person per granary for a single fumigation, 19 200 Yuan in 32 depots would be necessary for 6 persons.

The total costs would reach 78 200 Yuan. If two fumigations would be necessary per year, increased labor and much more phosphide product would be required without the guarantee of complete control of pests with possible side effects towards man and environment.

Cost for application of methyl bromide

Most of the money for this type of cylinder based fumigation with methyl bromide has to be spent for the chemical itself. If fumigation with this gas was carried out in 32 demonstration depots, the total fumigated volume would reach 206 629m^3. On the base of one yearly fumigation with methyl bromide with 25g/m^3, 5 165kg of gas had to be used. While the recommended price of this gas is 28 000 Yuan each ton, the fumigation would totally cost 145 000 Yuan RMB. Though it could help to control grain storage pests, it would bring the risk to cause destruction of the ozone layer.

It was shown that the application of alternative technologies did not only control pests completely, but also reduced costs clarified by actual fumigation validation experiments in 32 demonstration depots. The costs were 80% of conventional fumigation with phosphine and 44% of fumigation with methyl bromide.

Environmental Benefit

Grain storage industries brought pollution into environment, which accounted for yearly chemical consumption for grain storage and the risk of destroying the ozone layer with methyl bromide. The less chemicals are used, the less pollution to the environment will occur. When alternative technologies were taken in 32 demonstration depots, the consumption of aluminium phosphide added up to 414.7kg. During fumigation with phosphine the consumption of Aluminium phosphide got to 2 279 kilogram. If the fumigation was carried out with methyl bromide, the consumption would be 5 156kg and the ozone layer would be damaged too. Obviously, there were significant environmental benefits linked to the application of alternative technologies in grain storage industries.

Social Benefit

There were three aspects to reflect social benefits when applying alternative technologies in grain storage industries. The first one was the reduction of consumption of chemicals and decrease of pollution into the environment. Therefore, Chinese industry protected man and environment from application of ozone depleting methyl bromide for protection of grain against insect pests. The second one was to avoid storemen from entering into granaries to open gas bottles or distribute phosphides and come into direct contact with fumigants. A side effect was the decrease of labor and an improved healthy situation for storemen. The last one was that the application of alternative technologies increased the scientific level in the grain storage industries, improved the application of toxic chemicals and ensures the sustainability of use of phosphine in grain storage industries by decreasing the risk of development of phosphine resistance. The described approach has ensured sustainable developments for pests control in Chinese grain storage industries and ensured

Chinese grain storage safety.

Factors Influencing the Use of Alternative Technologies

Four factors influenced the application of alternative technologies, such as the difference of application alternative technologies, such as the hardware condition (granaries and equipments) , resistance of some grain storage pests, and the cost for investment.

Huge Difference of Application Alternative Technologies

All 32 demonstration depots improved their technique of applying alternative technologies for pest control in grain storage. However comparing all depots, there were differences concerning the application of alternative technologies.

The first difference was that many depots were very familiar with fumigation technologies with phosphine under film and fumigation technologies by combination of phosphine with carbon dioxide. The second one was that it was the first time for many depots to learn about and apply alternative technologies. Despite training by experts that had been sent to the local demonstration depots by the State Grain Administration, still some application techniques remained weak.

Constraints for Good Gumigation Practice in Granaries and Equipments

Due to different conditions in each depot, especially concerning the age of some buildings, difficulties could be observed to keep up with maintenance. There were leakages in windows, doors and equipments leading to weak gas tightness performance and resulting in effects typical for bad fumigation. As examples there was leakage between films, windows and doors in the HuCun grain supply center of HanDan county and between centrifugal fans with silo in JunLiangCheng State grain reserve depot in TianJing.

Phosphine Resistance

During these validation experiments, some pests could not be controlled in few depots. There were two kinds of conditions, such as bad gas tightness performance leading to surviving adults and F_1 progeny. When only the F_1 generation was found alive, there could have been two main reasons, such as grain reinfestation after fumigation, accidental errors or others factors. For proper application of chemicals, several ways were recommended, such as the use of sleeves with phosphide products, increasing the daily efforts to prevent pest infestation by intensified inspection and using traps and other devices, paying attention on pesticide resistance and strengthening inspection, especially to such grain storage pests with risk of high resistance as the rusty grain beetle.

Cost, such as Start-up Investment

There were many advantages for alternative technologies, such as decreasing labor intensity, improved pest control and preventing storemen from inhaling poisonous gas. However, it would take more start-up investment to apply these technologies by distributing lots of pipes, devices and apply other modifications. Meanwhile, during application of alternative technologies, it would be necessary that phosphine generators outside buildings should be equipped with carbon dioxide cylinders to decrease temperature during chemical reaction of the phosphide and prevent ignition before application chemicals. But in practice, many depots could not buy carbon dioxide cylinder in the local market, and it would have increased the application investment if they were bought in other places. All these constraints restricted the promotion and application of alternative technologies to some extent.

Prospects

By validation of the application technique and later evaluation of alternative technologies for phase out of methyl bromide in grain storage industries, it was shown that the alternative technologies was more appropriate, reasonable and competitive. Beside of economical, social and environmental benefits, there was technical support for the establishment of permanent mechanisms to phase out methyl bromide. However, in many depots the application of technologies was still weak, some strains of pests were resistant to pesticides, weak gas tightness granaries was observed, rules for the application of fumigants were missing or too simple. Therefore, the assistance project of phase-out of methyl bromide could be consolidated in many places. This could be enhanced by carrying out inspection on resistance and on technical support services. The relevant rules for good fumigation should be enacted as soon as possible and advanced pesticides and new technologies should be developed and promoted.

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Effect of Temperature and Humidity on the Potentiality of Sweet Flag(*Acorus calamus*) Oil against the Almond Moth,(*Cadra cautella* Walker,Lepidoptera :Phycitidae)

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Abstract: The effect of temperature and humidity on the potentiality of sweet flag(*Acorus calamus* Linn.) as fumigant against the Almond moth(*Cadra cautella* Walker) was studied under varying conditions of temperature(20℃,25℃ and 30℃) and relative humidity(60% and 70%) providing three exposure periods viz.,12 hrs,24 hrs and 48 hrs. The dosages were 0,5,10,25,50 and 100 μl/per volume,respectively,of 15cm x 7cm cylindrical glass jars. The result was that 50 to 100 μl dosages were very effective in suppressing the viability of two days old eggs,15 days old larvae,two days old pupae, and two days old adults. The higher dosages and higher exposure periods provided the higher mortality from 78.6% to 98.2% of eggs,larvae,pupae and adults of this insect. The increase in dosages,temperature,humidity and exposure periods provided increased mortality in all stages of the insect.

Key words: Almond moth,*Cadra cautella*,*Acorus calamus*, sweet flag

Introduction

The food range of Almond moth (*Cadra cautella* Walker) is wide viz., dried fruits and cereal grains either in whole or milled form posing a serious problem in India,Srilanka,Burma, Europe and America (Arbogast *et al.*, 2005)

The conventional use of insecticides leave the toxic residues for considerable periods in the treated commodities,which is dangerous to consumers. Plant products have been suggested as an alternative for the management of stored grain insect pests. (Mishra *et al.*, 1992; Singh,1993; Jood *et al.*, 1996; Arivudainambi & Singh. 2003 and Meena & Bhargava 2003). Plant products are less deleterious to human beings in manufacturing,handling and application especially in developing countries where no safe alternative methods are available. In present work,therefore,*Acorus calamus* extract was evaluated for the bio-efficacy on *Cadra cautella* Walker.

Material and Methods

Extraction of *Acorus calamus*

Rhizomes of *Acorus calamus* Linn. were collected and dried under shade for two weeks time. One kg of such rhizomes was powdered and extracted with soxhlet apparatus using petroleum ether(bp 40 – 60) as solvent. The extract was filtered through Whatman filter paper No. 1 and freed of solvent under reduced pressure at 50℃ to obtain the oil on the lines of Riar *et al.* (1990).

Mass culture of *Cadra cautella*

The laboratory culture of *C. cautella* was maintained on half-milled wheat grains alongwith 5 per cent dried yeast in plasticjars(20 × 10 cm) at 30 ± 1℃ and 70 ± 2% RH in Agust 2007 by releasing 20 freshly emerged adults(1:1 male,female ratio) and allowed to deposit eggs for mass culturing.

Obtaining of Known Aged Stages of *Cadra cautella*

In separate glass jars(10 kg capacity) having circular mouth were selected and 5 dates (preferred for oviposition) were kept inside. Ten pairs(1:1 male & female) of newly emerged moths of *C. cautella* were released inside it for oviposition. Five per cent glucose solution in the form of a wick was also kept inside the jar to supply food for moths. Mouth of the jar was tied with muslin cloth held by rubber band. The females preferred the ridges of dates for egg laying. Such eggs were daily brushed in a petridish and labelled for dates of egg laying to obtain known aged eggs & larvae for experimentation.

Experimentation

All experiments were conducted in controlled conditions of desired temperature and R. H. using thermostat & humidifier. For testing the effect of plant oil,the glass jars of 15 × 7 cm size were selected. The required dose of treatments (Table 1) was administered on a

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piece of filter paper, which was hanged inside the jar. Its lid was screwed and a wide cello tape was wraped around it to make it air-tight with the help of a thread fastening on muslin cloth tied on the mouth of jar. The per cent mortality of eggs, larvae, papuae and adults was recorded at different durations. There were three replications. The 'F' test was applied to calculate CD to find critical difference among treatments. The results are presented in Tables 1 to 4.

Results and Discussion

From the data presented in Table 1, it is evident that the maximum mortality of eggs (upto 90.0 per cent) was obtained after 48 hrs in 30℃ temperature & 70 per cent R. H. at 100 μl dose. It was significantly superior over all other dosages. In general longer exposure of 48 hrs was most effective in comparison of 12 hrs & 24 hrs time. Dosages below 50 μL were less effective.

There was a very slight effect of *Acorus calamus* on the mortality of 15 days old larvae of Almond moth when exposed for 12 to 24 hrs at lower dosages while it provided 90.6 to 98.2 per cent larval mortality at 100 μL dosage at 70 and 60 per cent RH, respectively in 30℃ temperature after 48 hrs of exporure (Table 2).

It is further evident that the significantly highest pupal mortality (86.2 to 90.6 per cent) was obtained at 100 μL dosage in 30℃ temperature under 70 and 60 per cent RH, respectively. It was followed by 50 μL dosage, but below this dose the mortality was not considerable (Table 3).

It is seen from the Table 4 that at 100 μL dosage after 48 hrs exposure period under 70 and 60% R. H. in 30℃ temperature provided significantly highest (78.6 and 81.3 per cent) adult mortality, respectively. The lower dosages, less exposure, less temperature and less humidity were less effective providing lesser mortality.

Arivudainambi and Singh (2003) have also found the effective dose of neem oil as 50 – 100 μL against the eggs, grubs and adults of Khapra Beetle, *Trogoderma granarium* (Everts).

Meena and Bhargava (2003) have tested some plant extracts/oils against Rice moth, *Corcyra cephalonica* Stainton and found that all the dosages (0.1 to 1.0%) were very effective in reducing the fecundity, egg viability and longevity of adults.

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Table 1. Effect of plant oils on the 2 days old eggs of Almond moth, cadra cautella at variying temperature and humidity

Treatment (dose) in μl.	Egg mortality (%)																	
	After 12 hrs exposure						After 24 hrs exposure						After 48 hrs exposure					
	20 ℃		25 ℃		30 ℃		20 ℃		25 ℃		30 ℃		20 ℃		25 ℃		30 ℃	
RH	1*	2*	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
100	15.5 (23.19)	18.6 (25.55)	10.8 (19.19)	12.3 (20.53)	18.6 (25.55)	22.4 (28.25)	40.2 (39.35)	45.3 (42.30)	55.8 (48.33)	67.1 (55.00)	70.3 (56.90)	72.5 (58.18)	66.3 (54.51)	67.8 (55.43)	70.6 (57.17)	85.6 (66.11)	85.6 (67.54)	90.0 (71.56)
50	12.5 (20.70)	8.4 (16.55)	14.2 (22.30)	6.3 (14.54)	12.6 (20.79)	8.4 (16.55)	32.7 (34.88)	34.8 (36.15)	38.2 (38.17)	40.7 (39.64)	56.2 (48.50)	54.3 (47.47)	50.0 (45.00)	55.6 (48.22)	62.2 (52.06)	66.3 (52.71)	70.5 (57.10)	66.7 (54.76)
25	2.5 (9.10)	3.8 (11.24)	4.2 (11.83)	3.2 (10.31)	4.8 (12.66)	5.6 (13.69)	11.5 (19.82)	16.4 (23.39)	7.5 (15.29)	10.2 (18.63)	10.2 (18.63)	15.5 (23.19)	22.8 (28.52)	20.2 (26.71)	25.3 (30.20)	28.5 (32.27)	30.6 (33.58)	34.5 (35.97)
10	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	2.8 (9.63)	6.6 (14.89)	0.0 (0)	2.5 (9.10)	0.0 (0)	4.4 (12.11)	5.5 (13.56)	10.8 (19.19)	10.5 (18.91)	8.5 (16.95)	10.5 (18.91)	5.2 (13.18)	12.8 (20.96)	15.7 (23.34)
5	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	5.5 (13.56)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	10.8 (19.19)	12.7 (20.89)	6.5 (14.77)	0.0 (0)	12.6 (20.79)	0.0 (0)	15.5 (23.19)	6.6 (14.89)
0	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	6.8 (15.12)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	5.6 (13.69)	0.0 (0)	0.0 (0)	0.0 (0)	10.5 (18.91)	0.0 (0)	15.0 (22.79)	0.0 (0)
SE(M) ±	2.71	3.62	2.56	2.95	1.98	1.12	2.44	2.09	2.33	2.47	2.52	2.76	1.62	1.85	1.16	3.47	2.46	4.92
CD 5%	5.53	7.31	5.78	6.08	4.23	2.33	5.11	4.75	4.95	5.13	5.98	6.01	4.05	3.92	2.76	6.94	5.01	9.96

1* = 60 % RH 2* = 70 % RH

Table 2. Effect of plant oils on the 15 days old larvae of Almond moth, cadra cautella at variying temperature and humidity

Treatment (dose) in μl.	Larval mortality (%)																	
	After 12 hrs exposure						After 24 hrs exposure						After 48 hrs exposure					
	20 ℃		25 ℃		30 ℃		20 ℃		25 ℃		30 ℃		20 ℃		25 ℃		30 ℃	
RH	1*	2*	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
100	22.1 (28.04)	18.6 (22.55)	27.2 (31.44)	22.5 (28.32)	29.3 (32.77)	24.6 (29.73)	27.5 (31053)	25.2 (30.13)	25.3 (36.45)	32.3 (34.63)	32.6 (34.82)	28.4 (32.20)	80.6 (63.87)	82.8 (65.05)	85.8 (67.86)	80.2 (82.29)	98.2 (82.29)	90.6 (72.15)
50	20.2 (26.71)	12.4 (20.62)	15.6 (23.26)	16.2 (23.73)	19.6 (26.28)	15.2 (22.95)	13.4 (21.47)	16.9 (24.27)	18.9 (25.77)	14.8 (22.63)	18.6 (22.55)	22.4 (28.25)	15.2 (22.95)	18.2 (25.25)	16.8 (24.20)	21.3 (27.49)	29.5 (32.90)	24.2 (26.47)
25	8.5 (16.95)	7.2 (15.55)	8.4 (16.85)	6.2 (14.42)	7.2 (15.55)	4.6 (12.39)	8.4 (16.85)	18.6 (25.55)	7.4 (15.79)	6.3 (14.54)	6.4 (14.65)	8.6 (17.05)	6.8 (15.12)	8.4 (16.85)	6.2 (14.42)	7.3 (15.68)	8.5 (16.95)	9.6 (18.05)
10	0.0 (0)	0.0 (0)	2.5 (9.10)	0.0 (0)	10.5 (18.91)	4.8 (12.66)	2.5 (9.10)	8.6 (17.05)	10.5 (18.91)	4.8 (12.66)	16.4 (23.39)	12.6 (20.79)	3.3 (10.47)	4.4 (12.11)	15.4 (20.2)	20.2 (26.71)	22.5 (28.32)	16.5 (23.97)
5	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	6.2 (14.42)	0.0 (0)	10.5 (18.91)	2.4 (8.91)	12.5 (20.70)	8.6 (17.05)	11.4 (19.73)	7.5 (15.89)	0.0 (0)	0.0 (0)	8.8 (17.26)	0.0 (0)	10.5 (18.91)	0.0 (0)
0	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	3.3 (10.47)	0.0 (0)	0.0 (0)	0.0 (0)	4.8 (12.66)	0.0 (0)	6.6 (14.89)	0.0 (0)	0.0 (0)	0.0 (0)	5.3 (13.31)	0.0 (0)	8.6 (17.05)	0.0 (0)
SE(M) ±	2.77	1.54	2.59	1.24	1.52	2.46	2.77	2.16	3.12	2.18	1.95	2.01	1.16	2.05	1.77	3.62	1.13	1.62
CD 5%	5.82	3.46	5.78	2.65	3.98	5.34	5.81	4.55	6.71	4.72	4.23	4.21	2.67	4.62	3.79	7.23	2.43	4.22

1* = 60 % RH 2* = 70 % RH

Table 3. Effect of plant oils on the 2 days old pupae of Almond moth, cadra cautella at variying temperature and humidity

Treatment (dose) in μl.	Pupal mortality (%)																	
	After 12 hrs exposure						After 24 hrs exposure						After 48 hrs exposure					
	20 ℃		25 ℃		30 ℃		20 ℃		25 ℃		30 ℃		20 ℃		25 ℃		30 ℃	
RH	1*	2*	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
100	34.2 (35.79)	35.5 (36.57)	45.2 (42.25)	38.4 (38.29)	40.2 (39.35)	44.6 (41.90)	40.6 (39.58)	36.6 (37.23)	49.1 (44.48)	44.6 (41.90)	48.5 (44.14)	48.5 (44.14)	75.8 (60.63)	70.6 (57.17)	82.2 (65.20)	75.5 (60.33)	90.6 (72.15)	86.2 (68.19)
50	25.7 (30.46)	28.4 (32.20)	27.4 (31.56)	29.5 (32.90)	34.6 (36.03)	30.5 (3.52)	35.1 (36.33)	30.4 (33.046)	34.8 (36.15)	40.2 (39.35)	40.2 (39.35)	38.6 (38.41)	52.8 (46.61)	48.6 (44.20)	61.8 (51.83)	59.3 (50.36)	75.6 (60.40)	72.4 (58.31)
25	15.6 (923.26)	13.9 (21.89)	16.6 (24.04)	14.2 (22.14)	28.6 (32.33)	24.2 (29.47)	22.4 (29.60)	18.6 (25.55)	21.3 (27.49)	19.5 (26.21)	28.1 (32.01)	30.2 (33.34)	31.2 (33.96)	30.3 (33.40)	38.2 (38.17)	30.6 (33.58)	34.5 (35.97)	32.2 (34.94)
10	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	12.2 (20.44)	0.0 (0)	0.0 (0)	0.0 (0)	8.6 (17.05)	0.0 (0)	12.3 (20.53)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	15.3 (23.03)	2.5 (9.10)
5	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	6.6 (14.89)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	9.3 (17.76)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	12.5 (20.70)	0.0 (0)
0	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	3.3 (10.47)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	3.3 (10.47)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	6.6 (14.89)	0.0 (0)
SE(M) ±	3.61	1.85	2.86	2.73	2.04	1.90	1.11	1.81	3.72	3.52	2.23	2.62	3.41	3.45	4.15	2.86	2.05	3.55
CD 5%	7.83	3.92	5.93	5.71	4.17	3.81	2.46	3.86	7.86	7.41	4.91	5.53	6.92	7.05	8.61	5.78	4.21	7.46

1* = 60 % RH 2* = 70 % RH

Table 4. Effect of plant oils on the 2 days old adults of Almond moth, cadra cautella at variying temperature and humidity

Treatment (dose) in μl.	Adult mortality (%)																	
	After 12 hrs exposure						After 24 hrs exposure						After 48 hrs exposure					
	20 ℃		25 ℃		30 ℃		20 ℃		25 ℃		30 ℃		20 ℃		25 ℃		30 ℃	
RH	1*	2*	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
100	28.4 (26.85)	24.3 (29.53)	26.2 (30.79)	20.4 (26.85)	27.5 (31.53)	25.4 (30.26)	35.6 (37.23)	28.3 (32.14)	40.5 (39.52)	38.2 (38.17)	52.6 (46.49)	48.8 (44.31)	42.6 (40.47)	44.3 (41.73)	70.6 (57.17)	68.6 (55.92)	81.3 (64.38)	78.6 (62.44)
50	15.3 (23.03)	12.3 (20.53)	21.4 (27.56)	17.8 (24.95)	20.2 (26.71)	22.5 (28.32)	32.8 (34.94)	15.6 (23.26)	35.7 (36.69)	34.3 (35.85)	32.8 (34.94)	40.5 (39.52)	34.2 (35.79)	31.3 (34.02)	38.2 (38.17)	41.3 (39.99)	68.6 (55.92)	69.2 (56.25)
25	9.9 (18.34)	6.6 (14.89)	12.3 (20.53)	9.6 (18.05)	12.6 (20.79)	8.4 (16.85)	12.6 (20.79)	10.2 (18.63)	23.8 (29.20)	18.6 (25.55)	15.8 (23.42)	14.3 (22.22)	15.2 (22.95)	12.4 (20.62)	32.6 (34.82)	34.8 (36.15)	30.6 (33.58)	29.5 (32.90)
10	3.3 (10.47)	0.0 (0)	3.3 (10.47)	3.3 (10.47)	6.6 (14.89)	3.3 (10.47)	6.6 (14.89)	3.3 (10.47)	9.8 (18.24)	9.6 (18.05)	9.6 (18.05)	12.3 (20.53)	8.2 (16.64)	9.1 (17.56)	16.6 (24.04)	14.4 (22.30)	22.2 (28.11)	20.3 (26.78)
5	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	3.5 (10.28)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	9.2 (17.66)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	9.2 (17.66)	0.0 (0)
0	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	3.3 (10.47)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	6.7 (15.00)	0.0 (0)	0.0 (0)
SE(M) ±	2.15	3.62	0.92	1.05	2.12	0.98	1.17	1.85	1.13	1.14	2.14	1.11	1.83	1.52	1.15	1.16	1.96	1.93
CD 5%	4.63	7.85	2.13	2.52	4.56	1.92	2.46	3.72	2.31	2.35	4.60	2.21	3.64	3.98	2.38	2.42	4.25	4.11

1* = 60 % RH 2* = 70 % RH

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Application Researches on Fumigation by Combination Sulfuryl Fluoride with Carbon Dioxide in Cereals

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Abstract: Based upon that median lethal dose(LD_{50}) of such beetles against Sulfuryl Fluoride (SF) as *Sitophilus zeamais*, *Oryzaephilus surinamensis*, *Rhizopertha dominica*, *Cryptolestes ferrugineus* and so on have been tested, while fumigation for 14 – 16 days by combination SF , which at different concentration such as 3g/m^3, 5g/m^3, 10g/m^3 and so on, with Carbon Dioxide at the concentration of 100g/m^3, egg, larva and pupa of *Sitophilus zeamais* in wheat and maize bulks could be killed completely. Through fumigation by combination SF with Carbon Dioxide in grain stored granaries, it showed that the residual of SF left lower after fumigation, and meanwhile it also could control effectively adults, eggs, larva and pupa of some main grain storage pests, such as *Tribolium castaneum* (with phosphine resistance or sensitivity), *Rhizopertha dominica* (with phosphine resistance or sensitivity), *Sitophilus zeamais* and so on.

Key words: Sulfuryl Fluoride, cereals, fumigation, grain storage pests

Preface

As early as 1901, Sulfuryl Fluoride (SF) has been produced in laboratory by H. Morssan in France. In 1957, it has been developed as commodity by Dow Elan Co. in America, which named as Vikane. In China, it was Mr. Xu GuoGan who raised to develop SF in 1975, and in 1978, it has been classified as emphasis research project by Ministry of Agriculture. Presided, leaded to organize and cooperate by Mr Xu, it was developed into product in 1983.

As one of Nerve Agents Poisoning, SF has excellent activities against insects, murine, Amphioxus, plants trichite and so on, which has been used for many industries, such as healthy quarantine, plant quarantine, commidity maintenance, products of a culture protection, pests control in ancient building and herbarium museum and so on. Since its excellent physicochemical properties, including difficult to burn, no influence on the ozone layer, large vapor tension, strong penetration capability, easy to diffuse and so on, it has been gotten application and promotion as fumigants. Besides of these, it also was safe to metal instruments, nature fiber or textile products, paper, leather and others chemical fibra. For middle toxicity, which there was no carcinogenic, teratogenic and mutagenic problems by animal testing, it could be applied in safety.

Rudimental researches on application with SF to control grain storage pests and wood insects have been carried on before 1983 by Mr Xu et al, and in 1989, it was used for controlling grain storage pests in crops seeds granary of of Crop Germplasm resources, Chinese Academy of Agricultural Sciences. Since 2004, application researches on SF to control grain storage pests have been carried on systemicly by Mr Xu and LongKou Chemical Plants.

In America, SF has been allowed to registrate for controlling storage pests in grain and their products storage, or in 14 kinds of dry fruits and nuts storage. In December 2005, World Healthy Organization (WHO) and Food and Agriculture Organization of the United Nations (FAO) held the international conference on developing the standards of pesticide residual. There were several biochemistry properties of SF which have been pointed out in this international conference. The first one was the direction of SF in people and animals bodies. While automatic tracer of ^{35}S tagged SF at the concentration of 30μL/L and 300μL/L (that was 0. 1365g/m^3 and 1. 36g/m^3), the tracer would be ingested quickly. It took 4 hours to get the terminal maximum concentration in blood plasma and akaryocyte. By determination the content of fluorosulfuric acid and sulfuric acid, it showed that SF would hydrolyze into fluorosulfuric acid and fluoride, and went on hydrolization into sulfuric

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acid and fluoride further. As the content of fluoride in rats' blood and urine increasing after fumigation SF, it also showed that SF would be decomposed and excreted. As fluorosulfuric acid residual was produced by SF hydrolyzation, it would means nothing than the nature producing. It was addressed in the international conference that residual was the total amount of fluorion in cereals. After fumigation with SF in wheat, maize, paddy, barley, oats bulks, the maximum residual concentration of SF was 0.05mg/kg, and the hazard residual (HR) was 0.08mg/kg. The fluorion hazard residual in cereals was 21mg/kg, Supervised Trial Medium Residue (STMR) in cereal was 3.5mg/kg.

Testing Indoors

Experimental Materials

SF Fumigants, produced in LongKou city Chemical Plant. barley

Experimental Methods

Experimental container

The volume of glass bottle was determinated by weighing method with water. Gubber bung should be fit suitably in glass bottle mouth and its bottom debouch and jam – pack, a piece of glass tube was plugged tightly into the middle of rubber bung in glass bottle mouth, its inferior extremity located in the place of grain surface, the superior extremity exposed out of bottle mouth and coated into latex tubing. As the same method as glass bottle mouth, a piece of glass tube was plugged tightly into the middle of rubber bung in bottom debouch, which strenching into the center of glass bottle, and there were few pore in its extremity, the copper tube, which out of bottle, should be coated latex tubing tightly. The hose in glass bottle mouth was used for application and air exhausting to analyze the concentration of SF and Carbon Dioxide in the upper part, the copper tube in bottom debouch was used for determination the concentration of SF and Carbon Dioxide in the bottom.

Inspection methods

The concentration of SF and Carbon Dioxide matched, the residual concentration would be inspected by conductance system in gas chromatogram and electron capture system.

Grain species

According to the situation of grain storage, wheat or maize in the bottle filled about 60% of its capacity, including wheat 14 kilogram weight, maize 12 kilogram weight.

Pests sample and their placing

Sitophilus zeamais tested which purified for 2 generations, adults after hatching for 16 days, eggs and larvae which produced in maize or wheat for 6, 8 and 14 days, mixture pests which consisted of adults, eggs, larvae and pupa.

Sweetmeats insects (adult, larva, pupa), *Tribolium castaneum* (adult, larva), *Rhizopertha dominica* (adult), longhorned flour beetle (adult), *Cryptolestes ferrugineus* (adult).

All of adults, larvae and pupa should be put into glass tube with feeds together, and sealed with nylon cloth and adhesive tapes at both extremities. To wheat or maize or rice with *Sitophilus zeamais* eggs, larvae and pupa, they should be stored in micro-nylon bags, which mouth should be tightened with strings. While pest samples tested lying in the bottom of glass bottle, it should be filled up with such filling-materials as wheat or maize.

Method on determination temperature

While thermometer was plugged into filling-materials, temperature should be made record at 8:00 a. m, 13:30 p. m, 16:30 p. m everyday. At end of experiment, the maximum temperature, the minimum temperature and accumulated temperature should be recorded in experimental period.

Calculation on dosage

Both of SF and Carbon Dioxide calculated their weight by volume of mole vapor, and the necessary volume applied by the volume of bottle and glass syringe of 100mL. At 25℃, these gas densities of SF and Carbon Dioxide was 0.0045mg/mL and 0.0018mg/mL respectively.

Chemicals application

When pressure regulator and sebific ducts contacted with the mouth of SF cylinder gradually, spigot should be opened slowly. After modulating the pressure regulator, plugged the glass syringe into the sebific ducts for collecting the necessary Sulfuryl Flulride, and then poured them into the bottle. The method for collecting 100mL of Carbon Dioxide was as the same as collecting SF. When collecting carbon dioxide, please exhaust and inlet gas again and again for well-distribution. At last, the total gas consumption would be made up with collecting carbon dioxide.

Effect inspection

After taking pests tested out from experimental bottle and gas exhausting for several hours, pests should put into constant temperature and humidity chamber for culture (temperature at 28℃, humidity at about 60%). The first check should be begun when adults pests were observed at emergence in control group. For re-

ducing interference, adults should be killed and checked out every time. There would be checked for several times and the last should remain 53 days, 54 days, 71 days before the end of experiment.

Results

determination results of median lethal dose which grain storage pests adults against SF

When fumigatiom with SF alone for 16hours in empty bottle at 15 – 10℃, median lethal dose (LD_{50}) of *Sitophilus zeamais*, sawed flour beetle, *Rhizopertha dominica*, *Cryptolestes ferrugineus*, longhorned flour beetle, Sweetmeats insects and *Tribolium castaneum* equals to 2.512g/m^3, 1.4125g/m^3, 1.972g/m^3, 1.274 g/m^3, 1.274g/m^3, 3.715g/m^3, 7.446g/m^3 respectively.

Determination effect when the lethal ratios against *Sitophilus zeamais*, Sweetmeats insects, *Tribolium castaneum* were 100% by fumigation by combination SF with Carbon Dioxide.

When experiment was taken in 20L of glass bottles and filled with 60% of grain, the results were as follows(Table 1):

Table 1. Pests control effects while fumigation by combination SF with Carbon Dioxide

concentration		Exposure time(h)	Temperature (℃)	CT value(gh/m^3)		effects(at different CT value)		
SO_2F_2 (g/m^3)	CO_2 (g/m^3)			SO_2F_2	CO_2	*Sitophilus zeamais*	Sweetmeats insects	*Tribolium castaneum*
14.32g/m^3	12.40g/m^3	6	20	85.94	74.39	79.00%	40.62%	/
15.38g/m^3	73.58g/m^3	6	18 – 19	92.29	441.48	100%	96.78%	86.00%
14.59g/m^3	12.33g/m^3	8	19 – 20	116.70	98.64	100%	100%	96.77%
15.24g/m^3	33.21g/m^3	8	20	121.95	265.69	100%	100%	100%
17.79g/m^3	17.79g/m^3	8	19 – 20	142.32	184.16	100%	100%	100%

According to CT value of SF, it showed that the CT value of *Sitophilus zeamais*, Sweetmeats insects, *Tribolium castaneum* increased gradually.

Here were data of effect while fumigation for 14 days and 16 days against *Sitophilus zeamais* eggs, larvae and pupa by combination SF at different concentration (3g/m^3, 5g/m^3 and 10g/m^3) with Carbon Dioxide at high concentration. (Table 2)

Table 2. Effect while fumigation by combination SF with Carbon Dioxide against *Sitophilus zeamais* eggs, larvae and pupa

concentration		Filling – materials	temper -ature ℃	Expo -sure time (h)	CT value(gh/m^3)		The amount of adults		The amount of adults	
SO_2F_2 (g/m^3)	CO_2 (g/m^3)				SO_2 F2	CO_2	Weight of wheat (g)	amount	Weight of rice (g)	amount
10	108.0	wheat maize	11.0 ~ 15.5	16	3 840	41 472	215	0	/	/
5	116.0	wheat	12.0 ~ 19.0	14	1680	38976	115	0	133 133	0
3	115.0	wheat maize	11.0 ~ 15.0	16	1152	44160	202	0		0
3	120.9	wheat	12.0 ~ 18.0	14	1008	40622	115	0	133	0
0	148.5	wheat	11.5 ~ 18.0	16	/	57024	135	9	133	107
0	170.5	wheat maize	12.0 ~ 13.5	14	/	57288	176	220	/	/
0	0	wheat	12.0 – 19.0	14	/	/	115	20	133	150
0	0	wheat maize	11.5 ~ 13.0	16	/	/	163	251	/	/

From experimental results in Table 2, *Sitophilus zeamais* eggs, larvae and pupa could be

killed completely at low temperature while fumigation for 14 – 16 days by combination SF at different concentration (3g/m^3, 5g/m^3 and 10g/m^3) with Carbon Dioxide at 100g/m^3 of concentration. However, there were no effects while fumigation with 148. 5 – 170. 5g/m^3 of Carbon Dioxide for 14 – 16 days alone.

Here were controlling effects against *Sitophilus zeamais* eggs, larvae and pupa while fumigation with SF at 5g/m^3, 10g/m^3 alone, or with 30g/m^3 of Carbon Dioxide for 7 days or 10 days.

Table 3. Effect against *Sitophilus zeamais* eggs, larvae and pupa while fumigation with SF alone or by combination SF with Carbon Dioxide

concentration		Filling – materials	temper -ature (℃)	Expo -sure time (h)	CT value (gh/m^3)		Amount of adults	
SO_2F_2 (g/m^3)	CO_2 (g/m^3)				SO_2 F2	CO_2	Average amount of feeds (g)	amount
10	0	maize	13 – 14	7	1680	0	105.4	1
10	30	maize	13 – 14	7	1680	7200	101.5	0
5	0	maize	13 – 14	7	840	0	126.1	4
5	30	maize	13 – 14	7	840	5040	101.5	0
10	0	maize	13 – 14	10	2400	0	107.5	0
10	30	maize	13 – 14	10	2400	7200	114.8	0
0	0	maize	13 – 14	10	0	0	108.2	223.5

From the Table 3, many results could be concluded, such as (1) one *Sitophilus zeamais* adult has been observed at emergence after culture for 53 days while fumigation with 10g/m^3 of SF for 7 days without carbon dioxide, however, no adults appeared with 30g/m^3 of Carbon Dioxide furthermore. (2) four *Sitophilus zeamais* adults have been observed at emergence after culture for 53 days while fumigation with 5g/m^3 of SF for 7 days without carbon dioxide, however, no adults appeared with 30g/m^3 of Carbon Dioxide furthermore after 53 days. (3) if fumigation with 10g/m^3 of SF postponed exporsure time for 10 days, there would be no adults appearance after 53 days whether adding Carbon Dioxide or not. Therefore, carbon dioxide addition had synergistic action, but there also were redeeming and ensuring effects by postponing exposure time.

Influence on germination ratios of wheat and maize while fumigation by combination SF with Carbon Dioxide

Here (Table 4) were determination effects of influence on wheat or maize germination ratios while fumigation by combination SF at 5g/m^3, 10g/m^3 with 30g/m^3 of Carbon Dioxide for 15 days and 90 days, and while fumigation by combination SF at 5g/m^3 with 30g/m^3 of Carbon Dioxide for 30 days.

Table 4. Influence on germination ratios of wheat and maize while fumigation by combination SF with Carbon Dioxide

concentration		Exposure time (d)	CT value (gh/m^3)		Grain species tested	Germination ratios (%)	influence or not
SO_2F_2 (g/m^3)	CO_2 (g/m^3)		SO_2F_2	CO_2			
10g/m^3	30g/m^3	15	3600	10800	wheat	98.5	No
5g/m^3	30g/m^3	30	3600	21600	wheat	99.0	No
10g/m^3	30g/m^3	90	21600	64800	wheat	99.5	No
Control group		90			wheat	96.5	
10g/m^3	30g/m^3	15	3600	10800	maize	97.0	No
5g/m^3	30g/m^3	30	3600	21600	maize	94.5	No
10g/m^3	30g/m^3	90	21600	64800	maize	90.0	A little
Control group		90			maize	96.0	

From the table 4, there were no influence on wheat germination ratios while fumigation by

combination SF with 30g/m^3 of Carbon Dioxide, however, there were a little influence on maize while fumigation by combination SF at 10g/m^3 with Carbon Dioxide at 30g/m^3 for 90 days. Certainly, further experiment should be taken next time.

Discussion

Qualities of products: as there were such by-products as sulfonyl fluoride chlorine in process flow, some high-purity SF should be taken.

technical parameter: there were reference indexes while fumigation by combination SF with Carbon Dioxide at common temperature: fumigation by combination SF at 6g/m^3 with Carbon Dioxide at 10g/m^3 for 20 – 30 days, fumigation by combination SF at 8g/m^3 with Carbon Dioxide at 10g/m^3 for 15 days, fumigation by combination SF at 10g/m^3 with Carbon Dioxide at 10g/m^3 for 10 days. If taking fumigation with SF alone, pests also would be controlled. However, Carbon Dioxide addition could be favor of penetration abilities to assure pests control effects, and it would decrease the cereals absorption to SF.

Influence on grain seeds: as fumigation with SF at low dosage for long time could not only control grain storage pests effectively, but also there were no influence on germination ratios, the influence on grain seeds while fumigation with SF would be made researches further. Beside of some factors, such as purity of SF, dosage, fumigation time, diffusion or not, there were others including fresh degree of seeds, moisture content and reserving qualities which could make influence on seeds germination ratios.

Roles which Carbon Dioxide played in the fumigation: SF could penetrate under 6 meters in grain bulks whether mixing Carbon Dioxide or not. Cereals absorption to SF could be decreased by combination with Carbon Dioxide. It also was favor to SF well-distribution and promote penetration abilities by increasing pressure. In addition, it had synergistic effect on control pests by 10 g/m^3 of Carbon Dioxide addition.

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0311

Measuring New Fumigants with Dräger Tubes®

Bettina Runge *

Abstract: For more than 65 years, Dräger Safety is offering the well-known Dräger-Tubes®. The detection of gases and aerosols is the main focus with this system. Today, Dräger Safety offers more than 220 different tubes.

The Dräger Tubes® program works in an easy way, combining a precise pump with a glass tube filled with analytical chemicals. The measurement is made by drawing a defined volume of sample gas through the tube. Immediately after the measurement is finished, the concentration of the measured gas can be read on the detection layer of the tube. The chemical reaction inside the tube has left an easy-to-read stain.

A new application for our tubes is the testing of fumigated transport containers. There are strong regulations for the fumigation of containers in the worldwide shipping of goods in order to protect the importing countries from foreign insects. Harbour workers, truckers and customs people who have to open the containers for inspection may come into contact with significant concentrations of fumigants.

Dräger offers a lot of different tubes for the measurement of phoshine, methyl bromide, sulfuryl fluoride, chloropicrine, ethylformiate and MITC with different range of measurement. Also simultaneous measurements of different gases are possible.

Key words: Draeger tubes, gas detection

Introduction

Many containers are sprayed with different kinds of fumigants to protect them against pests or fungi. If such containers are not declared as fumigated, or are declared incorrectly, serious damage to health can result when the container is opened. The Dräger Safety fumigation kit is a quick and reliable means of detecting fumigants, allowing appropriate protective measures to be taken.

The Dräger Fumigation-Kit makes the inspection of containers for fumigation agents easy (figure 1). Even while the container is still sealed, the measurement can be performed quickly and safely-without the hazard of being exposed to the gases within the container. A measuring strategy, specially developed for this application, enables to determine the fumigant, even if the container has not been marked properly.

Portable and Fast

Based on the proven Dräger Tubes, the Dräger Fumigation-Kit contains all the essential equipment to find out whether a container has been fumigated. Within a few minutes, a statement can be made about the gas concentration within the container, and whether it has to be aerated. Even if the container has not been marked, or the labeling is not legible, Dräger Safety has mapped out a fast and reliable measuring strategy as shown in figure 5. which enables to verify the fumigation agent. as shown in figure 2 and 3 and table 1, 2 and 3.

Fig. 1 **A worker during inspection of a container with Dräger Fumigation – Kit**

Easy to Use

For decades Dräger Tubes are renowned for their simplicity to use and their high accuracy. Without any training, the use of the Dräger Fumigation-Kit in combination with the especially for this application developed Dräger Tubes, leads to reliable results as shown in figure 2 and 3 and table 1, 2 and 3. Dräger Tubes are always on standby. All that is required are the suitable tubes, the hand-pump and the container-probe. Along with a color change within the tube, and the imprinted scaling, the actual concentration inside the container can be determined after a few strokes of the pump.

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Large Number of Fumigants

Additionally to the top 3 fumigants(Formaldehyde,Methyl Bromide and Phosphine),

the Dräger Fumigation-Kit can be equipped with the newly developed Sulfuryl-Fluoride tube. The Dräger Fumigation-Kit makes the inspection of containers for fumigation agents easy. Even while the container is still sealed, the measurement can be performed quickly and safely-without the hazard of being exposed to the gases within the container. A measuring strategy,specially developed for this application,enables to determine the fumigant, even if the container has not been marked properly.

ST－4324－2003

Fig. 2 **Simultaneous Tests Set Container Aeration I**(81 03 380)

Table 1. **detectable fumigants using Simultaneous Tests Set Container Aeration I**(81 03 380)

Tube No	Substance	Sensitivity	Colour change
1	Formaldehyde	1 ppm	white à pink
2	Phosphine	0.3 ppm	yellow à red
3	Hydrocyanic Acid	10 ppm	yellow à red
4	Methyl Bromide	0.5 ppm	green à brown
5	Ethylenoxide	1 ppm	white à pink

Strokes:50/measurement time:approx. 4min

Fig. 3 **Fumigation Tests Set I**(81 03 410)

Table 2. **detectable fumigants using Fumigation Tests Set I**(81 03 410)

Tube No	Substance	Sensitivity	Colour change
1	Formaldehyde	1 ppm	white à pink
2	Phosphine	0.1 ppm	yellow à red
3	Hydrocyanic Acid	10 ppm	yellow àred
4	Methylbromide	5 ppm	green à brown
5	Ammonia	50 ppm	yellow à blue

Strokes:50/measurement time:approx. 3 min

Example for Single Gas Tubes

Dräger Tube Sufuryl Fluroide 1/a (81 03 471)

· Lowest detection range 0.8 ppm
· Scale from 1 to 5 ppm
· No cross sensitivities to other fumigants
· The only tube for Sulfuryl Fluoride in the market
· Unique technique with a hot layer in the pre tube

Draeger Tube Methyl Bromide 0,2/a (81 03391)

· Lowest detection range in the market
· No cross sensitivity to other fumigants, except Ethylen Dibromide
· Detection range is required by European countries(Netherlands)

Draeger Tube Chloropicrin 0.1/a (81 03421)

Lowest detection range in the market

No cross sensitivity to other fumigants, except Ethylen Dibromide Detection range is required by European countries(Netherlands)

Draeger Tube Methylisothiocyanate 0.1/a(81 03485)

Field of application is the replacement of Methyl Bromide used in the ground fumigation for pest control

Draeger Tube Ethyl Formate 20/a (81 03541)

The first ethyl formate tube in the market

No cross sensitivity to 500 ppm CO_2 and 10 ppm CO

Summary

All important fumigants and TIC can be detected by Dr ger Tubes. This is the simplest and easiest way of detection. Table 3 gives an overview about the possibilities for single gas measurement.

Table 3

Fumigant	Range of Dräger Tubes
Ammonia(NH_3)	0.25 ppm – 10 Vol %
Benzene(C_6H_6)	0.5 – 60 ppm
Carbon Dioxide(CO_2)	100ppm – 60 Vol%
Carbon Monoxide(CO)	2 ppm – 7 Vol %
Chloropicrin(CCL_3NO_2)	0.1 – 2 ppm
1,2 Dichloroethane ($C_2H_4Cl_2$)	2 – 10 ppm *
1,3 Dichlorpropene ($C_3H_4Cl_2$)	0.1 – 10 ppm
Ethylene Oxide (C_2H_4O)	1 – 500 ppm
Ethyl Formate ($HCOOC_2H_5$)	20 – 500 ppm
Formaldehyde(HCHO)	0.04 – 40 ppm
Hydrocyanic Acid(HCN)	2.5 – 150 ppm
Methylbromide(CH_3Br)	0.2 – 50 ppm
Methylisothiocyanate (CH_3NCS)	0.1 – 6 ppm
Phosphine(PH_3)	0.01 – 10 000 ppm
Sulfuryl Fluoride(SO_2F_2)	1 – 5 ppm

* tube Methylbromide 0.2/a

Fig. 4

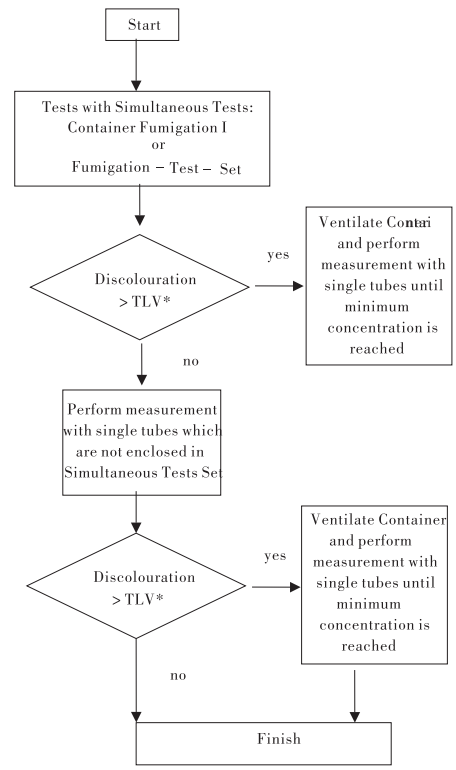

Fig. 5 **Measurement strategy to detect unknown fumigants; * TLV = Threshold Limit Values**

0312

Evaluation of 'Closed Loop Fumigation' in Large Steel Unsealed Silos in Western Australia

Christopher R. Newman

Abstract: Many large bolted silos with a capacity greater than 500 m are constructed unsealed in Australia unless full sealing is specified by the client. These silos may have a sealing compound inserted between each wall sheet and the base concrete but have open eaves,no sealing between the roof lap joints and unsealed access ports.

The fumigant phosphine is used routinely to control stored grain insects in this type of silo. The efficacy of these fumigations is questionable and gas may be lost before all life stages of the insect are controlled and with the potential to select for phosphine resistance. To control all life stages of the target species the gas must be retained within the grain for the period specified in the protocol.

To achieve full control of insects in these silos sealing is recommended but it is a more complicated and costly procedure to seal after construction. The addition of a recirculation(Closed Loop)system in conjunction with additional joint sealing and sealing of access ports may enhance distribution of gas throughout the grain profile.

This paper reports on an investigation into fumigations in bolted steel silos ranging from 835 m to 1 729m^3,ambient weather effects on the gas values and the impact of direction of airflow within the silo.

Fumigation results varied according to the ambient conditions and the air flow direction created by the recirculation fan.

A protocol of 100 ppm for seven days for susceptible grain beetles was reached in some storage under low ambient wind conditions but failed to reach this protocol under high wind conditions.

Fumigant loss was high when the gas was blown into the headspace and there was improved retention when the gas was extracted from the headspace and blown into the base.

Key words:closed loop fumigation,phosphine

Introduction

In very large squat sealed grain stores (height is less than twice the width) gas applied to the surface will diffuse over time to the lower parts of the silo. Friemel(1983 [6]) reported on the work by Allen et al(1979 [1]) on the surface application of Detia bag blankets to a 14 000 m squat welded silo in the USA. Phosphine concentrations > 100 ppm were achieved by day 8 in all parts of the silo.

To predict a successful fumigation the gas must be contained within a storage structure with minimal gas loss such as a sealed silo. A sealed structure is determined by a pressure halving test (P) of greater than five minutes from an initial pressure of 25 mm of water gauge for(i) an empty or part full silo and(ii) three minutes for a full silo. (Andrews et al 1994 [2])

In an unsealed silo,surface application of phosphine will more likely result in a greater part of the gas being lost to the atmosphere from a venturi effect(Reed 2006 [8]) created by wind blowing over the silo and causing a low pressure zone above the silo. The air/gas mixture within the silo will rise and leak from open joints before it can penetrate to the lowest parts of the silo.

In Australia a large number of squat grain silos > 500 m capacity have been constructed over the last 20 years. A majority of these silos were not constructed to the suggested sealed standard although the walls may have had a sealant product applied as the sheets were bolted together. The roof sheets and wall to roof joint are generally close coupled in the more recent constructions while older silos have open eave venting. These silos do not constitute a sealed storage and in terms of a successful fumigation are considered to be unable to reach a criteria suggested by Banks(1984 [3]) that "an average concentration greater than the minimum effective against insects be present at the end of

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the exposure period". In Western Australia the protocol for farm based fumigations is > 100 ppm for at least seven days > 25℃ and 10 days < 25℃. (Newman 2008 [5]).

Many of these large unsealed silos are regularly dosed with phosphine by the grain manager, most often by surface application of tablets and sometimes through aeration ducts if fitted. Many novel and informal methods are adopted by grain managers to reduce the numbers of visible insects during the storage period according to Andrews in 1994. This type of repeated fumigation can be considered a control failure because it has not eliminated all insect stages and has the potential to select insects for resistance to phosphine. In the absence of strictly policed regulations it would be useful to be able to recommend a technology that would assist a more effective fumigation.

Evidence from grain storage engineers in the USA has shown fumigations in large unsealed silos can be successful if the structure is fitted with recirculation ductwork and low power blower providing at least six air changes within the silo per day and minimising gas loss by sealing open joints, hatches and aeration ports. (Noyes, 1994 [7])

Method and Materials

This paper reports on work carried out to gauge the effectiveness of closed loop fumigations, the impact of recirculation direction and the effect of ambient heat on the concentration values at different times of the day in large unsealed silos in Western Australia (WA).

Ambient Wind Effect on Fumigations

The trial was conducted at Wongan Hills (WA) on 835 m 10 year old silos which had sealant applied to the vertical and horizontal lap joints and the concrete base during construction, the side entry door and the roof side hatch were fitted with rubber seals. The top hatch is attached to a permanent gravity tube sealed to the hatch during construction but remains open for grain flow allowing air leakage. The roof-to-wall joint and the roof sheet joints are close mated but not sealed. A 0.375 kW powered blower attached to the side of the silo draws the air/gas mixture from the headspace through a 100 mm PVC pipe which re-enters the silo through the lower wall. (Fig. 1)

The first fumigation in the summer of March 2006 was conducted under hot conditions of 26 – 36℃ with winds gusting between 23 – 30 kph. The silo contained barley at 75% fill and 9.3% moisture content. Blanket formulation of Quickphos aluminium phosphide at 1.19g/m^3 was laid on top of the grain and the recirculation fan was operated for the first three days of the fumigation period.

Fig. 1 Recirculation system fitted to 835m silo

A second fumigation was conducted in May 2006 under more moderate winds in an adjacent silo of the same capacity with the same construction and sealing techniques, 85% filled with wheat at 9.3% moisture content. Fumigant and fan operation was identical to the first fumigation.

Observations and discussion

Gas values were recorded with a Canary Company Silo Chek' and in the first fumigation (Fig. 2) increased rapidly in all monitored points reaching a peak of about 500 ppm by the second day.

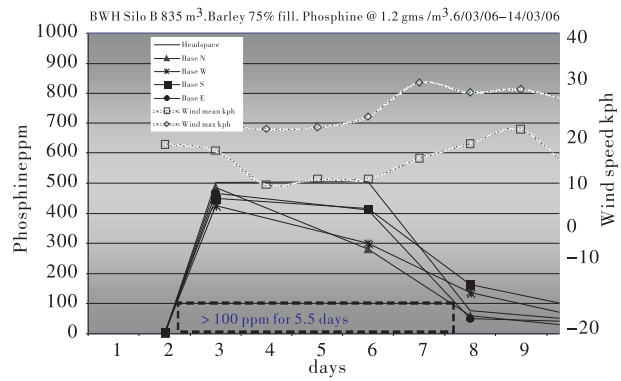

Fig. 2 Record of gas concentrations under high wind conditions

The gas values were trending downwards in line with the usual decay pattern seen in phosphine fumigation until day 5 when the wind speed increased, gusting up to 30 kph creating a venturi effect which drew the gas out of leak points in the silo. The result of 5.5 days > 100ppm is most likely to have been considered a success by the manager because there would be no insects visible in the grain. It is unlikely eggs and pupae were killed by this treatment and the grain would have required re-fumigation when adult insects became visible.

In the second fumigation (Fig. 3) under

lighter wind conditions (5 – 25 kph) gas values rose more slowly but reached 100 ppm in all parts of the silo except Base N in <44 hrs. Values remained above 100 ppm in all monitored points in the silo for seven days. Base N furthest from where the gas was blown in reached 100 ppm later but remained above it for >7 days. The fumigation period was considered successful according to Bank's criteria (1984 [4]) and corresponded with DAFWA recommendations (Newman 2008[5]).

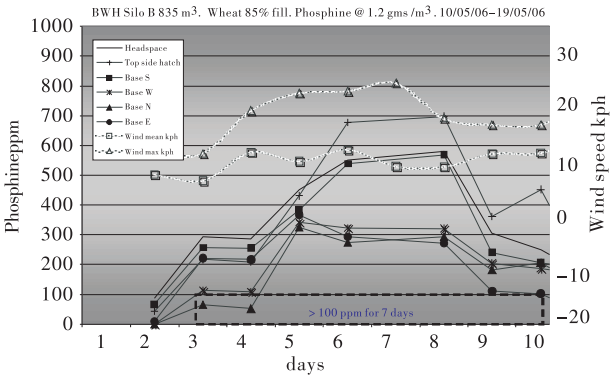

Fig. 3 Record of gas concentrations under low wind conditions

Effect of Airflow Direction within an Unsealed Silo

A trial on an unsealed 1 729 m silo loaded to 85% with Oats at 11% mc and 28℃ commenced in January 2007 at Wagin, WA.. The wall had been sealed during construction and additional sealing with polyurethane adhesive sealant was applied to the base joint, to the wall and roof joint internally, the roof seams externally and to the peak loading port. The roof top auger hopper was covered with a double layer of PVC sheeting, the bottom unloading auger was fitted with a seal plate and the auger void under the silo was blanked off at both ends. A dedicated fumigation hatch was created in the roof and equipped with hooks onto which to suspend the phosphine blankets. A low pressure fan (unknown capacity) was connected to a 100mm PVC pipe, entering the silo in the headspace and the auger void beneath the silo. Initially air was drawn from the auger void and blown into the headspace but re-configured during the fumigation to reverse the flow.

Observations and Discussion

Fumigation commenced by suspending two Quickphos blankets (1. 16g/m^3) of AlP in the fumigation hatch (Fig 4) and switching on the recirculation fan. Phosphine gas values rose in all points in the silo reaching 630 ppm in the headspace and 100 ppm at the base. (Fig 5)

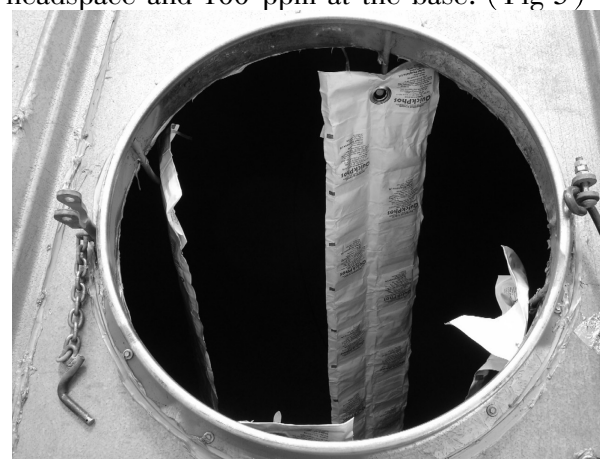

Fig. 4 Phosphine blankets suspended in fumigation hatch

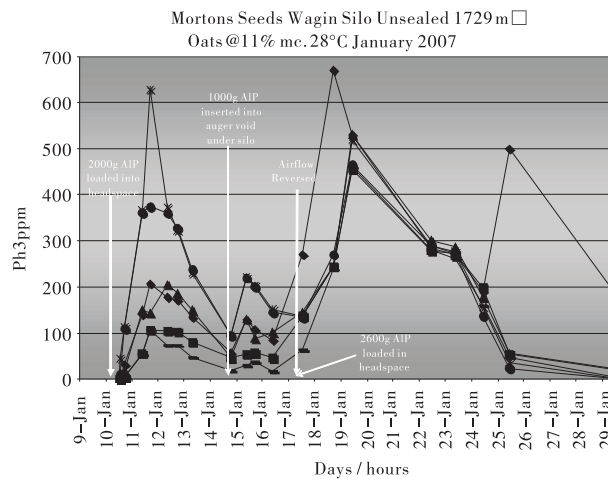

Fig. 5 Chart of fumigation showing the effect of redosing the headspace after reversing recirculation flow.

Gas concentrations were boosted by inserting 1 000g AlP tablets into the auger void when it was <20ppm, producing a short-lived elevation of gas values to a maximum of 220ppm in the headspace before falling rapidly.

The PVC pipe work was modified to reverse the direction of the air movement drawing from the headspace and injected into the auger void. With the recirculation fan off the headspace was re-charged with 2 600g of AlP in blanket and Bagchain formulations and gas values escalated rapidly at all points remaining above 100 ppm for 7 days reaching a peak of 670ppm at the SE base monitoring point.

Solar radiation effect on gas values Adjacent to the Wall in Steel Silos

The fumigation at Wagin produced slight evidence of gas movement attributed to the heating by the sun on the south east side of the

silo and rapid movement of air up the inside of the wall drawing gas across the floor recording a high value at the base monitoring point. This wall heating effect was observed in a CLF on 1 171 m unsealed silos at Cunderdin WA. (Fig. 6)

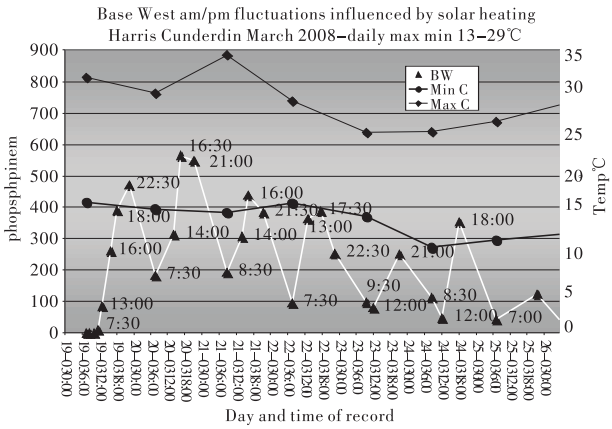

Fig. 6 Effect of wall heating on phosphine gas values, high diurnal temperatures

The monitoring was more frequent enabling more precise observations and it can be seen that the morning readings on the shaded west side (BW) were lower on average than the readings at the same point later in the day when the sun was heating that side. The effect was stronger when the ambient temperature varied between 13 – 29℃ then a month later when the daily average ranged between 11 and 25℃. (Fig. 7)

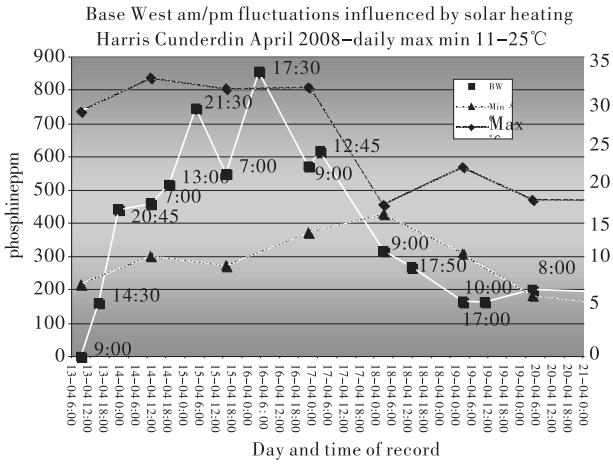

Fig. 7 Effect of wall heating on phosphine gas values, medium diurnal temperatures

This has implications for the timing of the reading suggesting the manager be aware of this effect during hotter periods. One reading a day could provide an incorrect measure of the fumigation. In this type of fumigation it is suggested there should be multiple readings taken to more effectively manage the gas loss and provide a clear indication of fumigation success or failure.

Conclusions

From these experiments it appears that fumigation in unsealed silos can be assisted with recirculation but ambient wind conditions will have a direct bearing on the success or failure of the fumigation. It is recommended by DAFWA to fumigate soon after harvest when high wind conditions are most likely. However in unsealed silos it may be more appropriate to manage the insects initially by reducing the grain temperature with aeration to prevent rapid population development and timing the end point of fumigation to coincide with extraction and sale of the grain.

Gas values achieved in the most successful of the fumigations were insufficient to control phosphine resistant insects had there been any present. Fumigators must be aware of the resistance status of the insects in their grain before considering a fumigation of this nature.

In the Wongan hill silo gas loss was recorded at a lower wall entrance hatch with a Silo Chek at up to 70 ppm while the fan was switched on and from the peak of the silo when the fan was switched off. Additional sealing would improve the result of future fumigations

This technique is not compatible with good grain storage practice and is contrary to recommendations promulgated in documents since the 1980's but this information has not prevented fumigation in unsealed silos. Further investigation would be valuable to make recommendations on reducing gas loss by fumigation timing, identifying common leak points sealing techniques and developing air systems to balance recirculating airflow around the silo.

While it is acknowledged that full sealing is the most appropriate method for control of grain insects, recommendations to retro-seal has had limited success, most likely because of the cost. Recommendations to add a recirculation system and apply sealing at critical points may be more acceptable and assist to improve the standard of fumigation in these silos. Part of such recommendations should include a benefit cost analysis to encourage the sealing of large squat silos during construction.

The observations of air direction effect in unsealed bolted steel silos shows that the roof is a vulnerable area most likely to leak gas and any additional pressure applied by a fan will ex-

acerbate the gas loss.

Acknowledgements

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SESSION 4

APPLICATION TECHNOLOGIES AND SAFE PRACTICES OF CA AND FUMIGATION TREATMENTS

Chairpersons:
Yonglin Ren, Australia
Zhang Hongyu, China
Ronald Noyes, USA

0401

Ground Level Phosphine Application Systems—— Towards a Safer Workplace

Christopher R. Newman

Abstract: Phosphine generating metal phosphide products have been in use in grain storage around the world for nearly 50 years. Early label recommendations suggested admixture into the grain stream as the most convenient method of application. A recent label change in Australia reflects the need to prevent admixture of spent phosphide residues with grain. This requires tablets or pellets applied in the headspace of the storage be placed onto a tray or similar device to capture the powder residue. This application method requires the fumigator to work at the top of some very tall silos and often in a confined or restricted area.

Techniques have been developed in Western Australia that enables sealed steel silos to be dosed at ground level, removing the need to climb the silo. The systems include: (i) a thermosiphon (temperature driven) distribution system in small farm silos, (ii) a fan operated recirculation system with an external phosphine generating system attached to larger sealed silos, and (iii) in unsealed silos, using a top up technique to maintain phosphine concentration at the required levels.

The results from trials with these techniques demonstrated the thermosiphon distributed phosphine gas safely and effectively throughout the grain bulk. All fumigations in the sealed silos reached the Department of Agriculture and Food Western Australia recommended protocol for elimination of all life stages of stored grain insects. In the unsealed silos, fumigation success was directly related to ambient wind conditions.

Key words: thermosiphon, phosphine, distribution, ground level application

Introduction

Recent changes to the phosphine label do not permit direct admixture (Pratt & Desmarchelier 1998 [9]) of phosphine generating products with grain, requiring the application of aluminium phosphide (AlP) formulation to the headspace of the storage. To retain the use of aluminium phosphide it must be applied in a manner that prevents contamination of grain. This may be onto a tray or similar device designed to contain the powder residue or by using a blanket or bagchain formulation. This means the fumigator must climb at least six metres and often work in a small or difficult area to apply a toxic product.

This paper reports on ground level generation and application systems using: (i) thermosiphon and (ii) an electric fan to distribute the phosphine in the grain bulk and avoid the need to climb the silo to insert AlP.

ⅰ) In 2005 Bird's Silos (Popanyinning, Western Australia) developed the first commercial thermosiphon (Cook, J. S. 1980[5], Boland 1984[3], Banks 1985[2], Cooper and Marszal 2000[4]) designed specifically to deliver phosphine generated in a reaction chamber' at the base of a sealed farm silo into the headspace. This system allows the fumigator to load the required AlP dose at ground level providing a safer, open air working environment.

Trials using a thermosiphon demonstrated phosphine gas can be safely and effectively distributed using a thermosiphon throughout the grain bulk in small farm silos up to 91mcapacity (Newman2006[7]). The trials were conducted with prototype equipment which demonstrated its efficacy but was vulnerable to damage and produced gas concentrations up to 7000 ppm in the phosphine chamber. A second prototype produced lower concentrations up to 4000 ppm and was of a more robust design. Further testing of the thermosiphon system was suggested.

ⅱ) Electric fan powered recirculation systems to move other fumigant gases into a product had been in use in the grain storage industry for many years. In 1980 a low flow/low pressure centrifugal fan recirculatory system was patented by Cook[5] to move phosphine gas through a grain bulk. The system has been emulated in many grain stores around the world with all relying on the insertion of tablets or pellets

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of AlP to the headspace. To achieve this many devices have been created to insert the formulation and capture the spent residue. The development of the aluminium phosphide blanket formulation removed some of the difficulty of applying loose tablets but still requires the fumigator to climb to the top of some very tall silos.

Powered ground level application systems have been in operation for many years, for example Siroflo and more recently Diluphos using gaseous formulation of phosphine. Other phosphine generating devices use metal phosphides activated in water to create phosphine and then move it into a grain bulk using a centrifugal fan. These systems provide rapid generation of phosphine to reduce fumigation time but are relatively high cost and require technical knowledge to maintain them.

Banks (1985 [2]) predicted that gas could be generated outside the grain store and delivered into the grain. A fan able to move a high concentration of phosphine gas very quickly to all parts of the silo without creating an explosion is outlined in AFHB/ACIAR (1989[1]). This states that fans should not exceed 10k Pa differential pressure or have a tip speed greater than 40 m/s.

A large amount of grain is stored in unsealed silos on farms in Australia, presenting difficult conditions to control grain insects. An external ground level application system will provide safer AlP application and also install a low cost recirculation system with the ability to maintain gas values by topping up low concentrations throughout the fumigation. Fumigation success in unsealed storage is highly dependant on the number of leak points and external wind conditions. Label recommendations in Australia now require application of phosphine to the headspace and prohibit the application of phosphine to a grain stream but surface applied phosphine gas concentrations will not persist in a leaky headspace.

The system described in this paper draws air from the headspace, passes it through the phosphine gas release chamber and into the base of the grain store and is suitable for sealed and unsealed stores. It is simple, can be manufactured with local materials and uses the most readily available aluminium phosphide formulation. Gas evolution is controlled by the moisture content of the recirculating air and is more suited to long term storage. The trials described in this paper were conducted under the previous label conditions that allowed the use of phosphine in unsealed silos.

1 Thermosiphon Powered Ground Level Phosphine Application Systems

1.1 Trial 1

1.1.1 Materials and Methods

This trial was conducted on a property near Kojonup, WA, February 14 – 22, 2006. A sealed silo with a pressure test (P) >180 seconds halving time and a capacity of 91m , was loaded to 98% fill with oats at a moisture content of 10.2%, and a temperature of 28℃. A thermosiphon made of black 90 mm internal diameter PVC pipe extending from the silo roof (close to the peak) down to the base of the sidewall where a water trap was built into it to collect any condensate that might accumulate inside the pipe.

Fig. 1 Black thermosiphon pipe connected to reaction chamber at base

The thermosiphon was connected to a 'reaction chamber' at the base of the silo using 32 mm internal diameter (id) flexible pipe attached to the underside of the cone base. (Figure 2) In this silo the 100 mm deep space between the lower seal plate and the butterfly valve' grain control device (flat steel disk swivelling on its axis in the outloading port) was used as the reaction chamber.

The grain in the silo was treated at 1.5g/m^3 using 130 aluminium phosphide tablets placed on the lower seal plate.

A Spectros Instruments Non Dispersible Infrared (NDIR) fixed filter phosphine monitor with the ability to measure phosphine concentr-

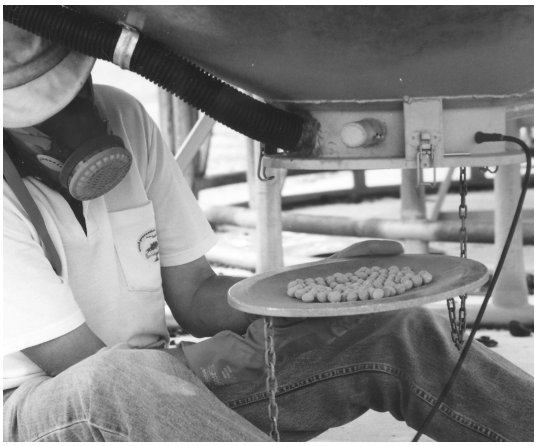

Fig. 2 Loading AlP tablets into reaction chamber at base of silo and showing pipe that carries the gas to the thermosiphon tube.

ations >3 000 ppm was used: (i) to ensure the safe operation of the system during the trials, and (ii) for automatic recording of gas concentrations at four points in the silo, sampling every 15 minutes in sequence for eight days. Sample tubes for this instrument were connected to the phosphine chamber, headspace, NE wall and the cone base using 5 mm id tubing attached to plastic fittings inserted through drilled holes.

1.1.2 Results

The first eight days of the fumigation period were monitored when gas values are most likely to peak in the phosphine chamber. (Fig. 3) The daily maximum and minimum temperatures were obtained from a weather station on the property.

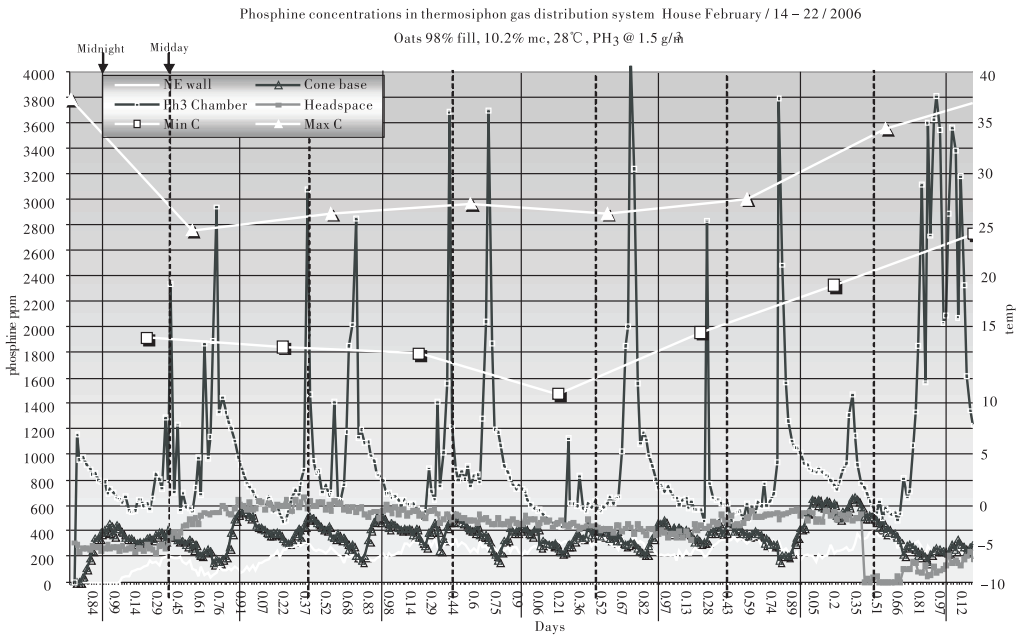

Fig. 3 Gas value record for 7 day fumigation on a thermosiphon powered silo.

The NE wall, the Cone base and the Headspace showed good distribution of the gas with all points above 100 ppm after 20 hours and reaching 200 ppm shortly afterwards. The concentrations were nearly consistent during the 7 day recorded period, which is in contrast to a top loaded non-recirculated fumigation which has high initial peaks and gradually falling concentrations. The sudden drop in concentrations on day six in the headspace and slow rise over 12 hours cannot be explained apart from possible equipment malfunction.

The marked rise and fall of the concentrations in the reaction chamber is characteristic of thermosiphon fumigation. This happens when air flow is halted in the thermosiphon and occurs when the commodity temperature is approximately equal to the ambient temperature. This situation has been observed to continue for up to 4 h (Newman 2006 [7]).

Some interesting changes of concentrations in Figure 3 demonstrate the influence ambient temperatures can have on the movement of air in the thermosiphon pipes.

For example the comparatively low morning peak on the fifth day is most likely Vthe result of cool air moving more rapidly down the thermosiphon under the influence of 8℃ overnight and early morning, pushing gas out of the phosphine chamber and into the cone of the silo.

On the seventh night the ambient conditions were warm from late evening until the early morning, close to the internal temperature of the grain bulk. It appears the air in the thermosiphon has stalled or was moving very slowly

through the phosphine reaction chamber giving rise to peak gas values of almost 4 000 ppm. These gas readings oscillated between 2 000 and 3 500 ppm until early morning which could be attributed to slight air movement or partial vapour pressure moving gas past the butterfly valve into the cone base of the silo.

1.2 Trial 2

1.2.1 Materials and Methods

A second trial was carried out at Balaklava in South Australia 27 May to 10 June 2008 under cool winter conditions on an elevated cone based sealed silo ($P^{1/2} = 60s$) with a capacity of 149m^3 loaded to 60% fill with oats at a temperature of 19.5℃ (moisture content unknown). A thermosiphon constructed of 90 mm id PVC pipe painted black was attached to the west side entering the roof near the peak and terminating in a water trap at the base of the wall. A 50 mm id PVC pipe was attached from the thermosiphon to the reaction chamber at the base of the silo.

Monitoring points using 1.5 mm id tube were inserted at the base of the silo wall at North, South, East and West sides, through the cone just above the butterfly valve and close to the reaction chamber and inserted into the roof near the peak with the tube running down the outside of the wall to the ground. A stainless tube carrying a monitoring line was inserted into the centre of the silo through the wall two days after fumigation commenced. A deeper reaction chamber 100 mm deep fitted to the bottom outlet grain control butterfly valve was trialled to hold the tablets. The reaction chamber contains a mesh platform which allows the powder to fall away as the gas evolves and prevents delay in gas release. (Fig. 4 & 5)

Fig. 4 Deep phosphine chamber – tablets loaded onto mesh tray.

Fig. 5 Deep phosphine chamber – tablets spent and powder under mesh.

The oats in the silo were treated at 1.5g/m^3 using 228 aluminium phosphide tablets which were placed in the reaction chamber. The gas concentration was measured with a Draeger Miniwarn that has a value limit of 1000 ppm.

1.2.2 Results

Phosphine concentrations passed the 100 ppm threshold two days after dosing and reached 200 ppm in all parts of the silo two days later (day 4). They remained above 200 ppm for nine days at which point monitoring was terminated. (Fig. 6)

The cooler ambient conditions compared to Trial 1 created a different pattern of gas concentrations. The concentrations were highest at the base monitored point indicating the air is travelling mainly down the thermosiphon and pushing the gas up into the cone. This is caused by an ambient temperature lower than the commodity temperature. Maximum concentrations of 1 000 ppm were recorded in the base point two days after fumigation commenced. An ambient temperature spike of 21℃ after noon on the fourth day demonstrates the air flow reversing in the thermosiphon pipe when the base values drop to 550 ppm and the top values rise to 550 ppm. The base value rose back to 1 000 ppm as the ambient temperature cooled overnight. The sudden fall and rise of all points on day 12 and 13 are unexplained and it is speculated the gas was moving to other parts of the silo. This could only be fully examined with more intensive monitoring in future trials.

1.2.3 Discussion

1. It has been demonstrated in these trials that a thermosiphon system effectively delivers phosphine gas into the grain bulk from the phosphine reaction chamber and produces rapid equalisation throughout the profile. The system has the advantage of not needing expensive ele-

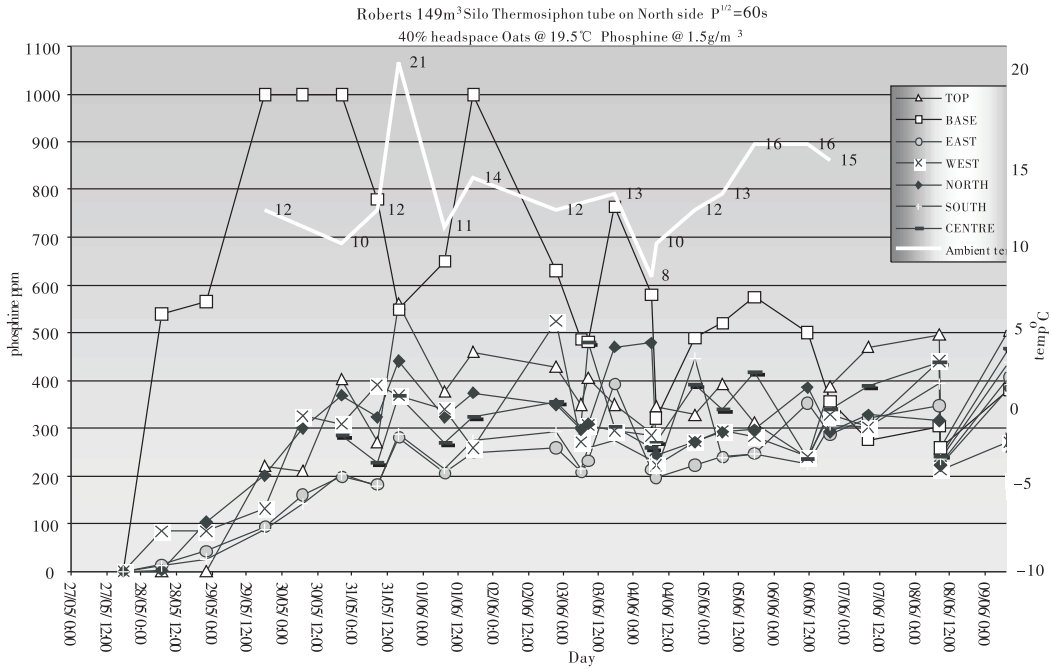

Fig. 6 Thermosiphon fumigation dataBalaklava South Australia℃

ctricity connection but it will only be effective in a well sealed silo. Application of phosphine tablets under the silo ensures there can be no admixture of the residue with the grain. In these trials despite the air stall within the thermosiphon and subsequent rapid escalation of gas concentrations in the phosphine reaction chamber, the phosphine concentrations remained well below the lower flammability threshold.

2. These initial trials were conducted with limited resources and further testing is needed to investigate fumigations under a greater range of commodity and environmental conditions. For example the effect of higher moisture grain on the speed of evolution of the gas, fumigation under cool conditions, placement of the thermosiphon pipe in relation to sun movement and use on larger silos.

3. While thermosiphon application addresses the safety concerns for climbing and working at heights with a dangerous product, it also places the worker in close proximity to the AlP tablets under the silo. Care must be taken when opening the reaction chamber that the spent powder is not blown over the worker. Full protective clothing and full face mask is recommended for these situations.

2 Electrically Powered Ground Level Application System in Sealed and Unsealed Steel Silos

2.1 Trial 1 Sealed silos

2.1.1 Materials and Methods

The first trial of a powered ground level application system in WA was at the Quaker Oats storage complex in Forrestfield from 1226 July 2006 on a 3 200m sealed steel silo with a below ground concrete cone, which exhibited $P^{1/2}$ = 180 seconds (500 Pa to 250 Pa). The silo was filled to 95% capacity with oats at 8.8% moisture content. A ground level pressure relief valve was connected to the headspace by a 200 mm steel pipe. The pipe was fitted with Camlock couplings to accept 100 mm i. d. flexible food quality PVC reinforced hose to move gas between the headspace, the reaction chamber and the base of the silo. A 3.2m × 0.8m × 0.47m phosphine reaction chamber was constructed with Camlock fittings on each end to receive the flexible pipes and sliding mesh trays inside the chamber to allow the rollout of three 3 metre AlP blankets.

Gas values were recorded using a Canary Company, Silo Chek' gas monitor through 1.5 mm i. d. nylon tubing inserted through the silo wall at four points around the base and one in the headspace and at the gas point of entry and exit from the silo.

2.1.2 Gas blown into headspace

The fumigation commenced by loading the reaction chamber with three AlP blankets providing 3 000g of phosphine gas in the silo, at a dosage rate of 0.95g/m. (Fig. 7) The released gas was blown into the headspace and the air/gas mixture drawn from the bottom of the silo.

Fluctuating gas values in the headspace after day 8 and no detectable gas in the below gr

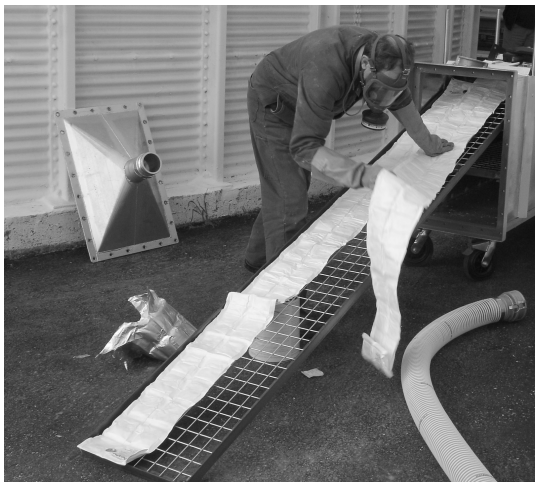

Fig. 7 Loading AlP blankets into the reaction chamber

ound cone compromised the fumigation and it was decided to reverse the air flow to try to increase the gas concentration in the cone. (Fig. 8) An additional monitoring point was inserted in the unloading auger.

2.1.3 Gas blown into the base

A trial in an adjacent silo of the same capacity and with the same pipe work fitted was commenced with the AlP reaction chamber reversed and the hoses connected to draw gas from the headspace and blow it into the base. This fumigation demonstrated lethal gas values for seven days in the cone auger but recorded variable concentrations in the headspace. (Fig. 9)

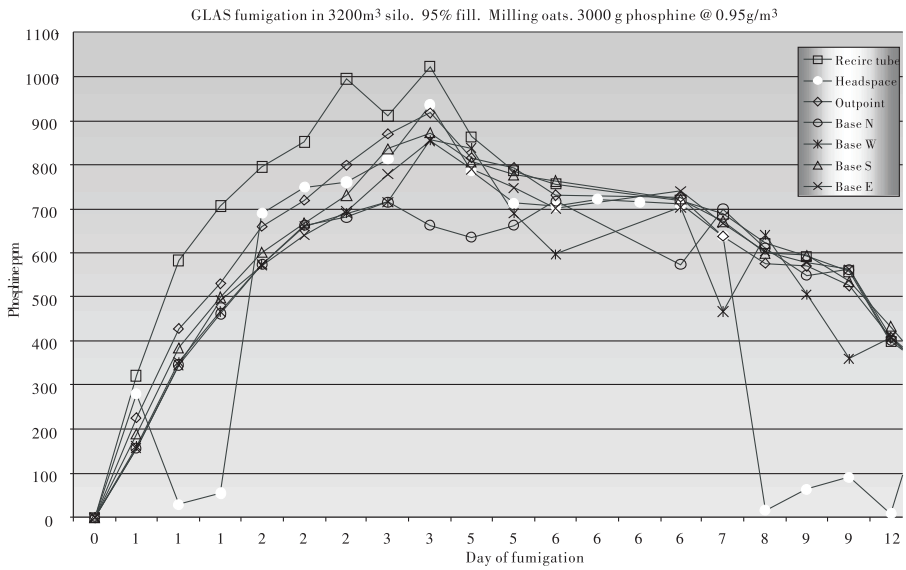

Fig. 8 Chart data from initial 3000g fumigation

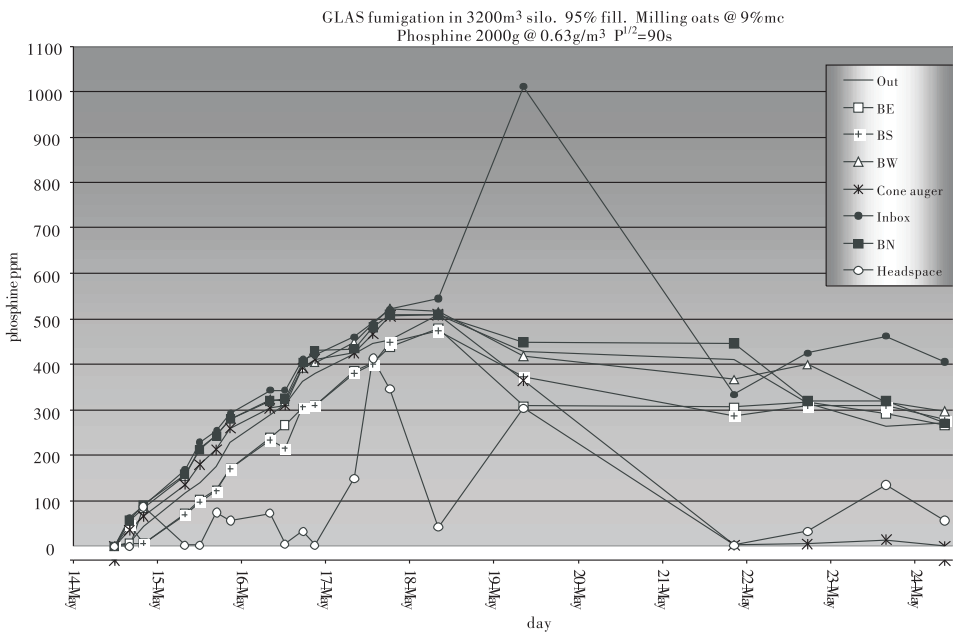

Fig. 9 Reversed flow fumigation

2.1.4 Discussion

Fluctuations in gas values in the headspace of these silos are most likely caused by strong winds creating a chimney or venturi effect' on the silo drawing gas out through any leak points. The slide valve under the top drag chain was a suspect leak point because it has to be sealed by applying mastic and dust makes it difficult to seal effectively.

From this trial and complimentary work on Closed Loop Fumigation (Newman 2008[8]) it is suggested that blowing the gas into the base of the storage is more likely to result in an effective fumigation than pressurising the headspace with a greater risk of gas loss through leak points.

In a sealed silo the outcome is more predictable and monitoring may be conducted once a day, however two readings per day will provide a better indication of the success of the fumigation and in particular give more accurate start and finish times of the protocol period.

This powered recirculation system contributes to a safer workplace, reduces the physical stress on employees, decreases the time to reach threshold gas concentration in all parts of the silo and reaches equalisation of the gas more rapidly than a top loaded fumigation relying on thermal currents and partial vapour pressure to distribute the gas.

2.2 Trial 2 Unsealed silos

2.2.1 Materials and Method

A trial was commenced at Cunderdin WA in March 2008 to control *Tribolium castaneum* (Herbst) on a farm in four 1 171m unsealed silos. The safe option to apply 1 900 AlP tablets into the silo was to construct a phosphine reaction chamber at ground level to hold the tablets on trays and blow the gas into the base of the silo. Loading the tablets onto trays in the headspace though a 500 mm entry hatch 13 metres above ground level and afterwards retrieve the spent powder is much more hazardous.

A sealed phosphine reaction chamber was constructed from 3 m × 0.45 m × 0.45 m steel plate and fitted with wheels to enable it to be moved easily to all silos on site. The fan bolted to the chamber had a differential pressure of 500Pa and fan tip speed of 25.4 m/s and was measured to draw 41.8 litres per second of air from the headspace providing three air changes in the silo per day.

A 90mm diam PVC pipe was inserted into the roof of the silo, continuing to ground level and connected to the reaction chamber by a flexible 80mm reinforced hose. The gas from the reaction chamber was injected into the base of the silo via a 10 cm × 90 cm perforated steel pipe inserted into the grain.

In this case the silo wall sheets were partially sealed during construction but it was technically unsealed. Some effort was made to reduce the number of gaps prior to the fumigation in the roof to wall joint and the peak loading point. (Fig. 10)

Fig. 10 Sealing the roof to wall joint

The roof lap joints were not sealed and the chain conveyor housing in the base remained a leak point.

2.2.2 Results

2.2.2.1 High wind fumigation

The fumigation commenced by loading 1900 AlP tablets into eight trays made from 20 litre plastic containers sawn in half and with a plastic mesh support for the tablets. (Fig. 11 & 12)

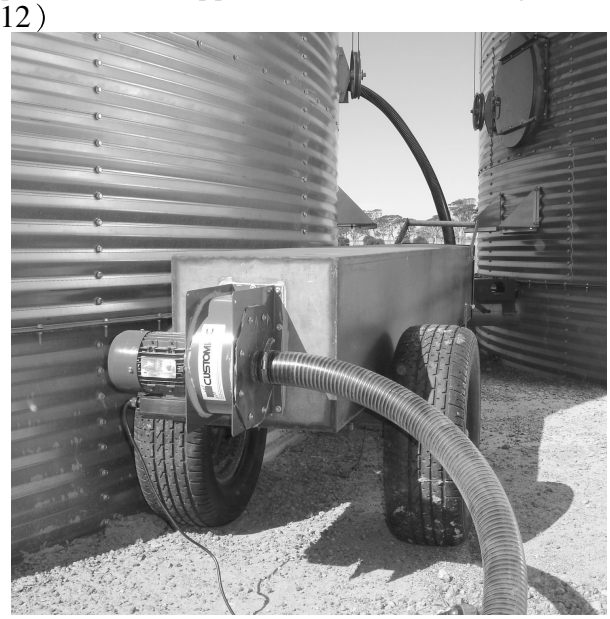

Fig. 11 Farm constructed ground level application system

Fig. 12 **Internal view showing AlP trays with plastic mesh**

The fan on the fumigation chamber was turned on and left running for most of the fumigation period.

This period was characterised by strong easterly winds with a mean ranging from 12 to30 kph prevented the gas values on the windward side from rising above 16 ppm except for a brief period.

This finding coincides with earlier work by Newman [8] on the gas loss caused by strong ambient winds.

The fan was turned off at day three and the gas values at the sampling points fell rapidly, demonstrating the need to keep the fans running in unsealed silos to hold the gas within the grain. The fan was re-started 21 hours later.

This fumigation did not achieve the fumigation protocol outlined by DAFWA of > 100ppm for 7 days and was deemed to have failed.

2.2.2.2 Moderate wind fumigation

The same silo was fumigated the following month with a new load of grain during a period of more moderate wind conditions. (Fig. 13)

All monitored points of the silo rose above 100 ppm after 36 hrs and stayed > 100ppm for a further 60 h under very low ambient wind conditions. On day five a 25 kph southerly wind demonstrated the vulnerability of unsealed fumigation when values dropped rapidly at the south monitored point. An additional 1 000g of AlP was added 24 hrs later raising the gas concentrations at three points while two points on the south and east remained below the 100 ppm target value coinciding with a rise in wind speed from the south.

Even though two points in the silo failed to reach the protocol gas values, and the warm grain remained in the silo for a further three weeks before outloading commenced, the grain was outloaded and sold over a period of seven weeks with no live insects detected.

(Harris, E. 2008 pers comm. [6])

2.2.3 Discussion

Moving the gas into the base of an unsealed silo places it directly into a more protected zone in the grain bulk and as the gas exits from the surface of the grain it is drawn quickly back to the base reducing the gas loss caused by external winds.

The grain in the test silos ranged between 8.5% and 11% and observations show peak gas values usually occur after 36 – 48 h which is comparable to the most common top loaded' farm fumigations. Equalisation throughout the bulk is more uniform and occurs much faster than in standard fumigations. Peak values in the headspace tend to be lower because the gas is drawn back into the return pipe soon after it emerges from the grain bulk.

Fumigations on farms in Australia are usually not monitored and rely on the label rate to reach the recommended c × t product. This will work effectively in a sealed silo up to 200t, but above this size there is a need to recirculate, and ideally the silo should be sealed. In reality a small percentage of large grain silos on farms in Australia are sealed to a fumigation standard or have a recirculation system installed. The cost of sealing can be significant, up to 20% of the initial construction cost. If there is an unwillingness to commit funds, poor fumigations will continue.

A ground level application system attached to these silos enables the concentration to be manipulated by topping up the AlP charge in the chamber as needed during the fumigation to maintain the designated gas concentration × time protocol.

Using a ground level application system in these types of silos provides a level of control over the fumigation and with selection of appropriate calm weather conditions by referring to the meteorological forecasts, good monitoring, and management of the AlP recharge in the reaction chamber, it has the potential to eliminate all stages of insects that are strongly resistant to phosphine.

To enable fumigation in the many unsealed silos in Australia there is a need to develop a phosphine label that gives instruction on main-

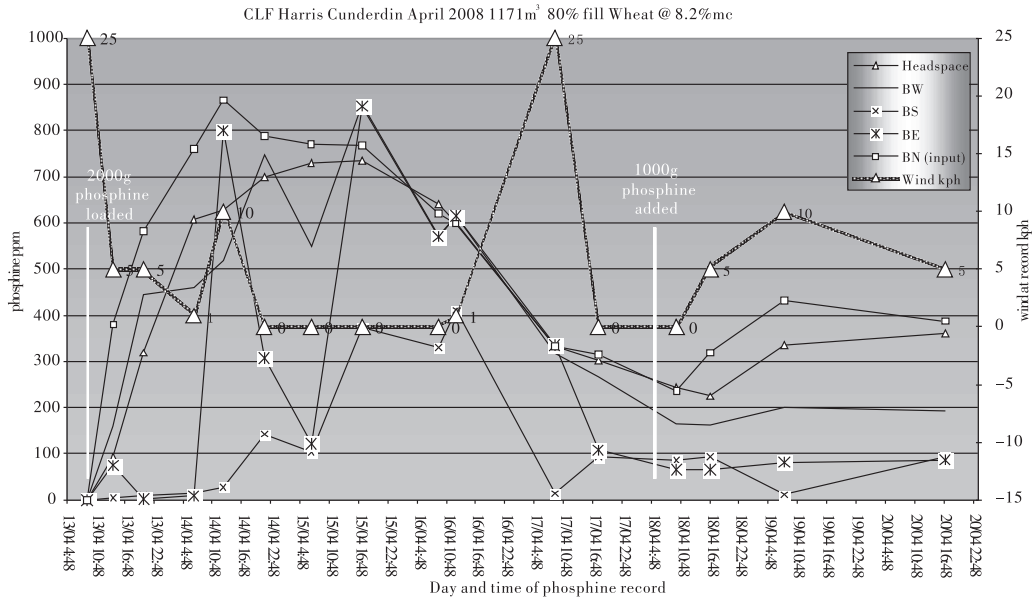

Fig. 13 **Influence of low wind conditions on internal gas values**

taining a protocol above a minimum concentration. The most recent phosphine label provides for fumigation only in sealed silos and for application of AlP to the headspace. This type of fumigation is highly susceptible to fumigant loss in unsealed silos and will result in selection for resistance in the insects present. A formal trial to assess techniques to distribute gas evenly throughout the silo is needed to provide data for a label stating required gas concentrations.

Conclusion

The trials reported in this paper have demonstrated the success of thermosiphon attached to sealed silos and an external ground level fan powered phosphine generating and recirculating device attached to large sealed silos. Both systems produced lethal phosphine concentrations to eliminate all life stages of the insects present.

Using an external phosphine generating device on large unsealed silos is subject to external weather conditions with the risk of fumigation failure and requires a much higher level of management for success.

The new Australian Standard that requires silos that are constructed as sealed, regardless of size, to conform to a pressure test of three minutes or longer from 500 Pa to 250 Pa will impact on the quality of the construction of farm silos in the long term. However there are large numbers of existing grain silos constructed many years ago that remain structurally sound but the cost of retro sealing is not economical for the remaining life of the silo.

Fumigation using an external generating and recirculation device is an attractive short term option to continue to use the silo. This system offers the chance to eliminate all grain insects in unsealed storages at a lower cost than complete retro sealing the grain store to the current grain industry standards.

Future trials should aim toward producing recommendations that will enable effective management of this system.

Acknowledgements

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0402

Hermetic Storage of High Moisture Corn under Tropical Conditions

Arnold R. Elepaño[1] and S. Navarro[2]

Abstract: Shelled corn of 26% moisture content (m. c.) was stored in a Cocoon™ under hermetic conditions for 96 days to demonstrate the effectiveness of maintaining its quality prior to subsequent drying or processing into feeds or ethanol. The Cocoon™ was made of a plastic liner and capable of holding 11 tonnes of corn stored in standard bags. Both the Cocoon™ and the control consisting of 3 bags were stored outdoors at the University of the Philippines Los Ba os, Philippines. Corn was sampled before and after storage for determination of m. c., starch, ethanol, aflatoxin, and germination rate. Temperature, oxygen and carbon dioxide concentrations were monitored throughout the storage period. The initial corn temperature in the Cocoon™ reached 45℃ and then equilibrated with that of the ambient at 30℃ after the first week of hermetic storage. The initial oxygen concentration dropped within one day and remained at an average of 0.54% throughout the storage period. Average m. c. at the end of storage increased to 29%. No significant change in starch content was observed throughout the storage period. Corn in the control bags deteriorated after three days and temperature increased to 55℃. The high moisture corn in the Cocoon™ initially had 59 ppb of aflatoxin due to a logistical delay of about 3 days for acquiring the corn from several farmers. Aflatoxin level increased to 90 ppb after one week of storage and remained at that level for 96 days. No presence of insects was observed in the corn samples stored in the Cocoon™. Feeding trials indicated that the corn from hermetic storage was palatable to cows and swine. Results of the study indicate that wet corn can be safely stored for extended periods of time without significant increase in aflatoxin, and without significant changes in starch and ethanol content.

Key words: hermetic storage, high moisture content corn storage, aflatoxin, oxygen, carbon dioxide, starch, ethanol

Introduction

In tropical climates like the Philippines, corn is often harvested under unfavorable conditions. While the need for high capacity mechanical dryers has been recognized to reduce post-harvest losses, investments have been high and in between. An alternative to immediate drying is to store freshly harvested corn in a hermetic storage, thereby maintaining its quality prior to subsequent drying or processing into feed or ethanol.

The present study was undertaken to develop an affordable system for long term storage of high moisture corn under tropical climates using the Cocoon™ hermetic storage system. Specifically this study aimed to: 1) demonstrate the effectiveness of storing of high moisture corn under gas tight conditions with minimal losses of weight and quality; 2) to determine the effects of modified atmospheric storage, i. e. oxygen depletion, on the storage environment produced by the biological activity of the commodity and its effect on the control of microflora to prevent development of mycotoxins and the level of ethanol and starch production.

Materials and Methods

Corn Samples

About 11 tons of Monsanto's Bt – corn Dekalb 818 samples were procured from 5 different farmers in Calamba City. Corn was planted between November 28 and December 1 2006, manually harvested between March 11 and 14, 2007 and shelled between March 14 and 15, 2007. The AMDP double – drum corn sheller, suitable for high moisture corn, was utilized in shelling the 25.6% wet basis kernels. Bulk density of the shelled corn is 690 kg/m^3. Corn was delivered and stored in the GrainPro 10 ton Cocoon at ABPROD on March 16, 2007. The second replicate totaled 7 400 kg of Dekalb

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818 which was harvested 25 – 27 June and was procured 28 June 2007.

Only one variety was used in the experiment as Simacha[1] indicated that there is no significant differences in fungal infection and aflatoxin accumulation among varieties.

The experiment was replicated in two periodsone during March to June and the other during July to December 2007. Freshly harvested corn was bought from farmers in the neighboring areas of University of the Philippines Los Ba os (UPLB).

Corn Setup and Sampling

During the first replication, samples of about 1 kg were needed for the various physical and chemical analyses every week. Thirteen bags containing wet corn samples were prepared and placed near the opening of the zipper. One bag was pulled out of the cocoon every week.

After a day of storage, the cocoon ballooned due to the production of carbon dioxide. To regulate the pressure a simple pressure relief consisting of a glass jar, copper tubing and water was installed. The cocoon was also placed under shade to prevent direct radiation which might induce excessive moisture migration and condensation on the corn inside. Visual inspection and simple bubble leak tests were undertaken on a weekly basis to determine the integrity of the cocoon, thus ensuring that there is no leak in the system.

Gas Concentration

Oxygen (O_2) concentration was measured using a portable GrainPro HGA – 11 – B oxygen monitor. Carbon dioxide (CO_2) was measured using a compact Bacharach CO_2 Analyzer 2 820 that displays the detected level of CO_2 in the range of 0 – 60%. The analyzer operates on the infrared-absorption principle to detect the presence of CO_2. Gas concentrations inside the cocoon were measured twice daily.

Ambient conditions

Temperature and relative humidity were measured using Lascar EL – USB – 2 which stores relative humidity and temperature readings in the range of 0 to 100% RH and – 35°to +80℃.

Grain composition and quality

Moisture content was determined at the Agricultural and Bio-Process Division (ABPROD) using the ASAE Standards for Moisture Content Determination. Starch content was analyzed by Lipa Quality Control Center using AOAC Official Methods of Analysis, 17th edition, 2002. Ethanol content was determined at ABPROD using the Ebulliometer method. Germination rate was determined at ABPROD using the International Seed Testing Association's Rolled Paper Method. Aflatoxin content was determined by the National Institute of Molecular Biology and Biotechnology (BIOTECH) using the Monoclonal Antibody Based ELISA. Some of the analyses were also undertaken by the Lipa Quality Control Center using R-Biopharm AG, Ridascreen, Fast Aflatoxin Enzyme Immunoassay ELISA.

Results and Discussion

Oxygen and Carbon Dioxide Concent – ration inside the Cocoon

After an hour of loading the wet corn inside the cocoon, the CO_2 Analyzer indicated a concentration above the maximum analyzer capacity of 60% CO_2 content. Throughout the experiment, the CO_2 concentration was over the limit of the equipment. With the dramatic production of carbon dioxide during the first four days, the cocoon ballooned, requiring pressure relief.

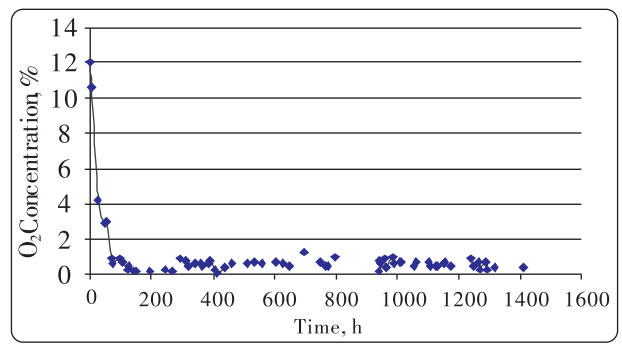

Fig. 1 O_2 concentration in the cocoon with time.

Figure 1 shows the level of O_2 in the cocoon which was at the first hour after sealing the cocoon 12%, and subsequently dropped to lower levels. During the first couple of days after sealing, excessive pressure accompanied by the production of CO_2 from respiration was observed. For three months, O_2 levels in the cocoon (Mar – Jun 2007) had an average value of 0.54%. The second replicate exhibited the same pattern where O_2 concentration greatly decreased after the first two days of storage. In trials carried out by Weinberg et al.[2] similar observations were made; as the moisture level increased the time for O_2 depletion below 1.0% shortened, from 600 h at 14% moisture to 12 h at 22%.

Temperature and Relative Humidity inside the Cocoon

Temperature and relative humidity dataloggers were placed in the top corner and middle portions of the cocoon. Another datalogger was used to measure the temperature and relative humidity of the ambient air. All dataloggers were set to record data every hour (Fig. 2).

Initial observations indicated that the cocoon had a higher temperature compared with the ambient temperature during the first several days of storage due to the accumulation of heat of respiration. During this time, the temperature inside the cocoon reached 45℃. After the first week, however, germination of the grains inside the cocoon dropped to zero and respiration ceased. Without respiration, there was no heat generation. Temperature of the air inside the cocoon approximated the temperature of the ambient air.

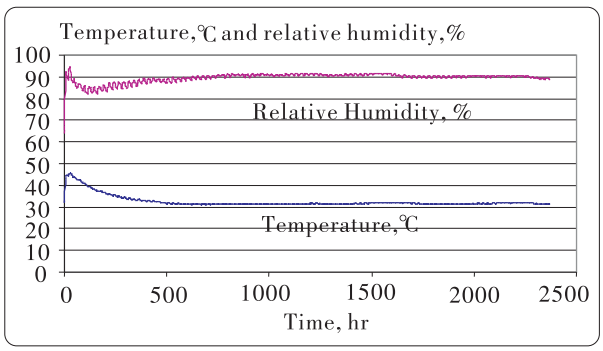

Fig. 2 Temperature and relative humidity at the center of the cocoon.

Moisture Content

Initial average moisture content of the corn was measured as 25.6%, wet basis. Figure 3 shows that the moisture content was initially constant then increased with storage time. The increase in moisture content is attributed to the water produced as a result of respiration of the organisms on corn. After three months of storage, average moisture content of the whole cocoon taken from 27 samples, was 29.1% which is an increase of 3.5 percentage points from the initial moisture content.

Final moisture content of corn samples throughout the cocoon ranged from a low of 26.2% to a high of 38.5%. It was noted that corn at the top of the pile had higher moisture content compared with those samples in the middle layer of the cocoon. This was attributed to the condensation of moisture on top of the cocoon during the early part of the day. Corn at the top of the stack subsequently absorbed the

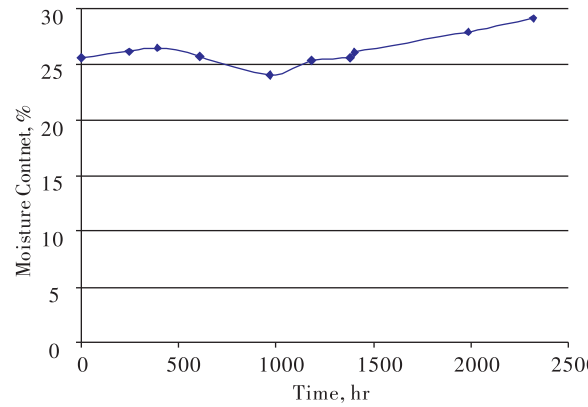

Fig. 3 Moisture content of corn with time in the cocoon

moisture. Corn at the bottom sides of the cocoon has been observed to have higher moisture content than the initial average moisture content. Some of the water condensing on top of the cocoon dripped down along the outer lining to the bottom panel where it was absorbed by the corn in bags on the bottom layer.

The second cocoon had an average initial moisture content of 31.6 % wet basis. After six months of storage, the moisture content was reduced to 28.9%. There was some variation in moisture content of the kernels inside the cocoon, as observed in the first replicate. It was also observed that condensation took place along the cocoon lining due to temperature fluctuation of the ambient air. Standing water was observed on the bottom portion of the cocoon, which explains the loss of moisture from the kernels.

Starch and Alcohol Content

During the three months of storage, starch content in the corn varied from 60% to 65%. Figure 4 shows that there was only minor change in starch content with storage time, stabilizing close to 60% after 600h.

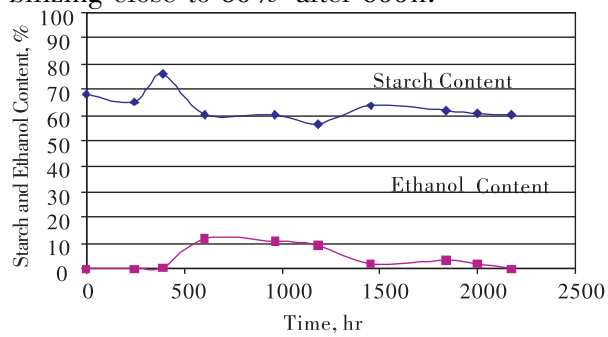

Fig. 4 Starch and Ethanol Content with time during storage in cocoon

The initial ethanol content of the kernel was found to be 0%, but it showed an increase

after 600h, then a subsequent decrease to 1% – 2% occurred after 1400h. After three months of hermetic storage at the opening of the cocoon, 27 samples were collected and subjected to ethanol content analysis. Average value was 0.14% indicating a marginal amount of ethanol in the corn. The data indicates that there is an insignificant conversion of starch into sugar, and to ethanol. However, after six months (4 320h) of storage, the ethanol content increased to an average of 4.4 % by volume. According to Weinberg et al. [2] the major fermentation product in the hermetically sealed corn was ethanol (0 to 5 g kg^{-1} DM), along with lower concentrations of acetic acid (0 to 1 g kg^{-1} DM). The familiar smell of lactic acid during the storage, especially during sampling, was present. There was no attempt to determine the actual concentration of lactic acid in the samples.

Molds

Three bags of freshly harvested corn were left near the cocoon to serve as a control sample. It was observed that after three days, the kernels deteriorated with the presence of black spots. It was also observed that the temperature of the grains increased to about 55℃, while the ambient temperature was only about 30℃. Moisture content of the grain in the control sample decreased to 19.9% during the first week of storage. Germination was also significantly reduced. Mold identification of the control sample revealed the presence of *Rhizopus spp.* and *Penicillium spp.*

Aflatoxin Levels

The high moisture corn inside the cocoon initially had about 59 ppb of aflatoxin, having been exposed to ambient temperatures for about 3 days after harvesting. Figure 5 shows that the aflatoxin level increased to 90 ppb after one week of storage and remained at that level during the storage. After 96 days of storage, 27 samples at different locations in the cocoon were analyzed for aflatoxin content. Results show that the average aflatoxin of the corn was 98.3 ppb with a sample standard deviation of 54.3 ppb. Although there was a large variability in measurement, we observe in Fig. 5 that the aflatoxin level in the cocoon did not increase significantly with storage time.

Retrieval of weekly bag samples for analysis necessitated opening of the cocoon, which provides opportunity for oxygen ingress. Hocking[3] noted that atmospheres containing high CO_2 levels are more effective in controlling fungal growth than those which exclude O_2 by replacement of N_2. Many spoilage fungus species are efficient scavengers and are capable of near normal growth in O_2 concentration of less than 1 percent. Hocking[3] concluded that atmosphere containing about 20% CO_2 generally inhibits mold growth but greater than 80% CO_2 may be required to prevent deterioration of high moisture commodities. Only when oxygen is completely unavailable, are microorganisms inhibited [4].

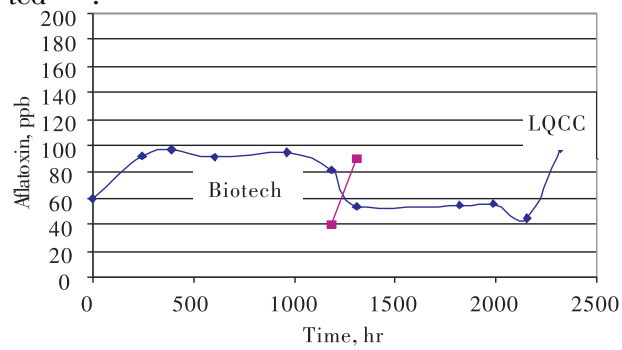

Fig. 5 Aflatoxin levels in the grains with time stored in the cocoon.

In the second replicate, the initial aflatoxin level of corn was found to be 53.9 ppb. After six months of storage, the level increased insignificantly to 72.9 ppb. At this level the grain can still be used for feeding – cattle and swine.

The present experimental data indicate that even after 96 – 150 days of storage, high moisture corn can be used to feed breeding – cattle and swine, mature poultry, finishing swine and beef cattle as presented in Table 1.

Insect Infestation

No presence of insects was observed in the corn samples stored in the cocoon.

Proximate Analysis

Table 1 shows the results of the proximate analysis of the kernels stored in the cocoon after drying. Kernels were dried in a flatbed dryer to 14 percent moisture. Since crude protein is a major constituent of ruminant diets, a high protein level is desirable in the feeds because it requires less supplementation and therefore results in lower feed costs. The data indicated that storage of wet kernels in hermetic storage increases its protein content.

Crude fiber which is a measure of the fiber content of the corn is less desirable since it is less digestible than the non-fiber constituent of the kernel. Samples with high fiber content have lower energy. That is the reason low fiber content is desirable for animal feeds. In wet corn

storage, carbohydrates are converted into organic acids predominantly lactic and acetic acids during the hermetic storage of corn.

The odor of the samples was light and pleasant with no indication of putrefaction. Although preliminary feeding trials on cows did not show differences in palatability and digestibility of the animals an additional feeding trial is recommended to determine the palatability and its effect on the growth of cattle and swine.

Table 1. Proximate analysis of corn after 3 months of hermetic storage compared with AACC values

	Moisture Content, % wet basis	Ash, % dry basis	Crude Protein, % dry basis	Crude Fat, % dry basis	Crude Fiber, % dry basis
Experiment	10.06	1.87	12.23	5.35	2.70
AACC	16.0 (7 – 23)	1.4 (1.1 – 3.9)	9.5 (6 – 12)	4.3 (3.1 – 5.7)	9.5 (8.3 – 11.9)

Note: 1. AACC values from White PJ & Johnson LA. 2003. Corn Chemistry and Technology. American Association of Cereal Chemists. Range values are in parenthesis.
2. Wet corn was hermetically stored for 3 months, and then it was dried using a flat bed dryer.

Conclusions

High moisture corn can be stored hermetically without reduction of quality. The results of the study indicated that the moisture content increased with storage due to respiration. Temperature of the corn in the cocoon increased during the first few days but dissipated after reduction of respiration and equilibrated with the ambient temperature after a week of storage. Starch and ethanol contents were relatively constant with time. Despite a large variability of measurement for aflatoxin, it can be concluded that the aflatoxin level in the cocoon did not increase significantly. An added benefit of hermetic storage of corn is the increase of protein of the kernels. Feeding trials on cows did not show significant difference in palatability and digestibility of the animals.

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0403

Research on Prevention Effect of Grain Pest by CO_2 Controlled Atmosphere

Zhang Funian[1], Chen Defa[1], Liu Qiang[1], Chen Biren[2] and Li Hongyang[2]

Abstract: The total 30 million kilos grain reserves project by CO_2 controlled atmosphere in our warehouse is invested and built by the state and was entirely finished in December 2002. CO_2 controlled atmosphere warehouse test was conducted from May to August in 2004, so as to give full play to the advantages of grain storage by CO_2 controlled atmosphere, which are pest disinfestations, bacteria inhibition, extension of the quality of reserved grain, without residue, pollution and public hazard. The test indicated that applying above 35% CO_2 in high flat warehouses for 15 days, grain can effectively be disinfested of stored – grain pests, postpone the rate of deterioration of reserved grain and realize the safe reserve of Grain Reserves of Central Government.

Key words: CO_2, Controlled atmosphere, Stored – grain pests, Prevention and control

Introduction

CO_2 controlled atmosphere storage technology is the grain storage method to output liquid CO_2 from fixed CO_2 gas storage, increasing the air tightness seal of warehouses, adopting a centralized gas supply method and utilizing manufacturing facilities of controlled atmosphere, then conveying the gas into controlled atmosphere warehouses through gasification and decompression. Then raise the CO_2 level in the warehouse to the treatment concentration rapidly and evenly through forced recirculation piping systems. Finally, using computerized technology to automatically check CO_2 concentrations in warehouses to keep it within the specified treatment range, so as to preserve grain by a suitable, clean controlled atmosphere. CO_2 can control stored – grain pests, inhibit occurrence and development of mold of reserved grain, postpone deterioration of reserved grain, decrease the cyclic frequency of insect infestations and phosphine fumigations, abate the harm to humans and damage to ecological environment, reduce pollution of reserved grain, increase the economic value of reserved grain, and realize the environment – friendly and safe storage of grain.

Lu'an Warehouse carried out stored warehouse test of grain reserves by CO_2 controlled atmosphere, which focused on carrying out primary research in terms of pest disinfestations effect of CO_2 controlled atmosphere, variation situation of grain quality and safe operation, compared to regular grain reserves. The test situation is as follows:

1 Basic Situation of Tested Warehouse

Select the No. 18 CO_2 controlled atmosphere warehouse to conduct stored warehouse gas charging test form May 26th, 2004 to the end of August, 2004. The 60 24m arch large flat warehouse reserved medium and late indica rice produced in 2003 with 6.0m stack height, moisture 13.4%, impurity 1.0%, fatty acid value 17.6mgKOH/100g, upper grain temperature of 13.6℃, lower grain temperature of 6.8℃, and average temperature of 11.6℃. The stored – grain pests are sitophilus zeamais and cryptolestes pusillus, with the total density of 6 insects/kilo.

2 Test Materials and Preparation

2.1 Safe operation and notes

Spray 5cm polyurethane foam on the surface of the lower panel of the arch panel of the warehouse to insulate heat before the test. Adopt special hermetic heat preserving door and window and vent sleeve with the silicone rubber sealing strip and the lever locking device. Instead of the ordinary interior wall coating, adopt polyamide epoxy resin coating as the hermetic seal coating. Adopt sealing materials such as caulking glue, polyurethane paint and epoxy resin to conduct sealing process for various slots such as holes in doors and windows, access of

1. *Liu'an Grain Reserves Storehouse Directly under the State, Liu'an* 237011
2. *China Grain Reserves Anhui Branch, Hefei* 230041

air pipe and the slot on structure base slab, connecting joint between interior wall and concrete base slab top, and internal angle of interior wall. The warehouse air tightness test on May indicated that the half-life period of decompression from 500Pa to 250Pa is 314 seconds.

2.2 Carry out System

From March to May, 2004, our warehouse cooperated with the project design institute and construction institute to carry out system adjustment for the gas transmission system, gas supply system, detection system, decompression system and computer automatic control system, no-load linkage trial run and stored warehouse linkage trial run of CO_2 controlled atmosphere warehouse. All the systems were in stable operation according to the test.

2.3 Pre-embed the Test Pests

On May 26th, 2004, under the guidance of the technicians in Chengdu Grain Storage Research Institute, insect test sample containers were pre-embeded according to the pest control test plan of CO_2 controlled atmosphere storage. This included a total of 10 groups of stored – grain pests separately containing 3 kinds of sensitive species and resistant species in each pest state (adult, egg, larva, pupae) with the appropriate food type and quantity for the insects during the test period.

2.4 Controlled Atmosphere System Map

The debugging of pipe, gate valve, monitoring system software and appliance in CO_2 air holder was normal (Figure 1).

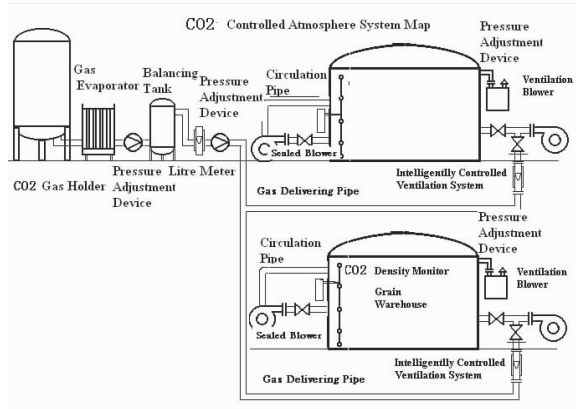

Fig. 1 CO_2 Controlled Atmosphere System Map

Table 1. Testing Record of Air – tightness of Empty CO_2 Controlled Atmosphere Grain Warehouse

Warehouse No.: 18 | Half Life Period of Pressure: 536 sec | Warehouse Type: arched – slab flat warehouse
Variety of Grains Stored: / | Height of Grains Stored: /m | Surrounding Wind Speed: 1 m/sec
Relative Humidity: 67 % | Warehouses Temp.: 16 ℃ | Surrounding Temp.: 12 ℃
Testing Method: EmptyP – T Positive Pressure Method, test the pressure half life period of 500Pa – 250Pa Air Pressure: 1.013×10^5 Pa

	First Sealing Press. Test			Second Sealing Press Test			Third Sealing Press. Test		
1	2	3	1	2	3	1	2	3	
P_{max} (Pa)	700	700	700	700	700	700	700	700	700
t_{mn} (s)	523	532	532	512	538	556	542	550	541
$t_{m/ave}$ (s)		529			535			544	
Δt (s)	6	3	3	23	3	21	2	6	3
ψ (%)	1.13	0.57	0.57	4.3	0.56	3.93	0.37	1.10	0.55
$t_{1/2}$ (s)					536				

Conclusion and Analysis: The air – tightness of the warehouse conforms to the design requirements at 5 minutes.

Table 2. Filled Testing Record of Air – tightness of Stored CO_2 Controlled Atmosphere Grain Warehouse

Warehouse No.: 18 | Half Life Period of Pressure: 317 sec | Warehouse Type: arched-slab flat warehouse
Variety of Grains Stored: Late indica Rice | Warehouses Temp.: 5 ℃ | Surrounding Temp.: 3 ℃
Height of Grains Stored: 6.0 m | Relative Humidity: 84 % | Surrounding Wind Speed: 2.7 m/sec
Testing Method: P – T Positive Pressure Method, test the pressure half life period of 500Pa – 250Pa Air Pressure: 1.013×10^5 Pa
Testing Method: P – T Positive Pressure Method, test the pressure half life period of 500Pa – 250Pa

	First Sealing Press. Test			SecondSealing Press. Test			Third Sealing Press. Test		
1	2	3	1	2	3	1	2	3	
P_{max} (Pa)	580	580	600	600	600	600	580	600	600

	First Sealing Press. Test			SecondSealing Press. Test			Third Sealing Press. Test		
t_{mn}(s)	324	318	316	312	324	320	308	320	311
$t_{m/ave}$(s)		319.3			318.7			313	
Δt (s)	4.7	1.3	3.3	6.7	5.3	1.3	5	7	2
ψ (%)	1.5	0.4	1	2.1	1.7	0.4	1.6	2.2	0.6
$t1/2$(s)					317				

Conclusion and Analysis: the air – tightness of the warehouse conforms to the design requirements at 5 minutes.

Notes: meaning of symbols in the table and calculation formulas

1. P_{max} – test the max value of the warehouse bulging pressure, unit: Pa;
2. t_{mn} – pressure half life period of No. n test in No. m hermetization, unit: sec;
3. tm/ave – average value of pressure half life period in No. m hermetization, tm/ave = (tm1 + tm2)/2, unit: sec;
4. Δt— deviation of pressure half life period, $\Delta t = |t_{mn} - t_{m/ave}|$, unit: sec;
5. ψ — deviation coefficient of pressure half life period, $\psi = \Delta t / t_{m/ave}$;
6. $t_{1/2}$ — effective value of warehouses pressure half life period, $t_{1/2} = \sum t_{mn}/n'$, unit: sec;
7. When calculating the $t_{1/2}$ value, bring the tmn value of $\psi \leq 10\%$ calculated in each measurement into the summation (∑) of t1/2, n′ means the times of tmn value's entering t1/2 summation, and $n' \geq 3$.

2.5 Calculation of Theoretical Gas Consumption

$$Mco_2 = [1.1Cco_2(NVb + VHS) + SVbPb/\rho co_2]/[571(m^3)/ton] = 13.39 \text{ ton}$$

2.6 Gas charging procedure

Table 3. CO_2 Density Change Data table during Pest Killing by Charging Gas in Stored Warehouse

Testing date	Minimum CO_2 density %	Testing date	Minimum CO_2 density %
2004 – 6 – 2	32.0	2004 – 6 – 11	42.8
2004 – 6 – 3	35.8	2004 – 6 – 12	46.6
2004 – 6 – 4	39.2	2004 – 6 – 13	45.1
2004 – 6 – 5	45.1	2004 – 6 – 14	42.4
2004 – 6 – 6	46.3	2004 – 6 – 15	45.7
2004 – 6 – 7	44.5	2004 – 6 – 16	42.5
2004 – 6 – 8	45.9	2004 – 6 – 17	39.6
2004 – 6 – 9	45.0	2004 – 6 – 18	42.1
2004 – 6 – 10	43.5	2004 – 6 – 19	42.0

Test grain condition—open valve of charged warehouse — start charging—stop charging when minimum density is more than 35%—open gas recirculation system — recharge gas slowly when average density is less than 75% – regularly test and ensure the time when minimum density is over 35% shall be over 15 days.

3 On-site Operation and Testing

3.1 Formally started first gas charging at 17:00 pm on May 26, 2004 and stopped at 16:00 pm on May 27 2004; 8.39 tons gas discharged in first filling period with CO_2 density of 1% – 86%; due to the minimum density < 35%, continued charging 4.1 tons gas from 20:00 pm, June 1 to 7:00 am June 2, 2004 with CO_2 density of 32% – 91%; supplement 1.1 tons CO_2 gas in small fills twice – on June 3rd and 12th with 35.8% CO_2 density at the minimum point. The test in this time totaled 13.59 tons CO_2 gas charged into No. 18 warehouse.

3.2 After gas charging, immediately start fixed internal gas recirculation system to evenly mix the CO_2 air density in the warehouse.

3.3 Finish test data record before and after the gas charging of stored warehouse separately, record the operation data of each appliance every two hours during the process of gas charging, mix evenly after gas charging, and record test data one time each day. The specific method sees to Table 1 and Fig. 2.

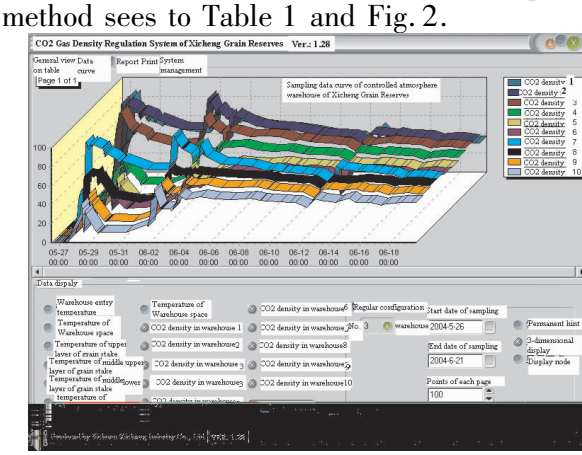

Fig. 2 CO_2 Density Change Curve during Controlled Atmosphere Storage

3.4 Delivered the pest samples to technical supporting institute, Chengdu Grain Storage Research Institute, for test on June 18th 2004 when the lowest CO_2 density in the warehouse had remained above 35% more than 15

days.

4 Analysis of Test Effect

4.1 During controlled atmosphere grain reserves test, the air tightness of stored warehouse was 5 min. and 14 sec. which is longer than the designed standard of 4 min., indicating that the air tightness of this warehouse exceeded the designed requirement.

4.2 The test data indicated that the time when CO_2 density remaines above 35% in each point of the warehouse reached 22 days, which was 7 days longer than the technical standard of over 15 days.

4.3 Pest killing result in stored warehouse: The test conducted by Chengdu Grain Storage Research Institute indicated that 10 – group trial stored grain pests (including each pest status of sensitive species and resistant species) were completely killed, and that the pest killing effect for stored grain pests reached 100% by using CO_2 with 75% – 35% density for more than 15 days.

4.4 The influence of controlled atmosphere storage on grain quality: Opened the warehouse to disseminate gas for 3 months after gas charging. Fatty acid value was 18.1mgKOH /100g by testing selected samples, which increased 0.5, and fatty acid value of regular warehouse at the same time increased relatively fast from June to August, increased 1.5 average, which indicated that under the same storage conditions, the increase of fatty acid value in late indica rice stored by controlled atmosphere storage was 1.0 less than regular warehouse.

5 Benefit Comparison Between CO_2 Controlled Atmosphere Storage and Regular Storage

After the test, we carried out comparison and analysis on the operation expenses of CO_2 controlled atmosphere storage in stored warehouse and that of regular grain storage, and found that gas consumption quantity for controlled atmosphere stored grain is 2.72 kg/year per ton, operation expenses was RMB 2.56/year per ton, which reached the anticipated target of = 3 kg/ton per year and criteria for evaluation of = RMB 4/ton per year.

The average price of grain that is stored by adopting new technology can increase RMB30/ton; realize newly-added benefit above RMB 25/ton. Ten thousand tons grain stored by controlled atmosphere for 3 years can newly add benefit of RMB 83.2 thousand.

Table 4. Benefit Comparison Table between CO_2 Controlled Atmosphere Storage and Regular storage

Serial No.	Compared items		Regular warehouse	Controlled atmosphere warehouse
1	Species		Paddy	Paddy
2	Tonnage (ton) of grain reserves		5016	5000
3	Main materials	CO_2		10880
		PH_3	864	/
4	Assistant materials		4500	500
5	Health care (assistance by medicine)		1000	/
6	Electricity Consumption		50	850
7	Warehouse repair	Material	/	400
		Labor expenses	/	150
8	Subtotal of operation expenses		6414	12780
9	Annual gas consumption (Kg) for grain reserves		0.0072	2.72
10	Annual operation expenses for grain reserves		1.28	2.56
11	Custody expenses per ton		78	78
12	Operation expenses/ custody expenses per ton		1.64%	3.28%

Note: (1) The additional materials needed for regular warehouse during grain storage are mainly sealing film for sealing doors, windows and grain surface, attached gland materials and fumigant pipe for grain stack. Convert to the expenses in one year according to their service life; doors and windows were sealed and grain surface was not sealed in controlled atmosphere warehouse.

(2) The AlP fumigation for regular stored grain was accounted as once per year, and that for controlled atmosphere stored grain was accounted as per CO_2 gas charging once every year.

(3) Circulation fumigation for regular stored grain adopted AlP under film dynamic deliquescence method, without using CO_2 gas in steel cylinder. AlP was RMB 24/kilogram; liquid CO_2 for controlled atmosphere storage was RMB 800/ton.

6 Analysis and Discussion

6.1 Analysis of pest killing effect

Compared with regular grain storage method, CO_2 controlled atmosphere storage can effectively kill stored-grain pests so as to achieve the purpose of inhibiting breath of grain and postponing quality change, and solve the difficult problems of excessive reliance on aluminium phosphide fumigation agent and increase of pest resistance to AlP at present in conventional grain storage which result in significant increase of AlP quantity. Use of CO_2 can resolve pest control difficulty caused by human behavior of ineffective AlP fumigations, while effectively

preventing grain and environment pollution from AlP , and provide new method for inhibiting the AlP resistance of stored – grain pests.

6.2 Analysis of Economic Benefit

The newly-added cost for controlled atmosphere storage technology is less than RMB 4/ton. year(minus fumigation cost of RMB 1/ton. year). Compared to regular storage technology, controlled atmosphere technology can greatly increase the added value of product as it can keep the application quality of grain and inhibit the damage losses from pests and molds.

6.3 Analysis of Social Benefit

CO_2 controlled atmosphere storage technology is one of the environment-friendly technologies generally recognized by the world, so that the parent company has determine to realize the target of above 50% environment-friendly grain storage. Controlled atmosphere storage is one of the effective methods in the high-temperature and high-humidity regions of South China. The successful application of this technology not only can upgrade the technological storage level of China Grain Reserves Corporation, but also can enhance and fully establish the image of China Grain Reserves Corporation as a world grain leader, and promote the status of our country in worldwide grain markets, import and export trade, and technology exchange.

6.4 Problems and Experiences

CO_2 controlled atmosphere storage can kill all life stages of pests and minimize the deterioration of grain quality; however, when compared to regular warehouse, first, operation cost is relatively high, second, it increases CO_2 emissions, third, repair expenses for maintaining high levels of air tightness of warehouse is high. In our opinion, advantages are much greater than disadvantages. Killing pests by controlled atmosphere must ensure that warehouse is provided with favorable air tightness and adopt the safety measures such as effective gas protection.

6.5 Safe Operation and Notes

To get excellent air-tightness of warehouse, it is necessary to be strict during the construction of the main project and supervise the construction quality of key parts like sealing junctions of walls, floors and arched slab as well as around doors, windows, piping, electrical wiring, etc. When the installation of CO_2 storage and vaporizing system is finished, airtight experiments with dry and oil-free air or nitrogen shall be performed on it, to check if there is leakage of pipes and valves under working pressure. The experiment procedure shall comply with *Pressure Vessels Safety and Technical Supervision Regulation* of the State Administration of Quality and Technical Supervision.

All pressure equipment and electrical installation are classified as high-voltage, hazardous equipment, so the relevant operation and maintenance should be conducted by trained, licensed and certified professionals. There should be the obvious warning sign on the site of installation and operation.

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0404

CTP Model for Optimum Efficacy of Closed Loop Fumigation (CLF) Systems in Partially Sealed Storages

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Abstract: Phosphine fumigations often use larger dosages than necessary, yet fail to accomplish satisfactory insect control because gas concentrations are not maintained in storages at sufficient levels long enough for complete kill of all life stages of stored grain insects. This CTP CLF model (concentration-time product) uses "half-life loss time" of storage structures. It recommends use of aluminum phosphide tablets rather than pellets for slower gas release, lower peaks, which extends time of higher gas concentrations which improves efficacy.

An alternative dosage method recommends using partial pellet dosage (15% – 25%) for fast early gas release with the balance of the dosage (75% – 85%) in tablets for slower release with lower peak concentrations. This split dosage method, "flattens" peak gas levels, slows losses and sustains higher overall gas concentrations for longer fumigation times with lower dosage.

Lower peak concentrations reduce gas leakage rates from storages. This CLF process allows a fumigator to calculate total dosage required for future fumigations for each storage structure based on the half-life loss time from peak gas levels to maintain concentrations above 200 ppm for 100 hours or more for maximum efficacy, minimizing insect resistance to phosphine.

Key words: closed loop fumigation (CLF), partially sealed storage, CTP, half-life, gas concentration, efficacy, phosphine resistance

Background

Dry aluminum phosphide pellets and tablets have been used as an inexpensive source of grain fumigant in Oklahoma grain elevators for more than 60 years. Pellets have been the primary fumigant of choice due to its fast response. Many concrete elevators in the U. S. A. use automatic dispensing machines to apply pellets at preset application rates when "turning" grain from one silo to another.

Phosphine dosages often exceed maximum recommended levels, yet fail to achieve maximum efficacy due to poor sealing, non-uniform distribution and insufficient concentration for long enough times. Due to fumigation failures, insect resistance to phosphine has increased dramatically in the past two decades.

Entomologists have found from research that if phosphine is maintained at levels of 200 ppm or higher uniformly throughout a structure for 100 hours or longer, all life stages of all stored product insects are killed. Engineers and entomologists have found that phosphide tablets generates peak gas levels about 2. 5 times slower than pellets, and although tablets sustain a lower peak level of gas for the same AI dosage, the gas duration is much longer with tablets.

Tablets vs pellets gas release time

Under identical air temperature and humidity conditions, phosphine gas release from pellets is approximately twice as fast as gas release from tablets. Under warm conditions, 24 – 30C (75 – 85F) in wheat with 11 – 12 percent moisture, peak gas release from thin layers of pellets will typically occur in 12 – 24 hours with maximum gas release in 36 – 48 hours. Under similar temperature and moisture application conditions, gas production from tablets will peak in about 40 – 60 hours with maximum gas release in 70 – 90 hours. Thus, gas generation time ratio for tablets vs pellets is about 2. 5∶1.

Structure 'half-life time' gas loss

The time when the gas concentration drops to half the peak gas level is the "***half-life time***" (HLT) of the structure. During calm stable weather, leakage is slow and relatively steady-the HLT is longest. Gas losses increase with higher wind velocities due to lower barometric pressure outside the structure, causing faster gas outflow from the differential pressure. The fumigation HLT in poorly sealed storages is often less than 24 hours.

Gas concentrations are more uniform and

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HLT is easier to determine using closed loop fumigation (CLF) in partially sealed structures. With forced recirculation by CLF fans, relatively uniform gas concentrations after the first day of CLF fumigation can be checked at headspace or base location to determine structure HLT for current and similar future weather conditions. Structural sealing tightness and resulting gas leakage rates will determine the HLT for each structure under existing grain and weather conditions during fumigations. Be aware that structural HLT during summer fumigations may vary significantly from HLT during fall or winter fumigation due to weather differences.

Example 1: The headspace gas level in a 'partially' sealed steel silo using CLF dosed with pellets peaked at 1200 ppm, 18 hours after dosage. With periodic recirculation the headspace gas reading dropped to 600 ppm, 54 hours from the start. The first HLT of that silo under that fumigation weather condition was 54 − 18 = 36 hours. With stable weather conditions, the second HLT of that silo, when the recirculated gas level dropped from 600 to 300 ppm, for a total of 18 + 36 + 36 = 90 hours from the start of the fumigation. The third HLT gas reading of 150 ppm would likely occur about 90 + 36 = 126 hours (5 1/4 days) from fumigation start

Note: CLF fans should not run continuously throughout a fumigation-leakage rates are higher when gas is continually recycled because the headspace is continually pressurized-run CLF fans just enough to keep headspace gas levels within 50%-75% of gas readings in the grain bulk or to maintain at least 200 ppm in the headspace-about 1 − 2 hours per 24 hours.

Example 2: In September, 2000, Mid − Oklahoma Coop Elevator sealed a flat grain storage warehouse and installed a CLF system in it. During their first fumigation in October, 2000, they recorded a recirculated peak gas concentration of 2 050 ppm after 24 hours. Seven days later (8^{th} day of fumigation), the gas concentration during recirculation was 850 ppm. Assuming uniform gas loss, the HLT of that flat storage of 1025 ppm can be computed. HLT = (2050 − 1025)/(2050 − 850) × 7 = 5.98 or 6 days (144 hours). During the fall 2001 fumigation, Mid − OK Coop reduced the dosage by 50% in the flat storage. The 24 hour headspace gas reading was 1 050 ppm. Seven days later, the recirculation headspace reading was 450 ppm. The 2001 HLT = (1050 − 525)/(1050 − 450) × 7 = 6.125; still about 6 days.

Gas Loss Rates Vary with Concentr Ation Levels

An important factor to consider when deciding whether to use pellets or tablets is that in any structure, higher gas vapor pressures at high gas peak concentrations will result in faster gas leakage from pellets than tablets in a given period of time.

Example 3: If a pellet dosage results in a peak concentration of 1000 ppm in 18 hours, the same AI tablet dosage may peak at 850 ppm in 48 hours. If the partially sealed structure sustains a HLT of 30 hours, the gas concentration for pellets would drop to for about 500 ppm, 48 hours from the start of the fumigation. From a peak of 850 ppm in 48 hours, tablet fumigations would drop to about 425 ppm 78 hours after dosage. The pellet gas level would drop to about 250 ppm after 78 hours during its second HLT while the tablet second HLT would be 108 hours at about 212 ppm.

Gas concentration vs Time @ 200 ppm for 100% Efficacy

The ***half − life time*** (HLT) from peak gas concentration is a useful guide for characterizing a structure for fumigation. Stored grain entomologists generally feel that if phosphine concentrations remain above 200 ppm for a minimum of 100 hours in all points in the grain mass, **all life stages of all grain insects will die**. Thus, an important grain management tool for steel, concrete or flat storage fumigators is to determine the length of time that all parts of the grain storage stays above 200 ppm.

Thus, combining the HLT concept and the principle of maintaining gas concentrations above 200 ppm for a minimum of 100 hours provides a very useful tool for predicting the appropriate dosage for partially sealed CLF systems. Once a storage structure HLT has been characterized during the first CLF using a conservative initial dosage, the grain manager can calculate the level of dosage expected to maintain gas levels above 200 ppm for 100 hours. This allows fine tuning of target dosages for one − dose fumigations. Keep in mind that this HLT is based on weather conditions during that fumigation. If the weather forecast during a fumigation predicts adverse weather, based on long range (10 − 14 day) forecasts, it may be prudent to increase dosage, unless a convenient re − dose method is available.

Example 4: The steel silo in Example 1 was fumigated the next year under similar

weather conditions and the manager wants to determine how long the gas level might be expected to remain above 200 ppm (for more than 100 hours).

If gas generation from pellets was straight line from initial dosage to peak gas concentration, then the point where CLF gas recirculation reached 200 ppm would have been about 200/1200 × 18 hours = 3 hours from the start of the fumigation. Thus, the structure would be above 200 ppm for 15 hours before peak concentration.

From Example 1, the gas level at the third HLT (at 126 hours) was 150 ppm. To determine the time when the gas concentration dropped from the peak of 1200 ppm to 200 ppm, one can calculate the second HLT of 90 hours at 300 ppm.

Solving for the lapsed time for the gas drop from 300 ppm to 200 ppm, or a 100 ppm loss would be: 36 hrs/(300 − 150) = HLT/(300 − 200); inverting, HLT = 36 × (300 − 200)/(300 − 150) = 36 × 100/150 = 36 × 0.67 = 24 hours after the second half − life time. So, 90 + 24 = 114 hours from start of fumigation. Since 3 hours was calculated to elapse before CLF gas concentration reached 200 ppm after fumigation started, total time above 200 ppm would be 114 − 3 = 111 hours. This should provide about 10% more time beyond the minimum 100 hours at 200 ppm for 100% efficacy of all life stages. Unless fumigation is during adverse weather, the dosage that provided the 1200 ppm peak concentration in Example 1 should provide 100% efficacy.

Length of time above 200 ppm concentration time should be a guiding factor for maximum efficacy-killing of all life stages of all grain insects in fumigations. Because of the longer release time of tablets, a factor of at least 2.5:1 slower than pellets, this guideline definitely favors use of tablets for initial dosages.

If the storage unit HLT is characterized during stable weather conditions, gas losses during fumigations in stormy weather will be faster. If inclement weather is predicted, dosage can be increased by 10% −25% to compensate for expected increased gas losses.

Optimum Efficacy CTP-CLF Model

A one-dosage CTP CLF model for optimum efficacy of fumigation can be developed for each grain storage structure by combining the following five fumigation management strategies: (1) use phosphide tablets (or other forms of slow release phosphine packaging) for slower, longer duration gas release; (2) replace tablets with 15% −20% of dosage as pellets for fast early gas release to shorten time when initial gas concentration reaches 200 ppm; (3) install closed loop fumigation (CLF) for uniform gas distribution in well sealed or partially sealed structures; (4) maintain 200 ppm or more for 100 hours for 100% efficacy of all life stages of stored product insects; (5) apply the principle of HLT from peak gas concentration to calculate minimum future dosages for each structure for weather extremes (examples: windy vs calm, hot/dry vs cool/rainy).

To estimate the optimum CTP dosage for a partially sealed storage with CLF, fumigate using a conservative dosage which is estimated to end up with a gas concentration at 100 hours that is well above 200 ppm. Document the point at which 200 ppm is first reached after dosage, document the time and gas level at peak concentration, monitor the time when the first HLT occurs (peak gas ppm/2). Then monitor gas levels at the predicted second HLT, and see how close it is to the first HLT when the gas level isof peak level. Document the additional HLT increments until gas concentrations are below 200 ppm. Example 4 can be used as a model to compute the time when 200 ppm is reached.

Large CTP − CLF field Demonstration Comparing Pellets Vs Tablets

In the Fall of 2000, Peavey Company, Tulsa Port of Catoosa installed a CLF system (designed by R. Noyes) in their 3.3 million bushel, 500 ft × 150 ft by 30 ft sidewall, flat storage barn, Figure 1. The CLF system design on the pre-cast concrete sidewall structure included a 4-inch ID PVC pressure pipe manifold on each side. The aeration system had 18 vane − axial fans (26.3 ft spacing) per side, each connected directly to a straight 65 − ft perforated duct that extended toward the center. Figure 2 illustrates the CLF blower design.

CLF plumbing was divided into three equal 1.1 million bushel sections with 6 − inch ID PVC suction pipes extending from the roof at the center of each section to 3 − HP (2.25 Kw) CLF fans, which deliver 1300 cfm/fan. Full-flow PVC ball valves were placed in the 4 − inch PVC pressure pipe between the three CLF fans per side. During normal fumigations, all ball valves were open. When the warehouse is full, the 6 CLF fans supply 7 800 cfm to 3 300 000 bushels of wheat. The gas flow rate is

7 800 cfm/3 300 000 bu = 0.00236 cfm/bu = 1/425 cfm/bu. This provides one gas exchange in about 3.5 hours, or 6.8 gas changes per day.

Data taken at the 3.3 million bushel flat storage at Peavey Grain Company, Tulsa Port of Catoosa in the Fall, 2000 and Fall, 2002 provides contrasts pellets and tablets. The 2000 fumigation used a one-dose application of phosphine pellets. The 2002 fumigation used a one-dose application of phosphine tablets. Some re-sealing of the structure took place before the 2002 fumigation to reduce gas losses experienced in 2000 and 2001. Dosage each year was 42 cases of aluminum phosphide at 7 000 grams AI per case for a computed warehouse volume of 6 000 000 cubic feet. This would provide a theoretical concentration (in a perfectly sealed structure): Total ppm = 42 cases × 7 000 gm/case × 25 ppm/gram/1 000 cu ft × 1/6 000 (1 000 cu ft units) = 1,225 ppm. The average peak reading from pellets in 2000 was about 740 ppm; in 2002, with tablets, peak gas level was about 650 ppm (Figure 4).

How Did the CLF System Perform

Figure 3 shows gas concentration data taken at the six CLF fan locations for both fumigations. Figure 4 compares the average of gas concentrations at the six fans for 2000 and 2002. The elevator superintendent was not pleased with the gas leakage during 2000 and 2001 fumigations, so the warehouse was partially re-sealed before the 2002 fumigation.

From Figure 4, in 2000 the average pellet gas concentrations peaked at about 740 ppm in 18 hours compared to the tablet dosage peak of 650 ppm about 53 hours from the start in 2002. The time from dosage until average concentration reached 200 ppm in 2000 was about 8 hours; for tablets in 2002 the time at 200 ppm was about 10 hours. The warehouse HLT for both pellets and tablets were about 32 hours.

These fumigations were under similar grain moisture and temperature conditions. Peak gas concentration time ratios were about 2.9:1.0 for tablets versus pellets. Using tablets in 2002, this facility reached the first HLT from 650 – 325 ppm about 86 hours after start of the fumigation. At that point, the entire warehouse had a gas concentration above 200 ppm for 76 hours. If the fumigation had continued at the same percentage loss of gas, the second HLT at 162 ppm would have been about 86 + 32 = 118 hours. Subtracting 10 hours, when the entire facility was at 200 ppm, assuming a steady decline in gas loss, the time when gas concentrations reached 200 ppm near the end of the fumigation would be: 86 + (325 – 162)/(325 – 200) × 32 = 86 + 162/200 × 32 = 86 + 26 = 112 hours. Subtracting 10 hours for the 200 ppm initial time, gives 112 – 10 = 102 hours above 200 ppm. This tablet fumigation should have killed all life stages of all insects.

Combined Tablet and Pellet Dosage Strategy

Use of 15 – 25% of the dosage AI as pellets for quick early release and 75 – 85% of the dosage as tablets would be a way to capitalize on the different release rates and loss rates of tablets vs pellets. This facility needs additional sealing to assure reaching the condition of 100 hours above 200 ppm at the present dosage levels for each future fumigation. With improved sealing, this structure HLT could be increase to 45 – 60 hours, and dosage AI could be further reduced. Savings in dosage could pay for tighter sealing, improving efficacy.

Fig. 1 Peavey Company's 3.3 million bushel warehouse at Tulsa Port of Catoosa, showing concrete silos an steel bins. Company installed CLF system in warehouse in 2000

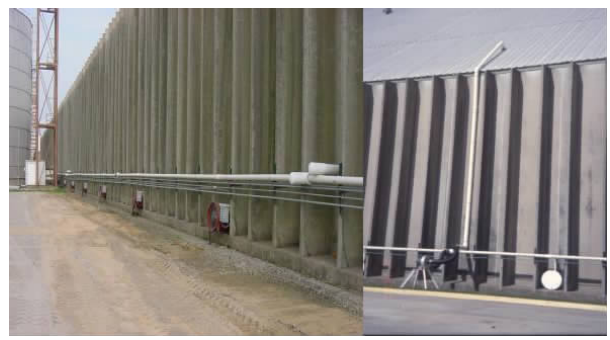

Fig. 2 Left photo shows closed loop fumigation (CLF) system PVC pressure piping system above aeration fans along one side. Right – Suction pipe from roof headspace and pressure pipe connected to CLF fan on movable base. Note vertical pressure pipe to aeration transition duct, with seal plate where aeration fan was removed.

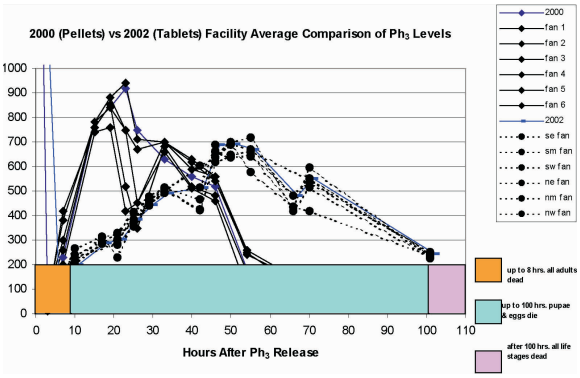

Fig. 3 Plot of gas levels at six CLF fan locations comparing pellets in 2000 and tablets in 2002

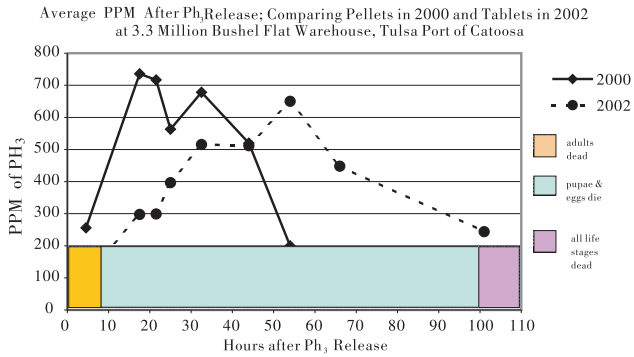

Fig. 4 Plot of average phosphine gas concentrations at six CLF fan locations comparing pellets in 2000 with tablets in 2002

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Test on Recirculation Fumigation under Film with Mixed Gas of $PH_3 - CO_2$ in Steel Cylinder

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Abstract: In order to research the applied technology of film sealing technology of the grain surface combined with recirculation fumigation of a mixed gas of $PH_3 - CO_2$ [PH_3 2% + CO_2 98% (w/w)], we performed field tests on recirculation fumigation under film in a warehouse. The warehouse was 54.36m long, 35.26m wide and the loaded grain height 6m. There were 7650t of bulk wheat stored in the warehouse, and five test points of gas concentration and five test-insect cages were located in the warehouse. Supply 250kgs of mixed gas of $PH_3 + CO_2$ and open recirculation ventilator to run for 12 hours, and then stop. Begin to test the gas concentration three hours after application, after that test the gas concentration of PH_3 in the warehouse once a day, degas after 21 days and take the test-insect cages out to check mortality. Culture the tested insects for 30 days (25℃75% RH) and then check mortality. In this research, we used No. 23 warehouse with the same conditions as the control warehouse which used normal aluminum phosphide fumigation with application on grain surface and application on buried bags. The No. 23 warehouse is the same as the tested warehouse in terms of locations of test points of gas concentration and test-insect cages, and sampling and testing methods. The result shows that the pressure half-life of the tested warehouse were 4s (−30Pa to −15Pa), and the average, Min. and Max. concentration of PH_3 at each location of the tested warehouse on the 7th day, 14th day and 21st day were 198 (136 − 266), 132 (86 − 170) and 86 (58 − 115) mL/m^3 respectively, the ratio of Min. concentration and Max. concentration of each gas sampling point was 1∶2. The distribution of PH_3 concentration at each location in the warehouse was uniform and four kinds of tested pests of full-stages (adult, egg, larvae and pupa) pre-buried in grain piles before test were killed completely; the pest control was 100%. The dosage applied to the control warehouse was three times as much as that of the recirculation fumigation under film with mixed gas of $PH_3 - CO_2$; on the 7th day and 14th day after fumigation, the tested gas concentrations of PH_3 at the same location and same depth were 173 (45 − 251) and 101 (26 − 156) mL/m^3 respectively; the ratio of Min. concentration and Max. concentration was more than 1∶5, and the distribution of PH_3 concentration was not very uniform; degas 14 days after end of fumigation and there was no live adult found after sampling and checking, the death ratio of adults being 100%. But after three weeks, live adults were found again in parts of the warehouse, and the density was 7 adults/kg, in which there were two *Rhizopertha dominica* (Fabricius) and five *Cryptolestes ferrugineus* (Stephens); the pest control was worse. Therefore, pest killing technology of recirculation fumigation under film with mixed gas of $PH_3 - CO_2$ not only improves air-tightness and uniformity of fumigation, reducing dosage and improving efficacy, but also saves time and work. It is an easy and safe operation.

Key words: high and flat warehouse, stored − grain pests, hydrogen phosphide, carbon dioxide, recirculation fumigation

Introduction

Our country has invested and built new warehouses with capacity of 50 billion kg in three batches since 1998. The new warehouse has the characteristics of big span, large volume and more loaded grain but it is not very ideal in some aspects such as air-tightness and pest killing by fumigation. There are a good many problems in fumigation:

(1) The duration of effective concentration of PH_3 is short.

(2) Pests can not be killed completely and resistance of pests to fumigant is increased.

At present, our country is using normal pest prevention and control with aluminum phosphide fumigation, whose use ratio is around 80%; because of long-term and illegitimate use of aluminum phosphide, the resistance for PH_3 of stored-grain pests has increased, and the ma-

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jor reason is the blind increasing or decreasing of dosage of aluminum phosphide during use and ignorance of air-tightness of warehouse and duration of effective concentration of PH_3. After repeated failed fumigations, the pests can not be killed completely, even the resistance of pests increased. Therefore, normal pest prevention and control of aluminum phosphide fumigation can not be applied to the new high and flat warehouse any more. If the pest control can not be resolved in time, it will result in considerable reproduction, spread of stored-grain pests and heat-releasing, mildewing of stored grain, and will influence safety of stored grain directly. Therefore, in order to ensure safety of stored grain, increase technical level of storage, improve and research use method of PH_3 and prolong its service life, the systematic and practical pest killing application experiment should be performed. This research performed the experiment for the application effect of mixed gas of $PH_3 - CO_2$ in steel cylinder in a high and flat warehouse and performed the comparison with normal PH_3 fumigation in a high and flat warehouse to provide control for effective application of PH_3 in high and flat warehouse.

1 Materials and Methods

1.1 Materials

1.1.1 Base situations of the tested warehouse and the control warehouse

We used No. 19 warehouse as the tested warehouse and used No. 23 warehouse as the control warehouse. No. 19 warehouse and No. 23 warehouse were all high and flat warehouses which were brick and concrete structure, and whose roofs were aerated concrete slabs, frames were steel frames and floors were concrete, east to west; the lengths were 54.36m, widths were 35.26m, loading heights were 6m, eaves heights were 9.8m, top heights were 11.6m. There were two gates and two wickets of upper warehouse equipped on the north and south separately; and there were two mobile PH_3 recirculation fumigation systems equipped on the north and south separately; the ventilation systems were cages on the gourd, one machine one passage, and there were 10 passages on the north and south separately, total 20 passages. Their geographic positions, trends of warehouses and construction materials were the same. The volume, kinds, quantities and stacking forms of stored grain were same basically. (Table 1)

Table 1. Stored grain status of the tested and control warehouse

No. of warehouse	kinds	Quantity (kg)	Grain storage volume (m^3)	Stacking form
19	wheat	7650	9563	Bulk
23	wheat	7530	9256	Bulk

1.1.2 Test – insects

The test-insects were *Cryptolestes ferrugineus* (Stephens), *Sitophilus oryzae* (Linnaeus) and *Oryzaephilus surinamensis* (Linnaeus) provided by Academy of State Administration of Grain.

1.1.3 Mixed gas of $PH_3 + CO_2$ in steel cylinder

Developed and produced by Hangzhou Tongyi Gas Research Institute. 25kg of mixed gas of $PH_3 + CO_2$ in each steel cylinder. The ratio of gas concentration was PH_3 2% + CO_2 98% (w/w).

1.1.4 Equipments and Devices

Pipelines of recirculation fumigation under film for grain surface in No. 19 tested warehouse were designed and provided by Henan Weilai Machine and Electric Project Co., Ltd. The layout of pipelines is shown in figure 1. Related equipments used in the experiment are shown in table 2.

Table 2. Equipments used in the experiment and the manufacturers

Name	Manufacturer
Pipelines of recirculation fumigation under film for grain surface	Henan Weilai Machine and Electric Project Co., Ltd.
Pipelines of recirculation fumigation	
Application cars and recirculation machines special for steel cylinders	Beijing Zhonggu Grain and Oil S&T Research Institute
Measuring device of PH_3 concentration	Drager, Germany

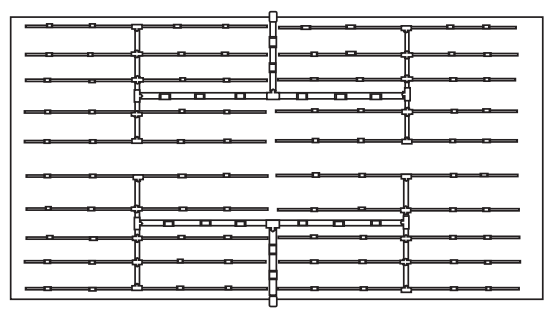

Fig. 1 Layout of pipelines of recirculation fumigation under film for grain surface in No. 19 tested warehouse

CQMY air – tightness determination device consists of car body, ventilator, U – type pressure gauge, butterfly valves, seconds-counter, connection soft tubes, connection flanges and etc., produced by Henan Weilai Machine and Electric Project Co., Ltd. The main parameters are shown in table 3.

Table 3. main parameters of CQMY air – tightness determination device

Item	Type of blower	Wind pressure (pa)	Air volume (m^3/h)	Diameter of wind pipe (mm)
Parameter	4 – 72 – 123kw	989 – 1578	2664 – 5268	200

1.2 Method

1.2.1 Culture of full – stages test – insects

\Put test – insects into 250g of related feed separately and cultured them under the conditions of 28 ± 1℃ and 70% ± 5% RH for 42 days, then put the test – insects and feed into cages for use.

1.2.2 Locating of pre-buried cages and PH_3 test points

Placed the sampling points for measuring of PH_3 concentration as quincunx, i. e. set one point at each corner (four corners) and one center point, which are 0.5m far from surface of grain mass. Gas sampling tube connected the outside of the warehouse to measure the concentration of PH_3.

There were five cages pre-buried in the warehouse, and their locations corresponded with that of test points of gas concentration, i. e. 0.5m far from surface of grain pile.

1.2.3 Sealing of the warehouse

No. 19 warehouse: installed recirculation pipelines at 30cm under the surface of grain, and performed plastic film sealing of grain surface after locating of cages and test points was finished. Both sides of warehouse body were sealed with PVC plastic film and the recirculation pipelines under the warehouse were sealed with adhesive tape.

No. 23 warehouse: performed film sealing of pipe chases of windows and doors.

1.2.4 Determination of air-tightness of warehouse

No. 19 warehouse: used CQMY determination device and U-type pressure gauge, performed air-tightness determination of negative-pressure half-life by suction type. Opened blower to run for about 60 min., then closed the butterfly valve and measured the static pressure of gas in the warehouse with U-type pressure gauge, repeated these processes for three times and used the average value as the result of air-tightness determination.

No. 23 warehouse: used L4 – 72 – 11 No. 4.5A centrifugal blower produced by Shijiazhuang Blower Factory, whose power was 7.5 kw; performed air-tightness determination of positive-pressure half-life by press-in type. Opened blower to run for about 30 min., then measured the static pressure of gas in the warehouse with U-type pressure gauge which was connected with gas sampling tube of fumigation. Repeated these processes for three times and used the average value as the result of air-tightness determination.

1.2.5 Application and recirculation outside of the warehouse

No. 19 warehouse: charged the mixed gas of $PH_3 + CO_2$ into application car special for gas in steel cylinder, and connected to application mouth of recirculation blower with high-pressure soft tube (pressure > 10kg); opened the recirculation blower at first, then opened on-off valve on the steel cylinder to apply. Applied at both sides of warehouse for 15 minutes each bottle. During application, opened the recirculation blower at both sides of warehouse to recirculate under the film for continuous 24h, and then turned off the recirculation blower.

No. 23 warehouse: used normal whole-warehouse fumigation, and application methods were grain surface and pipe outlet medicament tray methods, each tray applied 150g. Total dosage of aluminum phosphide was 46kg. Tested the concentration 24 hours after apply and tested once a day.

1.2.6 Measuring of PH_3 concentration

During the first week of fumigation, measure the PH_3 concentration of each point every four hours and every 24 hours after the week.

1.2.7 Checking of death of pests

Degas 21 days after application and fumigation, then take the pre-buried cages out and separate the adults out, put the adults and 10g of feed into insect culture room to culture (temperature: 28 ± 1℃, relative humidity: 70% ± 5% RH), check the death status of adults in each point after 14 days and record the data.

The remaining wheat feed after separation of adults was put into other culture bottles, 15g of fresh feed was added into each bottle and bottles were cultured for 42 days under the same conditions, then checked if there are adults appeared.

2 Results and Analysis

2.1 Result of Air-tightness Determin Ation in the Warehouse

Air-tightness determination results of No. 19 tested warehouse and No. 23 control warehouse are shown in table 3. For the tested warehouse, used film sealing of grain surface and used CQMY air-tightness determination device to perform negative pressure determination. Although we took some measures, the air-tightness was not very good, and the negative pressure only could reach −30Pa and pressure half-life was only 4s. For the control warehouse without film sealing and determined air-tightness by positive pressure method, and increasing of pressure was very small when determined with CQMY air-tightness determination device. The other fun with L4 −72 −11 (7.5kW) used for determination, and the pressure only could reach 350Pa and the half-life was 20s. From above, we can get that the air-tightness of these two warehouses are not good for fumigation. Overall improvements of warehouse should be performed to improve the air-tightness.

2.2 Changes of PH_3 concentration

The average, Min. and Max. concentrations of PH_3 measured at each location in warehouse on 7th day, 14th day and 21st day after fumigation of the tested warehouse were 198 (136 −266), 132 (86 −170) and 86 (58 −115) mL/m^3 separately, and the ratio of the Min. concentration and Max. concentrations of each sampling point was basically the same at each position which was 1∶2; the distribution of PH_3 concentration was relatively uniform. In the control warehouse, the average, Min. and Max. concentrations of PH_3 measured at each location in warehouse on 7th day and 14th day after fumigation were 151 (79 −202) and 31 (10 −72) mL/m^3 respectively, and the ratios of Min. concentration and Max. concentrations were 1∶2.6 and 1∶7.2; the uniformity of PH_3 concentration of each sampling point in the warehouse was worse than that in the tested warehouse.

Table 4. determination status of air −tightness in the tested warehouse

No. of warehouse	Pressure measuring method	Timing pressure (Pa)	Pressure half −life (s)			
			1	2	3	Average
19	Negative pressure	−30	4	5	4	4
23	Positive pressure	350	21	20	18	20

From the average concentration of PH_3 measured every day, the PH_3 concentration in the tested warehouse under the test on recirculation fumigation under film with mixed gas of PH_3 − CO_2 in steel cylinder is always higher than that of the control warehouse which uses normal fumigation, and from the 6th day after fumigation, the PH_3 concentration in the control warehouse reduced rapidly; see figure 2 and table 5. After 14 days, the concentration reduced to 31 mL/m^3. Degas and stop fumigation. But in the tested warehouse, the decreasing trend of PH_3 concentration become slow and the PH_3 concentration is still at 86 mL/m^3 after 21 days. It shows that the air-tightness of the warehouse after film sealing of grain surface is clearly improved.

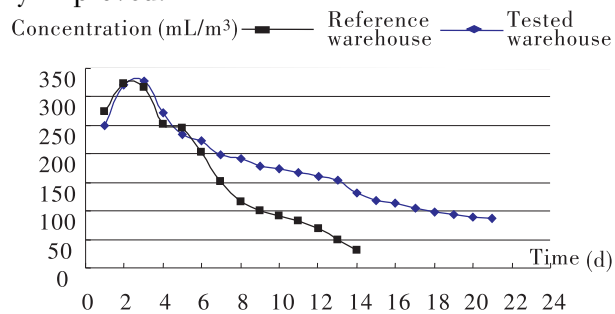

Fig. 2 changes of PH_3 concentration in the tested and the control warehouse

2.3 Efficacy offumigation

For the tested warehouse, 21 days after treatment by recirculation fumigation under film with mixed gas of PH_3 + CO_2 in steel cylinder, various pests in each pre-buried cages in the warehouse were killed completely. 30 days after culture (25℃ and 65% RH) of pests and feed in each cages, there was still no live pest found; pest mortality was 100%. At the same time, there was no live pest found when samples from the grain warehouse were checked.

Pest status before fumigation in the control warehouse; in field inspection, the density was 30 pests/kg, in which there were eight *Sitophilus zeamais* Motschulsky, thirteen *Rhizopertha dominica* (Fabricius) and nine *Cryptolestes ferrugineus* (Stephens) (no test-insect cage placed) founded on grain piles of which the surface was above 1.5m. Degas 14 days after sealing, checked the fumigation effect, there was no live adult found in checking, and the estimated death ratio of adults was 100%. But after three weeks, the live adults were found again in parts of the warehouse, and the density was 7 adults/kg, in which there were two *Rhi-*

zopertha dominica (Fabricius) and five *Cryptolestes ferrugineus*. (Stephens). It shows that the efficacy of fumigation is not good. The major factors are non-uniform distribution of PH_3, poor air-tightness, relatively low gas concentration short treatment period and rapid loss of concentration in the control warehouse which uses normal fumigation.

Table 5. the average, Min. and Max. concentrations (mL/m^3) of PH_3 in two warehouses

days (d)	No. 19 warehouse	No. 23 warehouse
1	249 (166 – 346)	274 (324 – 151)
2	320 (250 – 500)	324 (382 – 266)
3	327 (255 – 430)	317 (331 – 288)
4	272 (202 – 318)	252 (281 – 202)
5	233 (192 – 297)	245 (281 – 202)
6	224 (183 – 290)	202 (238 – 166)
7	198 (136 – 266)	151 (202 – 79)
8	191 (141 – 248)	115 (180 – 36)
9	178 (140 – 220)	101 (137 – 58)
10	175 (124 – 214)	92 (115 – 43)
11	167 (116 – 204)	83 (94 – 36)
12	160 (110 – 196)	68 (72 – 22)
13	153 (98 – 196)	50 (78 – 25)
14	132 (86 – 170)	31 (72 – 10)
15	119 (73 – 161)	
16	114 (71 – 153)	
17	105 (68 – 142)	
18	97 (65 – 133)	
19	94 (64 – 125)	
20	89 (63 – 121)	
21	86 (58 – 115)	

3 Discussion

Under the condition of not good air-tightness in high and flat warehouse, and under the basically same warehouse and grain storage conditions, we had used two different methods of recirculation fumigation under film with mixed gas of $PH_3 + CO_2$ in steel cylinder and PH_3 normal fumigation to perform practical application comparison experiment. In the experiment, the PH_3 concentration in the warehouse measured by recirculation fumigation under film with mixed gas of $PH_3 + CO_2$ in steel cylinder is effective for the storage pests, which shows that the major reasons for improving pest killing effect may be improving the air-tightness and the synergism of CO_2 for PH_3 fumigation. This experiment proves that: use recirculation fumigation under film with mixed gas of $PH_3 + CO_2$, reticulate for 5 hours in high and flat warehouse; the distribution of PH_3 concentration in each location of the warehouse is uniform and the ratio of Min. to Max. concentration of each sampling point is 1∶2. The average, Min. and Max. concentrations of PH_3 of each location measured on 7^{th} day, 14^{th} day and 21^{st} day are 198 (136 – 266), 132 (86 – 170) and 86 (58 – 115) mL/m^3 separately. Four kinds of full – stages (i. e. adult, egg, larvae and pupa) pests preburied in grain piles before experiment were killed completely. If the PH_3 normal fumigation method is used, the uniformity of distribution of PH_3 concentration in the warehouse is worse and the ratio of the Min. to Max. concentration is 1∶7. The dosage in normal fumigation is three times more than that of recirculation fumigation under film with mixed gas of $PH_3 - CO_2$ and also the air-tightness of the warehouse is worse; the PH_3 concentrations measured at the same location and same depth on 7^{th} day and 14^{th} day after fumigation were 173 (45 – 251) and 101 (26 – 156) mL/m^3 separately; although the adults in grain piles have been killed completely, but the larvae and pupa may have not been killed completely, and there were adults found again after three weeks. It shows that the pest killing effect is worse. If one used CO_2 (no PH_3 added) only to prevent and control stored-grain pests, the concentration of CO_2 should be determined by kinds of pests, stage of pest and fumigation duration in practical application; generally, the concentration of CO_2 required is 35%; but for some kinds of pests or the pests which are in the growth stage, the concentration of CO_2 should be increased to about 80% to improve mortality rate. If there is 8% oxygen gas, some stored-grain pests still can survive and reproduce. Therefore, using CO_2 alone to prevent and control stored – grain pests is valuable in special places and departments only under the capability which can be supported by recent economy level of our country.

The experiment proved that recirculation fumigation under film with mixed gas of $PH_3 - CO_2$ can be applied to pest killing by fumigation in high and flat warehouse; it not only can improve air-tightness and uniformity of fumiga-

tion, reduce dosage and improve pest killing effect of fumigation significantly, but also saves time and work, and is an easy and safe operation.

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Field Trial Report on the Application Nitrogen (N_2) to Maintain Grain Quality

Luo Feitian, Tang Shangqiang, Ling Caiqing and Pang Zhen

Abstract: Since 1960's, nitrogen (N_2) – "green gas" has been used for grain storage to maintain grain quality, control of insect pests in our country, However, in order to annoy (N_2) the oxygen displacement, storehouse of the source, grain heap to annoy airtight, air to examine in the nitrogen and the safety operate protection's etc. a series of key technique a problem up found out to effectively resolve a method, so the green nitrogen (N_2) keep a grain technique, the research has been be placed in small scale of on trial investigate stage, it adjusts with carbon dioxide (CO_2) to keep a grain technique similar temporarily return hard carry on large-scale production expansion application. My database in 2005 started adjusting to keep a grain technique to the green nitrogen (N_2) of particularly item research, by my database engineering technical personnel gram bitterness offend a pass, in solving to perplex green nitrogen (N_2) to adjust to keep a grain technique expansion an applied series of key technique a hard nut to crack obtained breakthrough progress, carried on green nitrogen (N_2) to adjust to keep the grain technique storage to experiment with expansion application study also obtained success, currently our database carry on green nitrogen (N_2) to adjust to keep the grain production experiment of grain already 52990.141 tons, have whole databases to always keep a grain of 78.2%.

1 Technique Principle

Make use of a forerunner of nitrogen (N_2) produce equipments produce high-pure nitrogen spirit (above 99.5%), pass in advance build in the database area the underground of the appropriation nitrogen (N_2) transport the piping (N_2) the nitrogen the importation the spirit the airtight function the good spirit adjust the storehouse, nitrogen (N_2) can from the storehouse ground of well ventilated way perhaps from the grain noodles the circulation tube got into a closeness grain a heap of inside space, opened appropriation air a density equilibrium system to carry on compulsive circulation, make each part in the storage of nitrogen (N_2) density even consistent, through going in to high-pure nitrogen spirit after make the nitrogen in the grain heap spirit the density attain 98% to keep grain pest to cause death a density, and make the nitrogen in the storage annoy (N_2) density with long hours keep in the certain scope carry on high-pure oxygen to adjust to keep a grain, pass change closeness grain inside heap of air composition, making it become a disadvantage in keeping the grain pest and microorganism growth breed of higher – N & lower-oxygen ecosystem environment, attain to repress to keep grain pest stop endanger grain, can't grow to breed, until the asphyxiation die and reduce the grain oxygen (O_2) inside the heap material to the grain nourishment of depletion with oxidize inferior change, defer grain quality to be worse.

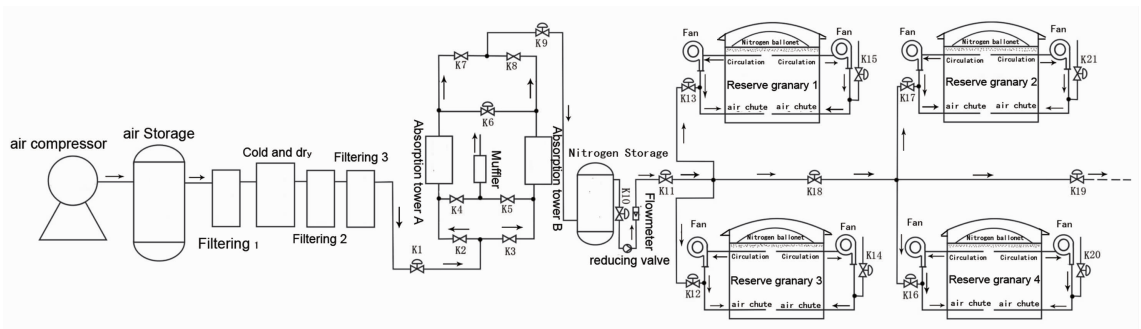


Fig. 1 green nitrogen (N_2) adjust to keep the grain technique work principle flow chart

Guangxi Fangchenggang National Grain Stores

2 Provide to Trying Storehouse

(1) Experiment a storage: to choose Guangxi national grain storage database on the 8th high & big one-storied storage is a green nitrogen(N_2) to adjust to keep a grain experiment storage, the storehouse grows 42 m, the breadth 30 m, pack the grain line high 6.1 m, after annoy air tightly reform, the storage annoys an airtight test to attain half-life ≥ 300(-300 Pa descend to -150 Pa) and keep grain to produce the United States for 2003 importing soft and white wheat, save grain: 5 120 tons, pack the grain height 5.1 m, permit heavy: 811 g/L, water 10.0%, the miscellaneous quality is 0.6%, and corn warm is normal.

(2) storage of checking against: to choose Guangxi national grain storage database on the 7th a high and big one-storied storage is a green nitrogen (N_2) so as to adjust to keep a grain experiment check against a storage, the storehouse length is 36m, the breadth 30m, pack the grain line high 6.1m, keep grain produced by the United States in 2003 importing soft and white wheat, save grain: 4 882 tons, pack the grain height 5.6 m, permit heavy: 811 g/L, water 10.1%, the miscellaneous quality is 0.6%, and the corn warm is normal.

(3) The expansion applies a storage: to choose Guangxi national grain storage database on the 1st, on the 3rd and on the 4th a high and big one-storied house storage is a green nitrogen (N_2) to adjust to keep the grain expansion application storage, 3 above storehouses all length 72m, the breadth 30m, pack the grain line high 6.1m, after annoy airtightly reform, the storage of above three storages annoy airtight the test all attains half-life ≥ 300 (the 300 Pa descend to -150 Pa: 00). The 1 among those numbers storage bulky cargo saves a grain: 9 307.08 tons, pack the grain height 6.15m, water is 13.2%, miscellaneous quality 0.8%; The No. 3 storage bulky cargo saves a grain: 9 271.24 tons, pack the grain height 6.15m, water is 13.2%, miscellaneous quality 0.8%; The No. 4 storage bulky cargo saves a grain: 9276.68 tons, pack the grain height 6.15m, water is 13.1%, the miscellaneous quality is 0.5%, above each storage grain be normal and have never had fever mildewed to change phenomenon.

(4) Experiment storage pest a circumstance:

Pest inside the storage: The grain samples were taken to examine No. 8 the insect of the storage density, such as *Rhyzopertha dominica* (Fbricius), *Tribolium castaneum* (Herbst), *Cryptolestes ferrugineus* (Stephens), *C. turcicus* (Grouville), the main insect the density is 35/kg. Experiment insect: According to experiment a project to request, combine a this database actual circumstance, select by examinations of experiment insect kind mainly with collect in this database to appear more, *R. dominica*, *T. cstaneum*, *C. ferrugineus*, *C. turcicus* (because of *R. dominica* individual be too small and can't pack into the insect cage), and be divided into its 11 groups & 10 *R. dominica*, 10 *T. castaneum*, 30℃. *ferrugineus*.

(5) The expansion applies storage pest a circumstance:

Used for carrying on large-scale nitrogen to adjust to keep the grain production research of the 1st, the high and big one-storied storage totally stores northeast corn 27 855 tons on the 3rd and on the 4th, should criticize corn to belong to produce in 2006, through Tieling-Liaoning Province, Tongliao-Nei Moggal, Siping, Jilin Province of northeast of our country of the corn habitat, the corn of above three storages arrives to defend city harbor to store in warehouse through a sea transportation in May, 2007, June and July respectively, the corn stored in warehouse headway to go in addition to miscellaneous. Through the check, should criticize the keep of corn grain insect to grow to mainly about: *R. dominica*, *T. castaneum*, *C. ferrugineus*, *C. turcicus*.

3 Experiment Material

(1) Make nitrogen machine a set: BE together developed completion by my database and the professional factory house, produce nitrogen(N_2) to measure to 30 square per hour, the design nitrogen (N_2) outputs the pure degree as 99.5% and actually measure nitrogen (N_2) to output a pure degree tallest can reach to 99.99%.

(2) One set of The oxygen quantity analyzes instrument: The model number is the NFY - Ⅰ C; Measure scope: 0.00 - 25.0% O_2.

(3) One set of the nitrogen (N_2) density circulation inside the storage equilibrium system. (BE developed by this database independence)

(4) One set of airtightness Various Types of Squat Silos

(5) The storehouse annoys airtight material: The nylon thin film, appropriation seals completely a slot tube, seal completely gum, appro-

priation to seal completely gum and change asphalt some.

(6) One set of Air Line Breathing Apparatus: breath mask, Multi-inlet Valve, Gasholder, An Alarm Apparatus, air compressor etc. use: Mainly to adjust to carry on a grain feeling check inside storage, if that breather used for the normal regulations chemistry medicine smoked steam to destroy insects Be a protection device effect better.

4 Experiment Methods

(1) Experiment storage (No. 8 storage) is provided to try pest to place: Before experiment, the grain samples will be taken to check the density of insect pest in the storage, try 11 sets to insect to pack into the insect cage before sealing completely a storehouse, press the figure 2 positions for mark to use bulk grain to pack insect cage to place the different part of grain heap.

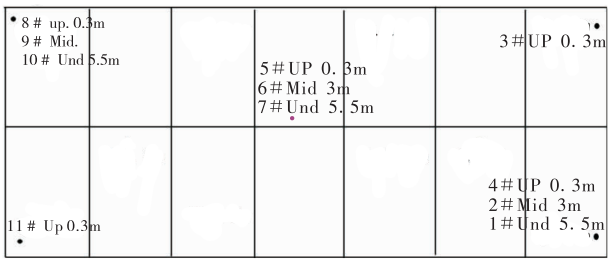


Fig. 2 **No.** 8 **Warehouse Insect cage Distribution**

(2) Expansion application the storage has never provide to try pest to place. The reason is the 1st which stores corn, the high and big one-storied storage belongs to an expansion application storage on the 3rd and on the 4th, and 3 storage all belong to an insect grain and need to be carry on immediately in addition to cure, our purposes are the grains which keeps nitrogen spirit to adjust to keep inside grain storage and keep the grain pest ecosystem and grain database normally keep a grain state of nature homology, adjust to keep a grain by the observation nitrogen spirit to the prevention and cure effect which keeps grain pest, so don't provide to additionally be provided to try pest.

(3) Experiment a storage, extension production storage closeness a processing: Wheat (experimental storage) and corn (the extension produce research storage) go into before the storage, should first storehouse ground the stretch and shrink of the ping sew, crack etc. the grain grain, dust, freestone within blind side clean off (the one who have condition can use compressed air to carry on tidy up), then infuse go into change sex asphalt cool stem need to be use; Closeness treatment of the wall in the warehouse: On all sides pack the wall of grain line in wall inside the storehouse up install appropriation to seal completely a slot tube (double slot tube), with the nylon thin film seal completely gum to press into the appropriation seal completely a slot tube to make it hang in the hand over of wall inside the warehouse, nylon thin film and ground connect place to use to seal chewing gum to glue to stick like then; The storehouse front door, air-vent adds appropriation to seal completely gum to carry on sealing completely a processing with the nylon thin film; The closeness method of grain heap in the storehouse surface is: First weld into the whole ratio grain heap surface to accumulate slightly big thin film with several nylon thin films, then seal completely a slot tube and seal completely gum to seal completely thin film with the appropriation, the whole storage become the closeness mode that five noodles thin films of a typical model seal. Storehouse closeness work completion behind have to be also carry on annoying an airtight sex examination, the storage annoys airtight and have to attain above square for 5 minutes is a pass, otherwise should continue to check to leak to repair hole until reach mark.

(4) Oxygen (the nitrogen annoy) examination: During the period of experimenting a storage to carry on nitrogen to adjust, the our usage NFY - I C amount of oxygen analysis the instrument carries on an examination to the air density in the grain heap, the oxygen (nitrogen spirit) density examination cloth orders plane chart to see figure 3.

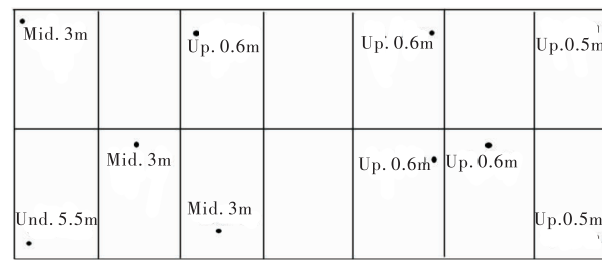
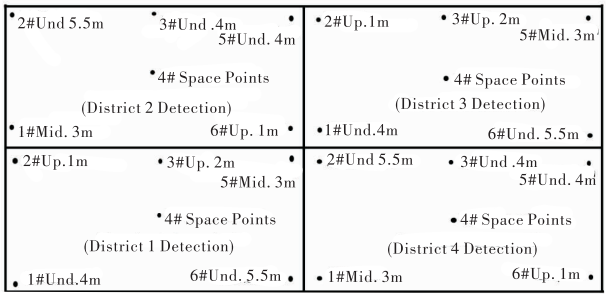


Fig. 3 **No.** 8 **Warehouse Distribution of gas Pai Layout**

(5) Experiment the storage (No. 8 storage) nitrogen (N_2) operation: Have carried on nitrogen (N_2) since September 12, 2006, will experiment the enter of storage spirit valve door to open first before the nitrogen, close row spirit valve door, then start to make nitrogen machine a set the beginning system for make nitrogen (notice; In order to making the nitrogen ma-



No. 1,3,4 **Warehouse Gas detection of Pai Layout**

chine nitrogen that set produce when just begin to work (N_2) a pure degree lower, generally only 90% be or so, this part of low and pure degree nitrogen spirits can carry on lining up a vacancy reason), the nitrogen that is on the observation system nitrogen machine set spirit density examination instrument, be the exportation nitrogen of appearance annoy (N_2) of when the density manifestation attain above 99.5%, open to make nitrogen machine a set a valve door to pass appropriation to lose a windpipe way a beginning to experiment storage go into nitrogen (N_2), this is that storage carries on first stage (time) of the nitrogen spirit adjust to keep a grain, go into nitrogen spirit of the amount usually takes signing a square rice as unit to compute. (according to the grain that warehouse keep of how much with storage inside space of size, each storage generally once can go into nitrogen annoy 1 000 – s 10 000 sign the square rice doesn't wait) Experiment storage when the first stage nitrogen spirit adjust to keep a grain work be over total go into a pure degree annoy for 99.9% nitrogen 1 937.32 sign a square rice, immediately after start the nitrogen in the storage (N_2) the density equilibrium system to carry on the nitrogen in the storage (N_2) density circulation balance, use the NFY – I C oxygen quantity analysis an instrument to nitrogen in the grain heap annoy a density (adopt nitrogen, oxygen density to pour calculate way) to carry on an examination, be the each examination point in grain heap in the storehouse of nitrogen (N_2) density bad value < 2%, then think the nitrogen in the storage spirit density already basic balanced consistent, can stop nitrogen (N_2) a density equilibrium system a work; The whole storage got at this time according to the examination in the meantime average nitrogen spirit density with keep grain pest to cause death nitrogen spirit a density 98% to carry on a comparison, if reach a mark can stop carrying on nitrogen to that storage, the nitrogen work namely tells be over; If the whole storage average nitrogen spirit density end attains to 98% ly keep grain pest with the result that the density has to then carry on the nitrogen (N_2) of next stage work, be each stage of nitrogen work completion after, nitrogen in the grain heap space spirit density will go up further, but the oxygen density will then descend further, the general building type storage usually needs through 4 and 5 stages (point a grain behind familiar period function not obvious or breath the strength be weaker to keep a grain) of nitrogen behind then can attain the cause death of pest nitrogen (N_2) density. (that density is 98%). When experiment the storage (8 storages) carry on arrive the fifth stage nitrogen (namely on October 3, 2006), the nitrogen in the storage spirit density through examination already 98.12%, immediately shut down stop nitrogen, that storage total goes into a pure degree to annoy for 99.9% nitrogen 9 849 sign a square rice, the nitrogen always uses is 351.75 hour, the nitrogen current of air measures to 28 to sign a square per hour.

(6) The expansion applied (the 1st, the 3rd, the 4th) storage nitrogen (N_2) operation: The 1st stored corn, the 3rd, No. 4 the storage belong to a high and big one – storied storage, the storehouse all grows a 72 ms, the breadth 30 ms, pack the grain line high 6.1 ms, the storehouse area reaches to 2 160 square meters and single storage is anticipated to be need every time above 99.5% nitrogen about 5 000 – 7 000 sign a square rice or so, is the storage nitrogen method to separately carry on nitrogen to what above three storages adopt, namely each time as to it's win a storage carry on nitrogen, according to the corn is into the dissimilarity of storage time, our arrangement above three storages carry on nitrogen of order of sequence is: No. 1 storage →No. 3→No. 4 storage. We carry on nitrogen an operation to the No. 1 storage on August 22, 2007: annoy the No. 1 enter of storage a valve door first to open, close row spirit valve door, and close the grain database other storehouses of enter spirit valve door, in order to prevent annoy nitrogen mistake into other storehouses. Start system nitrogen machine set make of beginning system nitrogen (N_2), observation system nitrogen machine the nitrogen on the set spirit density examination appearance, be appearance of the exportation nitrogen annoy (N_2) of when the density manifestation attain above 99.5%, immediately open make nitrogen

machine a set nitrogen spirit exportation a valve door make the nitrogen spirit pass appropriation lose a windpipe way toward No. 1 storage nitrogen (N_2), in the nitrogen the process we adopt every 24 hour respectively replace from the grain heap first floor and grain noodles nitrogen spirit of the method carry on nitrogen, so can make into a grain heap of nitrogen spirit density more even, go to No. 1 storage first stage nitrogen be over (namely grain heap the air sac can bear of safe tolerance) that storage total pure the degree annoy for 99.9% nitrogen 6 203.96 sign a square rice, the pass stop make a nitrogen machine set, close enter spirit valve door, and start the nitrogen in the storage (N_2) a density equilibrium system carry on nitrogen the density of the spirit (N_2) circulation balance, use the NFY - Ⅰ C oxygen quantity analysis instrument to grain heap inside of the air density carry on an examination and observe the oxygen density in the storage of variety circumstance, nitrogen spirit density whether have already attained to keep grain pest with the result that density with make sure whether next stage needs the nitrogen of continue or not. On top of that, we distinguish on September 21, 2007 and on September 30 to the carry on of storage of the No. 3 storage, No. 4 nitrogen of this database, concrete nitrogen operation and No. 1 storage completely same, this text no longer replies to say, go to nitrogen operation be over (namely grain heap the air sac can bear of safe tolerance) the No. 3 storage total pure degree to annoy for 99.9% nitrogen 5 952.8 sign a square rice, the No. 4 storage total pure the degree annoys for 99.9% nitrogen 6 440 sign a square rice.

5 Experiment Results

(1) Experiment the storage (No. 8 storage) destroys insects effect: Can prognosticate to start carry on more violent activity at grain heap surface and aisle plank top at this time, they express for the fluster and have no a purpose to crawl along everywhere; While carrying on the second stage nitrogen operation, the nitrogen spirit density in the storage rises to 91.74% from 86.83%, the above-mentioned pest starts more violently crawling along everywhere, also a little amount pest body be inside out, feet dynasty the God present to die appearance; While carrying on the third stage nitrogen, the nitrogen density in the storage rises to 94.82% from 91.74% at this time, keep the performance of grain pest as activity dilatoriness in the grain heap, walk to stop, have already can't endanger grain, body much is feet dynasty the God's inside out appearance. While carrying on the fourth stage nitrogen, the nitrogen density in the storage rises to 96.75% from 94.82% at this time and the above-mentioned pest continues to express for the appearance which is on the brink of to die, besides which, , present the appearance that will soon die; While carrying on arrive the fifth stage nitrogen, grain heap nitrogen the density has already rise to 98.12% and each kind of pest has already continuously died. Haven't discovered live insect while going to the tenth day (namely on October 13) to check into the storage. The storage's nitrogen annoys density to descend from 98.12% to 92.36% totally kept for 131 days (in the center don't add another nitrogen), Be done not discover live insects by check, until discover on the grain noodles aisle plank on February 21, 2007 that all pests have already been placed in to die appearance, we immediately carry on adding higher pure nitrogen (99.9%) to that storage about 1960 sign a square, nitrogen be over the nitrogen of the grain heap the density rises to 95.5%, in addition, for keeping higher nitrogen to annoy a density ($\geqslant$ 92%) inside storages, we still on November 16, 2007 give that storage complement higher pure nitrogen spirit (99.9%) about 1904 sign a square, close to February 16, 2008, through examination experiment storage 8 storages grain heap space of nitrogen the density remain about 92% and have no insect to expect as long as 491 days (namely since October 13, 2006 - February 16, 2008), till the day that this text cuts a draft experiment storage 8 storages still kept doing not discover to live an insect.

The pest experiments aspect and we choose each insect cage to put them into carrying on experimenting an observation. While carrying on first stage nitrogen (nitrogen density 86.83%), the pest performance is extremely nervous and without intermission carry on climbing to move; to discover insect cage while carrying on the second rank nitrogen (nitrogen density 91.74%) in equally have already had 30% pest death (the comparison, insect of death pest in each insect cage grow basic homology), survive down of try an insect to run about slow-moving, near to die; while arriving the third stage nitrogen to end (namely carry on the 11th day of nitrogen, the nitrogen in the storage density is 94.82%), the speed died from the pest to see, provide to try the pest in the insect

cage compare the pest in the storage death the speed want to have to be more quickly and investigate its reason us to recognize that the insect cage space is smaller, space oxygen the density be low, but provide density of try the pest again more big, two kinds of pest together entwine, squeezes depletion of accelerate its physique mutually, this is quickly to result in death main reason and in addition lay in grain heap, the death speed of the bottom layer insect cage pest ratio upper level insect the pest of the cage quick. Therefore, we think when doing nitrogen adjusts to keep grain pest death rate an examination, the death rate which provides to try pest probably also incompletely represents the death rate of storage pest, more at this time nitrogen in the storage annoy the density is a cause death of pest density, various measurements of data should with solid the warehouse grain death rate of the pest for 100% is standard for reference and we should specially notice this kind of special phenomenon.

(2) The expansion application (the 1st, the 3rd, 4 numbers) storage destroys insects effect: No. 1 the storage is from 2007 years after August 22 started nitrogen, we every morning 8: 30 – 11: 30 cent arrange specially assigned to the nitrogen storage carry on an oxygen density an examination, nitrogen go to the third weather discover that the storage appeared an oxygen density sharply descend of special phenomenon, then again always with quicker speed quickly descend, be the No. 1 storage first stage nitrogen end (9 days) the storage oxygen the density has already descended to 5.0% (but the theories decline an oxygen value just for 10.2%), to the 30th day (namely September 21) whole storage oxygen the average density has already descended to 1.7%, go to the 40th weather (namely October 1) whole storage oxygen the average density has already descended to 0.93%, go to the 50th weather (namely October 11) whole storage oxygen the average density has already descended to 0.5%, go to the 60th weather (namely October 21) whole storage oxygen the average density has already descended to 0.7% and have go to since the 70th day (namely October 31) 2008 years February 16 Japanese thesises cut a draft, that each oxygen of storage the examination order keep an oxygen density all for 0 time already 108 days, because of oxygen quantity analysis instrument already can't examine a grain heap of oxygen density, so we can treat it as unique oxygen or nitrogen spirit the density is for 99.9% (detailed see the No. 1 storage oxygen a density variety curve Figure) Owing to the No. 1 storage grain oxygen density inside the heap's exceeding the speed limit to descend, and have already attained the pest that the nitrogen keeps a grain to cause death a density 98% in advance. So our decision to that storage no longer carry on the 2 and 3rd stage nitrogen, change to continuing to observe an its oxygen density variety circumstance, can economize a great deal of nitrogen expenses so. No. 1 storage nitrogen spirit empress because oxygen density inside the grain heap quickly descends, various keep grain pest to cause because of the anoxia dispread from the grain heap, intermediate climb to a grain heap a surface layer, greatly part of keep grain pest gathering on the on all sides aisle plank of the warehouse wall, the parts of pest bodies are inside out, feet dynasty the god make to get close to die flounder form, continue to descend along with oxygen density inside the grain heap, go to 28 weathers through get into inside storage to grain each part of heap carry on sampling to sieve a check to discover inside storage of keep the grain pest has already all died. In addition, the No. 3 storage, No. 4 storage adjusts to keep at the nitrogen spirit the death circumstance of the pest within grain process and the variety circumstance of the oxygen density and No. 1 storage also greatly goes to a homology, this text no longer repeated, the oxygen density of these two storages variety detailed see the variety curve Figure of the 3rd, the No. 4 storage oxygen density.

In the No. 1 the storage nitrogen the spirit adjust keep the grain process is the reason why cause that storage of does the oxygen density exceed the speed limit to descend because according to compute that storage to carry on first stage after the nitrogen the its theories oxygen density at most can decline to 10.2%, but the oxygen density in the warehouse's grain heap has already all descended to 0 now, we analysis result in this kind of phenomenon of reason may be No. 1 storage, No. 3 storage, No. 4 storage store of the corn be the new corn which produces in 2006, corn go into storage empress because of afterward familiar period function and water be higher to cause breath quantity to enlarge but quickly finish consume the oxygen in the grain heap, cause thus after the nitrogen the closeness grain oxygen density within heap quickly descend. No. 1 storage's appearing this kind of oxygen density to exceed the speed limit the descendent phenomenon isn't accidental,

and close behind carry on g nitrogen spirit to adjust a No. 3 storage of keep the grain, No. 4, the storage also appeared a similar oxygen density to exceed the speed limit to descend phenomenon and also proved this kind of to calculate of accuracy, so we think such as the soybean, wheat, rice valley etc. of new results after familiar period obvious, breathe to have great capacity of grain when aftertime carry on nitrogen to adjust to keep a grain also very probably appear this kind of oxygen density to above exceed the speed limit the descendent phenomenon. Is exactly in order to appearing this kind of oxygen density to exceed the speed limit the descendent phenomenon, make to plan to carry on originally four-five stage nitrogen decline the work of oxygen to become to carry on first stage all completion, saved a great deal of nitrogen expenses, the normal nitrogen spirit adjusts to keep grain expenses of 20% –30%.

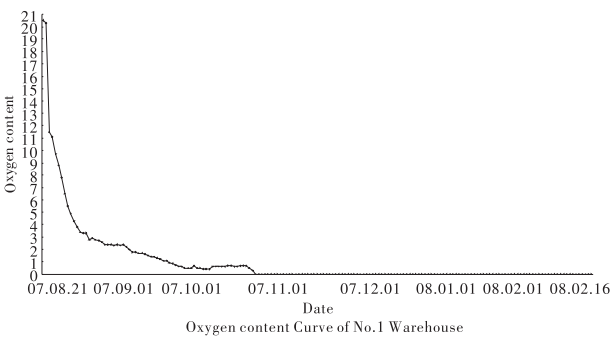
(2) Experiment the variety circumstance of the storage nitrogen and oxygen density

Table 1. Experiment the storage (No. 8 storage) nitrogen, oxygen density variety

Date	Times	Oxygen concentration	Concentration of nitrogen	time of Filling nitrogen time	Filled nitrogen
2006.9.10	0	21% (Air)	78% (Air)	0	0
2006.9.12	1	13.17%	86.83%	69.19	1937.32
2006.9.18	2	8.26%	91.74%	70.16	1964.48
2006.9.23	3	5.18%	94.82%	70.92	1985.76
2006.9.28	4	3.25%	96.75%	70	1960.00
2006.10.3	5	1.88%	98.12%	71.48	2001.44

Table 2. Expansion application storage (No. 1 storage) nitrogen, oxygen density variety

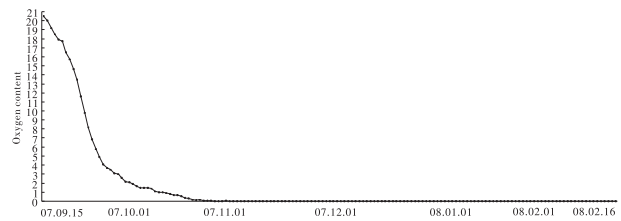
Date	Times of fillingn itrogen	Oxygen concentration	Concentration ofnitrogen	time of Filling nitrogen time	Filled nitrogen
2007.8.22	1	21% (Grain reactor)	99.9% (Filled)	221.57	6203.96
2007.10.18		2% (Grain reactor)	98% (Grain reactor)		
2007.10.29		0 (Grain reactor)	99.9% (Grain reactor)		



Oxygen content Curve of No.1 Warehouse

Table 3. Expansion application storage (No. 3 storage) nitrogen, oxygen density variety

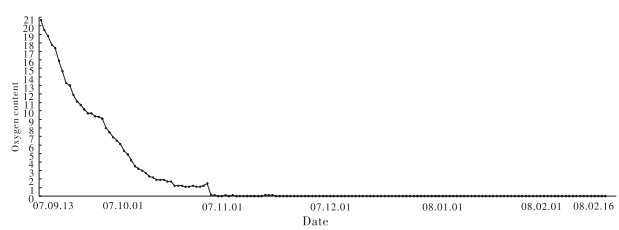
Date	Times of fillingnitrogen	Oxygen concentration	Concentration ofnitrogen	time of filling nitrogen time	Filled nitrogen
2007.9.21	1	16.5% (Grain reactor)	99.9% (Filled)	212.6	5952.8
2007.10.9		1.9% (Grain reactor)	98.1% (Grain reactor)		
2007.10.25		0 (Grain reactor)	99.9% (Grain reactor)		



Oxygen content Curve of No.3 Warehouse

Table 4. expansion application storage (No. 4 storage) nitrogen, oxygen density variety

Date	Times of filling nitrogen	Oxygen concentration	Concentration of nitrogen	time of filling nitrogen time	Filled nitrogen
2007.9.30	1	9.1% (Grain reactor)	99.9% (Filled)	230	6440
2007.9.15		1.9% (Grain reactor)	98.1% (Grain reactor)		
2007.10.29		0 (Grain reactor)	99.9% (Grain reactor)		



Oxygen content Curve of No.4 Warehouse

Note: table 1, 2, 3 and 4 of nitrogen parameter is: Output the nitrogen current of air measures to 28 to sign a square rice/hour and output the nitrogen pure degree is 99.9%.

Seen from the table 1, experiment a storage since September 12, 2006 first stage (namely stage 1) nitrogen to October 3, 2006 the fifth stage (namely stage 5) nitrogen be over, nitrogen spirit density from normal 78% of air rises to 98.12%; But the oxygen density then descend from 21% of normal air to 1.88%. Make nitrogen machine a set total work time is 351.75 hour, the total goes into a pure degree to annoy for 99.8% nitrogen 9849 sign a square. The storage's nitrogen time across the degree as 31 days and this be the cause that the nitrogen which all wants to carry on about 24 hours behind because of the stage be over of each nitrogen annoys density circulation bal-

ance.

Saw from the table 2,3 and 4, expansion application storage 1,3, No. 4 storage because the empress familiar period of corn mightiness store breathes a function, just through first stage nitrogen all of above 3 oxygen densities in storage grain heap descend to 0, keep grain pest death rate for 100%, because above 3 storage adopted the forerunner's grain storehouse to annoy airtight sex reformation technique, arrive this text to cut a draft above each storage keep an oxygen density is 0 of time already 96 day, the oxygen density in the storage rose of visible result, 1, 3, No. 4 the corn grain that storage keep feeling stability, have never appeared corn to have fever the phenomenon that mildewed change.

(3) Grain during the period of experiment of quality variety

Experiment storage (No. 8 storage) nitrogen (N_2) compared with check against a storage (No. 7 storage).

Table 5. The wheat stores quality variety comparison

Ware house	Inspection date	Moisture (%)	Bulkden sity(g/L)	Reinforc ement ofwater (%)	Scores of taste
No. 8	2006.8.29	10.0	811	195.9	82
	2007.5.31	10.2	811	194.8	81
	2007.9.7	10.1	811	197.2	84
	2008.2.9	10.1	811	197.0	84
No. 7	2006.8.29	10.1	809	196.2	81
	2007.5.31	10.2	809	191.1	78
	2007.9.7	10.2	809	192.7	78
	2008.2.9	10.0	809	191.5	76

From the table 5 we can see, experimented a storage (No. 8 storage) have carried on nitrogen since September 12, 2006 to adjust to keep after the grain wheat of the hoard quality had obvious variety, went to May 31, 2007 to carry on hoard quality an examination wheat of wheat gluten absorb water quantity from 195.9% just a little descended to 194.8% (not expel the examination error margin factor), carried on nitrogen to keep grain about after a year, its hoard quality not only descended to on the contrary have an obvious exaltation, wheat gluten absorb water quantity from 194.8% rise to 197.2%, go to on February 9, 2008 its wheat gluten absorb water quantity to still keep 197.0%, wheat gluten absorb water quantity and taste a grade point value respectively compare check against a storage (No. 7 storage) 5.5 high percentage points with 7 divide. But 2 checked against a storage (No. 7 storage) because of having never carried nitrogen to adjust to keep grain store quality indices to present straightly descend trend, its wheat gluten absorb water quantity from 196.2 descend to 191.5, taste a grade point value to descend from 81 to 76, descend of the ranges all have more and greatly.

By Table 6 we can know green nitrogen to adjust to keep grain expansion applied of 3 storage (1,3, No. 4 storage), in order to go into the nitrogen of higher pure to plus a corn oneself the breath function of the mightiness inside the storage, make the closeness grain oxygen density in the heap quickly descend to 0, and can with long hours keep, so the corn is under this kind of environment which looked like unique oxygen stored the fat in the grain grain to decelerate the progress that it oxidize because of anoxia, stored in warehouse to store more than 8 months. It mainly stores quality indices fatty acids a value ascension extremely slow – moving, equally only increase 1.2 fatty acids values, according to fatty acids value thus of growth speed, above 3 expansion application storage of the corn rotates its fatty acids value inside the period (in two years) in a hoard will probably can't over 50 this is proper to save with light the degree should not save this important boundary, if 60% storage corns in the whole country carry on green nitrogen spirit to adjust to keep a grain, will be able to significant exaltation storage corn of proper save a rate and save to rotate expenses, the hoard that even can also consider adjustment or prolong corn or other grain species in the future rotate a period, for the country house economy a great deal of storage the grain rotate expenses.

Table 6. The expansion application storage (1,3, No. 4storage. corn stores quality variety

Ware house	Inspection date	Moisture (%)	Bulk density (g/L)	Fatty acids	Scores of taste
No. 1	2007.6.29	13.2	722	43.6	86
	2007.8.15	13.2	722	43.7	86
	2008.2.5	13.3	722	44.5	85
No. 3	2007.6.29	13.3	728	42.2	87
	2007.8.15	13.2	728	43.6	86
	2008.2.5	13.3	728	43.9	86
No. 4	2007.7.26	13.2	726	43.2	86
	2007.8.15	13.0	726	43.5	86
	2008.2.5	13.1	726	44.1	86

(4) The economic performance contrast

The nitrogen adjust to keep grain to exper-

iment((No. 8 storage) and normal regulations to keep grain to check against storage (the 7th) to carry on to normally keep grain for a year of movement expenses comparison, the contrast sees table as a result 7.

Table 7. The nitrogen adjusts to keep grain toexperiment storage and normal regulations to keep grain to check against storage expensesto relatively analyze. Unit:(Yuan/Tonne of grain)

Warehouse	Capacity (t)	Amount (t)	Fumigation costs		Film grain costs	Nutritional supplement	Circulation electricity costs	Total costs	Costs/T
			N_2	ALP					
8	5500	5120	3940		1200		28	5168	1.01
7	5000	4882		2500	150	3255	55	5960	1.22

Note:The normal regulations keeps grain to check against a storage to press to carry on annually two times smoked steam to destroy insects the expenditure expenses needed.

According Table 7:expenses expenditures are analytical from the table more medium see, the nitrogen adjust to keep grain to experiment a year of storage (No. 8 storage) ton grain expenses 1.01 Yuan/year/ton and normal regulations keep grain to check against a year of storage (No. 7 storage) expenses 1.22 Yuan/year/Ton. Carrying on single storage expenditure expenses lower 0. 21 Yuan/Year/Ton, actually because of the nitrogen adjust to keep grain still have many storage nitrogen tail comprehensive the advantage of exploitation, significant decrease expenses expenditure 20% – 50%, therefore, the nitrogen spirit adjust actual grain expenses expenditure of keep the grain generally for 0.5 – 0.8 Yuan/year/Ton. To carry on normal regulations smoked steam to keep the expenses of expenditure that grain need to still want low.

Table 8. The storage (1,3,No. 4 storage) nitrogenof the expansion application adjusts to keep the grain expenses expenditure. Unit:(Yuan/Ton)

Ware house	Capacity (t)	Amount (t)	Nitrogen costs	Film grain costs	Circul ation electricity costs	Total costs	Costs/T
1	9000	9307.08	2041.8	2900	52	4993.8	0.536
3	9000	9271.24	2060.1	2900	52	5012.1	0.54
4	9000	9276.68	2228.7	2900	52	5180.7	0.56

From the table 8 data we can see, carry on green nitrogen to adjust to keep grain of expansion application storage 1, 3, No. 4 storage because the grain keep by belong to new results corn, the nitrogen lowered the oxygen density in the storage of in the meantime, corn oneself mightiness of after the familiar period breathe a function also consumed grain is a great deal of oxygen inside the heap, cause the oxygen in the storage density only descended to 0 by all of a nitrogen, expansion application storage 1, 3, No. 4 storage because of adopting the patent technique for possess singly to carry on annoying an airtight reformation, the storehouse have very and goodly annoy airtight function, so far above three oxygen densities of storage still keep to 0, have no ascension of trend, the nitrogen in the storage also didn't leak of phenomenon, the grain keep have no insect, have never had fever mildewed change phenomenon, grain feeling stability, keep grain safety, according to the green nitrogen spirit adjust keep grain of expansion application storage 1, 3, No. 4 storage current development trend, the corn that 3 storages keep is in this the hoard rotate the period (two years) won't need again additional complement higher pure nitrogen, as a result saved about 75% nitrogen expenses expenditures, so rotate a period (namely in two years) to only need the expenditure nitrogen expenses in this hoard:0.55 Yuan per ton.

6 Analyze with Discussion

(1) Destroy insects effect analysis:Store a method with the grain of the adoption normal regulations to compare, the green nitrogen (N_2) adjust to keep grain technique regardless to all have very big advantage in destroying insects effect and preventing and cure expenses, and availably avoided normal regulations keeping the grain chemistry medicine's pollution for the grain and the environment and provided for the drug – resistant which represses to keep grain pest new method. Experiment storage higher N_2

& with lower oxygen environment under the condition, successfully repressed the growth of insect, mildewed to breed. There is good prevention and cure effect, the insect pest death rate is 100%, such as *R. dominica*, *T. castaneum*, *C. ferrugineu* and *C. turcicus*. Destroy insects the effect outran our expectations, investigate its reason is to keep the grain pest basically can't breed under this kind of bad environment condition of higher N_2 & lower oxygen, existence, so adopt nitrogen green to keep grain technique prevention and cure to keep grain pest is a depopulation type of, but the adoption keep the prevention and cure pest of the grain chemistry medicine then to attain this effect. Time which checks against storage no longer than 10 months carried on to turn the aluminum chemistry medicine smoked steam to destroy insects.

(2) The spirit adjusts to keep grain function analysis: Occupy pass data introduction, foreign open the exhibition carbon dioxide (CO_2) or nitrogen (N_2) to adjust to keep with domestic currently the grain generally and all relatively is over-emphasize in spirit to adjust to destroy insects this function, but adjust to keep grain then give attention to both. But, we think no matter what it is adjust to keep grain technique, it has to have the following two functions: Since can adjust means to availably prevent and cure to keep through the grain pest adjusts means to keep through again the grain make grain of hoard quality can the biggest limit's keep (or protect fresh) just can be called real of the green keep a grain technique and lack a Our development, develop of the green nitrogen (N_2) adjust to keep technical biggest advantage of grain be-namely since can destroy insects and keep a grain, the storehouse which passes to adopt a forerunner annoys airtight patent a technique, adopt special closeness material and closeness method, it can make the grain heap keep disadvantage to breed at the pest growth over a long period of time, but be advantageous to the ecosystem environment of higher N_2 & lower oxygen of the grain hoard. It is a more perfect for green nitrogen to adjust to keep grain technique. In addition, adopting green nitrogen to adjust to keep grain technique can also solve many adopted a normal regulations method to keep in the past the grain can't solve of hard nut to crack, such as in the normal regulations method the hoard the process because of over a long period of time adopt hydrogen phosphide smoked steam a grain, usually result in the integrated circuit to monitor the system to grain feeling collect a machine and measure electric resistance to be decayed damage; Caused to keep grain pest to produce a serious drug-resistant, and we can not destroy the life of *R. dominica*, *T. castaneum*, *C. ferrugineu* and *C. turcicus*. So as to be result in the problems of medicine remains & pollute grain and endanger a human body's health and break environment etc.

7 The Green Nitrogen (N_2) Adjusts to Keep the Grain Technical Applied Foreground Outlook

Of development, research green keep the grain new technique is a local and foreign grain to store currently technical main development direction, and it is an irresistible general trend, but the green nitrogen (N_2) adjust to keep a grain technique doubtless again is various green keep to have to develop a potential most in the grain technique of one of the technique, pass to open an exhibition green to keep grain since can reduce to keep the grain chemistry medicine's pollution for the grain and the environment, and then can reduce a drug-resistant of keep the grain pest and keep a save of grain quality. Along with the progress in ages, it is every to will have green nitrogen (N_2) to adjust to keep from now on grain technique when the grain of the hoard carry on market sale, certainly will increase its merchandise additional value, this again for aftertime green keep grain of the expansion application got into the development orbit of virtuous cycle to create a beneficial condition and making the green nitrogen (N_2) adjust to keep grain technique will have vaster market foreground.

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0407

SuperGrainBag: A Hermetic Bag Liner for Insect Control of Stored Cocoa Beans in Ghana

W. A. Jonfia-Essien[1*], S. Navarro[2] and J. V. Dator[3]

Absrract: This paper reports the development of hermetic storage in Ghana by highlighting the scientific research conducted using GrainPro SuperGrainBag™ in controlling insects of stored cocoa beans as an alternative to chemical treatment. Three stacks, all of which were composed of hermetically sealed bags of SuperGrainBag™ of 40kg dry cocoa beans each inserted into standard cocoa jute sacks were built in the same warehouse close to each other for sampling and observation at the Research Department, Quality Control Division of Ghana Cocoa Board facilities in Tema, Ghana. Two additional stacks of 64 kg per bag of dry cocoa beans were also built for sampling and observation; one stack for conventional storage (without fumigation) the other for standard storage (fumigated with phosphine). Oxygen concentration inside the SuperGrainBag™ of cocoa beans was monitored daily. Sampling was done after 30 days to observe changes in insect density. There was a steep decline in oxygen concentration in all SuperGrainBag™ during the storage with the lowest concentration of 0.0% being recorded from the fifteenth day of storage. This depleted oxygen concentration was maintained throughout the storage period. The depleted oxygen atmosphere was attributed to activity of all living organisms within the SuperGrainBag™, including the micro organism activity due to the cocoa beans moisture content which was 7%. Although this level of moisture content reflects the normal storage conditions in Ghana, it still permitted the generation of a depleted oxygen atmosphere. After 30 days of storage, 100% mortality of high populations of insects were observed in all cocoa beans stored in SuperGrainBag™ liners except one single bag in which a few individual insects were alive. The insect population consisted of adults of *Carpophilus hemipterus* and *Tribolium castaneum*. The conventional stack containing the control bags remained highly infested with insect counts reaching 88 live insects/64kg bag of cocoa beans. The standard stack recorded 100% mortality of insects in each 64kg bag of cocoa beans. These trials indicate that hermetic storage provides an environmentally safe solution for preventing development of insects in cocoa beans and thus avoid the use of chemical fumigations.

Key words: SuperGrainBag™, hermetic storage, cocoa beans, *raecerus fasciculatus*, *Lasioderma serricorne*, *Carpophilus hemipterus and Tribolium castaneum*, storage insect control, conventional storage, standard storage

Introduction

In recent times airtight storage or hermetic storage, also known as sealed storage technology, has generated a lot of interest as one of the methods of quality preservation for stored products. Substantial literature on sealed storage technology or airtight storage has been summarized[1,2]. Its principle has been employed since ancient times in underground pits that are still used, particularly in semi-arid regions of the Mediterranean basin and Sahel[3,4]. The inherent advantage of the hermetic storage of dry grain lies in the bio-generation of an oxygen-deficient and carbon dioxide-enriched inter-granular atmosphere of the storage ecosystem, a condition produced by the aerobic metabolism of insects and microorganisms.

Stored product protection is enabled by the use of storage containers with hermetic seals[5,6], one of which is the SuperGrainBag™, which provides an airtight environment. The basic principle of protection[5,6] lies in the fact that from the time of sealing until the insects consume the volume of oxygen in the SuperGrainBag™, damage is negligible. If the insect population is low, the insects may survive causing minimal damage to the produce since it has been established that to obtain a complete kill, the oxygen tension must drop to two percent (2%) or below[7].

At present, the conventional and standard

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storage technologies are employed to preserve cocoa beans. These conventional storage methods involve stacking of bagged beans on wooden pallets inside the warehouse. The cocoa beans are then protected from pest infestation with a combination of hygiene, sanitation and chemical control. However, this method is inadequate in preserving beans quality for long periods of time. Hence, the need for alternative storage technology that is technically feasible and cost effective. Therefore, this project explores the " hermetic, gastight or airtight" storage technology as an alternative using the SuperGrainBag™ as liners.

Materials and Method

Three stacks, all of which were composed of hermetically sealed bags of SuperGrainBag™ (SGB) of 40kg dry cocoa beans each inserted into standard cocoa jute sacks were built in the same warehouse close to each other for sampling and observation at the Research Department, Quality Control Division of Ghana Cocoa Board facilities in Tema, Ghana.

The dry cocoa beans which were already naturally infested with *Araecerus fasciculatus*, *Lasioderma serricorne*, *Carpophilus hemipterus and Tribolium castaneum* were re-bagged into jute sack by placing the SGB inside the jute sack as liners before the cocoa beans were poured in. The upper free plastic portion of the SGB were twisted and closed with cable ties with the aid of a cable gun. These bags of cocoa beans with SGB liners were stacked on pallets and stored for 30 days.

A butterfly needle was connected to the inlet side of the pump for measuring oxygen (O_2) concentration in the SGB. The needle was permanently inserted in the SGB and sealed with packaging adhesive tape and epoxy to eliminate any possible air/gas leakage. The hose end of the needle has a built-in plug for quick-connection to the pump. Temperature and oxygen concentration inside the SuperGrainBag™ of cocoa beans were monitored daily. Temperature was measured using data loggers inserted into each bag and for oxygen concentration in air, a GrainPro Oxygen Meter, a portable oxygen analyzer using electro-chemical sensor was used.

Two additional stacks that served as controls and contained 48 bags each, with 64 kg per bag of dry cocoa beans were also built for sampling and observation; one for conventional storage (without fumigation), the other for standard storage (fumigated with phosphine). Insect sampling was done when the bags were filled, and again after 30 days to observe changes in insect density and mortality.

Results and Discussion

There was a steep decline in O_2 concentration in all SGB during the storage with the lowest concentration of 0.0% being recorded from the fifteenth day of storage (Fig. 1). This depleted O_2 concentration was maintained throughout the storage period. The depleted O_2 atmosphere was attributed to activity of all living organisms within the SGB, including the micro organism activity due to the 7% cocoa beans moisture content. Although this level of moisture content reflects the normal storage conditions in Ghana, it supports an equilibrium relative humidity (ERH) of about 67% at 27-29℃[8]. This ERH permits a moderate level of microflora activity[9] that eventually leads to CO_2 generation and a depleted oxygen atmosphere.

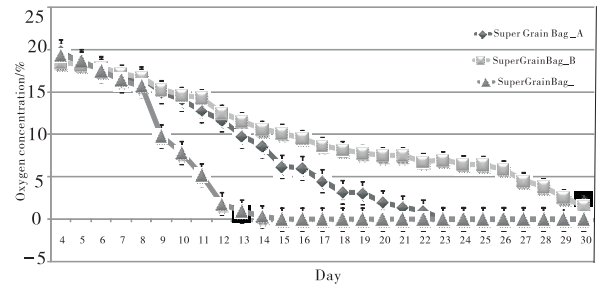

Fig. 1 Daily percent oxygen concentrations measured inside three hermetic Super Grain Bag™ over 30 days pf storage period.

Temperature of the cocoa beans inside the bags fluctuated throughout the storage period of 30 days within the range of 27℃ and 32℃. The temperature of the warehouse where the bags were stored was also within the same range.

After 30 days of storage, 100% mortality of high populations of insects were observed in all cocoa beans stored in SGB liners, except one bag in which a few individual insects were alive. The insect population consisted of adults of *Carpophilus hemipterus* and *Tribolium castaneum* (Table 1). The depleted oxygen concentration achieved in these trials was apparently due to microflora activity and not due to insect activity as investigated for dry grains[7]. In dry grains at ERH below 65% the microflora activity is negligible and the ERH values are below the critical levels for respiration and creating a depleted oxygen atmosphere in sealed stora-

ges[5]. In dry commodities like cocoa beans, depleted oxygen atmospheres can be generated in a short time as in Fig. 1, when the insect populations are sufficiently large, the temperature is adequate for insect development, and the gastightness permits hermetic storage[10,11] [12,5]. The reason for the insect survival in the sealed bag marked A might be due to the delayed reduction in oxygen concentration when it reached the level of less than 1% after 23 days. However, such low level of infestation and under the hermetic conditions achieved insect damage was estimated as below the economic threshold limit for practical uses. It is quite likely that extended exposure to such low oxygen levels would have caused complete mortality of these two species.

Table 1. Population of live adult *C. hemipterus* and *T. castaneum* in one SuperGrainBag™ – A

SGB-A	C. hemipterus			T. castaneum		
	Larvae	Pupae	Adult	Larvae	Pupae	Adult
1	0.0	0.0	2.0	0.0	0.0	1.0

The conventional stack containing the control untreated bags remained highly infested with insect counts, reaching 88 live insects/64kg bag of cocoa beans (Fig. 2). The standard stack was fumigated using phosphine at a dosage of 2 – 4 g/m^3 in which 100% mortality of insects was recorded in each 64kg bag of cocoa beans.

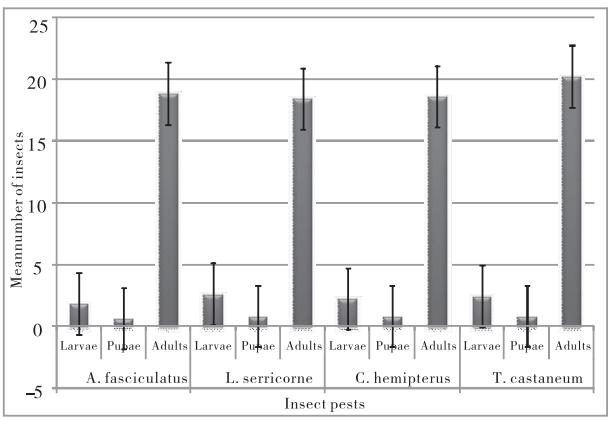

Fig. 2 Mean population density of live insect pests in a 64kg bag of the conventional stack.

At the end of the storage period, the cocoa bean samples taken from the hermetic and control bags were examined for quality control, and they were found within the standards set for commercial purposes by the Cocoa Board of Ghana. These trials indicate that hermetic storage provides an environmentally safe solution for preventing development of insects in cocoa beans[6] and thus avoid the use of chemical fumigations.

Conclusion

In view of the above findings, it is conclusive that SuperGrainBag™ is a good alternative to other pests control measures, especially during long term storage when it is used as a liner. The use of SuperGrainBag™ as liners thus avoids the use of chemical fumigations in storage.

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0408

Comparing Application of Slow-releasing Fumigation in Large Warehouse

Deng Zhonghua

Abstract: Recirculation fumigation, slow-releasing fumigation and recirculation, plus slow-releasing fumigation under film were compared in large warehouses with 6m deep grain bulk, the concentration change of PH_3 and killing effect were determined. The results showed that these three methods all can distribute PH_3 well. *Sitophilus oryzae* Motschulsky was killed in 5 days, while *Cryptolestes ferrugineus* (Stephens) was killed in 35 – 40 days.

Key words: large warehouse, recirculation, slow-releasing, fumigation

Slow-releasing fumigation has the advantages of operating simply, keeping insecticide effect long and killing insects completely, so it was widely used in small grain bulks against insects, but use in large warehouses (the depth of the bulk is greater than 6m) was seldom reported. The effective period of PH_3 distribution was compared among recirculation fumigation, slow-releasing fumigation and recirculation, plus slow-releasing fumigation in our test, and the insect killing effect was also observed.

1 Materials and Methods

1.1 Materials

1.1.1 Tested warehouse A1 – 1、A3 – 2 and A4 – 1 warehouses were chosen in Maoming Grain Depot for the test. They were all brick-concrete structure; length: 48m, width: 30m, grain bulk depth: 6m, wall thickness: 0.37m, bulk volume: 8640m^3; main air-ducts each with 3 branches (Fig. 1) and 6 air intakes installed in the warehouse which could realize aeration at two sides; fixed recirculation fumigation system in which fan power was 0.75kW, the fumigation-ducts under disposable film was expressed in Fig 1, air return tubes: PVC tube, ϕ160mm with flat holes.

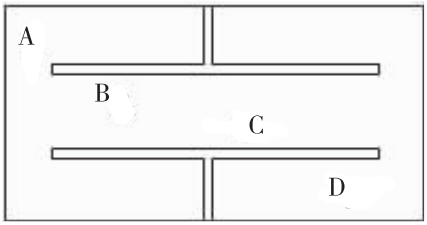

Fig. 1 The fumigation-ducts under disposable film layout floor plan (ichnography) of warehouses

1.1.2 Tested grainThe tested warehouses all contained stored bulk corn from Liaoning Province. Near the surface of grain bulk, the air outlet and near the door were found *Sitophilus oryzae* Motschulsky and *Cryptolestes ferrugineus* (Stephens) before test, where the population density was 2 – 4 insects per kg. The basic grain conditions are expressed in Table 1

1.1.3 Test instrumentsSampling device of deep grain layer (made in Chengdu); PH_3 concentration determine tube (made in Guangzhou); PH_3 gas detector (made in Beijing); PVC plastic film (0.08mm, bought in the market); cloth bag (25cm × 40cm); U type manometer (made in Guangzhou); stopwatch.

Table 1. The basic grain condition in warehouse

	A1 – 1	A3 – 1	A4 – 1
Quantity (t)	6033	5892	5987
Bulk density (g/L)	705	695	690
Moisture content (%)	13.1	13.2	12.7
Surface grain temperature (℃)	23.2	23.7	22.9
Highest grain temperature (℃)	24.7	25.5	24.9
Lowest grain temperature (℃)	15.5	15.0	16.1
Average grain temperature (℃)	18.2	17.5	18.7
Insect population density (heads/kg)	4	2	3
Storage style	bulk	bulk	bulk

1.2 Methods

1.2.1 Sealing method: Grain surface was

Maoming Grain Depot, State Grain Reserves

sealed by 0.14mm PVC film with sealed slot alongside the outside wall. Doors and Windows were sealed by 0.14mm PVC film too. The air outlet was a self – sealed type air outlet.

1.2.2 Test of air tightness: Air tightness was tested by negative pressure in reference warehouse after sealed. The pressure half – life of the A1 – 1、A3 – 2 and A4 – 1 warehouse was 41s,42s,45s separately. Testing was performed in accordance with the specifications of "Technical standards for PH_3 recirculation fumigation".

1.2.3 Location of gas detecting sites: There were 4 detecting points (A,B,C,D) positioned in the 4 corners at a distance of 0.5m to the end and side walls of the warehouse. Each testing point had sample tubes with inlets at upper, middle and bottom layers which were respectively at 0.5m, 3.0m and 5.5m below the grain surface. E detecting point was setted on the film. These 5 sites with 13 gas detecting points were fitted with plastic pipes which were brought out through the door.

1.2.4 PH_3 concentration determination: AHL – 210 type PH_3 gas detector (made in Beijing) was used for regular sampling the concentration of PH_3 after application. The detector was corrected by PH_3 concentration determine tube (made in Guangzhou) before used.

1.2.5 Insects inspection method: Sieve and visual inspection method were used for inspecting insects. The insects death condition was inspected by insect cage during fumigation, and inspecting the death condition of *Cryptolestes ferrugineus* (Stephens) on the footpath board by eyesight (visual). Insect cage making method was as follows: Select robust insects putting into nylon bag, filled with broken wheat and corn. In each warehouse were set 10 insect cages. In each cage were put 60 insects (50 of them were *Cryptolestes ferrugineus* (Stephens), 10 of them were *Sitophilus oryzae* Motschulsky), then we sealed and buried the cages (with a retrieval rope attached). 0.5m and 3m below grain surface and near the inspecting door respectively. The insects death condition in the cages was inspected after fumigant application and the warehouse was unsealed.

1.2.6 Application method: Recirculation fumigation under film was carried out in the A1 – 1 warehouse. Fumigant dosage was $2g/m^3$, 18kg AlP was applied for 50 intakes which were under the film, each cloth bag contained 60 tablets AlP. The rest of the AlP was put into 6 air outlets, each outlet bag contained 1.5 kg.

Slow-releasing fumigation was carried out in the A3 – 1 warehouse. Fumigant dosage was $2g/m^3$, sealed into 200 PVC bags, each bag contained 30 tablets. 139 bags were applied on the surface; 50 bags were put into 1.5m length, 120mm diameter PVC tubes, probed into the bulk, then the tube was taken out of the bulk, thus AlP was buried about 1m below the surface of the bulk. The remaining 11 bags were put in the air outlet and door respectively.

Recirculation + slow – releasing fumigation was carried out in the A4 – 1 warehouse. Fumigant dosage was $2g/m^3$. Every 30 tablets was put into a PVC bag, 100 PVC bags were put on the grain surface altogether. The rest of the AlP had been put into cloth bags in 6 air outlets, each air outlet contained 1.5 kg AlP.

Continuous recirculation was carried out for 48h after the fumigant was applied, then the fan was operated 2 ~ 3h each day for 7days in A1 – 1 and A4 – 1 warehouse. There was no recirculation in A3 – 1 warehouse.

2 Results

2.1 Change of PH_3 Concentration

The determination of results of PH_3 concentrations in tested warehouses during fumigation was listed in Table 2. The results from Table 2 showed that: gas release speed of PH_3 was slowest using slow-releasing fumigation; PH_3 distributed well in all three tested warehouses; PH_3 distribution was bad in the corner of the A1 – 1 and A4 – 1 warehouse in the horizontal direction. This was maybe because of the location of the air return tube. PH_3 distribution was bad in vertical direction in A3 – 1 warehouse. The concentration was low in bottom layer, that was because the natural vapor pressure diffusing ability of PH_3 was limited; the gas concentration retention time of PH_3 was longest in A3 – 1 warehouse. The tests showed while recirculation fumigation improved the diffusing speed of PH_3, at the same time, recirculation caused leakage of PH_3. So recirculation + slow – releasing fumigation could receive best effect considering PH_3 distribution and duration of concentration.

2.2 Results of Killing Insects

Insect survival condition of the tested warehouse was expressed in Table 2. The insects in the surface of grain was inspected by eyes, observe if there had any live insects on the footpath board. The results from Table 2 sh

Table 2. PH_3 concentration determine and insect killing condition

	T, d days	PH_3 concentration (mL/m^3)													Mortality (%)				
		A1	A2	A3	B1	B2	B3	C1	C2	C3	D1	D2	D3	E	IYM	IXB	IIYM	IIXB	surface
	1	75	63	92	104	93	72	73	93	86	97	68	107	25	30	0	30	32	Yes
	3	298	276	322	262	220	271	278	265	216	273	232	318	15	100	32	100	40	Yes
	5	426	350	366	411	392	355	415	395	428	396	322	411	19	100	50	100	60	Yes
	7	457	447	402	435	489	426	452	455	495	465	409	491	15	100	66	100	74	Yes
	10	423	321	361	365	378	388	402	401	459	356	423	478	25	100	78	100	88	Yes
A1 – 1	15	333	268	305	254	313	371	376	355	386	350	358	382	15	100	90	100	90	Yes
	20	241	201	213	227	244	297	289	324	321	267	321	323	19	100	100	100	100	Yes
	25	213	165	176	175	196	169	225	256	320	190	253	281	12	100	100	100	100	Yes
	30	160	102	121	132	144	135	189	191	279	175	189	243	11	100	100	100	100	No
	35	124	104	105	97	119	99	114	145	161	135	158	192	15	100	100	100	100	No
	40	52	64	74	61	72	86	64	71	84	80	89	90	15	–	–	–	–	No
	1	48	55	12	97	85	6	121	61	17	70	49	24	18	20	20	10	10	Yes
	3	155	131	35	157	163	71	147	114	62	179	182	69	15	100	36	90	20	Yes
	5	222	165	56	223	221	102	213	289	89	224	209	107	10	100	50	100	20	Yes
	7	289	225	98	267	273	116	231	298	98	298	264	152	12	100	60	100	50	Yes
	10	324	301	123	303	369	142	362	365	107	358	293	165	16	100	72	100	60	Yes
A3 – 1	15	426	386	164	346	411	153	402	316	122	401	360	184	5	100	84	100	72	Yes
	20	338	374	189	406	401	105	387	285	185	396	335	203	10	100	90	100	80	Yes
	25	270	309	205	355	305	161	321	246	186	302	284	179	9	100	100	100	98	Yes
	30	215	251	197	298	257	203	274	202	144	279	186	165	5	100	100	100	100	Yes
	35	146	206	177	243	199	166	222	261	123	264	142	156	7	100	100	100	100	No
	40	108	142	110	130	166	123	153	165	106	110	121	131	10	–	–	–	–	No
	1	102	78	115	99	85	76	67	77	87	73	88	94	23	20	40	20	10	Yes
	3	235	232	206	257	201	281	224	246	250	277	264	287	18	100	42	100	40	Yes
	5	324	306	315	266	221	256	313	289	298	307	291	375	10	100	64	100	52	Yes
	7	389	366	398	361	288	345	355	398	371	417	362	415	12	100	84	100	74	Yes
	10	425	405	485	453	402	442	419	469	427	489	396	459	15	100	86	100	98	Yes
A4 – 1	15	398	416	364	344	411	350	382	351	366	374	412	355	11	100	90	100	100	Yes
	20	385	354	355	313	392	321	358	279	342	268	387	263	16	100	100	100	100	Yes
	25	299	211	290	230	355	332	341	255	232	220	314	217	8	100	100	100	100	Yes
	30	176	172	208	174	216	245	274	204	209	199	265	185	5	100	100	100	100	No
	35	145	126	177	135	153	167	192	152	165	132	142	124	5	100	100	100	100	No
	40	99	105	89	103	105	106	103	113	120	111	121	101	5	–	–	–	–	No

owed that PH_3 enduring ability of different insects was not the same, *Sitophilus oryzae* Motschulsky was easier killed than *Cryptolestes ferrugineus* (Stephens) at the same fumigation condition, *Sitophilus oryzae* Motschulsky was all killed after 5 days fumigation, while *Cryptolestes ferrugineus* (Stephens) was still active in the earlier stage of fumigation, live insects could be found after 25 days fumigation, the mortality was 100% after 30 – 35 days. The results also showed that live insects could still found on the grain surface when the insects in the cage had all been killed, so the cage can only be a reference for inspecting fumigation results. In practice, when the insects in the cage all died, fumigation should be continuing for a period to strengthen the killing effect on those insects near the boundaries and surfaces of the storage bulk.

Sampling device of deep grain layer was used for inspecting upper, middle and bottom layer, especially sample the corner and impurity area after degassed, and no live insects were found. There was also no live insects found after continuous observing 2 months after the fumigation (the grain in A4 – 1 warehouse rotated after degassed 25 days).

3 Discussion

3.1 The practice showed that whether

recirculation fumigation, slow-releasing fumigation or recirculation + slow-releasing fumigation are used, all three methods can provide good effect on killing insect. Slow-releasing fumigation can be used in large grain bulk such us large warehouse for controlling insects, it was because: (1) grain temperature in large grain bulk had the advantages of outside surfaces were hot and the center was cool, thus, the cool center let PH_3 diffused deeply into the grain bulk; (2) insects in large grain bulk almost distribute at corner, near the door and air outlet, under 1m of the grain surface, PH_3 can naturally diffuse satisfactorily to these areas.

3.2 Recirculation + slow-releasing fumigation method was suggested to kill the insects in large grain bulk, considering the factors of slow-releasing fumigation can maintain long time effect, and recirculation fumigation can distribute PH_3 well, and this method or operation was simple.

3.3 According to many years practice, the keys of using slow-releasing fumigation method in large grain bulk we considered were as follow:

3.3.1 Under film fumigation should be used if possible, in order to enhance grain bulk airtightness, ensure fumigation effect.

3.3.2 High resistance insects should be killed as early as possible. The insects we observed by visual inspection were almost all adults, so when we find the insect damage seriously, it had many eggs and larva etc, these insect stage was lower susceptibility (higher resistance) against PH_3 than adults, so it was more difficult to control.

3.3.3 Different application method should be chosen against different insects. When the density of insects was large, recirculation + slow-releasing fumigation or regular + slow – releasing should be used; when the bulk had resistance insect, slow – releasing + interval fumigation should be used. Effective fumigation time should be prolonged as long as possible with whatever method you chose to insure all the insects are killed.

3.3.4 The dead corner such as impurity area and four corner of the bulk etc should receive pre-bury tube to strengthen killing effect.

3.3.5 DDVP can be sprayed to control insects around the warehouse.

3.4 Safety measures must be adopted before enter the warehouse when using slow-releasing fumigation method to kill insect. Start axial-flow ventilation fan first and operate for several hours, then determine PH_3 and oxygen concentration in the space to be safe, then enter the warehouse.

3.5 The dead bodies of *Cryptolestes ferrugineus* (Stephens) gathered concentrated at the area where PH_3 application dust residue was disposed, while no dead *Sitophilus oryzae* Motschulsky were found. The reason should be further researched.

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Report on Tests of Aluminum Phosphide Fumigation through Different Application and Recirculation Methods

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Abstract: In order to control the resistance of stored grain pests to PH_3 in the high temperature and high humidity areas of our country, improve the pest killing effect of recirculation fumigation and reduce the cost of control, Wuzhou Grain Depot, State Grain Reserves has performed the tests of recirculation fumigation in the whole warehouse and recirculation fumigation under sheet through the application of aluminum phosphide on the surface of grain mass and the application of aluminum phosphide in the vent of ventilation duct for many times since 2000, these tests were performed in three horizontal warehouses separately of which lengths were 51.2m , the widths were 20.5m, the eaves height were 7.8m, and there were eight fumigation tests performed with different application dosages, different application ways, different air-tightness, different recirculation ways early or late.

This article reports summary and analysis according to the results of these tests; test results showed that when air-tightness of the warehouse was not changed, holding time of the PH_3 concentration and unit dosage showed a positive correlation, i. e. the less unit dosage, the shorter holding time of certain concentration. Fumigation efficacy was not good when PH_3 fumigation time was less than 28 days; the recirculation fumigation through intermittent application had certain advantage under the same air-tightness condition compared with recirculation fumigation through single application, and the control effect of the former was better obviously; the air-tightness half-life time of the No. 6 warehouse was increased from 60s to 98s and the unit dosage of the fumigation was reduced from 6g to 5g; for the recirculation fumigation through single application, the holding times of the concentrations above 300 mL/m^3 and 200mL/m^3 were prolonged obviously and reached 21 days and 40 days, respectively. No live pest was found two months after the fumigation, the interval between two fumigations was prolonged to more than 257 days and obtained better effect of control; performed the test of the recirculation fumigation through intermittent application which combined application on the surface of grain with the secondary application in door of ventilation duct on the floor in the No. 10 warehouse, holding times of the concentration above 300 mL/m^3 and 200mL/m^3 were 38 day and 48 days, respectively. The interval between two fumigations was prolonged to 317 days, the fumigation effect was better than that of the No. 6 warehouse; for the recirculation fumigation under film in the No. 18 warehouse, the air-tightness (-300Pa) half-life time was 135s, when we used intermittent application by combination with the secondary application in vent of ventilation duct, the holding time of the concentration could be longer and the fumigation effect would be better; therefore, this method has useful value in management of the resistance of stored grain pests to PH_3. This article also presented the requirement for the operation of recirculation fumigation through application in vent to ensure safety and effectiveness.

Key words: aluminum phosphide, stored grain pests, recirculation fumigation

1 Preface

Wuzhou Grain Depot, State Grain Reserves is located in the centre of high-temperature and high-humidity South China region. Because of the humidity weather of plum rain in March and April and strong rainfall formed by tropical storm from May to September every year, the above 28℃ annual daily mean temperature and humidity between 60% -95% can reach to 120 days. This weather and environment condition is most beneficial for the growth and reproduction of stored grain pests. The common stored grain pests are *Sitophilus zeamais Motschulsky*, *Rhizopertha dominica F*, *Gelechiid moth*, *Cryptolestes ferrugineus*, *Cryptolestes pusillus*, Psocoptera. [1] Grain department in Wuzhou area has performed pest killing with PH_3 fumigation since late 1960s. Since PH_3 has high pest killing toxicity and less residual, it is safe for human and animals. PH_3 exhaust gas degrades naturally, so it is safe for the environment. Therefore, aluminum phosphide is still the stored gain pesticide which is used extensively at present[2]. Since the relationship between "threshold concentration" and "fixed death time" , i. e. relationship of Ct value when performed pest killing by PH_3 fumigation and influences of some factors such

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as limitation of facility condition of warehouses, inappropriate application dosage and holding time of effective concentration have resulted in big resistance of stored grain pests. The phenomenon that pests are not killed happens frequently. Generally, two fumigations are needed in one year. Three fumigations are needed for individual warehouses. The results of investigations for resistance of stored grain pests in this region performed by national experts such as Yang Xiaoping show that resistance multiple of *R. dominica* F was 70.5 – 260.7[3] and the resistance multiple of *C. ferrugineus* was 110 [3]; the research performed by Bai Qingyun and Cao Yang showed that the resistance multiple reached 165[4]. These kinds of stored grain pests belong to the high resistance and very high resistance series. Liu Chunhua reported test results of PH_3 fumigation for *C. ferrugineus*; the normal application dosage was 6.0g/m^3; 27 days after application with the concentration above 315 mL/m^3, the pests were not killed completely. [5] It showed that the PH_3 resistance of *C. ferrugineus* was very serious, and the improvement of fumigation technology and development of comprehensive controlling for resistance of stored grain pests were necessary.

The newly-built horizontal warehouse of Wuzhou Grain Depot, built in 1998, was put into use in March 2000. Outside-storage fixed recirculation fumigation equipment have been installed for each warehouse. From May 2000, in order to improve the effect of PH_3 fumigation, we have performed tests of recirculation fumigation in the whole warehouse and recirculation fumigation under film through the application of aluminum phosphide on the surface of grain and the application of aluminum phosphide in ventilation ducts many times to reduce dosage, fumigation cost, relieve labor intensity of chemical prevention personnel and improve fumigation efficacy.

2 Materials and Methods

2.1 Materials and Equipments

2.1.1 Tested warehouse

No. 6, No. 10 and No. 18 horizontal bulk storage warehouses of Wuzhou Grain Depot were selectedas test warehouses. Their structure and specifications were the same, with length 51.2m, width 20.5m, eaves height 7.8m and volume 8 186.9m^3. Tested field air-tightness of warehouses by positive pressure method: half-life pressure reducing from 300Pa to 150Pa. No. 6 warehouse was 60s before 2004 and increased to 98s after sealing improvement. No. 10 warehouse was 110s. Tested No. 18 warehouse with grain surface sealed by film by negative pressure, half-life was 135s.

2.1.2 Base situations of the stored grain in the tested warehouse

See Table 1 for test serial number, type of fumigation test, No. of tested warehouse, base situations of the stored grain in the warehouse.

2.1.3 Fumigant

56% (1.0kg/bottle) aluminum phosphide tablets produced in Jining, Shandong.

2.1.4 Equipments

Fixed recirculation blower produced by Shenzhen Dashi Co., Ltd.; power: 350W; air volume: 600m^3/h; static air pressure: 900Pa; tip speed of blade: 37m/s.

NHL(HL – 210) PH_3 concentration tester produced in Beijing;

HL – 200 PH_3 alarm system produced in Beijing.

DS – 97 computer detection control system for stored grain produced in Zhuzhou, Henan.

2.1.5 Ventilation/aeration ducts on the floor

There were three sets of one-sided reducing ventilation ducts for each warehouse, two of three ducts for one aeration blower and one of four ducts for a second aeration blower. There were nine air distributors for each duct, which are half-round, full perforated and shingle shaped to overlap. Size of outer joint of: 640mm 560mm; size of inner joint of each duct: 500mm 510mm; volume of: for three ducts for one aeration blower, about 9.5m^3; four ducts for second blower, about 13.0m^3.

2.1.6 Status of pests in the tested grain piles

The pests in the tested warehouses were the common insects existing in field warehouses. The sampling points were set at four corners of each warehouse and its centre of longitudinal central axis(two points), totally 6 sampling points. Performed sampling at 300mm below the surface of grain when testing, used the depth where density was maximal as the representative value. See Table 2 for the kinds and densities of pests before fumigation test in each tested warehouse.

2.1.7 Other tested materials

0.14mm and 0.08mm polyvinyl chloride film, plastic slot (outer width: 14mm, notch: 6mm, inner diameter of slot: 10mm), plastic

dish, cotton flour bag, woven bag, pest screen.

2.2 Method

2.2.1 Sealing and air-tightness improvement of the warehouse

Installed plastic slots on all gates, entry doors, grain inspection doors, and windows, and sealed them with 0.08mm polyvinyl chloride film. In order to improve the air – tightness of the warehouse, we have performed continued improvement on all warehouses in the whole depot.

Improvement process of the air – tightness for the No. 6 warehouse: At beginning of 2004, changed the glass windows with steel wood frame into double – faced colorful steel sandwich air tight windows, after improvement, there were rubber seal rings added all around the windows and doors. At the beginning of 2006, after the improvement of the vent of ventilation duct, the fixing of cover board by twenty screws (Φ12mm) was changed into fixing of leaf rotating type by four screws (Φ12mm), and it made the application in vent more convenient and changed the cover board from straight board type (seal rings were asbestos bars) to impacted rotating door (seal rings were tube-type rubber bars); changed 90^0 butt joint of the corners of plastic slots in gate, grain inspection doors and windows into circular arc butt joint; performed sealing of joints between plastic slot and wall body and cracks of walls and roofs of the warehouse.

2.2.2 Recirculation under grain surface cover film sheeting

Used single – face sealing with 0.14mm polyvinyl chloride film in the No. 18 warehouse, and fixed and sealed with the plastic slots on the surface of grain, see Fig. 1 for the PVC piping diagram buried at 300mm under the surface of grain.

The diameter of the major solid transverse pipeline in the centre was Φ150mm and the diameters of eleven rows of perforated pipes were Φ120mm; the diameters of pipes at the sides of wall (left, right, upper and lower pipes-not showen in the figure) were Φ80mm.

2.2.3 Setting of the test points of PH_3 concentration

There were 6 PH_3 gas sampling test points in the whole warehouse: at four corners (spaced 1.5m away from wall) and centre of grain piles, totaling 5 points which were buried at 300mm – 500mm under the surface of grain; the 6^{th} sample tube was located midpoint above grain surface in the warehouse to sample the headspace gas concentration.

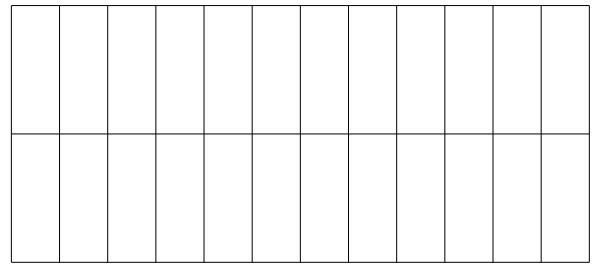

Fig. 1 Diagram of PVC piping pre – buried 300mm under the warehouse grain surface

2.2.4 Fumigation and application method for each tested warehouse

See Table 3 for application dosage and method of aluminum phosphide in each test.

2.2.5 Operation of recirculation blower during the test

See Table 4 for the opening status of recirculation blower in each test.

3 Result and Analysis

3.1 Control Effect of the Application Technology Improvement in the No. 6 Warehouse

See Table 5 and Table 6 for the test result of PH_3 concentration and control effect in each test. Since the air – tightness of the warehouse was not very good, the holding times of PH_3 concentration in the warehouse were different. In the same No. 6 warehouse, in the first normal fumigation test (serial number: A), the unit dosage reached $9g/m^3$, the holding time of the concentration above 300 mL/m^3 was 21 days; in the second and third tests (serial number: B and C), performed with recirculation, the methods for fumigant dosage application on grain surface and application in vent separately, the unit dosages were reduced to 6 and 3 (g/m^3) and the holding times of the concentration above 300 mL/m^3 were reduced to 15 and 5 days, the holding times of the concentration above 300 mL/m^3 were 28 and 20 days separately. The control effects were not very ideal after the three fumigations, where live pests were found the first week after degassing in the third test (serial number: C). This showed that when the air – tightness of the warehouse was not changed, the holding time of the PH_3 concentration and the unit dosage showed the positive correlation, i.e. the less unit dosage, the shorter holding time of certain concentration; the fumigation effect was not good when the PH_3 fumigation time was

less than 28 days (Table 6).

In order to improve the fumigation effect, we improved the application method from single application to intermittent application in the No. 6 warehouse, i. e. total dosage was divided into two applications. Serial number D, Table 3- for the same 6 g/m^3, although the holding time of the concentration above 300 mL/m^3 was 5 days less, the holding time of the concentration above 200 mL/m^3 was 14 days longer than that of serial number B. The efficacy was good and no live pests were found 2 months after degassing. The interval between two fumigations was prolonged to 236 days. Therefore, the fifth fumigation test, serial number E, also used intermittent application and its control effect was also good (Table 6). The recirculation fumigation with intermittent application has certain advantages and the control effect is obvious. We suggest that basic grain warehouses extend and apply this technology, including our excellent sealing technology.

In order to improve the control effect further, we performed air – tightness improvement in the No. 6 warehouse; the air pressure half – life time of the warehouse increased from 60s to 98s. Good fumigation effect was achieved after recirculation fumigation through single dosage application on grain surface. The holding times of the concentrations above 300 and 200mL/m^3 were 21 and 40 days separately (Table 5). No live pests were found 2 months after degassing and the interval between two fumigations was over 257 days (Table 6). The test results showed that improvement of air-tightness in the warehouse was the best way. We suggest that basic grain warehouses pay strict attention to detail and actively perform air-tightness improvement of horizontal warehouses, improving sealing and testing technologies of grain piles in the warehouses. Excellent sealing will provide twice the result with half the effort, with reduced dosages for major improvements of fumigation effect.

3.2.10 Control effect of the fumigation through intermittent application which combined application on the grain surface and a secondary application in vent.

In order to explore new application methods, we have performed recirculation fumigation through intermittent application combined with application on the grain surface and the secondary application in vent in the No. 10 warehouse. The 300Pa half-life of this warehouse was 110s which was better than that of the No. 6 warehouse. The test results showed that the holding times of the concentrations above 300 and 200mL/m^3 were 38 and 48 days separately (Table 5). No live pest was found within 2 months after degassing and the interval between two fumigations was prolonged to 317 days (Table 6). The fumigation efficacy was better than that of the No. 6 warehouse, test serial number G. The intermittent application combined with application on the grain surface and the secondary application in vent can improve the value of maximum pest killing concentration of PH_3 and prolong the time of the effective concentration further, realize outside-storage supplemental application, relieve labor intensity of operation personnel. The control effect is obvious, especially for the first fumigation of freshly stored grain. We, strongly suggest basic grain warehouses extend and apply this new technology.

3.3 Control Effect of Recirculation Fumigation under Film in the No. 18 Warehouse

In order to improve the air – tightness of grain piles further, we used film sealing technology on grain surface in the No. 18 warehouse. The test result of the air – tightness showed that the 300Pa half – life under the surface sealing PVC film was 135s. The test result showed that the holding times of the concentrations above 300 and 200mL/m^3 were 32 and 56 days separately (Table 5). No live pests were found within 2 months after degassing, the interval between two fumigations was prolonged to over 180 days, and secondary fumigation has not been required to the present. We see that using film sealing technology on grain surface can improve air-tightness of grain piles, fumigation efficacy is better. Higher efficacy has much value and important meaning in controlling resistance of stored grain pests to PH_3. We strongly suggest basic grain warehouses apply this new technology.

4 Discussion

In the high temperature and high humidity grain regions of China and other countries, when performing the first recirculation fumigation in the whole warehouse after fresh grain loading, we suggest using recirculation fumigation combined primary dosage application on the grain surface with the secondary dosage application in floor aeration ducts.

In the warehouse with less well sealed roof and upper sidewall conditions, we suggest that by using recirculation fumigation combined with film sealing technology on grain surface with the

secondary application in floor aeration ducts, the fumigation efficacy will be better.

When using application in floor aeration or ventilation ducts, start the recirculation blower immediately after dosage application. Do not stop the blower; test the PH_3 concentration in the warehouse every day, the recirculation blower should be not be stopped until the PH_3 concentration reaches the maximum concentration and begins to decrease. Carefully remove the residual phosphine dust from the aeration or ventilation floor ducts 24 hours after the recirculation blower has been stopped to ensure the operation is safe and effective.

Table 1. Base situation of warehouse and stored grain

Warehouse	Grain	Loadtime	Unload time	Quantity (t)	Pile Ht. (m)	Quality status
6	Latehsien Rice	2000.3	02.3	2430	3.6	Husked rice yield: 75.3%; Water Content: 12.8%; impurity: 0.8%
6	Late hsienRice	2002.5	05.2	3787	6.0	Husked rice yield: 75.7%; Water Content: 13.3%; impurity: 0.9%
6	Canadian Wheat	2006.1	In storage	4839	5.3	Volume: 808; water content: 12.8%; Impurity: 0.3%
10	Yellowcorn	2006.5	In storage	5011	6.0	Volume: 735; water content: 13.6%; Impurity: 0.8%
18	Yellowcorn	2007.4	In storage	4660	6.0	Volume: 712; water content: 13.8%; Impurity: 0.8%

Table 2. Kinds and densities of pests in each warehouse before fumigation test

Test No.	Warehouse	Kinds and densities of the pests in tested warehouse (kg)
A	6	Rhizopertha dominica F.: 3, Sitophilus zeamais Motschulsky: 6, Cryptolestesferrugineus: 23, Psocoptera: 63
B		Rhizopertha dominica F: 5, Sitophilus zeamais Motschulsky: 5, Cryptolestesferrugineus: 15, Psocoptera: 46
C		Rhizopertha dominica F.: 1, Sitophilus zeamais Motschulsky: 3, Cryptolestesferrugineus: 17, Psocoptera: 65
D		Rhizopertha dominica F: 1, Sitophilus zeamais Motschulsky: 1, Cryptolestesferrugineus: 28, Psocoptera: 53
E		Gelechiid moth: 2 Sitophilus zeamais Motschulsky: 2Cryptolestesferrugineus: 22, Psocoptera: 41
F		Sitophilus zeamais Motschulsky: 2 Indian grain moth: 2Cryptolestesferrugineus: 15, Psocoptera: 27
G	10	Gelechiid moth: 1 Sitophilus zeamais Motschulsky: 2Cryptolestesferrugineus: 12, Psocoptera: 62
H	18	Sitophilus zeamais Motschulsky: 2 Indian grain moth: 2Cryptolestesferrugineus: 18, Psocoptera: 49

Table 3. Application dosage and application way of aluminum phosphide in each test

Serial No.	Ware house	Applic. time (y/m/d)	Dosage (g/m^3)	Application method
A	6	2000.4.28	9.0	Application on grain surface: grain surface cloth application dish, 0.5kg/dish. Applied 1.0kg/vent, Applied 1.0kg inside film of earch warehouse door
B	6	2002.6.5	6.0	Application on grain surface: grain surface cloth application dish, 0.5kg/dish

Serial No.	Ware house	Applic. time (y/m/d)	Dosage (g/m^3)	Application method
C	6	2003.6.10	3.0	Application in vent, 8.0kg/vent, total 24kg. loaded dosage in cotton flour bags, 4.0 kg/bag, after application into vent, spread out flat with club, opened bag, closed vent door.
D	6	2003.10.10 2003.11.18	3.0 3.0	Applied dosage into vent twice: each dosage was same, .8.0kg/vent, total 24kg. loaded dosage in old woven bags 4.0kg/bag, after application into vent, spread out flat with club, opened bag and closed vent door.
E	6	2006.6.25 2006.7.19	3.0 3.0	Same as D。
F	10	2006.7.11 2006.8.6	4.0 3.0	Application on grain surface which was same as B. Secondary application and supplemental application into the vent was same as D. tested air – tightness, half – life = 110s
G	6	2007.2.28	5.0	Application on grain surface which was same as B. tested air – tightness; half – life = 98s
H	18	2007.7.24 2007.8.13	3.0 3.0	Applied dosage into vent at twice – same as D. tested air – tightness; half – life = 135s. performed recirculation fumigation under film.

Note: performed secondary application when PH_3 concentration reduced to $200mL/m^3$

Table 4. Operation status of recirculation blower during fumigation in warehouse

SerialNo.	Warehouse	Airtight time of fumigation(d)	Operation status of recirculation blower during test	Time of start-up(h)
A	6	22	Recirculation blower was not started – performed normal fumigation	0
B	6	37	Started recirculation blower 72 hours after application for 3 hours/day.	36
C	6	32	Started blower immediately after dosage finished – 24 hours continuously, stopped blower when PH_3 concentration started dropping	290
D	6	67	Started up after secondary application which was same as C	1st :241 2nd :332
E	6	56	Same as D	1st:243 2nd:403
F	10	55	The first application was same as B; the secondary application was same as C	1st:33 2nd:368
G	6	49	Same as B	42
H	18	74	Same as D	1st:432 2nd:384

Table 5. Test result of the PH_3 concentration in the tested warehouse

Serial No.	Warehouse	Dosage (g/m^3)	Max. gas level (mL/m^3)	Holding time of concentration(d)		oncentration when degassing (mL/m^3)	Test method
				Above 300 mL/m^3	Above200 mL/m^3		
A	6	9.0	>1000	21		421	Began at the second day after the application, tested once every day
B	6	6.0	>1000	15	28	100	
C	6	3.0	378	5	20	100	
D	6	6.0	365	10	42	100	
E	6	6.0	540	17	50	100	
F	10	7.0	835	38	48	100	
G	6	5.0	>1000	21	40	100	
H	18	6.0	558	32	56	100	

Table 6. Control effect of each test in the tested warehouse

SerialNo.	Warehouse	Efficacy	Inspection within 2 months after degassing (once every week)	Re – fumigation time after test	Interval between two fumigations
A	6	Mortality 100%	Live pests found at test points from 6th week: R. dominica F: 1; C. ferrugineus: 2; Psocoptera: 5	2000.8.24	96d
B			Live pests found at test points from 8th week: C. ferrugineus: 3; Psocoptera: 5	2002.9.29	79d
C			Live pests found at test points from 1st week: C. ferrugineus: 4; Psocoptera: 3	2003.10.10	90d
D			No live pests in 2 months	2004.8.19	236d
E			No live pests in 2 months	2007.2.28	192d
F	10	Mortality 100%	No live pests in 2 months	2007.7.18	317d
G	6	Mortality 100%	No live pest was found within 2 months	No secondary fumigation	Over 257d
H	18	Mortality 100%	No live pest was found within 2 months	No secondary fumigation	Over 180 d

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Effectiveness of Hermetic Storage in Insect Control and Quality Preservation of Cocoa Beans in Ghana

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Abstract: This paper reports the development of hermetic storage in Ghana by highlighting the scientific research conducted using GrainPro Cocoons™ in controlling insects and preserving the quality of cocoa beans as an alternative to chemical treatment. Three stacks for hermetic storage and one stack each for conventional storage (without fumigation) and standard storage (with fumigation) were built for sampling and observation. The Cocoon™ stacks were 3.40m (long) ×2.95m (wide) ×2.0m high and contained 220 bags (64 kg per bag) of sun-dried (average of 7.0% moisture content) cocoa beans. Oxygen concentration and temperature inside the stack of cocoa beans were monitored daily. Sampling was done at 0, 3, 6 and 9 week intervals to observe changes in insect density and product quality characterisation. There was a steep decline in oxygen concentration in all Cocoons™ during the storage with the lowest concentration of 0.0% being recorded in all the Cocoon™ from the fifteenth day of storage, maintained throughout the storage period. The temperature was almost uniform inside the Cocoon™ throughout the storage period. After three weeks of storage, the first Cocoon™ was found to be infested with live adults of *Cryptolestes ferrugineus*, *Cryptolestes pusillus*, *Araecerus fasciculatus*, *Lasioderma serricorne*, and *Ephestia cautella* larvae and some fruit flies. However, these live insects were few compared with the high insect population which was dead inside the Cocoons™. Most of the *Lasioderma serricorne* and *Tribolium castaneum* introduced at the beginning of the experiment were dead at the third week; 100% mortality was recorded for all insects at the sixth week of storage. All the cocoa beans inside the Cocoon™ maintained their quality level throughout the storage period; the grade remained the same after nine weeks of storage as it was at the beginning of the experiment.

Key words: GrainPro Cocoon™, hermetic storage, conventional storage, standard storage, cocoa beans, insect control, *Lasioderma serricorne*, *Tribolium castaneum*

Introduction

The production of organic Cocoa beans has been carried out on a small scale in Ghana since its inception some years ago due to certain problems associated with this production[1]. However great efforts are being made to export dry Cocoa beans to sustain and increase the production levels. Because of the desire to produce insecticide free Cocoa beans, much consideration is being given to storing Cocoa beans without or with minimal use of insecticides. Hence the need to investigate the possibility of adopting hermetic storage practices. Hermetic storage is based on the principle of modified atmosphere and no chemical.

Stored product protection is attained by the use of hermetic sealed containers, one of which is the Cocoon™, which provides an airtight environment[2]. The basic principle of hermetic grain protection lies in the fact that from the moment of sealing until the insects consume the volume of oxygen in the Cocoon™, the damage to the grain is negligible[2]. If the insect population is low, the insects may survive causing minimal damage to the produce since it has been established that to obtain a complete kill, the oxygen tension should drop to two percent (2%) or below[3].

Plastic structures suitable for long-term storage, as well as intermediate grain storage for cooperatives and subsistence farmers, for stored products in bags or in bulk, have been developed in Israel[4]. The influence of insulation materials in reducing the intensity of moisture migration under subtropical (Israel) and tropical (Philippines) climates has been investigated[5]. If the storage ecosystem can be sealed to prevent air from entering or leaving it, the respiratory metabolism of insects, moulds and the

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produce itself will lower the oxygen content and raise the carbon dioxide content of the intergranular atmosphere to a level where aerobic respiration is no longer possible[6]. This is the principle behind hermetic storage. However, although it sounds simple in theory, in practice, it is much more complex.

At present, the conventional and standard storage technologies are employed to preserve cocoa beans. This involves stacking bagged beans on wooden pallets inside the warehouse. The uses of deadly pesticides to combat pests of stored products have increasingly become unpopular because of health concerns and the havoc its indiscriminate use brings to the environment. In the past, traditional storage structures provided some protection against storage losses, particularly by insects and rodents, although annual losses that are estimated to be between 5 to 10 percent were previously considered as unavoidable. Attempts to reduce these losses through the introduction of modern storage technologies have frequently failed either because they were socio-economically unacceptable, or inappropriate to local climatic conditions and agro-technical practices[7]. The approach described in this paper involves the modification of existing structures or the construction of new structures in the conventional style but employing modified technologies to improve grain, seed or bean products conservation without being too disruptive to rural life, a concept termed by Guggenheim (1978) as "invisible" technology[8].

Materials and Methods

Dry cocoa beans that were harvested during the 2007/2008 main crop season used in the trials were mainly of grade I and II category. Cocoa beans were sun dried to an average moisture content of 7.0% after fermentation and then bagged in 64kg capacity jute sacks. Outdoor storage trials of 3, 6 and 9 weeks durations were carried-out in the compound of the Research Department, Quality Control Division (COCOBOD), Tema, Ghana. The method of 'airtight' or 'hermetic storage'[9] of grain quality preservation was followed.

Laboratory Rearing of the Target Insect Pests

The target pests were adult *Cadra cautella* (*Ephestia cautella*), *Araecerus fasciculatus*, *Lasioderma serricorne* and *Tribolium castaneum*. A stock of each species was raised in the laboratory of the Research Department of QCD at an ambient temperature range of 25 – 30℃ and relative humidity of 60% – 75%. *C. cautella* was raised on a mixture of ground maize, wheat bran and glycerol in a ratio of 8∶8∶1 (W/W)[10], *T. castaneum* on crushed dry cocoa beans, *L. serricorne* on a mixture of tobacco and dry cocoa beans and *A. fasciculatus* on cassava chips with moderate to high moisture content[11].

Storage Technologies Observed and Experimental Set-up

The storage technologies studied were hermetic storage (HS), conventional storage (CS), and standard storage (SS). The stacks built for HS to demonstrate the effect of these technologies had a uniform dimension of 3.40m × 2.95m × 2.0m length, width and height (LWH) while stacks built for CS and SS to serve as the reference stacks measured 4.0m × 1.0m × 1.50m LWH.

Hermetic Storage Set up

Three GrainPro Cocoon™ made of flexible Polyvinyl Chloride (PVC) plastic liner with a capacity of 220 bags, were set-up outside the building. Wooden pallets were laid-out on the ground and covered with pieces of plywood and cardboard which served as foundation materials to protect the bags during the set-up. Bagged cocoa beans were directly stacked or piled on the lower section of the Cocoon™ and when the desired height was reached, the stack was covered with the upper section of the Cocoon™. During stacking, 15 bags of cocoa beans were randomly selected and sieved to estimate insect pests present in the cocoa beans.

Ten laboratory reared adult *L. serricorne* and *T. castaneum* each were inserted into fifteen miniature jute sacks each containing 2kg dry cocoa beans, which were tied and the whole set up inserted into cotton cloth sacks and distributed randomly in the stack. Thereafter, the lower and upper sections were pulled together closely and zipped with gas-tight multiple tongue and groove zippers. In order to prevent condensation, a Grainshade was stretched over the Cocoon™ at a level of at least 20cm above the top cover. The three Cocoon™ were randomly assigned for destructive sampling at 3, 6, and 9 weeks, respectively.

Conventional Storage (CS) and Standard Storage (SS) Set Up

Two stacks were constructed for CS and SS with an individual capacity of 45 bags, also built outdoors, on double wooden pallets. Ten

laboratory reared adult *L. serricorne* and *T. castaneum each were inserted into fifteen miniature* jute sacks each containing 2kg dry cocoa beans which were tied closed and the whole set up inserted into cotton cloth sacks and distributed randomly in the stack. The stacks were individually covered with Herculite gas proof sheets. The CS had no chemical treatment at all but the SS was fumigated using Phosphine (PH_3) gas at an application rate of one tablet per 0.19 tonne cocoa beans.

Instrumentation

All stacks built for the storage trials were daily monitored for temperature and oxygen (O_2) concentration. The ambient temperature and relative humidity were recorded on a daily interval during the storage trials. A gas inlet valve was attached to the Cocoon™ to monitor oxygen (O_2) concentration inside the structure using a GrainPro oxygen analyser.

Sampling Procedure

Each stack was divided into three blocks and five marked bags per block were collected, sieved and distributed randomly within the stack. Thus, a total of 15 marked bags were sampled per stack. For CS and SS, wherein only one stack was built for observation, samples of two kg each were drawn from the same marked bags at time 0, 3, 6, and 9 weeks of storage. For HS, the marked bags in all three sealed stacks were sampled at time 0 after which only the assigned Cocoon™ for destructive sampling at 3, 6, and 9 weeks, respectively was opened.

Analyses of Samples

Samples gathered were analysed at the Laboratory of the Research Department, Quality Control Division (COCOBOD), Tema, Ghana, for insect mortality and changes in quality of the cocoa beans. Moisture content of gathered samples was immediately measured with an Aqua Boy moisture meter, which was earlier calibrated by oven drying.

Results and Discussion

Bean Quality Characteristics

All the dry cocoa beans stored in the cocoon retained their quality (Table 1). There were some reduction in the total purple color of some of the cocoa beans but differences were not significant enough to cause any change in the grade.

Table 1. Mean quality categorisation of dry cocoa beans after nine weeks storage in the cocoon

Cocoon	Period (weeks)	Sub – Plot	Quality characteristics					GRADE
			TM	TS	TP	AOD	PURITY	
		1	0.2 ± 0.1	5.2 ± 0.4	28.2 ± 1.2	2.4 ± 0.2	64.0 ± 0.9	II
A	3	2	0.6 ± 0.1	2.0 ± 0.2	20.4 ± 0.9	2.2 ± 0.2	74.8 ± 1.0	I
		3	0.0 ± 0.0	3.4 ± 0.3	16.0 ± 0.3	4.0 ± 0.3	76.6 ± 0.7	II
		1	0.0 ± 0.0	5.0 ± 0.4	26.0 ± 1.3	1.6 ± 0.2	67.4 ± 0.9	II
B	6	2	0.2 ± 0.1	2.2 ± 0.3	40.2 ± 0.6	2.8 ± 0.4	54.4 ± 0.4	II *
		3	0.0 ± 0.0	3.2 ± 0.3	38.6 ± 0.7	0.6 ± 0.1	57.6 ± 0.8	II *
		1	0.6 ± 0.2	1.8 ± 0.1	14.2 ± 0.2	2.4 ± 1.0	81.0 ± 1.0	I
C	9	2	0.2 ± 0.1	3.2 ± 0.1	12.2 ± 0.4	3.4 ± 0.9	81.0 ± 0.9	I
		3	0.2 ± 0.1	2.6 ± 0.2	19.0 ± 0.3	2.8 ± 1.6	75.4 ± 1.7	I

± SE; n = 5; TM – total mouldy beans; TS – total slaty beans; TP – total purple beans; AOD – any other defects

Oxygen Concentration

The control (conventional and standard) storage had 21.0% oxygen in the atmosphere. The hermetic storage, likewise, possessed the same concentration of oxygen during the start of the storage period regardless of the plastic enclosure.

There was a steep decline in oxygen concentration in all Cocoon™ during the storage with the lowest concentration of 0.0% being recorded in all the Cocoon™ (Fig. 1). The first 0.0% oxygen concentration was recorded in Cocoon™ "A" on the fifteenth day of storage followed by Cocoon™ "C" on the seventeenth day and Cocoon™ "B" on the eighteenth day. All the Cocoons maintained the 0.0% oxygen concentration till the end of the storage.

In both the conventional and standard experiments the oxygen concentration remained virtually the same. The steep decline in oxygen concentration in all Cocoon™ during the storage is an indication that the organisms including in

sects present in the Cocoon™ exhibited significantly high rates of respiration which is a major

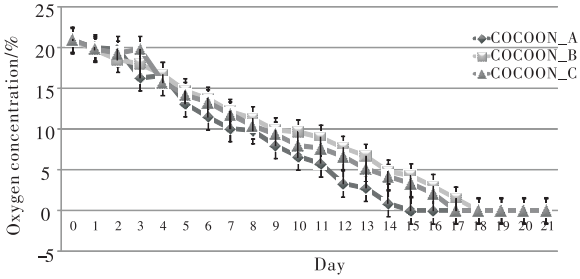

Fig. 1 Daily oxygen concentrations inside the Cocoon™ over the hermetic storage period

factor in the consumption of oxygen. The Cocoon™ prevented gas exchange between the plastic enclosure and the external environment and therefore a depleted oxygen atmosphere was created. Unlike the conventional and standard experiments, the Herculite gas proof sheets used allowed oxygen exchange between the plastic enclosure and the external environment thereby replenishing the oxygen used in the enclosure.

Moisture Content

Regardless of storage methods, the moisture content increased after three weeks of storage from a mean of 7.0% at the setting – up stage to a mean of 7.2%. However there was no change in moisture content with the prolonged storage of nine weeks.

Temperature

The temperature inside the Cocoon™ was not constant but it did not fluctuate significantly (Fig. 2). A mean temperature 28.3℃, 29.1℃ and 29.7℃ were recorded in Cocoon™ "A", "B" and "C" respectively. Comparably, temperatures in the Cocoon™ were lower than that recorded in the conventional and standard experiments.

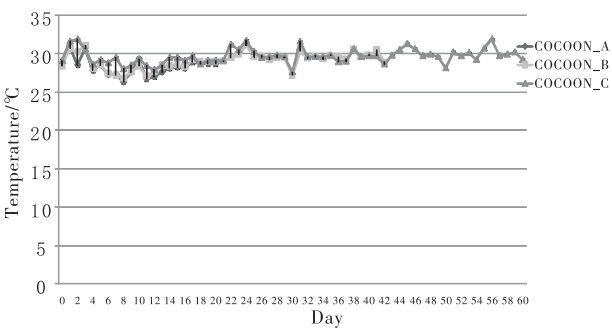

Fig. 2 Daily temperatures inside the Cocoon™ during the hermetic storage period

The temperature inside the conventional and standard stacks fluctuated significantly (Fig. 3) with recorded means of 30.5℃ and 30.6°C respectively. The significant difference in temperature between the two storage methods was likely due to the Grainshade that was stretched over the Cocoon™ at a level of at least 20cm above the top cover. The Grainshade cut off the sun from radiating on the Cocoon™ directly whereas the conventional and standard stacks were exposed to the sun during the day and the sky at night.

Insect Density

Just before building the stacks, the bags of dry cocoa beans that were sieved, then were infested with *Cryptolestes*, *Tribolium castaneum*, *Cadra cautella* (*Ephestia cautella*), *Oryzaephilus mercator Cryptolestes ferrugineus*, *Cryptolestes pusillus*, *Araecerus fasciculatus*, *Carpophilus dimidiatus and Carpopphilus hemipterus*. Predominant among them were *T. castaneum*, *C. ferrugineus*, *C. pusillus* and *O. surinamensis*.

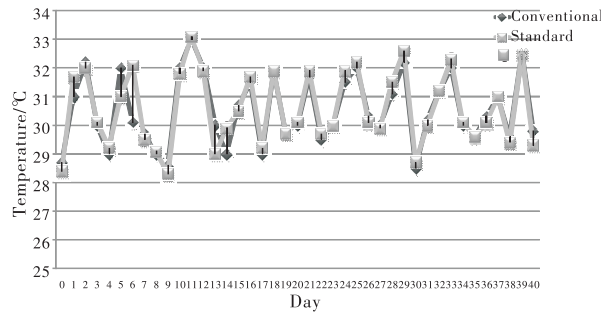

Fig. 3 Daily temperatures inside the control stacks over the storage period

Insect populations significantly increased with time of storage in the conventional storage and in addition to adults, larvae and pupae of both *T. castaneum* and *L. serricorne were found* (Table 2). Insect mortality of 100% was recorded in the standard storage which was fumigated with phosphine. In hermetic storage, not all the *T. castaneum* and *L. serricorne* that were introduced died (Table 3). In addition, a few live insects (adults of *C. ferrugineus*, *C. pusillus*, *A. fasciculatus*, *L. serricorne*, *and C. cautella* larvae and some fruit flies were found at the end of week three moving inside the Cocoon™. The insects were found to be weak and much stressed. It was also observed that high populations of the insects moved outside the bags of cocoa beans and were found dead inside the Cocoon™. However 100% mortality of *T. castaneum* and *L. serricorne* introduced into the Cocoon™ was recorded at the end of both weeks six and nine (Table 3). High populations of insects were found outside the bags of cocoa beans and dead inside the Cocoon™. Thus the Cocoon™ environment had effective control on the insect population in conformity to the findings of other researchers[12].

Table 2. Mean population live stages of *T. castaneum* and *L. serricorne* introduced into the conventional storage stack

Cocoon	Period (weeks)	Sub – Plot	Lasioderma			Tribolium		
			Larvae	Pupae	Adult	Larvae	Pupae	Adult
A	3	1	4.2 ± 0.3	0.0 ± 0.0	12.2 ± 0.3	6.2 ± 0.2	0.4 ± 0.2	15.8 ± 0.3
		2	2.0 ± 0.1	6.2 ± 0.1	12.0 ± 0.0	6.0 ± 0.4	6.2 ± 0.1	20.2 ± 0.1
		3	4.8 ± 0.4	0.0 ± 0.0	14.4 ± 0.1	4.4 ± 0.2	2.0 ± 0.0	18.6 ± 0.1
B	6	1	5.0 ± 0.6	5.0 ± 0.2	22.0 ± 0.2	8.8 ± 0.3	6.4 ± 0.1	32.2 ± 0.3
		2	10.0 ± 0.4	4.0 ± 0.2	26.0 ± 0.3	10.6 ± 0.3	6.0 ± 0.2	30.4 ± 0.2
		3	10.0 ± 0.3	2.0 ± 0.3	28.4 ± 0.4	10.0 ± 0.2	8.4 ± 0.1	36.2 ± 0.2
C	9	1	8.2 ± 0.1	8.4 ± 0.4	38.4 ± 0.2	12.2 ± 0.2	12.4 ± 0.2	58.2 ± 0.1
		2	10.2 ± 0.2	6.4 ± 0.1	38.0 ± 0.2	14.2 ± 0.1	10.4 ± 0.3	48.0 ± 0.0
		3	10.0 ± 0.4	5.0 ± 0.2	36.8 ± 0.1	14.0 ± 0.0	18.0 ± 0.0	56.4 ± 0.1

± SE; n = 5

The high oxygen concentration (21.0%), high temperature and high relative humidity promoted the growth of insects in the conventional storage. This was not the case in the hermetic storage. The much lower (0.0%) oxygen concentration in the three Cocoons made conditions unfavorable for insect growth, confirming the work of Bailey (1965).

Table 3. Mean mortality after nine weeks of storage

Cocoon	Period (weeks)	Sub – Plot	Lasioderma	Tribolium
A	3	1	90 ± 0.2	82 ± 0.2
		2	90 ± 0.1	84 ± 0.4
		3	88 ± 0.2	82 ± 0.2
B	6	1	100 ± 0.0	100 ± 0.0
		2	100 ± 0.0	100 ± 0.0
		3	100 ± 0.0	100 ± 0.0
C	9	1	100 ± 0.0	100 ± 0.0
		2	100 ± 0.0	100 ± 0.0
		3	100 ± 0.0	100 ± 0.0

SE; n = 5

Conclusion and Recommendation

Highly significant increases in insect population occurred in the conventional storage, especially after the week resulting in significant grain damage quality deterioration. Cocoon™ storage resulted in 100% insect mortality at the end of the nine week storage period with no change in product quality.

Based on the results, use of flexible hermetic structures made of gastight plastic liners can be a safe and viable alternative to permanent structures for organic protection of cocoa beans for extended periods and seems to be the most promising method for storing cocoa beans. Besides controlling insect population it is more economical and convenient. Its set-up is the least expensive compared to traditional methods (conventional and standard storages). Flexibility, transportability, ease of erection, simplicity of operation and maintenance and durability are distinct advantages. Their availability in various sizes, capacities and forms can suit a wide range of requirements to fit several levels of storage operations.

The usage of hermetic storage for cocoa beans is highly practical and technically feasible. Hermetic storage would adequately reduce problems on losses in storage and at the same time ensure cocoa bean quality preservation. The Cocoon™ can also be used as a fumigation chamber but will not require the use of sand snakes, gas proof sheets and residual insecticide.

It is recommended, therefore, that hermetic storage technology be promoted to solve the problem of organic storage of cocoa beans.

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An Antibacterial Test by Using High Concentration of Phosphine in South China Region on Corn Storage

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Abstract: Some practical methods in stored grain including grain cooling, mechanical ventilation, and local ozone fumigation conventional technologies such as anti – mildew have achieved safe storage of corn in Guangdong Xinshagang depot, but some economic and efficient mode of operation of the corn custody have not yet been formed. Because high concentrations of phosphine can inhibit the growth of mould, a pilot usage initially explored the effective antibacterial concentration of phosphine and feasible methods of operation. It summarized the economics of the current corn storage conditions in Squat Wharfs in South China region.

Key words: south China region, PH_3, antibacterial, storing, corn

Introduction

China Grain Reserves Guangdong Xinshagang Depot (Xinshagrain) locates to the east of the Longhai, near the entrance of the Pearl River to the sea, which is a typical subtropical monsoon climate. There is a small temperature difference between day and night, with an average temperature at 22℃, an average relative humidity 79% and an average 1 800 mm of precipitation. According to the statistics of Xinshagrain station in 2007, the highest temperature was 36℃, lowest was 5℃, the average temperature above 25℃ was about 169 days, about 11 days below 10℃, the relative humidity waved from 42% to 100%. About 205 days' relative humidity were over 80%, of which 79 days were more than 90 %, and only 50 days below 65%.

Heating is caused by micro – organisms including the majority of the bacteria, fungi, yeast and plant pathogenic bacteria that are the dominant ones in grain. The greatest damage to stored grain are microbial mycophenolate in almost all microorganisms and temperature microorganisms, such as Penicillium and most Aspergillus. Moisture transfer or uneven grain moisture caused the rapid growth of microorganisms, which is the main reason for heating of grain. In recent years, there are two main kinds of corn storage heating in Xinshagrain; one is the local grain-like heating and another is the level of heat at certain depths. Heating is irregular. Heating at depth usually occurs in spring and summer, more than in the grain heating regions at 0.5 – 1.5 m (with the grain size and warehouse temperature, the original grain moisture levels, the depth of different interface heating), is the main reason for the spring and summer. Temperature rises rapidly, resulting in a grain stack "*cold core with surface heating*" phenomenon, when water temperature gradients cause increased grain surface water leading to increased microbial activity, thus caused local heating. In addition, different stages of the new and old grain warehousing level interface are one of the main forms of fever.

According to the trial in southern China's natural hot and humid climate, through analysis of the stored grain storage indicators in squat warehouses and storage characteristics of maize, a high concentration of phosphine inhibits its microbial activities achieves safe storage of corn.

1 Materials and Methods

1.1 Test Warehouses and Control Positions

Test (Q43 storage) and control (Q45 and Q53 storages) are squat warehouse stores, diameter of 25 meters, 15.6 meters high with grain, with a total height 23.4 meters, design storage capacity 6,500 tons (in wheat). Experimental grain storage warehouses and control are 2006 Northeast-drying corn, moisture was 13.7%, 28.4 mg KOH/100g fatty acid value, bulk density of 720 g/L, 9.6℃ warehousing grain temperature, specific indicators and the corresponding methods followed as table1.

China Grain Reserves Guangdong Xinshagang Depot, Mayong town, Dongguan, Guangdong province, China 523147

Table 1. basic Comparison of Q43, Q45, Q53

No.	volume	moisture	density	Fat acid	Storage methods
Q43	5630t	13.7%	720g/L	28.4	fumigation
Q45	5584t	13.5%	723g/L	30.5	Fumigation, cooling
Q53	6504t	13.7%	720g/L	28.4	composite

1.2 Test Conditions and the Initial Preparations

1.2.1 Grain formation and keep a good janitorial positions. Corn warehousing in test positions had been ended in March 4 and grain formation had been end in March 10.

1.2.2 Additional grain surface temperature cables had been set. Under the grain surface temperature measuring 20 cm depth, an additional cable was set. Historical statistics show that local centres of grain with gathered impurities is the more regional site of heating, an additional temperature cable in the grain surface is helpful to strengthen the region's grain temperature tracking.

1.2.3 Development of antibacterial fumigation programme. The average grain warehousing grain temperature is 9.9℃, and basically no insects, pests and mold in accordance with the regularity, fumigation was planned in May.

1.2.4 Fumigant prepared. The warehouse fumigation plans to use active ingredients for 56% of the total 108 kilograms of aluminum phosphide tablets, fumigate three times, the first dose of 54 kg and 27 kg for the following times.

1.2.5 Ready for some fumigation bags. In order to facilitate addition of fumigant, some fumigation bags should be prepared in accordance with quantity and recirculatory fumigation.

1.2.6 Do a good job in the concentration of phosphine, complete concentration table (see Table 2 Q43, Q45, Q53 concentration of fumigation circulation records).

Table 2. Q43, Q45, Q53 concentration of PH_3 in recirculatory fumigation DateQ43

Date	Q43wharf	Q45wharf	Date	Q53wharf
May,22	drug dosage	drug dosage	May,17	drug dosage
May,23	333	359	May,21	613
May,29	923	840	May,24	660
June,5	928	730	May,28	492
June,11	763	462	June,4	267
June,19	603	383	June,11	155
June,26	452	267	June,18	79
July,3	392	180	June,25	24
July,10	788	122	July,2	gas scattered
July,16	710	12/07		
July,24	613	gas scattered		
August,1	467			
August,7	363			
August,14	264			
August,20	190	31/08		
August,28	111	drug dosage		
September,4	491	>1000		
September,11	653	708		
September,18	550	505		
September,25	478	367	October 12	drug dosage
October,2	399	276	October 15	558
October,9	308	193	October 29	600
October,16	225	12/10	November 5	407
October,23	156	scattered gas	Nov. 12	253
October,30	scattered gas		November 15	scattered gas

1.3 Test Methods

1.3.1 Dosing conditions and determination dosage time.

Dose when there are signs of moldy grain or the general development of grain pest and insects. On April 16, 2 insects/kg *Liposcelidate* were found near the west gate of the warehouse and 1 insects/kg *Tribolium castaneum* in the east gate. On May 14, mould had been sighted in the surface of grain with a slight odd smell; pest inspection found 4 insects /m^2 *Sitotroga cerealella*, larvae appeared on the warehouse wall. On May 22, a high concentration of warehouse fumigation operations had been implemented with a dose of 6g/m^3; the total dose was 54 kg per fumigation, administered mainly in the ventilation channels of the warehouse, airtight doors, and on the grain surface. The application methof for the dose was natural dynamic deliquescence.

1.3.2 Conditions, methods and the time set to increase the amount of drugs

When the concentration of PH_3 in the warehouse was below 350 ppm, fumigant should be added from the entrance of axial flow fan which was on the roof of warehouse, fumigant should be divided as parts by plastic bags, grain surface should be also uniformed. According to phosphine concentration reference data, about 27 kg fumigant packed in 18 bags had been put into grain surface on July 3 for the first, while another 27 kg fumigant had been added in the same way on August 31.

1.3.3 Concentration detection and tracking

For the first 12 hours after fumigant circulation, circulating about 24 hours, checking the concentration of stores in 36 hours, when the concentration of PH_3 were twice higher than 500 ppm while the gap was less than 100 ppm, circulation would be stopped. (It could be inferred that the concentration of phosphine in warehouse was nearly in balance, deliquescence of ALP began to slow down). After a week's circulation, each about 12 hours, the concentration had been checked in the following morning before shutdown as specific data in table 2. Data in the table shows that on May 22 for fumigant tests, on May 24 has risen to concentrations above 320 ppm, the scheduled effective inhibitory concentration is close to 350 ppm, to July 3, the concentration was attenuated to 380 ppm initially, according to weekly concentration decay rate, some more fumigant had been added on July 3. There was a maximum concentration of 780 ppm on July 10, after that, then began to gradually decline. On August 7, the concentration attenuated to 360 ppm. In order to find positions at the actual situation, no more drugs had been added then, and the attenuation concentration declined to 190 ppm on August 20. People with air respirator entered the warehouse and inspected grain. It turned out that grain was basically stable. On August 31, fumigant was added twice, the highest concentration of 650 ppm was on September 11, it declined to 220 ppm on October 16, gas had been scattered on October 30 until grain inspection (specific data Table 3).

1.3.4 Grain change tracking.

A combining method of temperature changes detected mainly through the grain system and inspection personnel in the silos had been used during the grain fumigation tracking. In this test, a tentative 38.0℃ is the extreme temperature, which reached the unconditional opening of temperature checks and handling grain. On August 22, temperature data shows that the pilot wharf cable 5#S7 – temperature warehouse slightly higher than that of warehousing, reach to 34.8℃. Since no gas had been scattered, for the sake of safety of stored grain, grain inspection done by person under the premise of security, there was a slight heating in the site about 3 – 5 m^2 grain surface with the remaining part of normal sensory quality. On August 31, 27 kg fumigant had been added for the second time. Temperature was controlled in the heating site and began to decline slowly on September 10.

The experiment began in May, ended in the early November, all – grain warehouses test measures had been carried out smoothly as planned. During the test, the whole situation was basically stable except there was a light heating when the concentration attenuated to 200 ppm in warehouse. As for the contrasts, there were many sites to be fever and the cooling machines had been used to reduce grain temperature. Test warehouse tracking and control positions have been shown in Table 3.

1.4 Results and Analysis

1.4.1 Test results

Data show that phosphine concentration attenuation to about 350 ppm on August 7; on August 22, cable 5#S7 point indicated abnormal warming; there are signs of microbial activity about 15 days after the concentration of phosphine attenuated below the effective inhibitory

concentration. If no additional aluminum phos-

Table 3. Q43, Q45, Q53 custody measures and cost comparison

Q43 Wharf	Q45 Wharf	Q53 Wharf
February 10 to March 2 grain warehousing		
On April 16, some liposcelidate and Tribolium castaneum	On April 23, an anarsia/m^2.	On May 17, the use of 42 kg drug for fumigation,
On May 14, sitotroga cerealella 4/m^2	On May 19, 2 # S7 point 35.1℃, handling grain artificially	On July 2, fumigation scattered gas.
May 22, fumigation, the use drugs of 54 kg	On May 22, fumigation, 45 kg drugs has been used	On July 5, grain fever, the first cold – energy consumption 2 580 kWh
On July 3 additional drugs 27 kg	July 12, gas scattering	On August 2, grain cooling twice, handling local warehouses fever, energy cost 6 038kW. h
On August 22, local minor fever	On July 12, local fever in grain surface, 3 540 kWh energy consumption	On August 7, water spray on warehouse roof for temperature control
On August 31, add 27 kg drugs	On August 6, 20, 27, three single – tube handle local heating ventilation	On August 15, September 21, 25, single tube ventilation handling local anomalies point of grain
On September 10, hot temperature began to drop	On August 31, fumigation, 36 kg drugs had been used	On September 27, the third times cold treatment, abnormal heat, energy consumption 2 280 kWh
In early October 5, gas concentrations below 350 ppm	On October 12, for the second time drug fumigation, the use of 52.5 kg drugs	On October 12, scattered gas.
October 17, unusual warming signs started in grain surface.	On October 12, daily entire warehouse centrifugal fan ventilation, effective	On October 29, roof insulation by water spray ended
Test basically completed by the end of October, fatty acid value was prepared to be checked on November 22		
PH_3 consumption: 108 kg of aluminum phosphide dosage:	81 kg aluminum phosphide	aluminum phosphide consumption: 94.5 kg
Grain cooling 0, electricity: 0	Grain cooling electricity: 3 540 kWh	electricity for three times grain cooling: 10 898 kWh
Single – frequency ventilation tube: 0	0 – frequency ventilation tube: 5: 00 meeting.	Single – frequency ventilation : three times. Water spray roof positions: 82 days.
Tons of grain cost: 0.58 ¥/ton	tons cost of grain: 1.27 ¥/ton	cost: 2.43 ¥/ton

Note: aluminum phosphide 30.00 ¥/kg; tariff 1.00 ¥/kWh; ventilation every single point, three one day 3 kW ventilation fan 36 hours about $3 \times 40 + 3 \times 36 = 228$ ¥; 8.3 tons per day water, 5.2 units of electricity, water priced 1.5 ¥/ton

phide had been added, some localized heating may have occurred in grain. Since more ALP was added twice by the end of August, with the rapid rise in phosphine concentration abnormal temperatures began to fall and were eventually brought under control. This shows that when the heating is still at the preliminary stage (low heat) and microbial activity has started, high concentration of phosphine gas can naturally penetrate into the heating region, so as to effectively curb the momentum of heating. On the contrary, if no dose of AlP had been added timely at this point, microbial activity could not be effectively controlled, with rising temperatures. Temperature control would then be very limited because of the greater difficulty of the gas in penetrating into heated regions. The practical experiences in maize storage work of 2003 in Xinshagrain had been fully confirmed.

On October 2, concentration of phosphine declined to 400 ppm; according to the decay rate, it was speculated it would be reduced to below 350 ppm on October 5. Actually, on October 17, there was an abnormal high temperature on the grain surface (The temperature of #14 cable S6 point did not drop with the warehouse temperature, which reached 32.3℃) and the time interval was 12 days. PH_3 gas had been

scattered until October 31 when the concentrations was below 100 ppm. The following grain inspection found a slight heating area within the radius of the central from the grain surface, about 50 centimetres depth, yet no obvious microbial activity could be seen. It was in an early heating period which was about 25 days after the PH_3 concentration attenuated below the effective concentration. The test had been completed in early November when temperatures gradually declined.

There were no phenomenon of grain storage temperature above the warning temperature (38℃) during the whole test and the whole situation in grain was basically stable. Both the local minor heat anomalies happened 15 days later after phosphine concentration had declined below 350 ppm. If the abnormal temperature was no more than 35℃, it could be effectively controlled by a timely replenishment of concentrations of phosphine.

By contrast, there were significant heating in contrast warehouse before phosphine concentration had been attenuated to 100 ppm.

Quality inspection was also included in test and control positions in warehouses in October and early December, data shown in Table 4: Q43, Q45, Q53. The fatty acid value in the pilot position was 3.3 units higher than in the control store Q53 Wharf, and 0.8 higher than inQ45 warehouse. Further follow-up testing should be conducted since the reproducibility was very poor.

Table 4. Q43, Q45, Q53 Wharf corn fatty acid value comparison

table Wharf	the detection time			
	2007.03	2007.08	2007.10	2007.12
Q43	28.4	–	38.5	41.9
Q45	30.5	38.0	40.7	41.1
Q53	28.4	36.5	38.5	38.6

1.4.2 Test results in accordance with the concentration changes in the drawing concentration of phosphine. According to Table 2 data, mapping changes in the concentration diagrams are as Fig. 1.

1.4.3 Cost analysis: Fumigant, water consumption, electricity consumption, services.

108 kg of AlP costs 3 240.00 ¥; no – cooling, single – tube ventilation and temperature control spray operations; total cost about 0.55 ¥/ton;

81 kg of AlP in Q45 wharf, cost 2 430.00 ¥; grain cooling operating once, power consumption 3 540 kHz, cost 3 540.00 ¥; single tube ventilation 5 – point times, with an average of 3 people/day/point, with 3W ventilation fan 36 hours, that is, each point of the 3 × 40.00 + 3 × 36 × 1.00 = 228.00 ¥ (circles), or 1 140.00 ¥; total cost 1.27 ¥/ton;

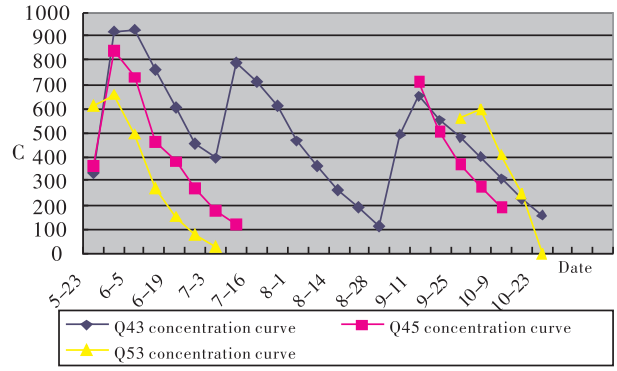

Fig. 1 Phosphine concentration curve

94.5 kg of AlP in Q53 Wharf, cost 2 835.00 ¥; grain cooling operating three times, 10 898 kHz, electricity cost 10 898.00 ¥; partial single tube ventilation 3 – point times, , and about 684.00 ¥; spray 8.3 tons of water daily, 1.5 ¥/ton, pumps, power consumption about 5.2 ¥, a total of 82 days Spray, 8.3 × 1.50 × 82 + 5.2 × 82 = 1 447.30 ¥, the total cost of individual 15 864.30 ¥, the total cost unit is about 2.43 ¥/ton. (Specific data in Table 3: custody measures and cost comparison of Q43, Q45, Q53)

2 Conclusions

2.1 Under the Experimental Conditions, It is Safe and feasible to Control Corn Heating By the High Concentration of Inhibitory PH_3

If the overall temperature is low in grain (about 10℃), there are no obvious mildews, moisture of the corn is about 13.7%, the use of high concentrations of phosphine inhibitory to control corn heating is safe and feasible. Although there were two slight local increases in temperature during the test, these were caused by the activity of microorganisms after the drug concentration decayed below the effective concentration. If the effective concentration of phosphine is maintained above inhibitory levels, safe storage of corn is feasible.

2.2 The Effective Concentration of Phosphine in corn (moisture 13.7%, Temperature about 10℃) to inhibit heating is about 350 ppm for Safe Storage in Summer

Preliminary tests indicate that the effective

inhibitory concentration of phosphine on corn is 350 ppm; when it is below the value, the inhibitory effect will be less or failure will occur. For example, on August 7, the concentration of phosphine is close to the critical value, after half month (on August 22), there appeared abnormal points (5 # cable S7) increasing in temperature, this concentration has been reduced to phosphine about 150 ppm. Also on October 9, the concentration has already begun lower than 350 ppm, to October 16, it has been close to 220 ppm; on the October 31 grain inspection small-scale heating was found.

2.3 With corn Moisture Storage, In Crease of Temperature, Impurity Content, Warehousing Grading Degree of Increased the Inhibitory Concentration Required for Control

There are about six years of experience in the storage of corn in Xinshagrain, which has shown that the higher the moisture in grain, and the quicker heating occurred in grain after storage, the grain surface level under the local aroma ly in the more shallow depth, the higher the concentration of PH_3 required for antibacterial control. The more extraneous matter and broken grains, the worse the effect of infiltration of phosphine; PH_3 inhibitory capacity will be worse, a higher concentration of phosphine is required to reach the same control of effective.

3.4 High Concentration of Antimicrobial Drugs Should be Accurately Used Timely

Practice shows that the inhibitory effect of high concentration and the time of application and fumigation methods are closely related. For example, if the local former fumigation has been obvious fever, even if multiplied increase dosage, antibacterial effects were not evident even failure, it is estimated that with the proliferation of poison gas by the temperature gradient effects.

3.5 High Concentration of Antibacterial Must Maintain the Continuity of Phosphine Fumigation

Historical data show that, partial abnormal heating during high concentration fumigation, and scattered gas treatment used again after the abnormal high concentration of antibacterial storage, often results in poor or worse conditions. The reason may be that the increased oxygen after gas scattering promotes the activities of microbes in grain.

Acknowledgements

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0412

The Study on Low Concentration Carbon Dioxide Controlled Atmosphere Storage

Liang Anyu

Abstract: The CO_2 controlled atmosphere granary in Shanghai Grain Depot (China State Grain Reserve) was built in 2003. The minimum value of gas impermeability test in empty/full granary is 5.75 min and 4.87 min respectively. These values meet the design requirement. Furthermore, a package of methods to deal with gas impermeability solves the problems of soft groundwork and arch bar rips, which result from the sedimentation of full granary. On April 4th 2005, we began a study on wheat reserve under CO_2 controlled atmosphere. Specifically, this work took placce in a CO_2 controlled atmosphere granary and a normal granary. Through comparison, it found that: 1. In the wheat granary with the content of more than 3000 tonnes, it took 56 days for the content of CO_2 to decrease from 81.3% to 34.1%. This data can meet the requirement of maintaining effective CO_2 concentrations over the required duration. 2. In the reserved wheat under CO_2 controlled atmosphere, no pests were found, including immature stages. Furthermore, no poison was left. Consequently, it was feasible to use CO_2 as an innoxious insecticide. 3. In a following study, CO_2 was decreased; under the environment of about 30% CO_2, if the duration was more than 60 days or 80 days it could still restrain insects effectively. As a result, insect pests could be inhibited with the long CO_2 duration and the cost could be reduced. In comparison with a normal granary, the CO_2 controlled atmosphere granary has longer-term prevention of insect pests and slower quality deterioration of reserved wheat. 4. The cost of a CO_2 controlled atmosphere granary is higher than in a normal granary. But if CO_2 is held in low concentration and long duration, the cost could be reduced. Compared with phosphine, CO_2 controlled atmosphere can not only kill insect pests but also avoid the harm and pollution of chemical fumigants.

Key words: carbon dioxide, controlled atmosphere, storage grain pest, control, stored grain quality, low concentration

Introduction

Carbon Dioxide gas used for stored grain truly meets the new green theme of application technology security, coordination, and development.

Shanghai locates in the centre of north-south coastline, the eastern part of Changjiang delta, and faces to East Sea at east. Its climate is semi-tropical seasonal wind and has high temperature and moisture for the whole year, which militates against grain storage. In 2003, the state grain reserves Shanghai depot began a study on CO_2 controlled atmosphere granary. It is very difficult to reserve corn in the Shanghai area, especially in high temperature, where corn will heat easily and cause quality deterioration. It is therefore more meaningful to study corn reserve under CO_2 controlled atmosphere in this situation, to make more rational use of facilities and solve the problem of the high temperature of reserved grain. In summary, it can increase the ability to reserve grain in the Shanghai area.

1 The Experiment on CO_2 Controlled Atmosphere to Store Wheat

The technology of CO_2 controlled atmosphere storage is applied, which lies in guaranteeing that the gas dense quality (gas-tightness) of the storehouse satisfies reasonable demands. The gas dense quality test of empty and filled storehouses was used to obtain the design requirements; after wheat is taken into the storehouse, the gas dense quality of the storehouse is maintained, and the contrasted research and application of CO_2 controlled atmosphere storage is conducted. .

1.1 Survey of Controlled Atmosphere Storehouse

The gas dense quality of empty and filled storehouse of CO_2 controlled atmosphere was tested: the lowest of the empty storehouse's gas dense quality is 345 seconds and the longest is 761 seconds, the gas dense quality of solid storehouse averages 308 seconds. It is provided with provision and equipment gas system out-

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side of the storehouse, and equipped with a grain storehouse CO_2 automation measurement system.

1.2 Methods on Treating with Gas Dense Quality of Storehouse

(1) The gas-tight treatment of controlled atmosphere storehouse emphasizes on choice, installation and sealing doors and windows. Make all equipment gas-proof.

(2) make gas-tight the chord juncture below the arch board of the controlled atmosphere storehouse. Chooses airproof seam glue, polyammoniaester materials to implement board sew fill and treatment to airproof the storehouse tip.

(3) Pay attention to treatment of craftwork hole, which is easily neglected in the controlled atmosphere storehouse, adapting to have good seal completely to glue knot and touching to change characteristic of high bear to the case of grain; examine pipeline and so on to make gas-tight. .

(4) Introduce general observation, audition, fire candle, instruments and solvent inspection and other measures to find a deficiency.

(5) When testing the gas dense quality of empty and filled storehouse, check the area of leak on the spot, analyze the reason, and clear up and modify at the time.

1.3 Test a Storehouse Circumstance

The trial storehouse is the No. 82 storehouse and No. 81 is the comparison storehouse. Each contained wheat (Table 1).

Table 1. the schedule of controlled atmosphere storehouse and comparison storehouse.

item (unit)	82Camalig	81Camalig
Camalig type	An one – storied house	An one – storied
The valid camalig (t)	3715	3715
Actual quantity(t)	3211	3771
The grain heap physical volume(m^3)	4623	4705
The food heap height(m)	5.6	5.7
Food species	White wheat	White wheat
Habitat	Anhui	Anhui
Go into camalig time	2003	2003
Food grade	2	2
Moisture (%)	13.5	13.1
Miscellaneous quality content(%)	0.9	0.9

1.4 Solid storehouse charge and CO_2 attenuation

The temperature of CO_2 is controlled at ± 5℃ below the storehouse temperature, using low pressure (50 – 150Pa) flow into No. 82 controlled atmosphere storehouse. In order to assure the equality of CO_2 in each quarter of the storehouse and good space distribution, circulate the CO_2 to make its concentration attain relative equality. Everyday we use CO_2 concentration automation check systems to note changes in CO_2 concentration. After charging, the initial average concentration is above 80.0%. After 56 days, the average concentration in the whole storehouse still reached above 34%. As fig. 1 shows, in 2005 and 2006 respectively the time of CO_2 concentration kept above 35% exceeded 15 days, satisfying the requirements of concentration and duration.

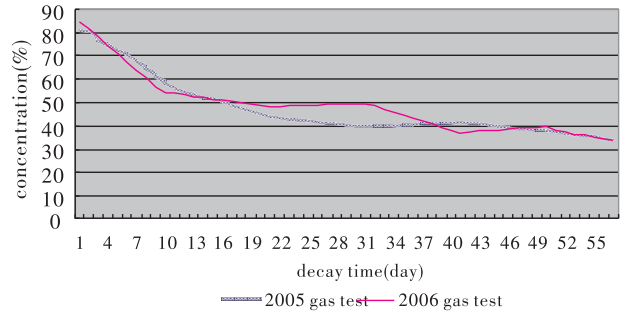

Fig. 1 The average concentration attenuation of CO_2 on wheat in a controlled atmosphere storehouse

1.5 Keep the Grain Pest Prevention and Cure Experiment

We adjusted a trial camalig to the No. 82 controlled atmosphere storehouse to carry on the sampling of the original sample and sample for insect efficacy.

(1) Experiment insect species

Three kinds of main stored grain insects are sensitive to phosphine-corn weevil, lesser grain borer and rust red flour beetle;

Three kinds of main stored grain insects are resistant to phosphine-rice weevil, lesser grain borer and rust red flour beetle; their pH_3 resistance factor is 196, 204 and 8 respectively.

Prepare 10 groups of above-mentioned 6 kinds of stored grain insects (imago and mixed insect form, such as ovum, Aurelia, grub). Every group has above-mentioned 6 kinds of standard imago (2 weeks age) of 20 tested insects respectively and mixed insect forms.

Furthermore, establishing a comparison group, every group contains above-mentioned 6 kinds of standard imago of 20 tested insects respectively and mixed insect forms. After one

month, examine their death rate.

(2) Methods

Place above-mentioned 10 sets of tested insects of 1 – 6 sets 2 meters apart from the wall. Group 7 – 8s tested insect to put in the air-vent neighborhood, Group 9 – 10s tested insect to hang one meter on the grains. Seal to put to go into before the camalig, take out after venting gas. Take out and examine the mortality on 6 kinds of tested insects (the PH_3 sensitive and resistant).

(3) Results

For 6 kinds of main stored grain insects of diversified stages (including imago and insect form, such as ovum, Aurelia, grub), the effect of prevention and cure of CO_2 controlled atmosphere storage attains 100% and has no F1 progeny. It destroys insects 100%; it will not produce harmful residues. CO_2 has no harmful effects.

1.6 Compare the Change of Stored Wheat Quality

Both in No. 82 and No. 81 wheat entered into the storehouse in 2003, and in 2004 was more than one year of the press camalig period. In 2005 – 2006, the change of stored wheat quality was compared in CO_2 controlled atmosphere with normal storage.

Table 2. Wheat quality index by CO_2 controlled atmosphere and normal storage

Examination Time	Gluten absorbs water quantity (%)		Viscosity (cSt)		Taste a grade point value	
	82#	81#	82#	81#	82#	81#
2004.3	206	202	8.4	8.0	76	76
2004.9	200	200	8.0	7.8	78	78
2005.4	216	220	6.8	7.1	79	79
2005.8	218	216	6.1	6.7	76	76
2006.3	208	194	4.4	4.3	76	74
2006.9	197	185	4.3	4.1	75	73

From table 2 we can see that: in the short time storage process, the quality of wheat has been improved to some extent through both CO_2 controlled atmosphere and normal storage because of wheat physiological and technical after-harvesting ripening. With longer storage, CO_2 controlled atmosphere will defer the decrease of wheat quality when compared with normal storage, for example absorption of water by gluten. In view of the good endurance to storage of wheat and better conditions in No. 81 storehouse, the pollution caused by chemical reagent will be avoided and thus the social benefit increased. There are benefits when CO_2 controlled atmosphere storage lasts three years, though there is no evident difference in the short term.

2 Test of Low Concentration CO_2 Over Long Durations

Some related researches indicated that under the condition of 20℃, CO_2 can kill all of the pests in the pile of grains when its concentration is above 60% for 10 days or above 35% for 14 days. We noted that most of the CO_2 controlled atmosphere storage kept the concentration of CO_2 above 60% for less than 10 days, but kept almost all the concentration of CO_2 above 35% for more than 14 days. The average concentration of CO_2 decreased quickly at first and then slowly after charging. In the first 10 days or within 25 days, the concentration of CO_2 decreased at a 2% – 5%/day rate, which made the average concentration of CO_2 decrease from 80% to a lower content, between 30% and 45%。 After that, the average concentration of CO_2 decreased at a distinctly slower rate, thus the average concentration of CO_2 maintained between 30% and 45% for a long time. In the subsequent storage process, we have tried the experiment of low CO_2 concentration and long duration.

2.1 Test Warehouse Situation

Gas test for the No. 82 warehouse stores, Reference the No. 83 warehouse stores, are stored grain corn varieties, casual forms of stored grain, as shown in Table 3.

Table 3. corn Gas warehouse stores basic information and the control list

item (unit)	82#	83#
Camalig type	An one – storied house	An one – storied house
The valid camalig (t)	3715	3715
Actual quantity(t)	3421	3332
The grain heap physical volume(m^3)	4891	4788
The food heap height(m)	5.9	5.8
Food species	corn	corn
Habitat	Jilin	jilin
Go into camalig time	2006.4	2006.4
Food grade	2	2
Moisture (%)	14.2	14.5
Miscellaneous quality content(%)	0.7	0.7

2.2 Low Concentration of CO_2 and CO_2 Attenuation after Long Pressure Test

By the end of March 2007, 82 positions were Kongcang air tightness test, through warehouse inflatable, the Warehouse 60 Pa pressure after the inflatable, attenuation period of 351 seconds. June 18, 2007, all 82 positions filling CO_2, to August 6, after 49 days, the average CO_2 concentration was 42.1%, the slow decay to October 4, the average concentration of CO_2 remains in the low concentration of 25.4% level (Fig. 2): After an inflatable, CO_2 concentrations remained in a relatively low concentration levels for more than 60 days, the average concentration of CO_2 was more than 30% up to 90 days.

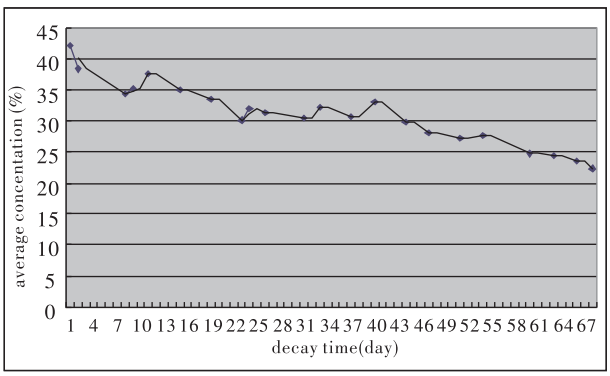

Fig. 2 Gas warehouses average concentration of CO_2 attenuation map

Main factors influencing air-tightness: the warehouse doors and windows sealed warehouses flattened performance and seamless. Improve sealing of windows and doors more easily done, spend relatively less cost and lower warehouse flattened crevice. So in the process of gas transfer, improving warehouse sealing of slowing the average concentration of CO_2 attenuation one of the important factors, but also conducive to the average CO_2 concentration to low concentration level of attenuation should be able to maintain a longer period of time to retard the decline in the quality of stored grain provide favorable conditions.

2.3 maize compared changes in the fatty acid value

82 warehouses and 83 stores are maize in April 2007 warehousing. In 2007, the CO_2 gas-storage and storage of conventional corn changes in the fatty acid value comparison found that the short-term storage, each average value of the fatty acid positions were little changed, their differences are not obvious.

Table 4. CO_2 gas storage and transfer of conventional corn storage fatty acid value test results

Detection time	Fatty acid value (mgKOH/100g dry basis)	
	82#	83#3
2007.5	40.9	41.3
2007.10	42.1	43.7

3 CO_2 Gas Cost Analysis for Stored Grain

Gas warehouse and choose the same kind of warehouse-stored grain costs for conventional warehouses. Analyze only operating costs during the trial period of the two storage; their common costs, such as management fees and staff salaries, are not analyzed.

3.1 Wheat ballast during the CO_2 gas-stored grain fumigation with conventional Comparative analysis of grain storage

Table 5. CO_2 gas-stored grain stored grain and conventional cost analysis table (yuan: RMB)

item (unit)		81#	82#
Variety		wheat	wheat
Grain stack height (m)		5.7	5.6
The actual number (t)		3 771	3 211
The main material costs (yuan)	CO_2	0	5 950
	PH_3	1 092	0
Supplementary material (yuan)		117	117
Grain-grain film costs (yuan)		1 708	0
Grant application (yuan)		48	0
Electricity costs		15	750
Warehouse maintenance costs (yuan)		/	/
Operating costs (yuan)		2 972	6 817
Annual gas consumption per tonne of grain (kg)		0	2.18
The annual cost per ton grain (yuan)		76	76
Annual operating costs per tonne of grain (yuan)		0.79	2.12
Annual operating costs per tonne of grain/The annual cost per ton grain		1.04%	2.79%
Expected proceeds tons of stored grain (yuan)		0	50
Annual Expected proceeds per ton of stored grain (yuan)		0	12.5

As gas was introduced after the new positions were put into use, the trial does not include warehouse maintenance costs and gas-testing equipment maintenance costs. If we consider the test itself, the warehouse maintenance costs 1 657 yuan/year, gas-testing equipment maintenance costs 8 704 yuan/year; conventional tons of stored grain warehouse, the operation cost will be 0. 79 yuan/ton, up from 1. 23 yuan/year tons of gas transfer tons of stored grain warehouse in operating costs will be 2. 12 yuan/ton, up from 5. 35 yuan/ton,

3.2 stored grain corn CO_2 gas fumigation and conventional comparative analysis of grain storage

Corn warehousing, maintenance of a warehouse to store two years of normal maize, produced a warehouse maintenance costs 2 485 yuan/year; Gas positions related equipment maintenance and test, a test maintenance costs 8, 704 yuan/year together were analyzed, as shown in table 6.

Table 6. grain CO_2 gas and conventional grain cost analysis table (yuan: RMB)

item (unit)		81#	82#
Variety		corn	corn
Grain stack height(m)		5.8	5.9
The actual number (t)		3 332	3 421
The main material costs (yuan)	CO_2	0	7 845
	PH_3	3 050	0
Supplementary material (yuan)		117	117
Grain – grain film costs (yuan)		1 708	0
Grant application (yuan)		48	0
Electricity costs		19.5	870
Warehouse maintenance costs (yuan)		2 485	2 485
Verification maintenance costs (yuan)		/	8 704
Operating costs (yuan)		7 427.5	20 021
Annual gas consumption per tonne of grain(kg)		0	2.29
The annual cost per ton grain (yuan)		76	76
Annual operating costs per tonne of grain(yuan)		2.23	5.85
Annual operating costs per tonne of grain/The annual cost per ton grain		2.93%	7.70%

In comprehensive tables 5 and 6 can be seen, consider the storage cycle of corn is shorter than that of wheat and the annual cost higher.

4 Conclusion

The state grain reserves Shanghai depot using CO_2 gas-stored grain, and through a series of warehouse hermetic security measures, three air tightness tests had good results. CO_2 gas – stored grain was 100% effective against to the six major insect pests in stored grain. A pollution-free pesticides method is a green way for grain storage. CO_2 gas used for long-term storage of wheat can delay the decline in the quality of stored grain, playing the role of food preservation. For short term storage of wheat and corn, there was no significant difference between controlled atmosphere storage and conventional storage in improving the quality of stored grain. By improving warehouse sealing of windows and doors, and other measures, raising gas-sealing of warehouses, stores can be maintained with an effective insecticide CO_2 gas concentrations; appropriate extension of the average concentration of CO_2 not only served the purpose of killing pests in stored grain, but also delayed decline in the quality of stored grain. As CO_2 gas costs are high, using CO_2 gas-stored grain storage costs more than conventional treatments. Take into account the normal short storage period of maize, compared to gas-storage maize has a high cost. By improving the air tightness of the warehouse, CO_2 gas consumption can be appropriately reduced, lowering the cost. As people gradually change the concept of consumption, there is increased demand for green products. Allowed under the premise of the policy, through allocation of reasonable market sales channels, the sale price of grain under CO_2 will have certain advantages. In Shanghai, and other high-temperature, high humidity areas, using gas-storage technology will not only bring social benefits, but some potential economic benefits.

Acknowledgement

We thank Dr. Jim Desmarchelier for help with the manuscript.

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0413

Research about the Technologies of PH_3 Recirculatory Fumigation in Squat Silos in the Ecological Regions of Medium Temperature and Low RH

Lu Xianli, Li Zongliang, Yu Jieqing and Luo Fang

Abstract: Because regions of medium temperature and low RH are suitable for growth of insect populations throughout the year, we need to treat stored grain according to the infected portions and time. In this paper we summarize three methods of integrated control to kill pests and prevent pest resistance.

Key words: fumigant, fumigation, squat silo, recirculation fumigation, integrated control

Introduction

Squat silos for long-term grain storage have been built in China since 1998 when the government invested to build many new grain warehouses and to extend existing grain ones. Because the grain bulk is high and the volume large, it is very difficult to achieve even diffusion of fumigant, where even, quick and effective fumigation is needed to control pests. Moreover, insects in the South of China and moisture content in the North are basic problems. The environment of high temperatures and the small temperature difference in the south, particularly in the ecological regions of the medium temperature and low RH, is very suitable for the growth and reproduction of insect pests throughout out the year. Therefore, the basic work in the warehouse is controlling stored grain pests. Although mechanical equipments in the squat silos are convenient for daily operations, they are also a good habitat for growth of stored grain pests. Here we summarize the fumigation technology which can kill pests quickly and completely at low dosage, and which can be used safely. At the same time, it overcomes the problems of uneven distribution of fumigant, high dosage and incomplete pest control. The technology will be ideal for fumigation in tall grain bulks.

Materials

Experimental Silos

No. 18, 20 and 21 silos have the same design dimensions, the stored grain is medium grade wheat produced in Henan. The diameter of squat silos is 25.0 m, with a height of 15.0 m grain; other detail contents are shown in table 1.

Table 1 Test basic information of stored grain warehouse

silo No.	Input time	Quantity (t)	Grain temperature (℃)	Moisture content (%)	impurity (%)	unitweight (g/L)	*Sitophilus zeamais*	*Rhizoperth adominica*, *etc.*	*Sitotroga cerealella*
							(number/kg)		(number/m^2)
18	2007	6000	21.6	12.5	0.5	778	25	0	0
20	2006	6000	15.8	12.2	0.6	785	9	0	3
21	2006	6200	19.2	12.1	0.4	788	5	6	0

Notes: *Rhizopertha dominica*, *etc.* in Table 1 includes high resistance pests such as *Cryptolestes ferrugineus* and *Tribolium castaneu*

Instruments

Beijing OPI Digital Stored Grain monitoring System, Shenyang 'Fengshou' 56% AlP tablet, PH_3 concentration detector (Beijing jiahua HL-210) and alarm device, phosphine-generator (Zhengzhou weilai), DSS fixed recirculation System of Shenzhen Automation Engineering Co., Ltd.

Method

Layout of Gas Detecting Point

The layout for detection of gas concentration are set between two cable lines in the external aeration tubes, and divided into three layers (upper, middle and bottom). The distance from the ground is 1.0, 6.0, and 12.0 m. The spatial concentration of gas is detected from the

Kunming Warehouse Directly under Central Grain Reserves, Kunming, Yunnan

test tube in the fumigant container next to the recirculation fan.

Airtightness of Silos

Air vents and axial flow fan mouth are sealed with covers, the doors of silos are sealed with 0.15 mm polyethylene approved for grain. Before loading grain, the manual and electric valves at the bottom of the silos must be well closed. The pressure test results of the pilots are that the hermetic time of No. 18, 20 and 21 silos are 120 s, 110s, 117s, which are longer than the 60s given in the Technical Regulations of grain storage.

Methods

Fumigation of New Arrival Grain in No. 18 silo

As the grain in No. 18 silo is inputted from July to October, its temperature is high, the quantity is large, the period of input is long and pest density is high. The pest density is 25/kg when the silo is full. The first thing we do is to drive the pests to the surface of grain bulk through aeration, and simultaneously to balance the temperature of grain to prevent condensation of water vapor. Then 16.0 kg tablets are distributed on the grain surface, and the air is recirculated to balance the phosphine gas concentration.

Fumigation during Grain Storage in No. 20 Silo

The grain temperature is uniform during grain storage in No. 20 Squat silo with a average temperature of 16.5℃. Fumigation combined the distribution of fumigant on the grain surface with PH_3 gas supplied from outside of the silo. The consumption of tablets was 8.0 kg for each stage.

Fumigation for the High Resistance Pests in No. 21 Silo

C. ferrugineus and T. castaneum infested the grain in No. 21 squat silo. Because they have high resistance, we applied intermittent recirculation fumigation to kill the pests. The method is supplying fumigant from outside the silo and maintaining effective concentrations for at least 18 days, then stopping supplying for a period of time, finally replenishing fumigation on the 25th day. The amount of fumigant in the first stage is 14.0 kg and 8.0 kg at the second stage.

Detection of PH_3 Gas Concentration

HL – 210 PH_3 gas concentration detector started to detect PH_3 gas on the second day after the first stage of finishing fumigant distribution on the surface of grain. When the concentration reaches effective concentration, the detection frequency changes with the phosphine concentrations (for example, when PH_3 concentration is higher than 500 mL/m^3, we will detect it once 2 – 3 days), until the concentrations is below the efficient concentration).

Ventilation and Pest Control Inspection

After finishing of the fumigation, four axial fans begin to ventilate grain bulk. Four days later, when the concentration is below than 0.2 mL/m^3, we inspect the result of fumigation through sampling the grain at points of high pest density or high temperature, and then inspect the points again a month later, making a detailed record at each time.

Conclusion and Analysis

PH_3 Gas Concentrations in Each silo

Fumigation of No. 20 and 21 silos took place in 2006, while No. 18 silo was fumigated in 2007. The maximum concentration in each silo was ⩾500 mL/m^3 (the instrument records 500 mL/m^3 for concentrations more than 500 mL/m^3).

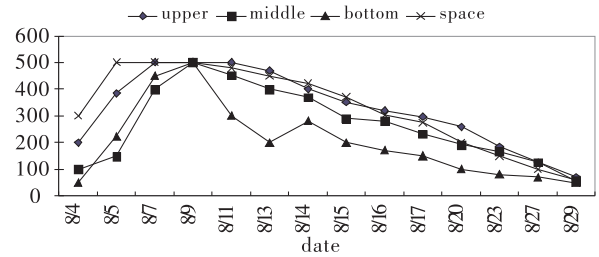

Fig. 1 Gas concentration in No. 18 silo

The concentration of PH_3 in No. 18 silo reached effective concentrations (Fig. 1) on the 2nd day, and peaked on the 4th days, then attenuated gently. The space and upper PH_3 gas concentration were always higher than others. Concentration of PH_3 was higher than 150 mL/m^3 for 14 days and higher than 100 mL/m^3 for 20 days. Because the grain temperature was higher, the AlP tablet evaporated easily. At the same time ventilation drove pests to the surface, which facilitated pest kill on the surface, which satisfies our purpose. This method is easy, efficient and saves costs.

In No. 20 silo, the concentration of PH_3 reached effective concentrations (Fig. 2) on the 1st day, and peaked on the 3rd days. The space and upper PH_3 gas concentration attenuated gently, and concentration was higher than 150

mL/m^3 for 16 days, and higher than 100 mL/m^3 for 20 days. At the same time CO_2 was used to help the fumigant diffuse into grain bulk quickly, so the airtight time is shortened, while the fumigant on the surface kept evaporating to maintain the concentration. This reached the desired aim.

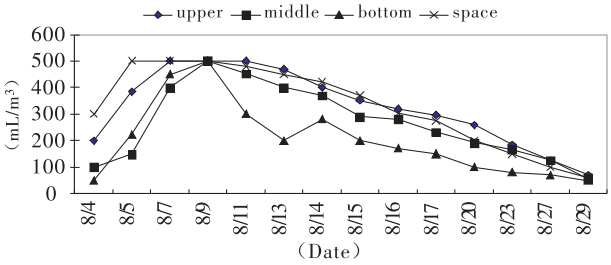

Fig. 2 Gas concentration in No. 20 silo

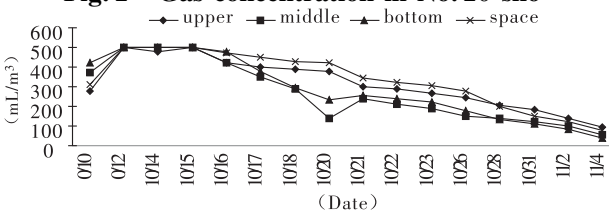

Fig. 3 Gas concentration in No. 21 silo

The concentration of PH_3 reached effective concentration in No. 21 silo on the 1st day (Fig. 3), and peaked at same time. The gas concentration at each layer attenuated quickly. The time of concentration higher than 150 mL/m^3 was 12 days, and that higher than 100 mL/m^3 was 16 days. During the replenishing period, concentration was higher than 150 mL/m^3 for 12 days. As the interval between two applications was 24 days, the eggs of high resistance pests hatched into larvae during this period, so the larval stage with low resistance was killed in the second fumigation period. This two-fold fumigation reduced the consumption of fumigant, and effectively solved the problem of incomplete control of resistant insects.

Controlling Effect and Benefit Analysis

Samples from 5 points in each layer (upper, middle, bottom and space) was inspected, and each silo (No. 18, 20, 21 silo) reached the desired control by different fumigation methods after more than 1 month airtight fumigation. Choosing different methods according to different situation will reduce the fumigation cost and labor intensity, at the same time solving the problem of incomplete kill of high resistance pests.

Table 2. Fumigation efficacy and costs

SiloNo.	Total amount(kg)	CO_2 gas(kg)	sampling afterdiffusion (number/kg)	sampling after 30 days(head/kg)	sampling after 60 days (number/kg)	Operating time(h)	labor (person)	Costs (yuan)
18	16	0	0	0	0	1	6	416
20	16	30	0	0	0	6	4	491
21	22	80	0	0	0	18	2	616

Notes: CO_2 gas 50 yuan/tank of 20 kg, CO_2 gases takes 2.5 yuan/kg, aluminum phosphide cost 26 yuan/kg, excluding labor costs.

Discussion

Choosing the best time to kill pests is important. As the activities of pests relates to stored grain temperature, normally they thrive in summer and reach their peak in autumn. As high temperature always relates to frequent activity, the best time for killing pests is from June to October.

Improving air-tightness of silos, monitoring concentrations of phosphine and replenishing concentrations will achieve the time required for effective concentration.

When grain at out-loading is infected, it can be treated by probing with phosphine formulations. If the grain on the surface is infected, it can be treated by 'Spot' fumigation, which is to cover the infested grain with gas-proof sheets like bell-shape, and to fumigate by probing formulations into areas. Normal more dosage is put on the top and the dosage is 6 – 15 g/m^3.

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0414

AlP Low Dosage Recirculation Fumigation under Film through PH_3 Dynamic Deliquescence

Wu Hongyan

Abstract: Three fumigation methods were investigated in this paper. The results showed that the recirculation fumigation under film through PH_3 dynamic deliquescence effectively reduced fumigation spaces, saved CO_2, decreased the moisture content losses of grain bulk, killed stored – grain insects effectively, and the phosphide residue in grain was lower than the national health standard. For the recirculation fumigation under film through PH_3 dynamic deliquescence, the storage cost was 53 % cheaper than that of whole warehouse PH_3 dynamic deliquescence, and 89 % cheaper than that of whole warehouse recirculation fumigation. In addition, the recirculation fumigation under film through PH_3 dynamic deliquescence realized the grain storage objective that is safer, economical, practical and in favor of environment protection.

Key words: low dosage, dynamic deliquescence, recirculation fumigation under film

Since 1998, all of newly built and extension depots have been equipped with new four grain storage technology which include grain inspection, mechanical aeration, recirculation fumigation, and grain cooling. Particularly, the recirculation fumigation greatly decreased the labor intensity during fumigation work, reduced the contacting time with toxic gas, improving grain storage scientific management level in depots. However, because of poor air-tightness of warehouses, inconvenient manipulation of gas-producing type recommended by new grain storage technology and higher application cost, the use of the warehouse and grain storage benefit was negatively affected. In order to resolve the poor air-tightness of the warehouses, the weak killing effect on pest insects, the overuse of fumigant dosage, the higher application cost, and so on, the joint key technologies R&D group between Zhoukou Grain Depot, State Grain Reserves and Zhengzhou Institute of Technology was established in March, 2003, and the program "AlP Low Dosage Recirculation Fumigation under Film through PH_3 Dynamic Deliquescence" was put forward, which harmoniously combined sealed grain storage technology and recirculation fumigation technology. The "AlP Low Dosage Recirculation Fumigation under Film through PH_3 Dynamic Deliquescence" can exert the advantages of recirculation fumigation technology which can realize the quick and even gas distribution in warehouses, keep excellent air-tightness of the grain mass sealed below plastic film, significantly reduce fumigation spaces and application dosage, and enhance the fumigation effect. By combining dynamic deliquescence, the technology is brought to perfection, and its manipulation is quick, convenient, economical, effective, safe and in favor of environment protection.

1 Experimental Materials and Methods

1.1 Experimental Materials

1.1.1 Experimental warehouses

The experimental warehouses included No. 27, 29 and 16 warehouses located in Zhoukou Grain Depot, State Grain Reserves. The former two warehouses were newly-built horizontal warehouses in 2000, whose specification was 60m × 30m. Warehouse air-tightness, namely pressure half-life time, was 43 seconds under 500Pa. There were 8 groups of in-floor U-channel main air – ducts each with 3 branches installed in the warehouse, 4 air intakes set at each side of the warehouse which could realize aeration at two sides. The air-ducts layout plan

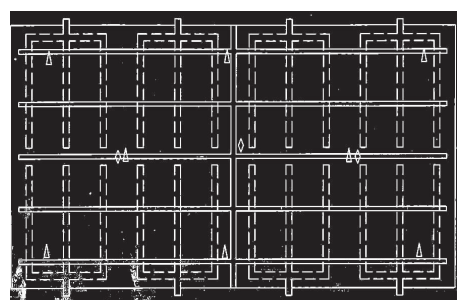

Fig. 1 The air-ducts, air return tubes, and air-testing sites disposal ichnography in warehouse.

Zhoukou Grain Depot, State Grain Reserves, Zhoukou Henan 466002, *China*

is shown in Fig. 1. No. 16 warehouse was a newly – built horizontal warehouses in 1998, whose specification was 58m × 24m. There were 3 groups of main air-ducts under floor each with 4 branches installed in the warehouse. The structure of the experimental warehouses was broken – line roof truss, and the filling high was 6m.

1.1.2 Experimental grain

The experimental grain was bulk mixed wheat. The grain surface was sealed by plastic film, and the other were expressed in table 1.

Table 1. The basic grain condition in No. 27, 29 and 16 warehouses

No.	Grain storage beginning time Year. month	Quantityt	Storage style	Bulk densityg/L	Moisture content %	Impurity %	Visciditycst	Gluten hygroscpic content %
27	02.2	8856	sealed	790	12.5	0.6	10.4	214
29	02.1	8916	bulk	786	12.5	0.5	7.7	209
16	01.7	6130	bulk	780	12.0	0.7	5.9	213

Note: The broken – lines indicate air-ducts, the solid lines indicate air return tubes; indicate air-testing sites in No. 27 warehouse; indicate air-testing sites in No. 29 warehouse.

1.1.3 Experimental instruments and materials

Recirculation system: the mobile recirculation fumigation system made in Shenzhen dashi stock Co., Ltd., whose power is ≤1kW, aeration ability 500 – 1 000m^3/h, full pressure 800 – 1 000Pa.

Outdoor PH_3 generator: XZL – IV type, made in Shandong Jinxiang grain machinery factory.

PH_3 gas detector – alarm: HL – 210 type, made in Beijing jialianglao technology and trade Co., Ltd.

Radiofrequency electronic welding machine: KW – 2500T type, made in Tianjin Beixin electronic equipment Co., Ltd.

Air pressure meter: YEP – 101 type, made in Handan xinyu instrument and meter Co., Ltd.

Plastic film: PVC press – extension film whose thickness was 0.2mm, made in Tianjin No. 4 plastic products factory.

Air return tubes: PVC tube, φ50mm, φ30mm, made in Shangshui county plastic tube factory.

Fumigant: 56% AlP tablet, baolianglin brand, made in Shandong Jining chemical and industrial experiment factory.

CO_2 gas: purity was 98%, 25kg/bottle, made in Pingdingshan fertilizer factory.

1.1.4 Experimental insects

The experimental insects were *Rizorpertha dominita*, *Tribolium confusum*, *Cryptolestes turcicus*, *Sitophilus zeamais*.

R. dominita and *S. zeamais* were collected in Zhengzhou. Both of them have been cultured with wheat for several years in stored – grain insects control laboratory in Zhengzhou Institute of Technology. *T. confusum* and *C. turcicus* were collected in Xinjinag and Zhengzhou respectively. Both of them have been cultured with whole wheat flour adding 5% yeast for several years in stored – grain insects control laboratory in Zhengzhou Institute of Technology.

1.2 Experimental Methods

1.2.1 Air return tubes disposal under plastic film

Because the grain would be stored by single surface seal with plastic film, the grain surface was sealed with plastic film after the grain condition was stable. The air return tubes was buried under the grain surface 300mm in advance. The air return tubes constituted 1 main tubes and 5 branch tubes arranged as "丰" type. The main tube diameter was 50mm, no holes on its surface. The main tube was arranged in the center of the warehouse, and its two ends were coupled with recirculation main duct on the warehouse wall. The diameter of 5 branch tubes was 30mm. The branch tubes were parallel arranged two sides of the main tubes, spacing 5m between 2 neighboring branch tubes. There were little holes on the branch tubes surface whose diameter was 2mm. There were 6 circularity holes every interval 1m in the branch tubes within 15m from the main tube, and 6 circularity holes every interval 0.5m in the branch tubes outside 15m from the main tube. The air return tubes layout pattern under plastic film is shown in Figure 1, and the gas recirculation fumigation process under film is shown in Figure 2.

1.2.2 The locations of gas concentration sampling sites and sampling times

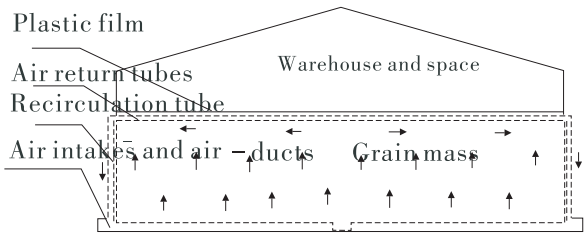

Fig. 2 Airflow circulation sketch map for recir culation fumigation process under film

8 sampling and detecting holes were placed on the grain surface film in No. 27 warehouse. Each hole had upper, middle and bottom layer depths which were located at 1.0m, 3.0m and 5.5m under the grain surface. The gas concentration detecting was conducted on the grain surface film in warehouse. A gas detecting site was respectively set in eastern and western side grain mass, locating at upper, middle and bottom layers in No. 29 warehouse. 5 gas detecting sites were placed in No. 16 warehouse. The PH_3 concentration at each detecting site and fumigation space in No. 29 and No. 16 warehouses was monitored on schedule by leading the gas through plastic pipes to an outdoor gas sampling instrument. The gas detecting sites layout patterns in each warehouse is shown in Figure 1.

The gas concentration was detected 20h after AlP application in each warehouse, two times each day for at the first 15 days, then once per day. If the PH_3 concentration was above 100mL/m^3, gas concentration detecting could be continued. One to two times gas concentration monitoring should be done before finishing film sealing, to check the PH_3 concentration maintaining background state in the warehouses.

Table 2. Fumigation concentration detecting values in No. 27 warehouse unit: mL/m^3

Day	1	2	3	4	5	6	7	8	Mean
1	131	248	323	168	274	322	204	205	234
2	291	314	328	311	363	403	369	365	343
3	364	359	401	381	442	489	432	468	417
4	390	427	459	423	488	516	512	493	464
5	318	316	354	330	392	413	376	374	359
7	296	291	316	306	376	383	355	344	333
14	230	239	273	226	305	292	270	243	260
21	163	179	202	159	219	210	192	166	186
28	116	134	151	118	155	153	139	118	136
34	73	90	100	75	90	100	89	73	86
55	45	56	60	49	56	60	55	45	53

Table 3. Fumigation concentration detecting values in No. 29 warehouse unit: mL/m^3

Day	Western site	Eastern site	Space site	day	Western site	Eastern site	Space site
1	135	61	50	14	173	196	177
2	211	121	231	15	161	175	150
3	262	256	242	16	143	153	128
4	346	292	270	17	133	142	108
5	318	324	300	18	124	133	103
6	312	316	298	19	109	118	87
7	306	310	293	20	102	112	82
8	279	299	275	21	96	106	84
9	263	281	273	22	90	97	89
10	244	271	270	23	87	99	91
11	227	263	266	24	70	80	73
12	205	237	220	25	55	57	25
13	183	212	175				

Table 4. Fumigation concentration detecting values in No. 16 warehouse unit: mL/m^3

Day	1	2	3	4	5	Mean	Day	1	2	3	4	5
1	62	78	69	58	67	12	264	270	259	200	185	236
2	153	160	138	151	152	150	13	210	225	189	169	153
3	362	367	345	345	357	355	14	163	170	144	131	120
4	433	434	438	406	440	430	15	137	152	125	125	113
5	320	337	309	300	285	310	16	118	128	103	119	100
6	264	279	243	257	232	255	17	96	107	85	113	89
7	206	211	158	197	168	188	18	88	99	78	101	78
8	136	149	108	110	95	120	19	79	93	70	87	65
9	295	264	252	301	290	280	20	72	87	64	76	59
10	452	410	480	395	406	429	21	63	80	57	62	48
11	370	333	388	289	260	328						

1.2.3 Sealing the grain surface

In order to further enhance warehouse airtightness, eliminate the effect of outdoor adverse environment condition, Zhoukou Grain Depot adopted grain surface film sealing method to store the grain. The detailed manipulation was the following: A batten, 7cm × 4cm width, was fixed alongside the inside wall at 6m filling height in the warehouses, and a sealed slot pipe was inlayed in the middle of the batten. The sealed slot pipe was 4cm spacing from the wall whose interior edge had a 25mm × 15mm rectangular groove, 25mm spacing from the wall, with its opening faced upwards. For the sake of conveniently sealing grain mass, alleviating the labor intensity, such as covering and uncovering film manipulation, the plastic film was divided into 8 pieces according to warehouse plane. The pieces of film was joined each other by 10cm

width wooden skeletal frames on whose two sides were mounted groved pipe. 4m width press-extension film was jointed together by radiofrequency electronic welding machine. Then, the film was pressed into the grove pipe using glue bar piece by piece, pouring the melted wax to seal the slot pipes on the ambient wall. The groove pipes in wooden skeletal frames were sealed by adhesive tape. This was simply called as "wooden skeletal frame piece sealing method".

To ensure the quality of film sealing, the air-tightness under the surface sealing sheet was tested by negative pressure method. The tested result showed that the pressure half life time was 147 seconds under 500Pa in No. 27 warehouse, according with the requirement for fumigation and grain storage.

1.2.4 The insect cages pre-burying

The insect cages were made beforehand from φ50mm × 120mm plastic pipes whose two ends were sealed by 120 eyes sieve silk. The cage was filled to 1/2 capacity with wheat in advance. Twenty standardized cultured testing insects, *R. dominita*, *T. confusum*, *C. turcicus*, *S. zeamais*, 7 – 14 day adults, respectively were put into the insect cage which was then sealed with sieve silk for experiment. The cages, with locater and retrieval cords attached, were buried 50cm below the grain surface at each gas concentration detecting site. The insects death condition was inspected after gas dispersing, and the cages with *R. dominita* and *S. zeamais* were again taken to incubation box (28 ± 2℃, 70% ± 5% r. h.) in the laboratory. Adult insects emergence condition from the fumigation test cages was again inspected in the laboratory after 40 days.

1.2.5 Fumigation scheme

The grain surface was sealed with plastic film in No. 27 warehouse. Adopted fumigation scheme was fumigant application by outdoor air intakes, dynamic deliquescence and low dosage fumigation under film. Fumigant dosage was 1.1g/m^3 (calculating by practical grain mass). Each intake was filled with 1.5kg AlP tablet, with 12kg AlP tablet for 8 intakes altogether. Continuous recirculation was operated for 48h after fumigant dosage application, then the recirculation blower was run 3h each day for at least 15 days.

Routine storage and whole warehouse recirculation fumigation was used in No. 29 warehouse. Adopted fumigation scheme was dynamic deliquescence recirculation fumigation combining grain surface and air – duct fumigant application. Fumigant dosage was 2g/m^3 (calculating by the whole warehouse volume). 16kg of AlP tablets were applied on the grain surface. Each intake was filled with 2.5kg of AlP tablets, or 20kg of AlP tablets for 8 intakes altogether. 36kg of AlP tablet was applied in the whole warehouse. The recirculation fumigation blower was started as No. 27 warehouse right after fumigant dosage application.

Routine storage was also used in No. 16 warehouse. However, the adopted fumigation scheme was whole warehouse recirculation fumigation using outdoor PH_3 generator to produce PH_3 gas. Fumigant dosage was 1.44g/m^3 (including supplement fumigant dosage). 20kg of AlP tablet and 1 000kg of CO_2 was applied in the whole warehouse as the initial dosage, and 6kg of AlP tablet and 300kg of CO_2 was added as a supplemental dosage after 5 days. Continuous recirculation was operated for 20h after initial fumigant dosage application, then recirculation was run 3h each day for at least 15 days.

If the fumigant concentration could not be maintained above 100mL/m^3 for 15 days after the first fumigant application in each warehouse, supplement fumigant application must be added depending on actual gas concentration conditions of each warehouse.

1.2.6 Organic phosphorus residues detecting in grain after fumigation

In order to compare the relationship between the fumigant application dosage and the organic phosphorus residues in grain, the organic phosphorus residue content in fumigation grain was detected after routine fumigant application dosage and low dosage under film.

1.2.7 The effect of film sealing on stored grain temperature change

In order to explore the effect of film sealing on stored grain temperature change, No. 27 and 29 bulk warehouses, which had the same warehouse structure, grain storage time and grain condition, with similar grain temperatures, were compared. When the air temperature was increasing in spring, the grain surface was sealed with film in No. 27 warehouse, and the grain mass was still kept as unsealed bulk with open headspace in No. 29 warehouse. The stored grain temperature change in the two warehouses was inspected by the same computer at the same time.

2 Results

2.1 Fumigation Effect of Different Fumigation Methods

PH_3 concentration sample monitoring re-

sults in No. 27,29 and 16 warehouses were listed in Tables 2 – 4. According to the detecting results, PH_3 concentration change trend was drawn in Figures 3 – 5.

Table 2 and Figure 3 had clearly showed that only one time 12kg fumigant application could maintain PH_3 concentration above 100mL/m^3 for

Table 5. Temperature change comparison betweenfilm sealing and bulk grain mass

Testing day	No.	Warehouse temperature ℃	Average temperature at each site, ℃				Average grain temperature ℃	grain temperature difference
			Upper	Upper – middle	Middle – bottom	bottom		
5.12	27	23.5	17.7	15.1	15.1	14.6	15.6	0.8
	28	24.1	18.7	16.1	16.5	15.7	16.8	
5.19	27	26	18.3	15.1	15.3	14.8	15.9	1
	28	25.8	19.2	16.2	16.6	15.7	16.9	
5.26	27	26.9	19.8	15.4	15.5	15	16.4	1.1
	28	27.4	21	16.5	16.5	15.9	17.5	
6.2	27	28.5	21.1	17	17.1	16.2	17.9	1
	28	29.2	22.5	18.3	18	16.9	18.9	
6.9	27	30	22.6	15.8	15.7	15.3	17.3	1.4
	28	30.8	24.1	16.9	17.1	16.8	18.7	
6.16	27	29.2	23.3	16.1	15.9	15.5	17.7	1.2
	28	31	24.6	17.1	17.3	16.7	18.9	
6.23	27	29.4	24.3	17.2	16.5	16.2	18.6	1.1
	28	30.7	25	18.4	18	17.3	19.7	
6.30	27	29.6	24.1	16.9	16.6	16	18.4	1.4
	28	30.3	25	18.5	18.2	17.3	19.8	
7.7	27	29.8	23.7	17.1	16.7	16.1	18.4	1.2
	28	30	24.8	18.3	17.9	17.2	19.2	
7.14	127	29.8	24.3	17.5	16.6	16.4	18.9	1
	28	30.9	25.7	18.6	18.1	17.4	19.9	
7.21	127	30.7	25.3	17.9	16.9	16.7	19.2	1.2
	28	31.5	27.3	18.8	18.3	17.1	20.4	
7.29	127	31.2	26	18.4	17.1	16.8	19.6	0.7
	28	31.8	27.1	18.9	18.4	16.9	20.3	

32 days because of the excellent air – tightness in No. 27 warehouse. PH_3 fumigation concentration reached the highest level on the fourth day, and the average fumigation concentration reached 464 mL/m^3. According to PH_3 fumigation concentration distribution on the second and third days, the rate between the highest and the lowest concentration were respectively 0.72, 0.73. Decreasing ratio per day was 2.7% – 3.5%, which showed that PH_3 concentration could reach even distribution in the warehouse after 2 to 3 days recirculation. The PH_3 concentration decreasing trend was stable at 8 detecting sites during sealing period.

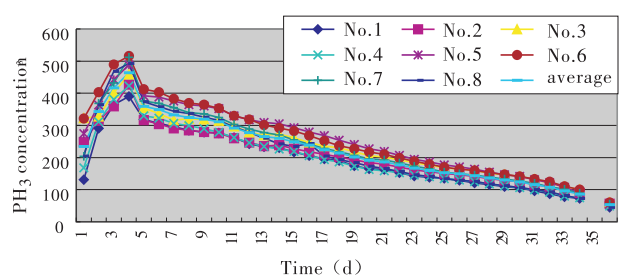

Fig. 3 PH_3 concentration change trend during fumigation sealing period in No. 27 warehouse

Table 3 and Figure 4 clearly showed that a one – time fumigant application can maintain PH_3 concentration above 100mL/m^3 for 18 days in No. 29 warehouse. The highest concentration

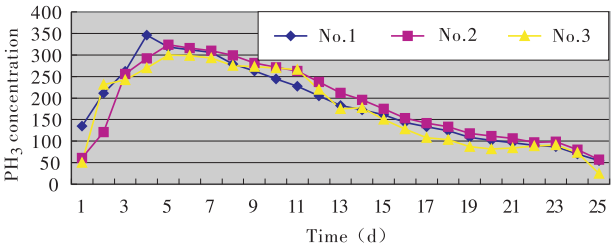

Fig. 4 PH_3 concentration change trend during fumigation sealing period in No. 29 warehouse

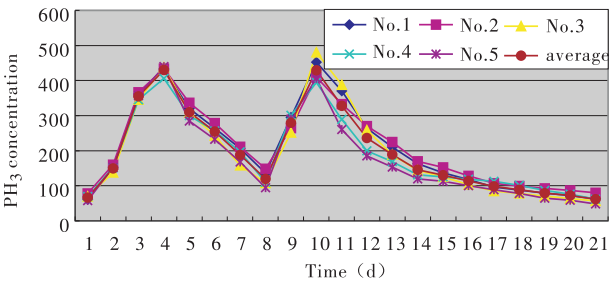

Fig. 5 PH_3 concentration change trend during fumigation sealing period in No. 16 warehouse

in eastern part, western part and space above grain surface in the warehouse were respectively 324, 346 and 300 mL/m^3. However, the PH_3 concentration in No. 29 warehouse decreased faster than that in No. 27 warehouse, and decreasing ratio per day was 8.5% – 10%.

After only 8 days after dosage, the PH_3 concentration decrease to 120mL/m^3 in No. 16 warehouse. In order to enhance the fumigation effect, an additional 6kg of AlP tablets and 300kg of CO_2 was added as supplemental dosage on the 8th day. Thus, 26kg of AlP tablets and 1 300kg of CO_2 was applied during two fumigant applications in Warehouse No. 16, then PH_3 concentration above100mL/m^3 was maintained for 16 days. Among the three warehouses, the PH_3 concentration in No. 16 warehouse decreased fastest. During the whole recirculation fumigation period, the highest average concentration respectively reached 429 and 430 mL/m^3 (see Table 4 and Figure 5).

2.2 Fumigation Effect Inspecting

The pre-buried insect cages in each warehouse were taken out of the grain mass after fumigation. Both the insects death condition and the adults emergence condition in the cages with *R. dominita* and *S. zeamais* and grain samples sampled at each detecting site after 40 days incubation in the laboratory were inspected, and no live insects were found. The results proved that all insects were killed in all warehouses, thus, the fumigation efficacy was excellent. However, one live insect was found at one side door in No. 29 warehouse and on two northern windowsills of No. 16 warehouse. Thus, although the whole air-tightness in the warehouses reached the lowest standard (40 seconds), there still existed local positions with poor air-tightness. The toxic gas leaked from these positions resulting in the PH_3 concentration decreasing, which allowed a few individual insects at some developmental stage of some species of insects survive the fumigation. The phenomenon did not occur in No. 27 warehouse which adopt recirculation fumigation under film. Further more, low dosage, relatively uniform concentration distribution and low fumigation cost were realized. Consequently, it could be speculated that the air-tightness standard in present warehouses only satisfied with the lowest requirement for killing insects. In order to enhance the fumigation effect and reduce fumigation cost, some effective measures should be further taken to improve the air-tightness standard. Additional inspections with workers using sensitive phosphine detectors immediately after fumigations begin to locate major points of significant leakage, then resealing those leaks immediately, may help fine tune the sealing of each warehouse to increase half-life times of 90 sto 120 seconds or higher, like the 147 seconds of the tightly sealed Warehouse No. 27.

Three key factors for high fumigation efficacy are excellent air-tightness, maintaining uniform fumigant concentrations above lethal dosage and retaining lethal concentrations for the desired exposure time. Thus, the air-tightness in warehouses is the primary requirement for maintaining fumigation concentration and its sealed duration. Only if the air-tightness in warehouses reaches higher level, can higher fumigation concentration and its sealed duration be maintained, and the ideal fumigation effect be realized. However, the present poor air-tightness in some warehouses can't completely satisfy that the fumigation will not kill the insects.

By sealing the grain surface with film in Zhoukou Grain Depot, State Grain Reserves, the air-tightness of the warehouses were strongly enhanced, allowing the harmonious combining of recirculation fumigation with grain surface sealing by film. This method could realize sealing grain surface, keep excellent air-tightness (even if serious leaks may exist in warehouse roofs, or walls above the 6 m grain storage levels), accelerate the gas quickly with even distribution in grain mass by recirculation, improve

the air-tightness in the grain storage part of the warehouses, reduce fumigation spaces and application dosage, and enhance the fumigation effect of recirculation fumigation under film. Using film sealing of 6m level grain surface can save much sealing expense of roofs and eaves of current warehouses, making air-tightness easier to maintain as warehouses weather and age during future decades.

2.3 Dynamic Deliquescence Producing Gas Method Resolved the Difficult Problems Such as Fumigant Residues Cleaning after the Whole Recirculation Fumigation, Inconveniently Buying CO_2 Gas, and Higher Fumigation Costs.

The recommended producing gas method in the three national standards and regulations relating to recirculation fumigation was PH_3 – CO_2 mixed gas filled in high pressure steel cylinders (steel cylinder formulation) and whole warehouse recirculation fumigation. However, the former application cost was very expensive, and the latter had the difficult problems such as fumigant residues cleaning after the whole recirculation fumigation, inconveniently buying CO_2 gas, higher fumigation cost, and the transportation inconvenience and costs, and handling safety of high pressure cylinders.

Three fumigation methods, the recirculation fumigation under film through PH_3 dynamic deliquescence, whole warehouse PH_3 dynamic deliquescence and whole warehouse recirculation fumigation, were investigated in Zhoukou Grain Depot, State Grain Reserves. The results showed that economic application cost and simple manipulation for the recirculation fumigation under film through PH_3 dynamic deliquescence was far better than that for whole warehouse PH_3 dynamic deliquescence and whole warehouse recirculation fumigation. The recirculation fumigation under film through PH_3 dynamic deliquescence properly resolvea the difficult problems such as higher cost of gas producing method recommended by national standards and regulations and tedious manipulation. Further more, considering safety and environment protection, the method saved man power, reduced cost, lessened environment pollution, enhanced fumigation effect, The fumigation under film method has great development and extension potential for warehouse storage across China.

Although the dynamic deliquescence gas producing method by applying fumigant in air ducts and grain surface was not recommended by related regulations, its economic application cost and simple manipulation made it be quickly extended. Particularly, since the dynamic deliquescence gas producing method did not need to add CO_2 gas. Then, the dynamic deliquescence gas producing method resolved inconvenience to buy CO_2 gas for backland and grass roots depots, incomplete reaction in PH_3 generator, inconvenient fumigant residues cleaning, long time fumigant application, and so on, meanwhile, reduced greenhouse effect generated from CO_2 gas in the world.

2.4 Advantages of the Recirculation Fumigation under Film through PH_3 Dynamic Deliquescence

2.4.1 Sealing the grain surface could improve the air-tightness in the warehouses

In view of the excellent fumigation effect, sealing the grain mass not only improved the air-tightness in the warehouses, reduced toxic gas leaking, lessened environment pollution, but also avoided the grain again being reinfested by insects, alleviated the negative effect of bad environment factors on stored grain, significantly reduced grain quality spoilage rate, cut down grain moisture content loss, debased fumigant contamination in grain, and enhanced grain storage benefit.

2.4.2 The recirculation fumigation under film could save fumigant, reduce fumigation cost.

The fumigation space was reduced to 3/5 of the warehouse volume and the air-tightness in the warehouses was improved after sealing grain surface. Then the low dosage fumigation could be carried out, and the fumigation dosage was greatly reduced to 1/3 of previous dosages. Because all the insects were killed during the fumigation, one fumigation may maintain no insects occurring for at least two years, and the storage cost was significantly saved.

2.4.3 Low dosage recirculation fumigation under film could reduce phosphide residues in grain

With a defined AlP dosage, the fumigation effect of long time sealing was better than that of short time sealing depending on PH_3 characteristics. Due to poor the air-tightness in the warehouses, to get an excellent fumigation effect, the AlP dosage must be increased which correspondingly increased grain storage cost and made the organic phosphorus residues in grain exceed standards. The grain detecting results in table 6 showed that high dosage fumigation

could increase the organic phosphorus residues in grain, while low dosage fumigation obviously decreased the organic phosphorus residues in grain, far lower than permitted organic phosphorus residues standards in the national health standard GB2715 – 81. Consequently, to get required fumigation concentration by using low dosage, good air-tightness in the warehouses or grain mass must be maintained or enhanced. In practice, excellent fumigation effect should mainly be realized by enhancing the air-tightness in warehouses or grain mass, not by increasing dosage.

Table 6. Phosphide residue content in grain detecting results after fumigation

Permitted phosphide residues content (calculating as PH_3)	≤0.05mg/kg
Low dosage recirculation fumigation under film in No. 27 warehouse	0.03
Whole warehouse PH_3 dynamic deliquescence in No. 29 warehouse	0.12
Whole warehouse recirculation fumigation in No. 16 warehouse	0.10

2.4.4 Sealing the grain surface could reduce moisture content loss, and enhance the depots' economic benefit.

In recent years, tracking comparative moisture content analysis for grain in sealed and unsealed grain mass taken out of the warehouses were conducted in Zhoukou Grain Depot, State Grain Reserves. The results showed that the moisture content of wheat in sealed grain mass was 0.5% – 1.0% higher than that of the wheat in unsealed grain mass when being taken out of the warehouses which had been stored for 4 years. For the 8 856t wheat in No. 27 warehouse, if the wheat price was calculated as 1.20 yuan/kg, just the reduced moisture content loss could increase economic benefit 53 136 yuan.

2.4.5 The effect of film sealing on grain mass temperature

Grain is very susceptible to high temperatures. The higher the storage temperature, the worse the effect on grain quality. The average temperature of grain mass sealed with film in No. 27 warehouse was 1.1 C lower than that of bulk grain mass in No. 29 warehouse (see Table 5). Film sealing decreased gas convection in the grain mass and the negative effect of bad outdoor environmental factors. Surface sealing enhances grain mass heat preservation ability, thus, improves maintenance of stored grain quality.

2.5 The Economic Benefit Analysis about AlP Low Dosage Recirculation Fumigation under Film through PH_3 Dynamic Deliquescence

The detailed expenditures of sealing grain mass, recirculation fumigation under film and whole warehouse recirculation fumigation were listed in table 7.

The table 7 showed that the detailed expenditure items of the recirculation fumigation under film through PH_3 dynamic deliquescence, whole warehouse PH_3 dynamic deliquescence and whole warehouse recirculation fumigation mainly included film purchasing cost, yearly depreciation cost, man power cost for covering the grain with film (covering the whole warehouse took 3 days by ourselves). The saved cost included artificially cleaning, fumigation cost (additional fumigation was not needed because there were no insects after one – time fumigation). Its social benefit was far better than whole warehouse recirculation fumigation. Benefits were reducing toxic gas leaking and organic phosphorus residues content in grain, maintained grain quality with higher selling price. Just the cost savings by reducing grain moisture content loss could make up the fumigation and film sealing cost. By calculating, for the recirculation fumigation under film through PH_3 dynamic deliquescence, the storage cost was 53 % cheaper than that of whole warehouse PH_3 dynamic deliquescence, and 89 % cheaper than that of whole warehouse recirculation fumigation.

Table 7. Fumigation cost comparison of different fumigation styles in three warehouses

No.	Gas method or position	Grain amount (t)	Dosage (kg)	Fumigant cost – (yuan)	CO_2 cost (yuan)	Film yearly cost (yuan)	Subsidy cost (yuan)	Total Cost (yuan)	Cost (yuan/t)
27	Recirculation fumigation under film – PH_3 dynamic deliquescence	8856	12	288	0	375	100	569	0.064

No.	Gas method or position	Grain amount (t)	Dosage (kg)	Fumigant cost – (yuan)	CO_2 cost (yuan)	Film yearly cost (yuan)	Subsidy cost (yuan)	Total Cost (yuan)	Cost (yuan/t)
29	Whole warehouse PH_3 dynamic deliquescence	8916	34	816	0	0	400	1216	0.136
16	Whole warehouse recirculation fumigation	6130	26	624	2730	0	200	3554	0..580

Note: The CO_2 price was 2.1 yuan/kg, AlP 24 yuan/kg. The recirculation fans operated for 60h; the small electricity cost was ignored. Sealing grain surface film, 300kg, actually cost 3000 yuan, but only 375 yuan per year when calculating as average for 8 years grain storage before unloading the grain. Air return tubes for fumigation under film actually had initial cost of 300 yuan; that cost can be spread over several years use, so the cost is so small, it could be ignored. For one fumigation every two years by the recirculation fumigation under film through PH_3 dynamic deliquescence, the average yearly cost per ton grain was $388 \div 2 + 375 = 569$ yuan.

3 Conclusions

AlP dynamic deliquescence, film sealing the grain surface, recirculation under film, low dosage fumigation, and other grain storage technologies, were harmoniously combined in Zhoukou Grain Depot, State Grain Reserves, commendably resolved present, practical and difficult problems, such as high recirculation fumigation cost, tedious manipulation, poor air-tightness, abd worker health and safety.

3.1 AlP dynamic deliquescence producing gas method did not require use of CO_2 gas, and the grain managers did not enter the warehouse to apply the fumigant, or cleanout fumigant residues. The method not only resolved high cost of buying CO_2 gas, inconvenient fumigant residues cleaning and other difficult problems, but also alleviated workers' labor intensity and higher fumigation cost, shortened the workers contacting time with toxic gas, simplified fumigant application manipulation procedure, and enhanced work efficiency.

3.2 Adopting single grain surface sealing and wooden skeletal frame piece sealing method

The method effectively resolved the difficult problem, i. e. extended grain surface not easy to be sealed in large-scale warehouse, and further greatly improved the air-tightness in the grain mass, reduced fumigation space and dosage, saved fumigation cost.

3.3 The technology, recirculation fumigation under film through PH_3 dynamic deliquescence, made organic phosphorus residues content low, reduced PH_3 gas leaking, fumigant contamination on grain and environment, and commendably maintained grain quality.

3.4 Storage characteristics of the sealed grain mass was stable after fumigation. It was not necessary to uncover the film and turn the grain, or operate mechanical aeration. The recirculation fumigation under film through PH_3 dynamic deliquescence avoided the grain being reinfested by insects. The complete efficacy (100% insects killed) from one fumigationresulted in no insects infestation in the warehouse for at least two years. Meanwhile, a mass of grain moisture loss during long-term open bulk grain storage, which resulted in significant grain market weight loss, was effectively resolved. The grain storage aim, that is safe, economical, practical and in favor of environment protection, was realized.

3.5 Because the technology used low dosage and reduced number of fumigations, saved CO_2 gas, integrative application economic and social benefit was greatly enhanced in depot.

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Characteristic of Fumigation Test on Stored – grain Insects in the Northeast Area of China

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Abstract: In this paper, the condition of geography and climate, main stored – grain species and stored – grain insects, the type of the warehouse, the fumigation equipment and medicine, and the main way of prevention and cure for stored – grain insects in the northeast area were briefly introduced. The results and characteristic of the typical field fumigation test in this area were analyzed in details. The fumigation field test includes phosphine recirculation and AlP deliquescence fumigation test in shallow silo, and AlP deliquescence fumigation test in large warehouse.

Key words: phosphine, AlP, fumigation

1 The Condition of Stored Grain in the North East of China

1.1 The Condition of Geography and Climate

The northeast area lies in the northeast part of China, which includes Liaoning, Jilin and Heilongjiang provinces. This region is situated between 120° – 135° east longitude and 40° ~ 50° northern latitude, which belongs to the cold and wet ecosystem area in the northeast Chinese stored-grain regions. Its climatic characteristic is that air temperature varies in great extent in spring and autumn. Sometimes the change exceeds 10℃ each time. In summer, it is very hot in this area with a long sunshine time and plentiful precipitation. The average air temperature is above 15℃. In winter, it is freezing in this area with a short sunshine time. The average air temperature is below 0℃.

1.2 The Main Grain Species and Stored-gain Insects

The grain species mainly includes corn (maize), paddy rice, wheat, and soybean in this area. The stored-grain insects mainly include Sitophilus zeamais, Tribolium castaneum, Ttibolium confusum, Sitotroga cerealella, Plodia interpunctella. Among these insects, the most serious insect pest are Sitophilus zeamais, Tribolium castaneum, Plodia interpunctella. The active time of stored-grain insects is between July and October every year. After October stored – grain insects get into winterization or incubation status.

1.3 the Main Type of Warehouse, Fumigation Equipment and Tablets

The main type of warehouse is shallow (short) silos, and large warehouses. The fumigation equipment mainly includes recirculation fan, recirculation pipe, ventilation pipe, fumigant gas concentration testing tube, phosphine generator, and phosphine concentration tester. The gas recirculation fans are divided into both the fixed and the movable type. The ventilation pipe is fixed. The ventilated passage is built underfloor or on the floor of the storage. The fumigation materials mainly include AlP tablets, chloropicrin and dichlovos. The residual pesticide protection chemicals mainly include Malathion and diatomite.

1.4 The Main Prevention Type of Stored – grain Insects

The main type of prevention and cure for stored – grain insects is by adding phosphine fumigant to the storage, then using the phosphine recirculation fumigation through the ventilation pipe, and by natural gravity phosphine fumigation by laying phosphine tablets and plates on the grain floor or in aeration ducts under the grain floor.

2 The Characteristic of Phosphine Fumigation Test

2.1 Recirculation Fumigation Test in a Shallow Silo

2.1.1 the material

The No. 3 shallow silo of Fuxin Xihe State Grain Depot was selected as a test silo. The capacity of the silo is 11 540 m^3 with 30 m in diameter, 14.5 m in eaves height, and 21 m in total height. In this silo, 7 173 tons of corn was stored with a moisture of 13.5%, a impurity of 0.5%, and temperature between 15℃ and

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25℃. Stored-grain insects include 3 Tineidaes per square meter, 1 Tribolium madens per kilogram, 1 Sitophilus zeamais per kilogram, 10 Troctes divinatorius Muller per kilogram. 56% AlP tablets are used as the fumigant. The recirculation fumigation system is fixed, and the phosphine generator is movable. The measure range of phosphine concentration tester is 0 – 500 ppm, with an accuracy of 1 ppm. The radial ventilated passage is underground.

2.1.2 the method

There are 6 gas sampling tubes in the silo; 5 of the tubes were placed at a depth of 0.3 – 1 m under the grain surface, each being placed in the east, west, south, north and center area. The last one was suspended within the silo to sample headspace gas. Copper mechanical components, instruments, electrical wires and switches were protected by grease coating or sheet plastic covering. The entrance of temperature-testing cable, natural ventilation, mechanical ventilation, entrance of person and large doors were sealed with plastic, gluey paper and glue. An airtight gate was placed over the inlet of the axial flow ventilator and intake of grain were sealed. Recirculation ventilation was done in the silo for 10 minutes to test for leaks, then the place of leaks was checked, and was sealed. According to the user manual of phosphine generator, 16.5 kg AlP and 20 bottles of carbon dioxide gas (25 kg per bottle) was transferred into the silo. Recirculation ventilation was done in the silo, starting within an hour of dosage and was operated during the first two days. The concentration of phosphine in the silo was measured everyday at 9:00 a. m. and 15:00 p. m., respectively, and the results was recorded in details. Recirculation fumigation was carried out for 14 days, followed by natural ventilation for 14 days. The fumigation effect was examined when the concentration of phosphine was below 0.2 ppm.

2.1.3 the results and analysis

No lived insects were found after the concentration of phosphine in the test silo was maintained above 100 ppm for 8 days. Insect pests weren't found in the storage during the following 4 months.

After the start of the fumigation, the uniform concentration of phosphine must be controlled in the silo by operating the fumigant recirculation fan. The length of time of the first recirculation period is ascertained by the measured curve of gas concentration from the 6 sampling tubes. Usually, the more stored-grain and the smaller the recirculation ventilator air volume, the longer the time of first recirculation is needed. Figure 1 gives the concentration curve of the phosphine in the south and north recirculated pipes after a period of time of the initial fumigation. It can seen that the time of first recirculation should be above 9 hours. The time of first recirculation and the total time of everyday recirculation must be controlled in the lowest level in order to diminish the leak loss because of the local positive pressure in the silo.

Because of better diffusibility using recirculation fumigation, it is considered that the leak of phosphine is one-dimensional, with simple and stable molecular diffusion in one direction when the phosphine fumigation of the certain silo is controlled in a uniform concentration. According to the integral of Fick's law $J = -Ddc/dz$, the concentration in the silo at "n" moment can be inferred, i.e., $C_n = (1-k)n-1C_1$, where C_1 is initial concentration, k is a constant. The value of Cn is decided by the air – tightness of the silo, the quantity of stored-grain, the species of stored – grain, everyday recirculation time and weather condition.

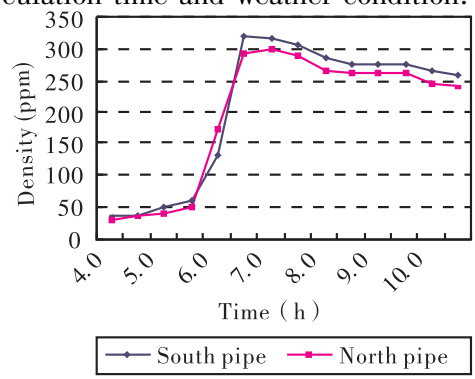


Fig. 1 Phosphine gas concentration in south and north sample pipes

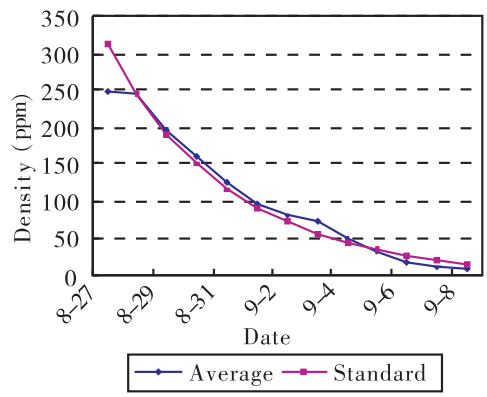


Fig. 2 Recirculation fumigant gas concentration loss rate comparing measured vs calculated rates.

Figure 2 shows the average concentration curve of phosphine and standard function curve

of $C_n = (1-k)^{n-1} C_1$, where $C_1 = 244$ ppm, $C_n = 72$ ppm, $n = 6$, $k = 21.7\%$, that is to say, the concentration of phosphine reduces about 21.7% everyday. It can be seen that the two curves fit well in Figure 2. There exists some deviation when the concentration of phosphine is below 70 ppm, but the actual measured value should be considered as an accurate value. The larger the concentration of phosphine is, the more the moare gas is dissipated in a certain time. Therefore, to minimize total dosage and fumigating by applying only one high concentration of dosage once should be avoided. According to k value of the silo, the concentration of phosphine, and assumed concentration of efficient killing insects, the duration under the concentration of efficient killing insects can be calculated approximatively using the standard function $C_n = (1-k)^{n-1} C^1$.

2.2 AlP Natural Gravoty Fumigation Test in a Shallow Silo

2.2.1 the material

The No. 1 shallow silo of Bayuquan state grain depot was selected as a test silo. The capacity of the silo is 11 540 m^3 with 30 m in diameter, 14.5 m in eaves height, and 21 m in total height. In this silo, 5 700 tons of corn was stored with a moisture of 13.5%, a impurity of 0.5%, and temperature between 1.8℃ and 20.6℃. The height of stored-corn without insects is about 11m. 56% AlP tablets are used as the medicine. The measure range of phosphine concentration tester is 0 – 500 ppm, with an accuracy of 1 ppm. The perforated ventilation ducting is under the silo floor.

2.2.2 the method

The recirculation fumigation method of this silo is the same as the one described in The gas sampling pipes were distributed with one places 1 m above the grain surface, 1 m and 3 m under the grain surface, respectively. Small AlP tablet pockets (pervious cloth bags) were made up outside the silo. According to the pre – arrangement, 8 – 10 persons with respirators came into the silo and probed AlP tablet pockets at depths of 50 – 80 cm under the grain surface through appropriative probe pipe tools. The total quantity of phosphide tablets was 30 kg. 10 kg AlP tablets were put inside 6 entrances of mechanical ventilators at the base of the silo. After the whole silo was obturated (sealed), the place of leak was checked through warning device and then these leak points were sealed. The concentration of phosphine and the temperature of grain were measured everyday. The obturated fumigation lasted for 35 days from 31 May to 6 July. After natural ventilation of 14 days, the fumigation effect can be examined when the concentration of phosphine is below 0.2 ppm by using warning device.

2.2.3 the results and analysis

No lived insects were found when the concentration of phosphine at the place of 3 m under the grain surface and the headspace in the test silo were maintained above 163 ppm for 35 days. Insect pests haven't occurred within 4 months after fumigation, especially undergoing active season for insects.

The concentration of phosphine in the silo headspace reached 105 ppm after 41 hours, which is an efficient fumigation concentration. And the concentration of phosphine at the place of 3 m under the grain surface reached 187 ppm after 17 hours. It has been indicated that velocities of AlP deliquescence and phosphine diffusion in the space are quite fast, and the penetrating ability of phosphine through corn (which is very porous), especially in downward direction, is very strong.

Figure 3 shows the average concentration curve of phosphine and standard function curve of the test silo. It can be seen that the concentration curve of phosphine is divided into two parts. The curve after T days is an attenuation curve of phosphine concentration, which coincides with Fick's law. The concentration of phosphine at "n" moment can be calculated, i.e., $C_n = (1-k)^{n-T} C_T$, where C_T is the concentration of phosphine at "T" moment when AlP gas release comes to end, where k is a constant when the condition of stored – grain is fixed. The curve before T days is an ascending curve of concentration of phosphine. According to the characteristic of phosphine attenuation, when the silo is airtight, the velocity of AlP gas release and the temperature of grain are constants, the concentration of phosphine of "n" moment can be calculated, i.e., $C_n = C_{n-1} + C_w/T - k(C_n + C_{n-1})/2$, where C_w is the concentration of phosphine came from total dosage without loss, k is the loss factor for phosphine concentration. It can be inferred further, i.e., $C_n = C_w/Tk(1 - ((2-k)/(2+k))^n)$, which can be simplified as $C_n = A(1-K^n)$, where A and K are constants. The curves can be drawn by using standard functions, i.e., $C_n = A(1-K^n)$, $C_n = (1-k)^{n-T} C_T$, and the average value of actual testing data. It can be seen that the two curves fit quite well in the Figure 3. However, during the period of AlP gas fumigation, the concentration of phosphine is higher near the

place of probing of the dosage in the silo. So the testing points of phosphine concentration should not be near the points where the phosphine tablets were placed.

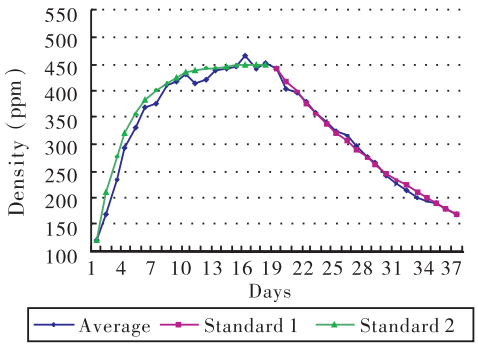


Fig. 3 Gravity phosphine gas concentration loss rate comparing measured vs calculated rates

2.3 AlP Recirculation Deliquescence Fumigation Test In Large Warehouse

2.3.1 the material

The No. 3 large warehouse of Jinzhoujinyang state grain depot was selected as a test warehouse. The capacity of warehouse is 16200 m^3 with 60 m in length, 30 m in width, and 7.88 m in eaves height. In this warehouse 4500 tons of corn was stored with a moisture of 13.5%, a impurity of 1.0%. The height of stored – corn is 4 – 5 m. Stored – grain insects include 13 Tribolium madens and Plodia interpunctella Hubner per kilogram. The ventilation cage passage is on the ground. Recirculation pipe is in a double – side fixed manner. 56% AlP tablets are used as the fumigation dosage. The measure range of phosphine concentration tester is 0 – 500 ppm, with an accuracy of 1 ppm. .

2.3.2 the method

The entrances of cable, mechanical ventilation, axial flow ventilator were sealed with adhesive tape. The doors and windows of the warehouse were sealed with plastic, adhesive tape and rubber strip. There are 8 pipes for testing phosphine concentration in the grain surface. Four pipes were placed in the four corners of the warehouse, the other four were distributed as lines at equal intervals along the center of the warehouse. In the afternoon of 18 September, 28 kg AlP tablets were divided into 6 portions, each of them was wrapped by gauze, put at 6 entrances of mechanical ventilation, and the entrance openings were sealed at once. The first recirculation fumigation lasted for 48 h continuously, then 4 h everyday. The total time of recirculation fumigation was 15 days. The concentration of phosphine in the warehouse was measured everyday. The warehouse remained sealed for 5 days after the recirculation fan was stopped. Natural ventilation was done with doors and windows open in the warehouse the night of 6 October, and then axial flow ventilator was used for 3 days. The concentration of phosphine was measured continually by warning device during the period of ventilation. The prevention staff with respirator began to examine the fumigation effect when the concentration of phosphine is safe.

2.3.3 the results and analysis

Lived insects, the phenomenon of grain fever and dew were not found when the concentration of phosphine in the test warehouse was maintained above 100 ppm for 16 days.

Figure 4 was drawn by using the data recorded at 9:00 a. m. everyday during the period of fumigation. From Figure 4, it can be seen that the concentration of phosphine increases continually after the dosage was added, attains equilibrium after 3 days. Then the concentration of phosphine was maintained between 100 – 160 ppm, which is an effective range where stored – grain insects can be killed. Meanwhile, this range of phosphine concentration has been proved to be favorable to maintain because it is not too high.

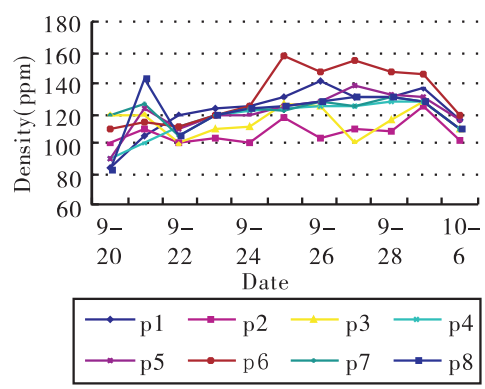


Fig. 4 Fumigation concentration for four corners and centerline of warehouse

The effect of fumigation can be controlled by changing recirculation time everyday and increasing the quantity of medicine. According to the response of grain depot, this method exhibits completed efficacy of fumigation, and it has a merit of simple operation and low cost.

References

[1] National grain bureau, study on construction of northeast grain granary

0416

Case Study: The Use of Low-Oxygen, ECO_2 Controlled Atmosphere Method to Control Insects in Sesame Seed and Dried Figs from Greece

Fred Bergwerff[1]* and Vasilios Sotiroudas[2]

Abstract: The international industry is under high pressure to seek a preventive method to control insects in food commodities that does not lead to any quality reduction of the products. Especially the organic food industry is seeking an effective method that does not leave chemical residues.

The ECO_2 Controlled Atmosphere (CA) treatment is known and used worldwide by many companies and is a very effective method to control insects in all stages of development in food commodities, without using toxic chemicals and negative effect on the quality of the products. CA is currently used to control insects in sesame seed from Greece. Both conventional as well as organic sesame seed is treated with CA. The CA method used is based on low – oxygen in combination with increased temperatures (e. g. 35 Celsius). To prove and document the effectiveness of this method, an experiment was conducted by controlling *Tribolium* and *Sitophilus* (Greek origin) in Sesame Seed and Dried Figs.

Introduction

The objective of this experiment was to prove and document the effectiveness of CA during exposure to sesame seeds and dried figs to control Tribolium and Sitophilus insects.

The experiment was carried out in one of the ECO_2 facilities in Antwerp (Belgium), where sesame seeds and dried figs from two different Greek companies where treated. Extra thermometers and oxygen sensors where placed in various spots to control and document the parameters of the experiment. After treatment the experiment showed 100% control of insects in all stages.

After proving the effectiveness of this experiment, the Greek company decided to construct 6 Controlled Atmosphere chambers (5 of 147 m^3 and 1 of 294 m^3) at their factory in Thessalonica for the treatment of their yearly volume of sesame seeds.

Materials & Methods

Equipment

The experiment was conducted in a climate controlled room, constructed by the company ECO_2 and connected to the ECO_2 converter system, creating the Controlled Atmosphere. The facility is located in Antwerp, Belgium. The volume of the room is 310 m^3. The experiment products and insects species were placed inside the room after which it was closed and hermetically sealed. Inside air is circulated through the ECO_2 converter which creates low – oxygen air of < 1% O_2. Inside room temperature was increased to 2 835 degrees Celsius for optimum insect activity. Extra temperature and oxygen sensors where used for extra data recording.

Products

Products of two different Greek companies where treated during this experiment; one pallet of sesame seeds imported from India and one pallet of sesame seeds from Greece and dried figs from Greece. All products came in normal packaging. The sesame seeds came palletized with external wrapping which was taken off the packaging (Fig. 1). The dried figs came in 2 boxes of 12 kgs each, commercially packed (Fig. 2).

Fig. 1 Pallets of sesame seeds

1. *EcO_2 B. V.*, *P. O. Box* 7488, 3280 *AG Numansdorp*, *the Netherlands*, *E – mail*: *fbergwerff@ eco2. nl*, *Phone*: +31 – 186 651010, *Fax*: +31 – 186 65784)

2. *AgroSpeCom Ltd*, 3, *N. Kountourioti str.*, 546 25 *Thessaloniki*, *Greece*

Fig. 2 Boxes with Dried Figs

Insects

The experiment was performed using most developmental stages (adults, larvae and eggs) of a mixture of several pest species that commonly infest the food products being tested, originated from Greece (Table 1). Insects were placed in the pallets of sesame seeds (Fig. 3.) and in the two boxes of the Dried Figs. Test insects (Fig. 4.) from the same species where held at the Laboratory of AgroSpeCom for control.

Table 1. Insect species, stage, placement during experiment

Code	Insect	Stage	Placement
1	Tribolium	Larvae	Sesame company A
2	Tribolium	Larvae	Sesame company B
2	Tribolium	Larvae	ASC Lab
4	Tribolium	Eggs	Sesame company B
5	Tribolium	Eggs	Sesame company A
6	Tribolium	Eggs	ASC Lab
7	Sitophilus	Larvae	Sesame company A
8	Sitophilus	Larvae	Sesame company B
9	Sitophilus	Larvae	ASC Lab
10	Sitophilus	Eggs	Sesame company B
11	Sitophilus	Eggs	Sesame company A
12	Sitophilus	Eggs	ASC Lab
13	Tribolium	Adults	Sesame company A
14	Tribolium	Adults	Sesame company B
15	Tribolium	Adults	ASC Lab
16	Sitophilus	Adults	Dried Figs box 1
17	Sitophilus	Adults	Sesame Cargill
18	Sitophilus	Adults	ASC Lab
O	Tribolium	Eggs	Dried Figs box 2
OO	Tribolium	Larvae	Dried Figs box 2

Fig. 3 Insects placed in bag of Sesame Seeds

Fig. 4 Insect tubes

Treatment Set up

The two pallets of sesame seeds were placed in the climate controlled room (Fig. 5 & 6.). One box of dried figs was placed on each pallet. Extra data recorders were placed at the following positions:

1 data logger per carton box of dried figs (applied in center of the product)

1 data logger per pallet sesame seeds (applied in center of pallet and bag of product)

After the treatment set up, the door of the climatic room was closed and treatment was started on – line via remote control.

The experiment was conducted in November 2007 which is winter season in Europe. Upon arrival of the experiment, temperature of the products was 11℃. In 2,5 days of CA treatment the product temperature reached the ideal treatment temperature of 32℃. Simultaneously during heating up of the products, the oxygen is decreased to < 1% in the room ensuring 100% control of the insects.

Results

Low-Oxygen, Controlled Atmosphere treatment of the sesame seed and dried figs, experi

Fig. 5 Climatic Controlled Chambers

Fig. 6 Experiment set up

mental infested with Tribolium and Sitophilus, caused 100% mortality of the three developmental stages tested. Total treatment duration (including heating and decrease of oxygen) was 5,5 days (Fig. 7). No negative effects on the quality of the products were observed or reported by the two companies.

After the treatment all insect bioassays were kept at 25℃ for a period of 2 months at the Laboratory of AgroSpeCom, showing 100% mortality.

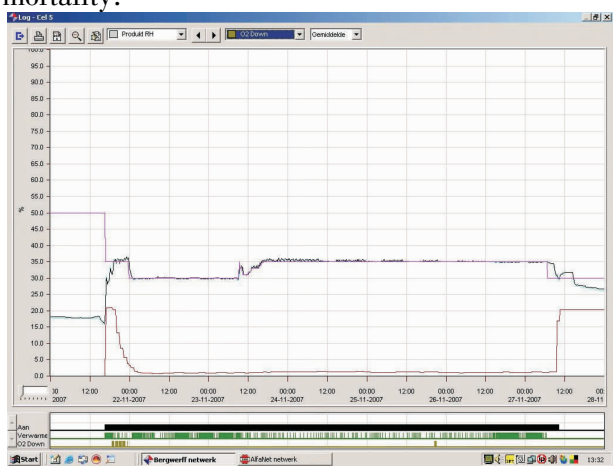


Fig. 7 Treatment graphic (black line = inlet temperature, blue line = room temperature, red line = O_2 concentration, pink line = set point temperature)

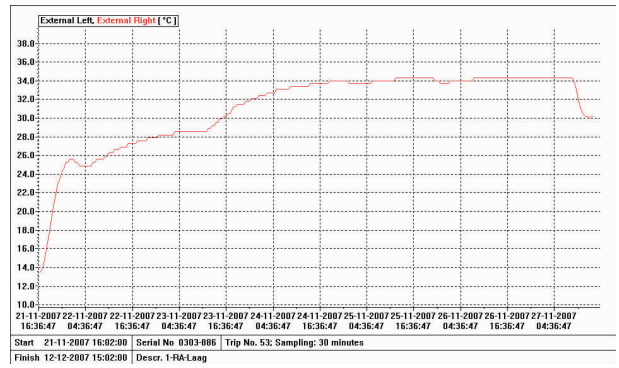


Fig. 8 Extra temperature data logger in box of dried figs

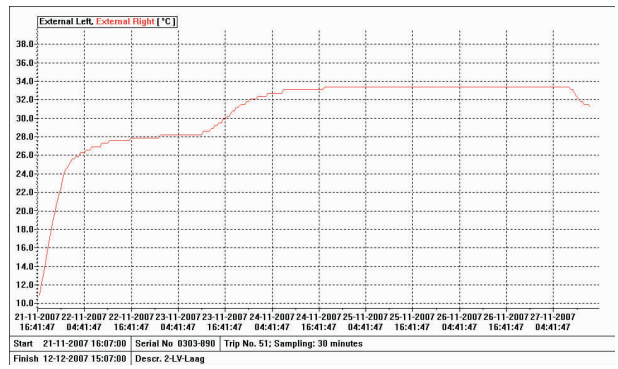


Fig. 10 Extra temperature data logger in pallet of sesame seed

Conclusions

The use of Controlled Atmosphere has several advantages compared with existing methods for chemical insect treatments on products:

Insects usually do not die inside the product. The insects try to escape the low oxygen atmosphere by moving out of the product toward the sides of the chamber, thus moving out of the product.

There is no use of insecticides and thus no residues.

The method is environmental friendly.

The system can be used without waiting for a fumigator.

Each treatment is certified by an internationally recognized certificate of treatment.

No insect resistance is found with the use of Controlled Atmosphere.

There is very low danger for the working personnel.

Controlled Atmosphere treatments are currently carried out by ECO_2 in ECO_2 facilities and customer facilities constructed by ECO_2 worldwide (14 countries) which totally have more than 105 treatment rooms.

0417

Research into the Pest Prevention of Stored Grain in Underground Warehouse with New Earth – Structure

Zhang Longchuan*, Sun Yuhua and Wang Guoli

Abstract: Make use of natural low temperature, low moisture, air tightness of the underground warehouse and take effective eco – methods to keep grain all the year round in the environment of low temperature, low moisture and low oxygen. Without chemical antiseptic and preventative, we've prevented pests and mildew and realized safe storage.

Key words: underground warehouse, stored grain, pest prevention, safe storage

Introduction

With the development of society and improvement of people's living standard, the demand is becoming higher that people should reduce the harmful remainders in grain and grain produce. Safety, sanitation, environmental protection and saving energy have become a necessary choice about the storage of grain. According to the demand-higher quality, more nutrition, more benefit, less loss, less pollution, lower cost-for the development of storage skills, since 2004 we've conducted the experiment with stored grain in the underground warehouse, in which we use eco-methods to prevent pests and mildew. We've made much progress.

Yuanbaoshan State Grain Warehouse lies in the dry area between Inner Mongolia and Xinjiang, where there is little rain and low temperature——the monthly average temperature is below zero centigrade for more than five months, and it has low water level. The water content in the top ten-meter earth-layer is only 11.8%. The whole underground warehouse is water-proof, moisture-proof and airtight. The platform at the top of the warehouse is hardened with concrete. The warehouse also has complete drainage equipment. The whole warehouse is deeply buried under the ground and its top is covered with an more than 4 – meter – deep layer of earth. So all the year round the temperature of the warehouse remains 8 – 12℃ and the warehouse air relative humidity remains 40 – 50%, which forms an ideal grain storage environment with low temperature, low air humidity, airtightness and no oxygen.

1 Materials

1.1 Experimental Warehouse

No. 51 warehouse was the one used for the experiment, was built in 1998. The roof is like part of a spheroid with an arch height of 3.6 meters and reinforced steel concrete structure. In order to prevent moisture, we spread, three layers of asphalt, each separated by a layer of felt, covering a layer of dry brick. The roof is covered with a four-meter thick layer of earth. The main storage compartment is like the frustum of a cone; its top diamete is 18 meters and bottom diameter is 12 meters. The storage compartment height is 15 meters, made of brick. In order to prevent moisture, we spread two layers of felt between three layers of asphalt like the roof. The bottom of the silo is like a cauldron whose depth is one meter. It's also made of brick, with two layers of felt between three layers of asphalt to seal out ground moisture. There are two openings in the underground silo, one at the top and the other at the bottom. The roof inlet is a hollow cylinder, one meter inside diameter and its height is 3.5 meters. The bottom outlet is a door with 2 meters in height by 1.2 meters in width. The door is made of metal and it's airtight. On the door is a grain discharge opening where grain slides out into an unload conveyor. The capacity of the warehouse is 2 500 ton (90 000 bushels).

1.2 Facility Test Instruments

One electrical oxygen-measuring machine; one carbon dioxide-measuring machine, one electrical instrument for measuring grain moisture and temperature.

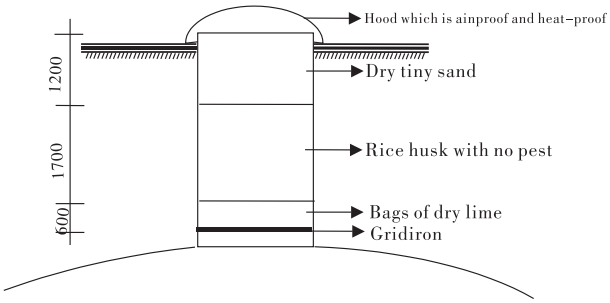
Yuanbaoshan State Grain Storeage, Chifeng, Inner Mongolia, 024070

1.3 Experimental Grain Seed Storage

Experimental or research seed corn which was locally produced in 2003 was stored in this high quality storage center because of the high standard and demand to maintain safe seed moisture content and temperature.

2 Research Methods

2.1 Make best use of natural low temperatures, low air moisture, and air tightness of the underground warehouse, and take storing methods of low temperature and airtightness, rather than applying chemicals and aerating to cool the grain for safe, insect free storage. Use the biotic factors in this hermetic storeage silo to consume oxygen by the physiological activities (storage insects and grain respiration) which reduced the oxygen and increased the carbon dioxide to control pests and prevent mildew.



2.2 The top outlet filler material is supported by a strucutural steel gridiron. Once the silo has been filled, the 1 meter diameter inlet is filled in proper sequence with bags of dry lime, sterilized rice husk with no insects, and dry fine sand. After compacting these three insulating products, we covered it with a rainproof, heat resistant hood then sealed it to make it airtight.

2.3 In order to make the warehouse more airtight, the grain discharge opening at the bottom outlet is sealed up by thin polyethylene film and pressed hard to seal it uniformly. The steel door has rubber gasketing all around to make it airtight.

2.4 Grain condition is examined totally by an electrical monitoring system which provides 24 – hour monitering of the grain temperature and moisture content at selected sampling points every day. In order to improve the accuracy of examining and increase the number of temperature-monitoring points, the whole warehouse is fixed with 17 cables, 3 circles, making a total of 119 temperature monitoring points and 2 sensors for measuring air humidity.

2.5 Dry the experimental grain to reduce grain moisture and disinfest pests and bacteria. Grain goes though the drier which opoerates at a plenum temperature between 100 – 120℃ to dry the grain to safe moisture contents. While drying the moisture, the high temperature will also kill pest eggs and mildew on the grain.

2.6 Chill the experimental grain to temper, and killing insect pests and mildew in low temperatures. After grain is dried, put it into a shed with a hood to make it cool and freeze slowly. Make sure that the grain temperature is lower and lay the foundations of killing pests and mildew by heating, then by freezing. .

2.7 Sterilize the empty warehouse and keep it cool. Sanitize and sterilize the warehouse according to the state grain storage warehouse rules before the grain is loaded into it. Open the top outlet and the bottom outlet on a cold day. With a large axial-flow ventilator blowing cold air downward through the top inlet, give the whole warehouse a non-stop 48 – hour mechanical ventilation to get rid of excess heat and air moisture so that the whole warehouse can be in a state of low temperature and low moisture, which is good for filling with grain.

2.8 Cool the grain a second time while loading it into the warehouse. The loading is all-weather and non-stop. Foreign substance is removed by a slide sifter before entering the warehouse. At the same time, use a ventilator to blow high velocity airflow into the grain slide sifter in the direction opposite of grain flow to remove trash and foreign substances and cool the grain.

3 Experiment in Underground Warehouse

On January, 13, 2004 the empty warehouse was aerated non – stop for 48 hours to remove excess heat and air moisture, when the temperature was 14.7℃ and the air humidity was 38%. After 48 hours, we loaded the whole storehouse with the grain. During the course of loading, we smoothed out the surface every one meter of the loaded grain to guarantee the grain was well-distributed and reduce particle size separation and self-grading. We non-stop loaded 2 347 tons of grain in 24 hours. During the course of it, the two outlets were sealed up on time so that the whole warehouse had an airtight and independent storing environment. And we installed the grain-examining equipment. Then the stored grain got in the normal charge. Having loaded the warehouse with grain, we determined the level of oxygen in the warehouse was 20.8%, carbon dioxide 0.03%, and the ware-

house temperature was 5℃, the grain temperature was 2℃ while the air temperature was 16.5℃ when the grain was loaded. All the grain in the warehouse had no pests. Six months later, we determined the level of oxygen in it was 16.3%, carbon dioxide 4.2%.

Rank	Capacity g/L	Foreign Substance %	Moisture %	Defects %		Color, Odor	Pests
				Total	Musty Grain		
1	716	0.4	14.2	3.8	0.1	normal	no

The normal arrangement of stored grain is carefully and systematically carried out according to the system of grain storage. Grain condition is tightly monitored and controlled by the electrical examining system for grain condition. Make a good collection and analysis of the data of the grain temperature and grain moisture. Strengthen the examination in pests and mold and strengthen the appraisal of the quality so as to ensure the storage is safe. In March, 2007, the experimental corn left the warehouse, when the level of oxygen in it was 11%, carbon dioxide 10% and there was no pest in the whole warehouse.

Rank	Capacityg/L	Foreign substance %	Moisture %	Defects %	Fatty acid value (KOH) mg/g	Taste evaluation	Color, odor	Pests
				Total	Musty grain			
No	715	0.3	14.1	4.1	0.2	35.3	81	Normal

4 Result and Analysis

4.1 The Grain temperature changes a little, and grain condition is stable. During the 38 month storage, the grain temperature was generally kept 6 – 11℃. The grain temperature has a little change, grain condition is stable and we didn't have to turn the grain because the temperature need dropping or the grain quality need adjusting.

4.2 There is no pest case. Judging from the grain condition, grain temperature is generally kept 6 – 11℃, the moisture is 40 – 50%. The whole warehouse is effectively sealed to cut off the influence on it from outside air temperature and moisture. Before loading, use high temperature to heat and cold temperatures to freeze and kill worm eggs and mold sticking to grains. The whole warehouse is sealed to form a condition of low temperature and humidity. Use the biotic factors of stored grain respiration to reduce the level of oxygen in the warehouse and increase the level of carbon dioxide. Destroy the environment in which insects and mold live and reproduce, so that there is no pest case. So we don't need chemical protection and prevention.

4.3 The quality of stored grain is good. Stored grain is in the airtight environment of low temperature, low moisture and low oxygen, so its activities are limited, it lies dormant, and it has little change in quality. We've realized the goal – keep stored grain fresh and guarantee the quality.

5 Conclusion

Use the natural low temperature, low moisture and natural cold source, cool and clean the grain while loading the warehouse, use the special features of the underground warehouse that it has few doors and no windows to strengthen sealing of the warehouse. Reduce the level of oxygen in the warehouse by eco – methods. Leave the stored grain in the environment of natural low temperature, low moisture, low oxygen and airtight store for long periods. Don't put any chemical antiseptic or preventative in the warehouse to control the growth and reproduction of insects and molds. Guarantee the quality, keep it fresh and store safely.

Month	2004			2005			2006		
Juanuary	Top grain Temperature ℃	3.1	Moisture %	Top grain Temperature ℃	6.5	Moisture%	Top grain Temperature ℃	8.8	Moisture%
	Middle grain Temperature ℃	2.8	39	Middle grain Temperature ℃	5.2	41	Middle grain Temperature ℃	6.7	42
	Bottom grain Temperature ℃	1.9		Bottom grain Temperature ℃	4.8		Bottom grain Temperature ℃	6.5	

Month	2004			2005			2006		
February	Top grain Temperature ℃	3.1	Moisture %	Top grain Temperature ℃	6.7		Top grain Temperature ℃	8.9	Moisture%
	Middle grain Temperature ℃	2.9	39	Middle grain Temperature ℃	5.2	43	Middle grain Temperature ℃	6.7	44
	Bottom grain Temperature ℃	2.1		Bottom grain Temperature ℃	4.9		Bottom grain Temperature ℃	6.5	
March	Top grain Temperature ℃	3.1	Moisture %	Top grain Temperature ℃	6.7	Moisture%	Top grain Temperature ℃	8.9	Moisture%
	Middle grain Temperature ℃	2.8	40	Middle grain Temperature ℃	5.3	42	Middle grain Temperature ℃	6.8	43
	Bottom grain Temperature ℃	2.1		Bottom grain Temperature ℃	4.9		Bottom grain Temperature ℃	6.7	
April	Top grain Temperature ℃	3.5	Moisture%	Top grain Temperature ℃	6.7	Moisture%	Top grain Temperature ℃	8.9	Moisture%
	Middle grain Temperature ℃	2.8	42	Middle grain Temperature ℃	5.4	44	Middle grain Temperature ℃	6.8	45
	Bottom grain Temperature ℃	2.4		Bottom grain Temperature ℃	4.8		Bottom grain Temperature ℃	6.6	
May	Top grain Temperature ℃	3.8	Moisture %	Top grain Temperature ℃	6.9	Moisture%	Top grain Temperature ℃	9.4	Moisture%
	Middle grain Temperature ℃	3.1	42	Middle grain Temperature ℃	5.5	44	Middle grain Temperature ℃	7.1	47
	Bottom grain Temperature ℃	2.8		Bottom grain Temperature ℃	5.1		Bottom grain Temperature ℃	6.6	
June	Top grain Temperature ℃	4.1	Moisture%	Top grain Temperature ℃	6.9	Moisture%	Top grain Temperature ℃	9.8	Moisture%
	Middle grain Temperature ℃	3.4	45	Middle grain Temperature ℃	5.7	47	Middle grain Temperature ℃	7.2	49
	Bottom grain Temperature ℃	3.1		Bottom grain Temperature ℃	5.3		Bottom grain Temperature ℃	6.5	
July	Top grain Temperature ℃	4.8	Moisture %	Top grain Temperature ℃	7.1	Moisture%	Top grain Temperature ℃	10.2	Moisture%
	Middle grain Temperature ℃	4.1	47	Middle grain Temperature ℃	5.9	49	Middle grain Temperature ℃	7.3	48
	Bottom grain Temperature ℃	3.7		Bottom grain Temperature ℃	5.5		Bottom grain Temperature ℃	6.4	

Month	2004			2005			2006		
August	Top grain Temperature ℃	5.2	Moisture %	Top grain Temperature ℃	7.3	Moisture%	Top grain Temperature ℃	10.4	Moisture%
	Middle grain Temperature ℃	4.5	49	Middle grain Temperature ℃	6.2	48	Middle grain Temperature ℃	7.3	9
	Bottom grain Temperature ℃	4.1		Bottom grain Temperature ℃	5.7		Bottom grain Temperature ℃	6.3	
September	Top grain Temperature ℃	6.1	Moisture %	Top grain Temperature ℃	8.1	Moisture%	Top grain Temperature ℃	10.7	Moisture%
	Middle grain Temperature ℃	4.7	48	Middle grain Temperature ℃	6.5	47	Middle grain Temperature ℃	7.2	47
	Bottom grain Temperature ℃	4.3		Bottom grain Temperature ℃	6.1		Bottom grain Temperature ℃	6.5	
October	Top grain Temperature ℃	6.3	Moisture %	Top grain Temperature ℃	8.4	Moisture%	Top grain Temperature ℃	10.7	Moisture%
	Middle grain Temperature ℃	4.7	45	Middle grain Temperature ℃	6.7	46	Middle grain Temperature ℃	7.3	45
	Bottom grain Temperature ℃	4.5		Bottom grain Temperature ℃	6.3		Bottom grain Temperature ℃	6.4	
November	Top grain Temperature ℃	6.4	Moisture %	Top grain Temperature ℃	8.7	Moisture%	Top grain Temperature ℃	10.8	Moisture%
	Middle grain Temperature ℃	4.8	42	Middle grain Temperature ℃	6.6	44	Middle grain Temperature ℃	7.3	43
	Bottom grain Temperature ℃	4.7		Bottom grain Temperature ℃	6.4		Bottom grain Temperature ℃	6.3	
December	Top grain Temperature ℃	6.5	Moisture %	Top grain Temperature ℃	8.8	Moisture%	Top grain Temperature ℃	10.7	Moisture%
	Middle grain Temperature ℃	5.1	42	Middle grain Temperature ℃	6.5	43	Middle grain Temperature ℃	7.2	42
	Bottom grain Temperature ℃	4.8		Bottom grain Temperature ℃	6.5		Bottom grain Temperature ℃	6.4	

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0418

Fumigation Activity of Plant Essential Oils against the Adults of *Rhizopertha dominica*

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Abstract: The fumigation activities of 29 plant essential oils against *Rhizopertha dominica* (F.) were determined by the method of treated paper fumigation. At 30 ± 1℃, relative humidity 70%, and concentration of 0.5μl/L, plant essential oils of Cinnamomum cassia, Ilex chinensis and Allium sativum had significance fumigant activities, whose corrected mortalities at exposure times of 24, 36 and 72 hours all reached 98%. Mortalities from Illicium verum and Syzygium aromaticum reached 50% after 72 hours exposure. The corrected mortality of other plant essential oils was less than 10%. The effect of temperature to fumigation activities of 5 plant essential oils was tested. At 20℃ and 30℃, temperature had no significance effect on the fumigation activities of 4 plant essential oils: Cinnamomum cassia, Ilex chinensis, Allium sativum, and Illicium verum, which had significance fumigation activities.

Key words: plant essential oil, fumigant, fumigation, *Rhizopertha dominica* (F.)

Preface

Rhizopertha dominica (F.) belongs to Bostrichidae of Coleoptera, it spreads all over the world and can be seen throughout the year in South China region. It can damage paddy, wheat, corn, potato and their processed goods; the loss in weight of the grain caused by *R. dominica* is 5 – 6 times of its weight, the injured grains are always eaten to empty shell, which will damage the quality and weight of the grain seriously, and it is one of the most common and most important stored grain pests in the grain storage process of our country[1-3].

Plant essential oil is an oleaginous secondary metabolite of plants distilled from natural plants and which has aromatic odor and can volatilize under normal temperatures; its main constituents are monoterpenes, sesquiterpenes and aromatic hydrocarbon derivates. Many reports at home and abroad demonstrated that plant essential oils have several activities on stored grain pests such as prevention, fumigation and killing.[4-6].

Twenty nine essential oils from normal fruits and vegetables, spices or traditional Chinese medicinal materials were selected to perform the research on fumigation activity against *R. dominica*. The aim was to find those oils which have stronger biological activities and to explore their potential effects in controlling stored grain pests. This provides a theoretical basis for control of stored grain pests by plant essential oils and for research of green grain storage technology.

2 Materials and Method

2.1 Tested Insects

R. dominica was provided by the Guangdong Foodstuff Research Institute. It was bred at 25 ± 1℃ and a relative humidity of 70% – 80%. Wheat was from national grain storage depot, and before use, heated at 60℃ to disinfest grain, cooled and adjusted to 14% moisture content. Wheat was placed in wide-mouth bottles and adults added. After seven days, adults were removed. About two weeks after exclusions of the adults from the next generation, adults were removed for testing.

2.2 Tested Plant Essential oil

The tested plant essential oils were self-extracted or provided by Guangzhou Xunyang Essential Oil Development Co., Ltd and Gaoshangmei (Guangzhou) Fine Chemical Co., Ltd. There were 29 oils, from the plants of 17 families and 22 genera. The names and sources of the essential oils are shown in table 1.

Self-extracted essential oils were obtained by vapor distillation. Plant samples purchased from the market were cleaned, dried, and broken into pieces and steam distilled, 12 hours after distillation, the obtained distillate fractions

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were extracted thrice with diethyl ether. The extract was dried with anhydrous sodium sulfate, concentrated under reduced pressure at 30 – 35℃ in a rotary evaporator and recovered in absolute diethyl ether. The obtained oleaginous liquid was the essential oil, stored at 4℃ prior to use.

2.3 Determination of Fumigation Effect

The method was based on an established procedure [7]. A 100mL Erlenmeyer flask was sealed with a cork and tin foil. 30 adult insects were added. A pin was inserted through the centre of the cork and a 1 cm × 1 cm filter paper was fixed to the end of the pin. Measured quantities of oils were dropped from a micro pipette onto the filter paper. The Erlenmeyer flask was sealed with film. The flask was stored at fixed temperatures. Mortality was assessed after 24, 48 and 72 hours, at 20 ± 1℃ and 30 ± 1℃ and 70% relative humidity. The tested concentration was 0.5μL/L. Flasks with no added oil were used as controls. Each control and treatment were replicated 7 times.

3 Results and Analysis

3.1 Fumigation Effects of 29 Plant Essential Oils on *R. dominica*

Results at 30℃ are shown in table 2; the essential oils of *Cinnamomum cassia*, *Ilex chinensis* and *Allium sativum* had significant fumigation effects on adults of *R. dominica*: the corrected mortalities at 24 hour, 36 hour and 72 hour were 98% – 100%; oils of *Illicium verum* and *Syzygium aromaticum* had corrected mortalities at 24 hour, 36 hour and 72 hour of 60.0%, 64.8%, 64.8% and 28.6%, 43.4%, 50.8% separately; for *M. piperita* and *Mentha spicata*, the corrected mortalities at 24 hour, 36 hour and 72 hour were 17.7%, 21.0%, 23.0% and 0.95%, 7.6%, 14.8% separately; the fumigation effects of other essential oils on *R. dominica* were weaker, the corrected mortality after 72 hours were all below 10%.

3.2 The fumigation Effects of Five Kinds of Plant Essential Oils on *R. dominica* under Two Different Temperatures.

Five essential oils which had significant effects on adults of *R. dominica* were tested at two temperatures; the test results are shown in figure 1. Under the test concentration of 0.5μL/L, 72 hours exposure, there was no obvious effect of *M. piperita* oil on *R. dominica* at 20℃, and the effect was significantly lower than at 30℃; however, temperature had no obvious effect on the toxicity of *Allium sativum*, *Ilex chinensis*, *Cinnamomum cassia* and *Syzygium aromaticum*. These four essential oils had significant fumigation effects on *R. dominica* at both 20℃ and 30℃.

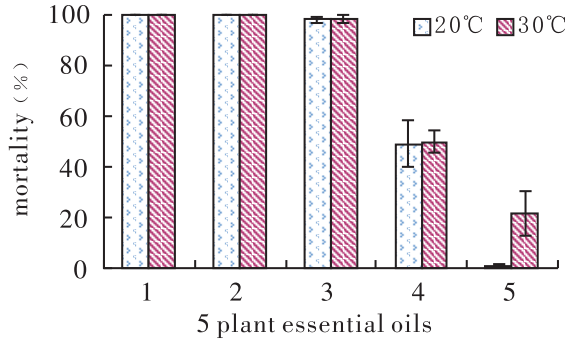

Fig. 1 The fumigation effects of 5 plant essential oils against *R. dominica* adults at temperatures (72h, 0.5μL/L) Plant essential oils: 1. *A. sativum*, 2. *I. chinensis* 3. *C. cassia*, 4. *S. aromaticum*, 5. *M. piperita*

4 Conclusion and Discussion

Green grain storage is a development trend in our country. Performing research and application of the plant source medicines with high effectiveness and low toxicity to replace the existed chemical medicines with high toxicity is an important way to realize green grain storage. However, there was no report on practical application of plant essential oils used as the grain fumigants at home and abroad. The article performed research on fumigation effects of 29 plant essential oils on adults of *R. dominica* initially, in which, under the test conditions, the corrected mortality of *R. dominica* by fumigation of *C. cassia*, *I. chinensis* and *A. sativum* essential oils can reach to 98% and above within 24 hours. The oils can also be effective at 20℃. Essential oils used as fumigants for stored grain pests have prospects, but how to utilize the fumigation activities needs to be resolved urgently.

Acknowledgement

We thank Dr Jim Desmarchelier for help with the manuscript.

Table 1. The source of essential oils for test

No.	Species of plants	Family Names	Genus Names	Part used	Country of origin	Source
1	*Capsicum annuum*	Solanaceae	*Capsicum*	Fruit	China	Xunyang

No.	Species of plants	Family Names	Genus Names	Part used	Country of origin	Source
2	*Zingiber of f icinale* Rosc.	Zigiberaceace	*Zigiber*	Root	China	Xunyang
3	*Citrus limon*	Rutaceac	*Citrus*	Peel	USA	Xunyang
4	*Citrus sinensis*	Rutaceac	*Citrus*	Peel	USA	Xunyang
5	*Citrus paradisi* Macf	Rutaceac	*Citrus*	Peel	Morocco	Xunyang
6	*Citrus reticulata* Blanco	Rutaceac	*Citrus*	Peel	Sichuan, China	Gaoshangmei
7	*Zanthoxylum bungenum* Maxim.	Rutaceac	*Zanthoxylum*	Fruit	Hungary	Gaoshangmei
8	*Piper nigrum*	Piperaceae	Piper	*Fruit*	India	Gaoshangmei
9	*Artemisia argyi* Levl. et Vant.	Asteraceae	*Artemisia*	Stem & Leaf	China	Xunyang
10	*Illicium verum* Hook. f.	Magnoliaceae	*Illicium*	Seed	Guangxi, China	Gaoshangmei
11	*Camellia sinensis*	Theaceae	*Camellia*	Leaf	Guangxi, China	Gaoshangmei
12	*Camellia sinensis*	Theaceae	Camellia	Fruit	*China*	Xunyang
13	*Cinnamomum cassia* Presl	Lauraceae	*Cinnamomum*	Bark	Guangxi, China	Gaoshangmei
14	*Litsea cubeba* (L.) Pers.	Lauraceae	*Litsea*	Fruit	China	Gaoshangmei
15	*Pinus massoniana* Lamb	Pinaceae	*Pinus*	Bark	China	Xunyang
16	*Ilex chinensis* Sims	Aquifoliaceae	*Ilex*	Leaf	Malaysia	Xunyang
17	*Styrax benzoin* Dryand	Styracaceae	*Styrax*	Stem	India	Xunyang
18	*Allium sativum* Linn.	Liliaceae	*Allium*	Fruit	Shandong, China	Gaoshangmei
19	*Allium cepa* Linn	Liliaceae	*Allium*	Corm	Guangdong, China	Extract
20	*Cyclosorus parasiticus* (L.) Farw.	Thelypteridaceae	*Cyclosorus*	Stem & Leaf	Guangdong, China	Extract
21	*Cymbopogon nardus* Stapf	Poaceae	*Cymbopogon*	Full	Guangdong, China	Xunyang
22	*Myristica fragrans* Houtt	Myristicaceae	*Myristica*	Fruit	India	Gaoshangmei
23	Syzygium aromaticum (L.) Merr.	Myrtaceae	*Syzygium*	flower	Indonesia	Gaoshangmei
24	*Eucalyptus tereticornis*	Myrtaceae	*Eucalyptus*	Leaf	China	Xunyang
25	*Agastache rugosus* (F. et M.) Kuntze.	Lamiaceae	*Agastache*	Leaf	China	Xunyang
26	*Rosmarinus officinalis* Linn.	Lamiaceae	*Rosmarinus*	flower	France	Xunyang
27	*Mentha haplocalyx* Briq.	Lamiaceae	*Mentha*	Leaf	China	Xunyang
28	*Mentha piperita*	Lamiaceae	*Mentha*	Stem & Leaf	USA	Xunyang
29	*Mentha spicata Linn.*	Lamiaceae	*Mentha*	Stem & Leaf	China	Xunyang

Table 2. Toxicity of plant essential oils to *R. dominica* adults at 30℃

No.	Essential oils	Corrected mortality %		
		24h	48h	72h
1	*C. annuum*	0.5 ±0.5e	0.9 ±0.6e	3.8 ±2.3ef
2	*Z. of f icinale*	0.5 ±0.5e	3.8 ±1.1e	5.7 ±1.9ef
3	*C. limon*	3.3 ±1.3e	5.7 ±1.7e	9.5 ±2.5def
4	*C. sinensis*	0.5 ±0.5e	2.4 ±0.9e	3.8 ±1.1ef
5	*C. paradisi*	0.00 ±0.00e	1.4 ±0.9e	4.8 ±1.2ef
6	*C. reticulata*	0.5 ±0.5e	0.5 ±0.5e	1.4 ±0.7f
7	*Z. bungenum*	0.9 ±0.6e	0.00 ±0.00e	1.4 ±1.0
8	*P. nigrum*	0.5 ±0.5e	0.00 ±0.00e	0.5 ±0.5f
9	*A. argyi*	1.4 ±0.7e	3.3 ±0.7e	7.1 ±1.3ef
10	*I. verum*	60.0 ±11.9b	64.8 ±10.8b	64.8 ±11.1b
11	*C. sinensis*	0.00 ±0.00e	0.5 ±0.5e	1.4 ±1.0
12	*C. sinensis*	1.4 ±1.0e	3.3 ±1.0e	8.100 ±3.2ef
13	*C. cassia*	98.1 ±1.9a	98.1 ±1.9a	98.1 ±1.9a
14	*L. cubeba*	0.9 ±0.6e	0.9 ±0.9e	6.2 ±2.2ef
15	*P. massoniana*	0.9 ±0.6e	3.3 ±1.6e	4.8 ±1.9ef
16	*I. chinensis*	100.00 ±0.00a	100.00 ±0.00a	100.00 ±0.00a
17	*S. benzoin*	1.9 ±1.0	10.00 ±2.30e	9.5 ±3.2def
18	*A. sativum*	100.00 ±0.00a	100.00 ±0.00a	100.00 ±0.00a
21	*C. nardus*	0.9 ±0.6e	0.00 ±0.00e	1.4 ±1.0
22	*M. fragrans*	2.86 ±1.13e	0.9 ±0.6e	2.9 ±1.3f
23	*S. aromaticum*	28.6 ±7.2c	43.4 ±7.0	50.8 ±4.5b
24	*E. tereticornis*	0.00 ±0.00e	1.4 ±0.7e	3.8 ±1.3ef
25	*A. rugosus*	0.5 ±0.5e	5.7 ±1.7e	3.8 1.5ef
26	*R. officinalis*	1.1 ±0.7e	1.7 ±1.1e	3.3 ±1.5f
27	*M. haplocalyx*	0.5 ±0.5e	1.4 ±0.7e	3.3 ±2.2f
28	*M. piperita*	17.7 ±7.3d	21.0 ±7.3d	23 ±0 8.7c
29	*M. spicata*	0.9 ±0.6e	7.6 ±2.1e	14.8 ±1.6cde

* Tested concentration is 0.5μL/L. Temperature:30℃. Means followed with same letters within the same column are not significantly different at 0.05 level by Duncan's multiple range test.

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0419

Two Stage System for the Destruction of Methyl Bromide from Fumigation Ventilation Streams

Peter J. Joyce * and Roman Bielski

Introduction

Large-scale emissions control of methyl bromide from fumigation vent streams presents a formidable scale-up problem. Industry and government representatives have stated that large-scale emissions control is not likely to be technically or economically viable [1,2] for large tarp fumigations typically done in warehouses. To date, small-scale emissions controls of methyl bromide are commercially done for individual containers of less than 150 cubic meters.

The problem of removing and destroying methyl bromide from large volumes of air > 7, 200 cubic meters, encountered in Quarantine and Pre-Shipment (Q/PS) operations, has been resolved. *Value Recovery*, *Inc.* has solved this problem by employing a sequential two-stage scrubber system that employs carbon adsorption in the first step followed by de-sorption and simultaneous chemical destruction in the second step (Fig. 1). Previously we have reported[3,4] on the ability of a chemical scrubber to instantaneously destroy methyl bromide from fumigation vent streams with a thiosulfate solution. The adsorption step removes methyl bromide from the relatively large volume of fumigation air at ambient temperature. The de-sorption step employs much lower air volumes at elevated temperatures to provide a feed stream compatible with the scrubber. Enough carbon is present in the adsorption cycle to ensure that all of the methyl bromide is retained on the bed without breakthrough into the vent stream. The de-sorption step is designed to remove the methyl bromide from the carbon bed with cycle times required for high throughput distribution characteristic of unloading ships at ports.

During the aeration step that follows a typical fumigation, the concentration of methyl bromide in the exhaust streams shows an exponential decay as the methyl bromide is "swept" from the fumigation enclosure with replacement air as diluted air – gas mixture. In most California Q/PS operations, aeration times are four hours with more than 95 percent of the methyl bromide removed in the first 30 minutes. In order to predict the correct sizing of the carbon bed, one must allow for 3.5 hrs of relative pure air displacing this initial methyl bromide concentration "spike." In order to quantify the performance of the carbon and ensure retention of the methyl bromide, experimental work confirming the carbon bed size was performed.

Experimental

Value Recovery built an apparatus (Figure 2) to quantify carbon performance at identical superficial gas velocities needed for industrial scale-up. It consists of a stainless steel insulated column (2.5 cm diameter) filled with carbon particles to a depth of 127.4 cm. Temperature control was maintained with circulating, pressurized hot water in an external heat exchanger jacket that envelopes the full length of the carbon bed. The inlet, outlet and jacket temperature was measured with multiple 3 – wire RTD's. Inlet and outlet temperature probes were welded into the pipe and protruded directly into the air streams. Adsorption and de-sorption air flow-rate was measured using factory calibrated mass flow-meters (Cole-Parmer). The outlet methyl bromide concentration in air was measured via Infra Red absorption with a Spectros Instruments (Hopedale, MA) methyl bromide analyzer. Calibration gas standards of 1.50, 0.76 and 0.18 volume percent methyl bromide in air (Scott – Marin Riverside, CA) were used to check the calibration of the IR analyzer. The calibration standards along with the mass flow-meters were used to simulate the exponential concentration decay flow loading of methyl bromide onto the carbon column. All of these instruments were connected to a National Instruments Labview® data acquisition system connected to a personal computer. Data points for all instruments were taken every 30 seconds and recorded and time-stamped in an excel

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spreadsheet.

Math Model

Professor Genarro Maffia[5] of Widener University provided a numerical solution for adsorption of an organic compound from liquid streams. The model was modified for gases since it is based on first principals, and the gas density change (<4 percent) is assumed negligible across the carbon bed. The same model was used to predict both adsorption and de-sorption cycle performance. Inlet gas concentration to the carbon bed was based on a typical exponentially decaying feed stream exiting the fumigation chamber[4].

Input data parameters for the model are:

Temperature, C

Initial Fumigant Concentration, c(0,0) g/m^3

Fumigation Volume, V, m^3

Gas Flow – rate, G, m^3/s

Carbon Bed Diameter, D, m

Carbon Bed Length, L, m

Gas Constant, R, 0.0821 Lit – Atm., Deg – Gmol

Carbon Particle Length and Diameter, m

Carbon Bulk Density, kg/m^3

Methyl Bromide and Air Critical Constants, Tc – Deg K, Pc – Atm

Fumigated Commodity Uptake – Fraction of "dead volume," dimensionless

Pressure, Atm

Diffusivity Parameter constants

Fruendlich Isotherm Constants for Methyl Bromide Adsorption on Carbon

Time Increment, dt, sec.

Column Differential Slices, n, dimensionless

Calculated parameters from the model are:

Space Velocity (V/G) time constant, seconds

Gas Phase Concentration, c, (function of time and column position), kg/m^3

Solid Phase Concentration, q, (function of time and column position), kg/m^3

Equilibrium Gas Phase Concentration, ce, (function of time and column position), kg/m^3

Carbon Particle Area, A, m^2

Number of Carbon Particles in a differential slice, Np, dimensionless

Gas Viscosity, μ, kg/m/s

Gas Density, ρ, kg/m^3

Diffusivity of Methyl Bromide in Air, *D*, m^2/s

Reynolds No., $N_{Re} = dv\rho/\mu$, dimensionless

Schmidt No., $N_{Sc} = \mu/\rho/D$ dimensionless

Mass transfer constant correlation coefficient, j_D, dimensionless

Mass transfer coefficient, kc, m/s

Superficial Gas Velocity, v, m/s

Mass of Carbon in Bed, MC, kg

dz – differential column length, m

The key equations for solving for the gas phase concentrations at all times t, everywhere in the bed, z are:

1. c(t,z) = c(t – 1,z) + vdz * dt * (c(t,z – 1) – c(t – 1,z)) – (k * A * dt/vd) * (c(t – 1,z) – ce(t – 1,z))

2. c(t,z) = c(t. z) + *D* * dt * (c(t – 1,z) + c(t – 1,z – 2) – 2 * c(t,z – 1))/dz^2

3. q(t,z) = q(t – 1,z) + (k * A * dt/(1 – vd)) * (c(t – 1,z) – ce(t – 1,z))

4. ce(t,z) = (q(t,z)/a)^(1/p)

where

c – gas phase concentration in kg/m^3

v – superficial gas velocity, m^3/s

dt – differential time, s

kc – mass transfer coefficient, m/s

A – Area of carbon particles in a differential slice, m^2

vd – particle void fraction, dimensionless

ce – gas phase concentration in equilibrium with adsorbed methyl bromide, kg/m^3

D – Diffusivity of methyl bromide in air, m^2/s

q – Adsorbed methyl bromide, kg/m^3

a, *p* – Freundlich Isotherm parameters

For every time increment, dt, the mass balance and equilibrium concentrations were solved for the entire column. The mass transfer coefficient was obtained from a correlation provided by Sherwood, Pigford and Wilke[6].

The Freundlich Isotherm parameters were obtained from a regression of methyl bromide gas – solid equilibrium data provided by Snyder and Leesch[7].

The model runs in True – Basic and prints out all inputs, calculated values and all three calculated concentrations for every bed position at the end of the run. It also plots the gas phase concentration vs. bed depth as a function of time on the computer screen while the calculations are progressing.

Experimental Results and Discussion

During the adsorption step, the mass of

methyl bromide loaded on the column was obtained by summing the flow-rate provided by the mass flow-meters times the known feed concentration while methyl bromide was being fed. During the de-sorption step, the mass of methyl bromide removed was obtained by summing the flow-rate times the concentration of methyl bromide provided by the IR Analyzer. The latter was obtained every 30 seconds over 16 hours (~1 900 data points).

Table 1 shows data for two corresponding adsorption & de-sorption cycles of methyl bromide on carbon. The loading of methyl bromide is very low, 2.5 wt%. The mass balance closure to within 96% (out/in) for both runs gives us confidence that the methyl bromide is well accounted for and comes off the bed at predicted rates. Temperature control was not used during the adsorption step and the temperatures shown for adsorption correspond to ambient temperatures. The de-sorption cycle time was approximately 4 times longer than the adsorption cycle time. The temperature was set at 98.5 to 101C.

Figure 3 compares the model and experimental data for the methyl bromide concentration in air (PPM) exiting the carbon bed for the de-sorption step. Figure 4 shows the cumulative amount of mass desorbed from the bed as a % of the amount loaded or charged during the adsorption step. The math model predicts that methyl bromide should come off approximately 15 percent faster than that shown by the experimental data. The two experimental runs were in relatively close agreement with regard to the mass balance and cumulative mass desorbed. The data suggests that the discrepancy comes from an extrapolation in the use of Freundlich equilibrium isotherm parameters. We believe that equilibrium data for methyl bromide adsorbed on carbon is needed at higher temperatures to eliminate the discrepancy. However, from a design point of view, knowing that the model and the data differ by only 15 percent still makes the model a valuable scale-up tool.

Conclusion

Experimental data combined with a model show that the scale-up of a two-stage process for destroying methyl bromide from very large scale Q/PS and structural fumigations is a technically and economically feasible option. Predicted mass adsorption and de-sorption of methyl bromide from a carbon bed provide methyl bromide concentration profiles that show that the de-sorption from carbon combined with a chemical scrubber will destroy methyl bromide in concentration ranges demonstrated in previous commercial trials. The time needed for the de-sorption cycle is four times that of adsorption and is easily allowed for in the design of sequential fumigation operations. Applying this system to large scale operations and smaller ones as well will make a major impact on protecting both bystanders and preserving the ozone layer. A commercial installation of this two-stage system is planned for start-up in 2009 for the Port of Stockton, CA, USA.

Table 1 Methyl Bromide Adsorption and De - sorption Data

Run No. 1	2	
Adsorption		
Grams MB Loaded	7.78	7.73
Loading %	2.50%	2.48%
Temperature, C	16.2	17.8
Time - Hours	4.0	4.0
De - sorption		
Grams MB Removed	7.50	7.49
Temperature, C	98.5	101.0
Time - Hours	16.2	15.1
Mass Balance (Out/In x 100)	96.4%	96.9%

Column Parameters:	
Diameter	2.5cm
Bed Depth	127.4 cm
Carbon Charge	311.8 gms
Carbon Density	0.457 Kg/m3

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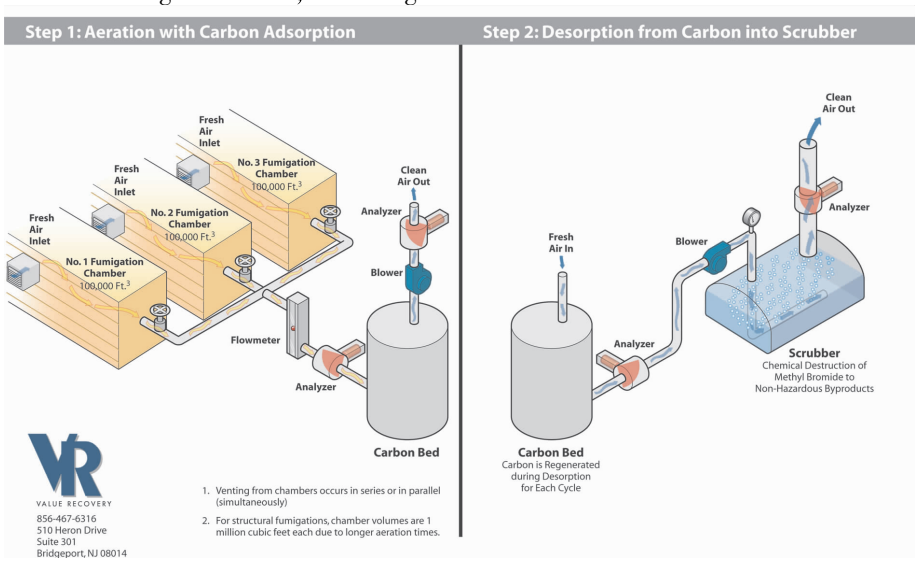

Fig. 1 Value Recovery Methyl Bromide Scrubber System For Very Large Q/PS and Structural Systems

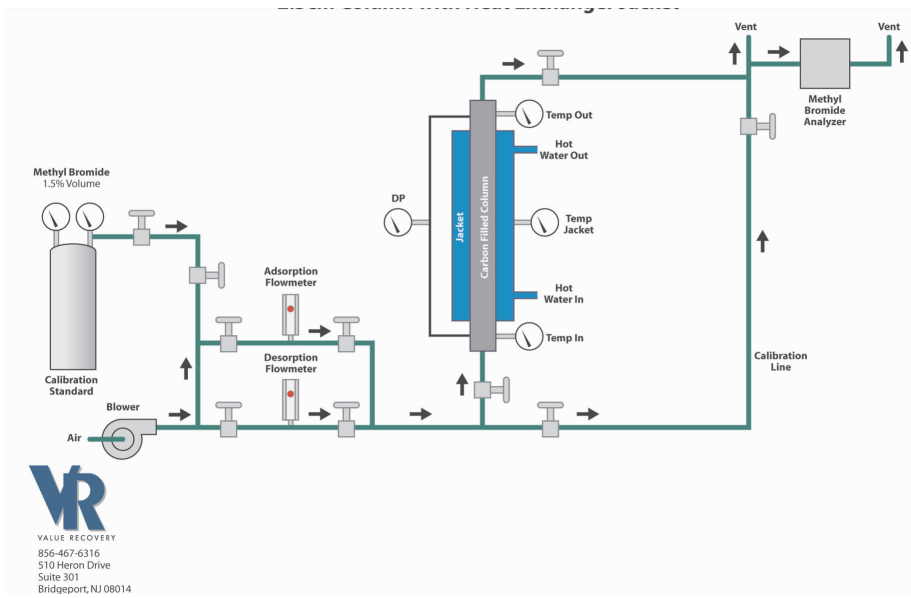

Fig. 2 Experimental Carbon Column 2.5cm Column with Heat Exchanger Jacket

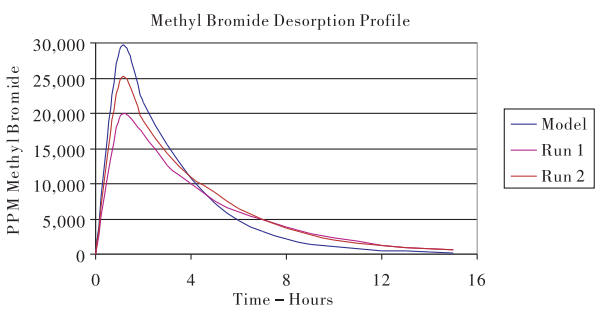

Fig. 3

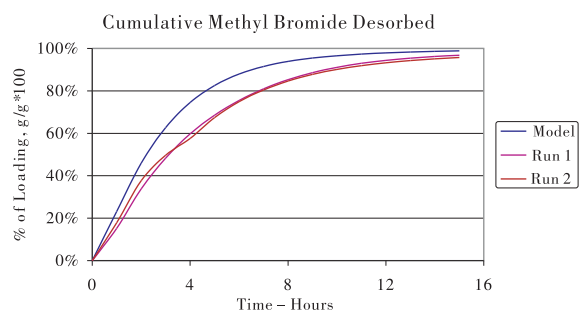

Fig. 4

0420

Research of Phosphine Re-circulation under Plastic Sheet for Grain Fumigation in Horizontal Warehouse with Aluminium Phosphide in Air-duct

Tian Hua[1], Zhou Shifa[1], Chen Mingwei[1] and Gong Qing[2]

Abstract: To effectively control the stored grain pests, the technology of phosphine re-circulation under plastic sheet for grain fumigation by placing aluminium phosphide (AlP) in air-ducts was researched. First, the aluminum phosphide tablet was placed in air – ducts, then reacted with moisture in the atmosphere and released phosphine. Meanwhile, phosphine was blown into grain bed by the circulation fan. In this way, the phosphine dispersed and distributed uniformly throughout the grain in the warehouse. All stages of stored grain pests were controlled by an effective concentration of phosphine held for enough time. In fact, this technology was the combination of "phosphine re-circulation fumigation under plastic sheeting" and "placing AlP at air – duct", which has many advantages, such as flexible and safe operations, functional, economical, reduced pollution to grain.

Key words: placing AlP in air – duct; re-circulation fumigation

Introduction

When we use phosphine re-circulation technology to control stored grain pests in horizontal warehouse, there are several ways of placing AlP, such as: (1) placing pellets or tablets on the grain surface, (2) using inserting tube or "probing", (3) packing and burying the phosphine under grain surface, (4) using on-site phosphine generator, and (5) high pressure steel cylinders of "2% phosphine + 98% carbon dioxide" gaseous mixture for grain fumigation. But, all these methods have drawbacks, such as heavy workload of methosds 1 – 3 methods; long duration of using (4) on-site phosphine generator, difficulty of purchasing carbon dioxide, complicated operation, or (5) the expensive steel cylinderized "phosphine + carbon dioxide"..

In this study, the phosphine gaseous generated by the AlP was placed in the air-duct of warehouse and was blown through the grain by the circulation fan, distributing the gas uniformly in the grain. All stages of stored grain pests were effectively controlled by the uniform concentration of phosphine for enough time for efficacy. This technology was combination of "phosphine re-circulation fumigation under plastic sheeting" and "placing AlP in air-ducts", which has advantages of flexible and safe operations, functional application, economical process, reduced pollution in grain, and easy recovery of phosphine residual dust from aeration ducts. This application method promotes the contintued development of phosphine re-circulation fumigation technology in horizontal warehouses throughout China and other countries.

1 Materials

1.1 Test Depot

Test depot: Rushan Depot of State Grain. No. 2, No. 3 (both warehouses placing AlP tablets in air – ducts).

Control depot: Rushan Depot of State Grain. No. 4 (using on – site phosphine generator).

Each of depots, equipping with above – ground ducting, five vent openings with 10 ventilation plants, fixed phosphine re-circulation fumigation system and microcomputer detecting system, was placed into service in 1999. See Table 1 for more details of depots.

Table 1. Detailed information of test depots in Rushan Depot of State Grain

No.	Grain	Quantity (t)	Height (m)	Volume (m^3)	Grain moisture content (%)	Depot air temperature (℃)	Grain temperature (℃)			Pests density (Insects/kg)
							Upper layer	Middle layer	Bottom layer	
2	Wheat	5763	6.02	6957	11.9	28	24	16	14	2^a, 1^b, 1^c

1. *China Grain Reserves Corporation, Shandong Branch (No. 29 East Wenhua Road, Jinan, Shandong, 250014, China)*

2. *Rushan Depot of State Grain (Station in Xiachu Town, Rushan, Weihai, Shandong, 2645014, China*

No.	Grain	Quantity (t)	Height (m)	Volume (m^3)	Grain moisture content (%)	Depot air temperature (℃)	Grain temperature (℃)			Pests density (Insects/kg)
							Upper layer	Middle layer	Bottom layer	
#3	Wheat	5661	6.0	6735	12.0	28	24	14	13	2^a, 1^b
#4	Wheat	5000	5.6	6284	12.2	26	23	10	11	1^a, 1^b

Note: The water content, depot air temperature, grain temperature and pests density was recorded on August 8 2007, before test, respectively. The a, b, c in "Pests density" row corresponding to *Sitophilus zeamais*, *Tribolium castaneum*, *Oryzaephilus surinamensis*, respectively.

1.2 Test Insects

Rhizopertha dominica Fabricius, *Tribolium castaneum* (Herbst), *Sitophilus zeamais* (Motschulsky), were reared in Chengdu Grain Storage Research Institute, State Administration of Grain, Sichuarn, P. R. China). Three insect specieis were all phosphine resistant strains.

The insects were placed in a small cloth bag, in which insects feed occupied one thirds volume. There were 30 insects of each strain in every bag.

1.3 Equipment and Insecticides

Fixed phosphine re-circulation fumigation system (Weilai Machines Engineering Co.; Ltd, Henan, P. R China); Phosphine monitoring device, HL-210 (New Hualao S&T Co., Ltd, Beijing); Home-made in-bin reverse flow gas distribution network under plastic sheet; 56% AlP tablets (Yongfeng Co., Ltd, Jining, Shandong, China)

2 Methods

2.1 Sealing and Gas Tightness Measuring

The home-made in-bin reverse flow gas distribution network was composed of Φ110mm main top suction (reverse flow) PVC pipe, Φ75mm branch suction (reverse flow) U-PVC pipes which contained no lead or cadmium, and the external blower and piping re-circulation fumigation system. Drilled holes on branch pipes A, B, C, D, E and F accounted for 10%, 12%, 14%, 16%, 18%, 20% of total areas of A, B, C, D, E and F, respectively. There were no holes on the main reverse flow pipe. The main and branch suction (reverse flow) pipes were connected and buried 30 centimeters deep below grain surface in advance. The main suction (reverse flow) pipe was connected with external re-circulation fumigation system. The level grain surface was firmly sealed with plastic sheeting connected to warehouse walls. The distance between the ends of branch pipe and the wall was 60cm. The interval between branch suction pipes was 4.9 m.

To detect the gas tightness of test warehouses, we measured the half-life time of negative pressure, -500 Pa to 250 Pa, in each warehouse. The negative pressure was made by centrifugal fan connected to in-floor ventilation ducts. The half-life times of No. 2, No. 3 and No. 4 warehouse were 124 s, 131 s and 114 s respectively. The values were in line with the standards of re-circulation fumigation set by the state. See Fig. 1 for more details of in-bin gas suction (reverse flow) piping network under plastic sheet.

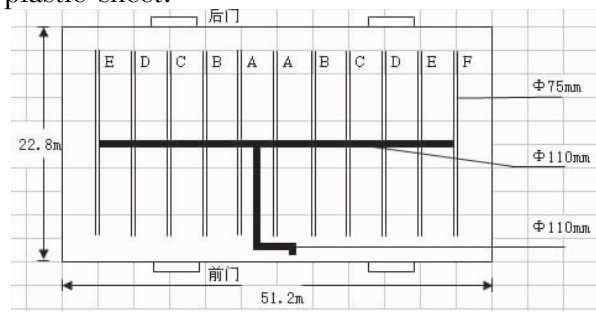

Fig. 1 The graph of in-bin suction (reverse flow) gas piping network under plastic sheet

2.2 Arrangement of Phosphine Monitoring Pipes and Test Insects

The arrangement of phosphine monitoring pipe and test insects grain depths and horizontal spacings are shown in Fig. 2. The points in the corner were at a distance of one meter from the wall. There were three kinds of test insects at each phosphine monitoring point. The phosphine monitoring pipe and test insects were placed to the specific depths by grain vacuum sampling probes.

2.3 Placing AlP at Air-duct

The quantity of AlP was calculated at 1.5g/t. The aluminum phosphide tablet was put into cotton bag (at most, 1 kg/bag). Each bag was tied with a long rope. The bags were placed in underfloor air-ducts. To prevent the tablets being too centralized to be safe, we spaced the bags along each air-duct using a long push-pole. The ends of the rope tied to the bags were tied together and put at the opening of the air-duct for convenient spent ash recovery after fu-

migation. Air-duct openings were sealed afters the AlP was placed.

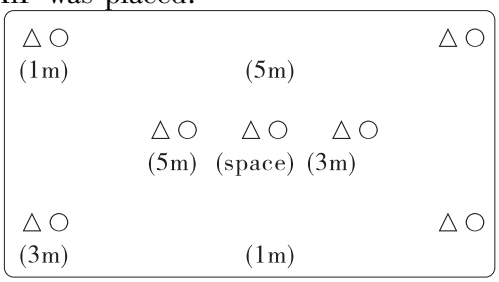

Fig. 2. The distribution of Phosphine monitoring points and test insects

Note: The symbols of "△" represent the sampling bag of Rhyzopertha dominica, Sitophilus zeamais, Tribolium castaneum, respectively. The symbols of "○" represent the phosphine monitoring point. The "*(m)" shows depth below grain surface of monitoring point and test insects bag.

In consideration of low moisture content of wheat, low relative humidity in warehouse and too dry air in air-duct, wet sand was placed in air-ducts to increase air humidity and promote faster reaction speed of phosphine generated from AlP.

2.4 Phosphine Re-circulation and monitoring

2.4.1 Intermittent re-circulation

Intermittent re-circulation fumigation was adopted after placing of AlP. It was supposed to turn off the electric power of the re-circulation device when phosphine concentration reached the level of basic balance (the ratio of highest concentration to lowest concentration should be at most 4:1). Twenty-four hours after starting the fumigation, we measured the concentration and made a decision of continuing re-circulation or not, according to the phosphine concentration we measured.

2.4.2 Phosphine monitoring

When re-circulation blower was going, the phosphine concentration was measured every 8–12 hours. After re-circulation blower was stopped, it was measured every 24 hours by HL-210 phosphine monitoring device. After the highest (peak) concentration was detected, gas levels were measured every 72 hours.

2.4.3 Inspection of insect mortality

To compare the insect density before and after this test, the grain was sampled at the same place in test warehouse before and after fumigation, and the insect in sampling grain was counted. At the same time, the insect sample bags, buried in advance, were taken out. Then, the adults were separated and reared on a diet of 10g wheat flour in an air-conditioned room at 28 ± 1℃, relative humidity (RH) 65%–75%. The adult insect mortality was detected and recorded after fourteen days, and was corrected according (compared) to the control mortality. To detect the growing of F_1, material left on the wheat floor was put into a new rearing bottle and reared under the conditions described above for 42 days.

2.4.4 Detection of Phosphine residue on wheat

The phosphine residue was determined using gas phase chromatography. Fifty kilograms of crushed fumigated wheat was put into a round bottom flask and then added to 150 mL water, and covered the flask by a grinding lid with rubber pad. Then, five ml concentrated hydrochloric acid was added into the flask at the location of silicon rubber. The flask was shook in a supersonic cleaner for five minutes. Then, the mixture in the flask was incubated for 30 min before measurement. The supernatant gas was used as residual Phosphine sources. The quantity of Phosphine residue on wheat was figured out according to the PH_3 standard curve regression equation.

3 Results

3.1 Insect Mortality

In our study, the mortality of all kinds of insect in test depot and control depot was 100% after fumigation. The mortality of resistant insects, buried in test samples in the depot warehouse grain during the tests, was checked after 30 days incubation, and was also 100%.

3.2 Development of PH_3 Concentration in Fumigation

In No. 2, No. 3 depot, average uniform concentration (over 80 mL/m^3) attained after 48 hours fumigation. Maximum concentration (281mL/m^3) attained in 144 hours in No. 2 depot, and maintained at 142 mL/m^3 26 days later. Maximum concentration (272 mL/m^3) attained in 152 hours in No. 3 depot, and maintained 138 mL/m^3 26 days later. In No. 4 depot (control depot), average concentration (over 80 mL/m^3) attained in 8 hours, maximum concentration (290 mL/m^3) attained in 30 hours, and maintained 124 mL/m^3 26 days later.

3.3 Phosphine Residue on Wheat

The PH_3 residues found on wheat of No. 2, No. 3 and No. 4 depot were 0.0054mg/kg, 0.0062mg/kg and 0.0048mg/kg, respectively. All residues were within the state's standards. However, compared to Phosphine re-circulation under plastic sheet fumigation using on-site phosphine generator, the residue of Phosphine re-circulation under plastic sheet by placing AlP in air-ducts was slightly higher.

4 Discussion

4.1 Safety of Phosphine re-circulation Fumigation under Plastic Sheet by Placing AlP in Air-ducts

For traditional phosphine fumigation (without Phosphine re-circulation system), the PH_3 generated from AlP tablets spread by the difference in gas pressure concentration. It was a static process. It was not safe because of high local concentration near dosage placements sites. So, the quantity of AlP tablets placed in each air-duct was limited. Compared to traditional phosphine fumigation, Phosphine re-circulation fumigation under plastic sheet by placing AlP in air-duct was safer for its flowing air, dynamic process and uniform PH_3 concentration. So, it's necessary to equip sites with alternate electric source using an emergency generator before fumigation to maintain operation of recirculation blower when needed if local electric power failure occurs during the fumigation.

4.2 Validity and environmental Protection of Phosphine re-circulation fumigation under Plastic Sheet by Placing AlP in air-ducts

According to our test, the PH_3 dispersed rapidly and uniformly and controlled the insects effectively. Three methods, re-circulation fumigation with on-site phoshpine generator, re-circulation fumigation with high pressure steel cylinder phosphine + CO_2, re-circulation fumigation under plastic sheet with placing AlP in air-ducts, were all effective for controlling insects with no fumigation trap. The method of re-circulation fumigation under plastic sheet with placing AlP in air-ducts was really a good technology to control insects for grain, because of reducing quantity of AlP, reducing fumigation cost, reducing discharge amount of CO_2 and poisonous gas, and no need of CO_2 for fire retardant, and easy recovery of AlP dust in cloth bags from air ducts by pulling all bags from ducts with ropes.

4.3 Comparison of Three Different Way of Generating PH_3 in fumigation

There were three different sources of phosphine in re-circulation fumigation, they were as follows: phosphine from on-site phosphine generator, high pressure steel cylinder phosphine + CO_2, phosphine from natural deliquescence of AlP tablets placing at air-duct. The first two methods were widely used nowadays. But, high pressure steel cylinderized phosphine + CO_2 was expensive and not convenient to purchase and transport to the site and return after fumigation. For the on-site phosphine generator, the CO_2 was essential to fire resistance of phosphine in fumigation. But, the efficient synergy concentration of CO_2 for controlling insects did not occur as the amount of CO_2 was not properly balanced with PH_3. The positive pressure, formed by large amounts of CO_2 under plastic sheet, promoted the leakage of poison gas during the fumigation, and shortened the PH_3 efficient concentration time. At the same time, the operation of on-site phosphine generator increased the labor intensity, exposure time to poison gas and costs of CO_2. However, phosphine re-circulation under plastic sheet fumigation with placing AlP in cotton bags in air-ducts was different from the first two methods. The negative pressure formed by re-circulation fan retarded the loss of PH_3 concentration and prolonged the hours of PH_3 at effective concentrations for high efficacy for all insect stages during the fumigation.

4.4 Economic Effect of Phosphine Re-circulation Fumigation under Plastic Sheet by Placing AlP in Air-ducts

Under the direction of the experts from Chengdu Grain Storage Research Institute, we compared the costs difference among fumigations of different phosphine resources (phosphine from on-site phosphine generator, high pressure steel cylinderized phosphine + CO_2, phosphine from natural air humidity generation of AlP tablets placing in air-duct) by fumigation tests in horizontal warehouses in August 2008. The result indicated there was no difference of controlling effect among fumigation with different phosphine resources. But, there was significant difference of costs among fumigation methods with different phosphine resources. See Table 2 for more details of costs of fumigation with different phosphine resources.

Table 2. Costs comparison of fumigation with different phosphine resources.

Fumigation method	Depot No.	Test time	Operation time	Labor force	Costs(yuan /ton grain)
A	2、3	2007.8	10min	2	0.070
B	5	2007.8	4h	2	0.210
C	2	2007.8	10min	8	0.256
D	3	2007.8	8h	4	0.337
E	1	2007.8	8h	4	1.080

Note:

a) The symbol "A" represents "placing AlP tablets in air-ducts + re-circulation fumigation under plastic sheet".

b) The symbol "B" represents "phosphine generated from on-site phosphine generator + re-circulation fumigation under plastic sheet".

c) The symbol "C" represents "placing AlP tablets on surface of grain stack + re-circulation fumigation".

d) The symbol "D" represents "phosphine generated from on-site phosphine generator + re-circulation fumigation".

e) The symbol "E" represents "steel cylinderized phosphine + re-circulation fumigation".

5 Conclusion

The greatest superiority of "Re – circulation fumigation under plastic sheet with placing AlP tablets in air-ducts" was that it promoted good phosphine dosage dispersion and distributed gas rapidly and uniformly through the grain bulk. Advantages of this method were as follows: flexible and safe operations, functional, economical, reduced pollution to grain, long efficient concentration, easy dust residue recovery.

In general, this technology was a combination of "phosphine re-circulation fumigation under plastic sheeting" and "placing AlP in air-ducts", which has many advantages, such as uniform distribution of PH_3, flexible and safe operations, functional, economical, reduced pollution to grain, decreased quantity of PH_3 used, good sealing property, and easy recovery of residual phosphine dust by pulling cloth bags from aeration ducts. The technology was particularly suitable for warehouse with broad space or plastic sheet.

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0421

Application of Phosphine Slow-releasing Fumigation

Lu Jianhua, Liu Shulun, Jia Shengli, Wang Sulin, Wang Fengqi and Liu Guoqi

Abstract: The paper introduced the current status of phosphine application, especially application of phosphine slow – releasing fumigation. For various warehouses (e. g large warehouse, horizontal warehouse and silo), storage ways (e. g bag or bulk storage), insect species and their developmental stages, the corresponding fumigation techniques, dosage and seal time were adopted. e. g. Phosphide aluminium 3g/m^3 was applied in bulk grain in large warehouse; 2g/m^3 and 5g/m^3 in stack of bagged grain and bulk grain respectively in horizontal warehouses, 3g/m^3 for bulk grain in silos. When average temperature of grain was 19 – 28℃, phosphine concentration can reach 200 mL/m^3 in all above warehouses at different time and in different seal ways, and can be kept not less than 30days. About 500 mL/m^3 for resistant insects also can keep not less than 30 – day. The lowest effective concentration was 20 mL/m^3 and can keep 75 – 170 – day with different dosage and seal ways.

In recent years, application of phosphine slow-releasing fumigation technique has obtained satisfactory effect against stored grain insects.

Key words: phosphine, slow – releasing fumigation, application

Introduction

Phosphine has been used as a fumigant for more than 70 years since 1930s. It has the advantages of safe, economic and satisfactory effect against insects. China has widely used it since 1960s. The research workers have researched some techniques of phosphine fumigation including conventional fumigation, fumigation with high dosage or low dosage, intermittent fumigation, slow-releasing fumigation, mixture fumigation, recirculative fumigation. Phosphine fumigation has played an important role in protection of postharvest grain. However, insects resistance develop day by day due to misuse of phosphine that fumigation fail. Therefor, we should master knowledge of phosphine, reasonably use to prolong life-span of its use and achieve satisfactory fumigation effect. Phosphine slow-release fumigation has the characteristics of effective concentration persistence and even distribution in grain bulk, effectively killing different species and their development stage.

1 Introduction of Phosphine Slow-releasing Fumigation Technique

Phosphine slow-releasing fumigation is to control releasing speed of fumigant by physical or chemical ways to slowly release phosphine and effectively control insects.

Aluminium phosphide tablets are put into film packets. Speed of resolution and release of phosphine can be controlled by film so that phosphine can be kept a long – term in grain bulk and get ideal effect against insects. The technique can not only kill adult and larvae, but also kill the adult and larvae which developed by more tolerant egg and pupae. Owing to less content of oxygen in film packets, flammability or explosion can be avoided due to phosphine concentration not excessive high.

2 Procedure of Phosphine Slow-releasing Fumigation

Aluminium phosphide 6 – 8 tablets (below 10 tablets) are put into polyethylene packets (0. 03 – 0. 07mm, 8 × 10cm) respectively. Tie up the packets. The packets are put into grain bulk about 0. 5m and about more than 3m from the surface of grain bulk respectively for over 6m – high large warehouse; about 0. 5m from the surface for about 4m – high horizontal warehouse. It is suitable for that the packets of aluminium phosphide are set in the middle and top of stacks of bagged grain. Space among applying spots is 1. 5 – 2. 5m for bulk storage, 3 – 4m for bag storage. 2 – 3g/m^3 is suitable for bag storage.

Thickness of polyethylene film depends on grain moisture content and storage period. Thick film can be used for grain of high moisture content and for long – term storage. , and vice – versa. For instance, for more than 13. 5% of grain moisture content, 0. 05 – 0. 07mm – thick

Tanggu State Grain Storage, Tianjin

film can be selected.

Slow-releasing fumigation should be carried out under the condition of well airtightness. Six-side of stack of bagged grain should be sealed with PVC membrane. For the bag stack with five-side sealed, the join between floor and the membrane must be sealed strictly. The surface of grain bulk need sealing with PVC membrane, walls, windows and doors must be also sealed. For warehouse in poor airtightness, effective concentration of phosphine can not keep for a long time and insect resistance will develop.

3 Notices in Slow-releasing Fumigation

3.1 Airtightness is the key to success or failure for slow – releasing fumigation. So seal work for warehouse, grain bulk and bag stack is very important. Pressure decay should not be less 40s from 500Pa to 250Pa for horizontal warehouse, especially for large warehouse, and not less 60s from 500Pa to250Pa for silo or squat silo. Six – side sealing should be adopted for bag storage, pump air out of the stack and then repair the leaks. The leaks between floor and covered membrane must seal well for bag stack with five-side seal.

3.2 Slow-releasing fumigation is suitable for grain stored for a long time. Sealing period is not less two months.

3.3 Slow-releasing fumigation is suitable for controlling the resistant insects to phosphine not serious. For the insects with obvious resistance, concentration of phosphine is at least more than 500 mL/m^3, and keep over 30 – day.

3.4 Phosphine concentration should be monitored to determine that the target concentrations are being achieved during fumigation. Phosphine concentration of different warehouses and stacks which have the same dosage and the same applied time will be compared. When concentration of phosphine in some warehouses and stacks lowers, the tablets will be made up in time.

4 Application of phosphine Slow-releasing Fumigation in Different Types of Storages

Trails of phosphine slow-releasing fumigation have been carried out since 2000 in different types of storages including bulk storage, bag storage in large warehouses and in horizontal warehouses, combination of cloth packets with film packets, and combination of film packets with different thickness in silos and the trials obtained good results.

4.1 Trial of slow-releasing fumigation with low dosage of phosphine in large warehouse

4.1.1 1Procedure Trial was carried out in a large warehouse in Ninhe storage in Tianjin. 3652t white wheat was in bulk storage. 2 – 3 populations of *Sitophilus zeamais* per kg were detected. Before application each of 18 cages contained 40 populations of insects was set in the top, middle and bottom of grain bulk respectively. 6 cages were set in each layer. Each cage contained 10 *Sitophilus zeamains* Motschulsky, *Cryptolestes ferrugineus*, *Tribolium confusum* and *Attagenus piceus* Oliv in different development stages respectively

Combination application was adopted such as combination of conventional application at low dosage with slow release application.: ① combination of cloth packets with 0.05mm – thick polyethylene packet ② combination of probe with 0.05mm – thick polyethylene packets. 300 application spots altogether were set in the top and low levels respectively (150 spots in each level). The spots distributed evenly and the space among the spots was 2 – 2.5m. The surface of grain bulk, doors and outlets were sealed with nylon film.

4.1.2 Measurement resultsApplying fumigant and seal for grain bulk were conducted in Jul. 8 – Sep. 21, 2000. Phosphine concentration was monitored at regular during trial. The results showed that average concentration of phosphine was 200 mL/m^3 after application 24h. Phosphine concentration tended to equilibrium after 5 – day, and achieved 680mL/m^3; the average highest concentration achieved 790mL/m^3 after 7 – day; 210mL/m^3 after 30 – day; 20 mL/m^3 after 75 – day.

4.1.3 Insect control effectNo live insects were found in 27 application spots (9 spots in the top, middle and bottom respectively) after ventilation. Average temperature of grain bulk was 29℃. There were also no live insects in 18 cages which were collected from grain bulk. Put the insects in cages into an incubator of 28℃, RH80%. The result showed that no live insects occurred after 30 – day. 100% controlling insect effect was obtained.

4.2 Trial of slow – releasing fumigation for stack of bagged grain in horizontal warehouse

4.2.1 ProcedureThe trial was conducted in Yujiabao State Grain Storage in Tanggu of Tianjin. A stack of bagged wheat of 1 000t was

used for the trial. Before applying insecticide, insect density was 3 and 5 populations per kg for *S. zeamais* and *C. ferrugineus* respectively. Each of 10 cage which contained 20 *S. zeamais*, *C. ferrugineus and T. castaneum* respectively were put into the tested wheat stack.. Five – side of the bag stack was sealed with PA/PE membrane. The packet was made of 0. 05mm and 0. 07mm polyethylene film. Dosage of aluminium phosphide was 2g/m^3, each packet contained aluminium phosphide below 30g. Applying spots were set evenly in the middle – upper stack.. Space among the spots was about 3m. Membrane around the stack bottom was pressed to seal with nylon bags of sand.

4. 2. 2 Measurement resultsAluminium phosphide tablets were applied in bag stack in May, 9 2002. Phosphine concentration in grain stack was determined regularly from May, 16. The results showed that average concentration of phosphine achieved over 40mL/m^3 after 7 – day, over 140mL/m^3 after 21 – day and kept 78 – day (the highest concentration was 410 mL/m^3); the lowest effective concentration 20 mL/m^3 kept 170 – day. (average grain temperature 15℃ in middle of May, the highest temperature 27℃ in Jul. – Aug).

4. 2. 3 Insect control effectNo live insects were found in 15 spots in the grain stack after ventilation. 10 insect cages were collected and also no live insects were found in the cages. Then the insects in the cages were put into the incubator (27℃ and RH75%) for 30 – day in which no live insects occurred. Effect of insect control achieved 100%.

4. 3 Slow-releasing fumigation in bulk grain in horizontal warehouse

4. 3. 1 ProcedureThe trial was carried out in the horizontal warehouse in Hangu State Storage in Tianjin which was 800m^2, grain bulk 4m-high. 2400 t white wheat was loaded into the warehouse from Jun. 15 to 20, 2003. The loaded wheat was infested by insects which the density was 3 populations, 2 populations and 5 populations per kg for *S. remainze*, *R. dominica* and *C. ferrugineus* respectively. So applying aluminium phosphide tablets was conducted with loading grain. Each pocket with 8 tablets aluminium phosphide was made of 0. 06mm polyethylene film. Dosage was 5g/m^3. After finishing loading grain, the surface of grain bulk, doors and windows of the warehouse were sealed.

4. 3. 2 Measurement resultsPhosphine concentration of grain bulk was measured at regular from beginning of Jun. 25, 2003. The results showed that phosphine evenly distributed in grain bulk. Average concentration of phosphine was over 300mL/m^3, over 500mL/m^3 in Jul. 5 and kept more than 30 – day. The lowest concentration was 20ml/m^3 and kept 120 – day. (average temperature 24℃ during applying insecticide)

4. 3. 3 Insect control effect15 samples were taken from 15 spots (east, west, south, north, centre, top, middle, bottom) in grain bulk. There were died insects but no live insects in the samples. Put 15 samples into the incubator at 28℃ and 70% and no live insects occurred after 30 – day.

4. 4 Slow-releasing fumigation in silo

4. 4. 1 ProcedureTrial was carried out in a silo in Yujiabao State Storage in Tianjin. The silo with 6. 6m – diameter, 9m – high grain bulk which contained 240tonne (t) white wheat. Turnover for the tested grain was conducted for lowering temperature in Jan. 2004. The grain temperature in the surface obviously changed with atmospheric temperature in spring and summer, and lower in middle and bottom in the silo. Applying insecticide was carried out in Jun 6, 2004. Each of pockets made of 0. 06mm polyethylene film which contained 30g aluminium phosphide tablets (dosage 3g/m^3). Five cages were put into grain bulk 1m from the surface in which there were different insect species including their different stages. There were 10 populations *S. zeamais*, 15 populations *C. ferrugineus*, 30 populations *Psocids* per kg in the cages respectively. All the entrances, outlets and exits were well sealed.

4. 4. 2 Measurement resultsPhosphine average concentration of grain bulk measured was over 100mL/m^3 in 16 Jun. 2004, over 280 mL/m^3 kept more than 30 – day, the lowest concentration 20 mL/m^3 kept 130 – day (grain temperature 22℃ in the top, 18℃ in the middle, and 16℃ in the bottom during applying insecticide).

4. 4. 3 Insect control effectAfter ventilation to disperse, 5 cages collected for inspection. There were no live insects to be found, then the insects in the cages were put into an incubator at 28℃ and RH80%, also no live insects occurred after 30 – day. The insect control effect was 100%.

5 Discussion

5. 1 Application of slow – releasing fumi-

gation should consider some factor, such as different types of warehouses, grain varieties, different insect species, storage period, seal conditions, and reasonably use film packets with different thickness and their combination use to achieve satisfactory killing effect.

5.2 Phosphine concentration change is related to grain temperature, when grain temperature is high, volatile speed of phosphine is quick, it is also related to grain moisture content, when grain moisture content high, the volatile speed is quick during slow – releasing fumigation.

5.3 Moving of phosphine gas is related to microcurrent in grain bulk during slow – releasing fumigation. Grain temperature is higher in the top than the middle and bottom in spring and summer, and phosphine gas move from top to the bottom. Under well seal condition, applying insecticide in the top of grain bulk, the ideal insect control effect can achieve with microcurrent effect for all types of warehouses.

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0422

Closed Loop Fumigation of a Small Rural Concrete Elevator in a Growing Urban Setting

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Abstract: As urban areas spread to adjacent rural countryside, small rural elevator managers are faced with increased safety challenges. While fumigation practices are closely monitored and documented, every effort must be made to contain gases within the structure and make the most efficient use with the least amount of chemical possible. This research project installed and tested a closed loop fumigation (CLF) system in a small concrete elevator in a growing downtown center. One half of the facility was included in the closed loop system while the remaining half was treated as conventional fumigated silo storage. Phosphine treatment rates were set by the elevator manager using his normal dosage rate for non – CLF treated bins. Phosphine levels were monitored at three locations in each silo: bottom, middle and headspace. Gas levels in the closed-loop bins were compared to a silo under standard fumigation. The closed-loop system maintained phosphine concentration levels as much as ten times higher than the standard gravity fumigated silo. An economic comparison between closed loop fumigation and traditional gravity methods is presented in this research. Economics strongly favor closed loop fumigation. This research concludes that closed loop fumigation in a concrete elevator provides better containment of gases which provides a safer environment through better control of fumigant, and a more economical method of achieving improved concentration over the required time to control all life stages of stored product insects. CLF will provide opportunity to greatly reduce dosage and manpower, greatly increase efficacy, and fumigate on short notice, compared to conventional silo fumigation. Better safety conditions for elevator personnel conducting the fumigation using a closed loop system are documented.

Key words: closed loop fumigation, CLF, concrete silos, phosphine, fumigation, efficacy

Introduction

Population growth has caused the urbanization of land area surrounding grain storage facilities located in areas that were previously considered rural. Elevator managers are more cognizant than ever of the need to pay strict attention to safety measures not only for their employees but also for the environment and the inhabitants surrounding the storage facilities. Conventional fumigation application techniques known as "probe", "probe and tarp", automatic dispenser, or gravity fumigation offer increased risk of exposure to fumigant during insertion of the fumigant into the grain and because of the time it takes to place a tarp over the grain[1]. In concrete silos, automatic pellet dispensers place fumigant pellets or tablets into the grain stream via bucket elevators or conveyor belts, requiring the grain to be turned or moved. Some of the pellets spill out of elevator cups and fall into the leg boot. This releases gas into the basement. A stalled leg, belt or drag conveyor can also increase fumigant concentration levels to the point that workers should be wearing self-contained breathing apparatus (SCBA) to work within the facility. In the above application methods, fumigant is left to travel throughout the storage facility without adequate control. The escape of the fumigant is dependent on structure sealing, weather, temperature, and humidity conditions.

Methyl bromide fumigation has been accomplished using closed loop fumigation (CLF) practices for many years. James Cook patented a recirculation process called The J-System® in 1980[2]. CLF is an example of the J-System. A typical CLF system consists of a low-pressure, low-volume centrifugal blower that moves a fumigant-air mixture through pipes from the headspace of grain bins and silos, and re-circulates the gas-air mixture into the base of the storage structure. Pressurized base ducts force the gas mixture to move upward through

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the grain bulk in a loop-like pattern (thus the name "closed loop") providing better gas distribution through the grain. Thus, the chance of fumigation failure is reduced due to better distribution of the gas in partially sealed structures, compared to conventional fumigation in unsealed storage. By providing suction from the storage headspace, fumigant leakage is minimized by the slight vacuum on the storage headspace. Storage base openings are easier to seal than roofs and silo roof decks, vents, man-entry doors and downspouts from legs or conveyors.

Without CLF in sealed or partially sealed storages, the gas fumigant in unsealed storage will escape into other areas of the structure and out to the environment through openings and leaks in the bin. In the case of phosphine fumigants, the weight of the gas is close to that of air. The fumigant will move in and out of the bin anywhere air is allowed to move or leak from the structure. The use of a CLF system necessitates better sealing of grain bins and provides better control of the fumigant's travel through the grain bulk. Fumigant pellets and/or tablets are normally placed on the top surface of the grain or, in some cases, can be inserted from outside the storage facility, thus reducing the exposure time for employees. Turning of grain in a conventional fumigant application increases the opportunity for grain dust explosion and also causes an economic loss due to the product shrinkage that occurs during the handling of the grain.

CLF does not require extra turning operations, thus reducing explosion risk, grain dust loss shrinkage, and labor costs. Grouping several concrete silos within a facility as a "closed loop unit" can reduce the amount of labor and the amount of exposure workers encounter to the gases and grain dust during fumigation. While the fumigant is eventually purged to the atmosphere outside the structure after fumigation, this purging can be scheduled when conditions are favorable for the least hazard potential.

Because CLF in sealed storages requires a much lower dosage, there is much less gas released to atmosphere during ventilation of the storage. If grain does not need to be moved after fumigation, the structures can be left sealed, allowing the gas to slowly dissipate for days or weeks, providing extended insect protection from the residual gas and sealed openings. This is legalas long as the "Danger" warning placards are left in place to warn people that the storage is still under fumigation.

When it's time to ventilate or purge the gas from the structure, by operating the CLF fan, purging can be accomplished quicker than by using natural air flow and gravity venting. Because gas fumigant is contained and re – circulated, better control of the fumigant is achieved and less fumigant is used.

Objective of Research

The objective of this research was to compare aluminum phosphide fumigation using a CLF system to traditional fumigation within the same concrete elevator under the same conditions.

Materials and Method

A CLF system was installed in a portion of a small, rural elevator that is now located in a growing urban downtown area in the eastern part of the state of Oklahoma, USA. One half of the facility was included in the closed loop system (Figure 1). This portion included six bins with approximately 8660 MT (120 000 bushel) capacity. The facility stores primarily oats and barley. The CLF system consisted of a 249 W (1/3 HP) blower which discharged the air/gas mixture at approximately 0.15 m^3/s (320 cfm) into the bottom of the bins and pulled air and gas out of the headspace above the grain. The bin structure was partially sealed using closed – cell expanding foam insulation to close the air vents at the top of the silos and the interior vents between CLF bins and adjacent bins not included in the CLF system, thus forming a composite storage unit of six silos that can be fumigated as one unit.

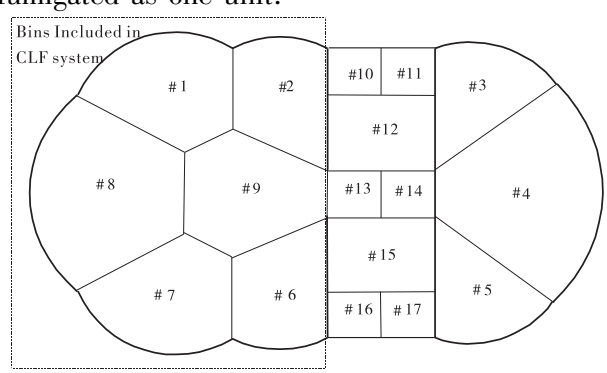

Fig. 1 Bin layout diagram for elevator fumigation. Left side was included in a closed loop fumigation system. Right side was treated with conventional fumigation methods

Duct tape and plastic sheeting (6 mm thickness) were used to seal covers at the top and bottom of each bin included in the CLF

system. Polyvinylchloride (PVC) and flexible plastic piping with ball valves from each bin bottom was connected into a manifold so that individual bins could be monitored or excluded from fumigation. Flexible hose and plastic pipe running vertically through the man-lift (elevator) shaft carried discharge air and fumigant to the manifold from the blower. The blower was mounted outside on top of one of the bins adjacent to the head house. The inlet of the blower was connected to a suction port opening in the steel man-entry door cover on one of the silos. After piping installation and sealing of the bins, the system was pressure checked for leaks and blockages.

The elevator manager recommended the dosage rate of phosphine fumigant according to the rate that he would normally use for the silos under conventional fumigation. The chosen rate was 17 pellets/m^3 (475 pellets/1000 ft^3). This suggested rate was used to treat the CLF part of the elevator. The same rate was applied at the same time by dropping the pellets into the non – CLF side while grain was being loaded into the bins. Both sides of the elevator were treated at the same time. The CLF bins were treated by placing pellets on the top surface of the grain in each of the bins. Care was taken not to stack or pile the pellets, thus decreasing possibility of explosion or fire. After dosage, all bin covers were secured and sealed with plastic sheeting and duct tape. The blower was switched on. Electronic gas detection meters were used to record gas concentration levels in each of the bins, both conventional and CLF, every 4 hours for the first 32 hours and then every 10 hours for the next 88 hours. Gas samples were taken from the headspace, the bottom of thebins and the midpoint of the bin through pre-installed gas sampling tubes. Readings in adjacent work areas and bins were recorded to identify escaping gas and to document safe working conditions. When gas concentration levels were sufficiently uniform throughout the grain bulk, the blower was turned off. When gas levels dropped significantly, the blower was turned on again for a few minutes to re-distribute gas from the CLF silos to the headspace of all six silos. At the end of the fumigation, the blower discharge piping was disconnected and the blower was used to evacuate the fumigant from the CLF side of the elevator by drawing gas from the base and exhausting it into the air above the silo roof deck, about 35 meters above ground level, where rapid dilution of the gas will take place, especially with the wind that is normally present on top of concrete elevators in the U. S. This gas purge evacuation was scheduled for the night time when area residents and elevator employees were least likely to be in the vicinity of the storage facility.

Results and Discussion

The CLF bins were compared to similar bins in the traditional side of the elevator. The gas concentration sampling data for two of those bins are presented in Figure 2. These results are typical of the data taken for the remaining bins. Research entomologists report that in order to kill all life stages of insects, fumigant concentrations of 200 ppm must be held for at least 100 hours[3]. The conventional side of the elevator never reached the needed concentration of 200 ppm for insect kill. The CLF side easily reached and exceeded the necessary concentration by almost ten fold. Areas adjacent the conventional bins encountered external gas concentration levels exceeding 20 ppm. Fumigation personnel were required to wear protective gear to monitor these areas for extended periods beyond the allotted fumigation time period until conditions allowed the gas to dissipate. The elevator manager reported low gas amounts leaked

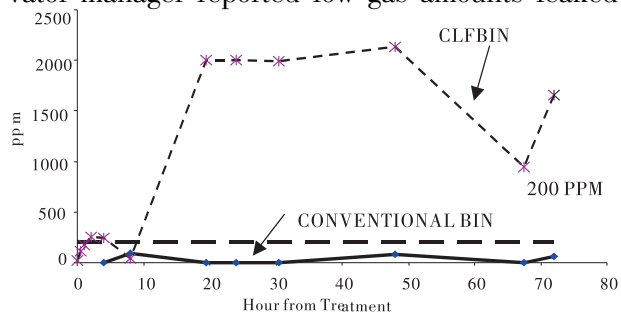

Fig. 2 Comparison of gas concentrations in closed loop fumigations versus conventional fumigation. Data points represent averages of three sampling points in each of two of the six silos.

from the sealed and piped CLF bins. The use of CLF can reduce cost by reducing the amount of fumigant required, the cost of turning the grain, grain dust weight losses, labor expense, health costs, and insurance costs. Table 1 shows general differences in the expense and benefits of CLF vs. conventional fumigation. The greatest economics savings in CLF is the lack of turning costs and the resulting shrinkage. The amount of savings depends greatly on the market price of the grain. Figures 3 and 4 show comparisons of the different cost factors for conventional fumigation and CLF. Figure 3 considers the price of

wheat to be 55USD/MT ($ 4/bu) while Figure 4 assumes 110 USD/MT ($ 8/bu). While CLF has a higher installation cost, the higher turning cost resulting in shrinkage, more fumigant required to try to achieve adequate efficacy, and the higher labor costs involved with conventional fumigation methods give CLF a major economic advantage. In the case of the elevator used for this research, the installation costs total approximately 0.18USD/MT ($ 0.013/bushel), primarily for plumbing and sealing, materials, and special equipment. This installation cost is a one-time expense while the turning and shrinkage costs in conventional fumigation occur each time the facility is fumigated.

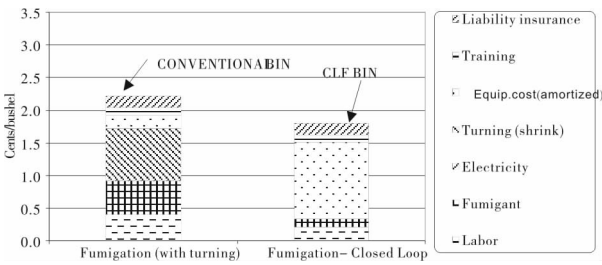

Fig. 3 Comparison of conventional and closed loop fumigation cost at 55 USD/MT ($ 4/bu wheat price).

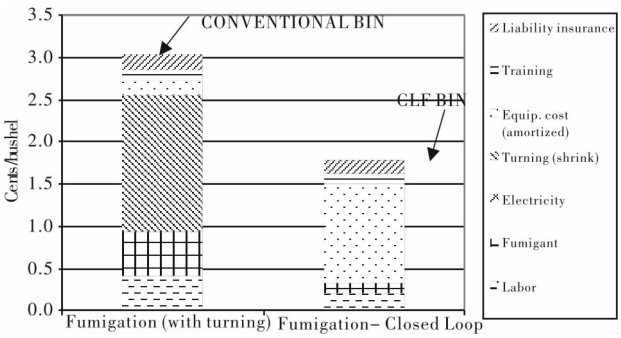

Fig. 4 Comparison of conventional and closed loop fumigation cost at 110 USD/MT ($ 8/bu wheat price) 1 bushel is equivalent to 0.07216 metric ton.

The results of this study indicate that not only was the fumigation more successful due to the much higher concentrations of fumigant sustained over the required period of time but it is much less risky for workers and the residents and businesses around this once – rural elevator. The CLF system will allow this facility to apply fumigant at minimum labeled dosage rates instead of the much higher rates this manager has traditionally used. Operating costs will be reduced due to approximately 75% savings in fumigant cost and larger savings in shrinkage costs as the price of grain continues to increase. Further research in this facility will validate this data and will also extend the CLF system to the entire elevator.

Table 1. Comparison of Closed Loop Fumigation general expense categories to Conventional Fumigation Methods

Closed Loop Fumigation	Conventional Fumigation
· Installation costs · Less fumigant (20 – 35% Conv.). · High Efficacy · Minimal labor (1 – 2 workers) · Less worker exposure, injury · Lower insurance/health costs · Precise timing (1 – 2 hours)	· Turning costs and product shrinkage · More fumigant – residual dust in grain · Low efficacy – phosphine resistance · More labor (4 – 10 workers) · More bin entry required of workers, · High health risk, higher insurance rates

1 bushel is equivalent to 0.072155 metric ton.

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0423

Partial Pest Fumigation Technology Research for Grain Piles

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Abstract: Partial pest fumigation technology for grain piles has always been a very complicated problem, by using exploring tube for dosage administration, with cloth bags of aluminum phosphide buried together and by means of the pipes for partial dosage administration to kill pests. However, it never covers all the fumigation vacancies, and solves uneven gas concentration problems. Through years of practice and contrast, we find that the recirculation fumigation method fixed with fumigation tubes is the most effective in the areas of pest infestation.

Key words: partial pest infestation, recirculation fumigation

In recent years, the technology applications for low temperature grain storage and balanced grain temperature have been widely promoted in grain depots, which have tremendously disrupted the pests' living environment and prevented the spread of pests so that the incidence rate for grain pests in large areas grows less. However, the partial grain insect infestation damages arising from various factors are still running rampant in many grain depots. If fumigation for the entire depot is adopted, it will not only cost more, but also labor intensity will be increased. At the same time, if there is no thorough fumigation, pests will spread to the whole depot causing other unfavorable factors. Thus, partial fumigation for grain pest treatment is the fundamental solution for this problem.

1 Causes for Partial Pest Spread

1.1 Poor Ventilation

In winter, the whole depot is not covered by mechanical ventilation, leading to big temperature differences with neighboring grain piles so that variations in grain temperartures within a grain pile can cause convection air movement which can cause increased moisture content for grain piles due to condensation of moisture. If this moisture shift phenomenon can not be controled in a short period of time, fungi in the grain piles will begin to propagate. Because the heat for microbial activities can not be released, it leads to the heat accumulation providing a favorite environment for the pest reproduction in stored grain piles. During the seasonal conversion periods between autumn and winter, between spring and summer the depot warehouse doors are opened often for wind ventilation, but a sudden temperature drop or increase in a large scale outdoors tends to cause condensation of moisture on the grain pile surface, on the warehouse doors and corners. If this phenomenon lasts, the partial grain pile will be subject to pest damage.

1.2 Wind net Design Defects before the Storage

Especially for the grain deposited during the hot temperature period in summer, although the grain depot has been disinfected, it keeps the temperature in individual parts of the grain depot still above 25℃ resulting from wind network design defects and bad winter ventilation. Because of the temperature difference within grain piles, the spread phenomenon of dampness and heat makes grain – storage temperature and humidity ideal for pest development and reproduction.

1.3 Halfway Disinfection

During phosphine fumigations, due to improper operation methods or inadequate dosage, some pests with phosphine resistance are not completely killed. After fumigation gas has dissipated, surviving pests will gather in warm humid areas favorable for living, feeding and breeding, which would trigger an outbreak for a new partial pest infestation, with increased phosphine resistance being bred by surviving insects.

1.4 Automatic Grading

After the grain enters the depot, because of

1. *Xinjiang Branch of China Grain Reserves Corporation, Urumqi* 830002
2. *Jinghe Branch Warehouse directly under China Grain Reserves Corporation, Jinghe County* 833300
3. *Yining Branch Warehouse directly under China Grain Reserves Corporation, Yining County* 835109

automatic grading in the grain bulk, the imbalanced distribution for internal impurities, water content and grain quality tend to result in the poor – quality grain accumulation with more impurities and high water content in a certain area. This kind of partial environment for grain piles is particularly suitable for pest growth and reproduction. Under appropriate conditions, it can become partially pest – prone.

Of course, apart from the above – mentioned pest – prone factors, improper comprehensive pest control can cause pest infection in partial grain piles. The grain stored in hot and humid season is prone to form partial hot and humid environments within grain piles, which is suitable for renewed eggs – hatchings and pest infestation. In short, on one hand partial pest damage in the grain bulk causes the partial heat, or even mildew, which is a direct impact on grain storage safety, On the other hand, pests spreading throughout the warehouse has increased the difficulty for grain storage. If the fumigation for the whole depot is adopted, the expense will be much higher. Sometimes the temperature where pests are infested is much higher than other parts, so that fumigation gas does not infiltrate sufficiently to kill pests[1]. For this, taking a scientific and rational partial fumigation approach for partial pest disasters, we not only achieve maximum results with reduced effort, but also it is more effective. The investigation into partial grain pest fumigations is as follows.

2 Partial Pest Fumigation for the Grain Bulk

Partial pest fumigation for grain piles technology has been a very complex problem, and exploring tube administration, concentrated burial of aluminum phosphide hop – pockets or sinusoid vessel partial dosage or other means still can not deal with the fumigation vacancy and uneven gas concentration problems. Through years of practice and contrast, at the place where pests run rampant, the recirculation fumigation method fixed with portable or moveable fumigation probe tubes are the most effective. Around and at the centre of the pest infestation area, with the grain surface covered with film sheets, gas tubes (Fig. 1) are arranged to transmit the fumigation gas to the center sites of the grain pest reproduction (pest hot – spots), so that the effective concentration of fumigation gas is confined to a stenotic area making for a three – dimensional gas siege for pests so as to annihilate the localized pests. However, during the fumigation process, the gas diffusion will reduce the gas concentration in the pest intensive concentration areas. For this, we must pay careful attention to the gas concentration of partial fumigation at the pest infestation areas. When necessary, more gas should be added to keep the insecticidal gas density above the effective concentration. The gas concentration can be measured by setting detector tubes in the grain mass area being fumigated. Fumigation gas tubes can be buried in grain piles by using sampling devices, and this technology in China has got almost mature. Developed and manufactured by Chengdu Grain Storage Research Institute under State Grain Reserve Administration, the processing machine of partial grain piles has been successfully applied in many of the granaries, which proves the maturity of the technology.

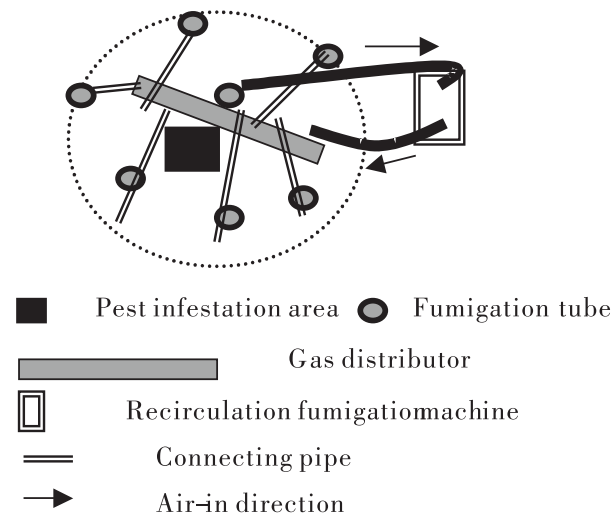

Fig. 1 Processing machine for partial grain piles (planform)

3 Do and Don't Partial Recir Culation Fumigation

3.1 Phosphine Concentration Measurement

The partial pest spread causes hot temperature spots in the grain pile, which creates temperature differences that causes hot and cold air convection air currents to flow within the grain piles. These slow moving air currents directly affect the concentration homogeneity of phosphine, so the concentration at the fumigation areas of phosphine can not be known at any given time. Because of this, it often leads to halfway, poor, ineffective fumigation, or excessive fumigation which can adversely affect grain quality. In the grain piles of fumigation, the scientific arrangement for phosphine detection points is

very necessary, so we can always know on a real-time basis what the phosphine concentration values are in the target fumigation area. With this valuable information, we can add dosage or suspend dosage delivery, as needed. This allows the partial fumigator the ability to keep an effective concentration of phosphine around the center of insect infestation in the grain piles, greatly improving insecticidal efficiency. The following arrangement of phosphine detection points is very scientific[2]

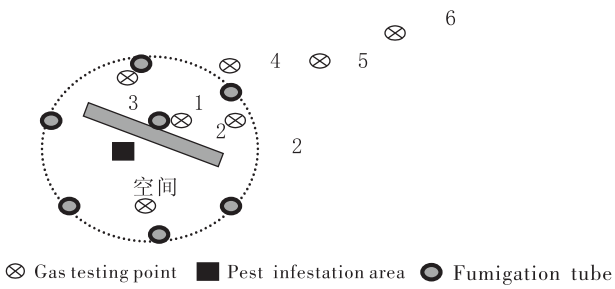

Fig. 2 Phosphine detection point arrangement (planform)

Table 1. Phosphine Detection point position in grain piles

Detection point	Vertical distance from the grain surface (m)	Distance from the central tube (m)
1 – 1	2.5	0.3
1 – 2	4.5	1.2
2 – 1	0.6	1.2
2 – 2	1.5	1.2
2 – 3	2.5	1.2
2 – 4	4.0	1.2
2 – 5	5.0	1.2
3 – 1	2.5	1.8
3 – 2	4.0	1.8
4 – 1	3.2	2.5
5 – 1	0.5	3.0
6 – 1	0.5	5.0
Airspace	0	1.0

3.2 Determination of Confinement Method and Time

On the grain surface, with film sheets covering the grain pile surface above target area, film sheet edge should keep distance from fumigation tubes at least 1m beyond the tube, and is vertically buried more than 50 cm under grain piles surface. The interface with tubes must be closely confined (Fig. 2, Table 1).

Phosphine fumigation is a very complicated process, not only requiring the effective phosphine gas concentration, but also making the effective gas concentration last long enough to kill all life stages of the pests. As for Phosphine imago and larvae pest fumigation, insecticide concentration and time requirement is lower than for eggs and pupae. Grain bulks are half-closed in the process of fumigation. Phosphine gas under the films spread around in the grain piles. If not promptly fumigated before pest populations expand, in the pest infestation area it is difficult to form an effective concentration for pest killing. Before the partial fumigation, we must know the location and size of the pest infestation area, grain bulk temperature, dosage concentration, and pest species state. As for comprehensive factors such as the pests drug resistance, a thorough investigation should be made to work out the best concentration and confinement fumigation timetable. Listed in " Phosphine Recirculation Fumigation Technical Specification" (LS1201 – 2002), for different temperatures of phosphine, effective concentration and confinement time for pest killing are given (Table 2). In the operation, based on some experiment results we may see the results as a reference to ensure the insecticidal effect.

Table 2. Recirculation fumigation different confinement time, temperature and pest species Phosphine concentration reference table

Pest species	Temperature (℃)	Confinement time (d)		
		14	21	28
1. Sensitive pests: corn weevil, gnawing beetle, siligua gnawing beetle and other sensitive species	>25	200	150	100
	20 – 25	250	200	150
	15 – 20	–	250	200
2. Drug resistance pest: lesser grain borer, rice weevil, oblate gnawing beetle, red flour beetle, Indian Meal Moth and other species of insects	>25	300	250	200
	20 – 25	350	300	250
	15 – 20	–	350	300

3.3 Phosphine Flowing Direction

Phosphine has advantages, such as strong penetrating property, strong diffusibility, effective pest killing, convenient use, low cost, low pollution and residual. It is currently one of the world's best fumigants[3]; During a local partial phosphine fumigation, to form an effective gas concentration, the fumigator must properly distribute recirculation fumigation tubes down through the grain pile into the pest infestation zone. Then, through the gas distributor, phosphine delivered by the recirculation fumigation machine can flow downward through the fumiga-

tion tubes which are pressed into the grain piles. Thus, the pests are surrounded by leathal concentrations of gas, and through suction created by the central fumigation tube (Fig. 3), phosphine is accumulated in the pest infestation area, improving insecticidal efficiency. The recirculation blower then continues to recycle the fumigant gas, keeping it flowing through the pest infestation areas.

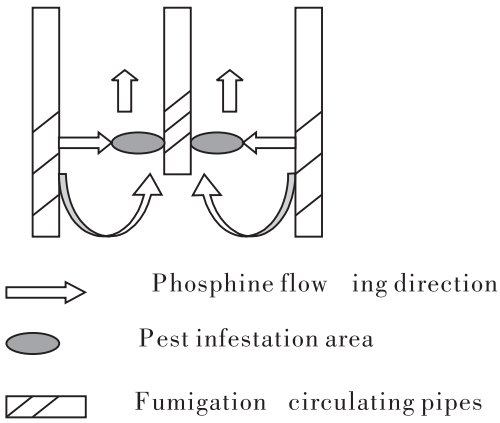

Fig. 3 Side view of phosphine's flowing in the grain piles during a local fumigation

4 Conclusion

4.1 By using the processing machine of grain piles, the partial pest fumigation has advantages, such as low dosage, low-cost, light labor intensity; rapidly making the phosphine of various parts maintain uniform concentration[4].

4.2 The partial pest infestation causes the hot sport temperature in grain piles, such as the temperature over 32℃, while at other parts of grain piles temperature is lower. Under this situation, we should conduct partial fumigation after cooling the grain[5], or else it would lead to condensation of moisture. Big temperature difference in grain piles forms strong interior airflow, and it easily lead to the loss of phosphine gas, affecting recirculation fumigation effects.

4.3 If large areas of pest infestations cover large areas near the grain pile surface, it is better not to use partial fumigation methods for pest control.

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0424

Phosphine Fumigation by Aluminium Phosphide Decomposing in Air Mixed with Dichlorvos

Tian Hua[1], Zhou Shifa[1], Chen Mingwei[1], Zheng Tianyang[2], Yan Shengli[2], Song Jinguang[2] and Gai Yuwei [2]

Abstract: A field trail of phosphine plus dichlorvos fumigation was carried out in a flat storage of wheat. The fumigant was distributed better by recirculation. Some insects common in grain stored grain in China were controlled more effectively.

Key words: phosphine, dichlorvos, fumigation, insect, recirculation

Introduction

Phosphine has been used as fumigant for stored grain fumigation in the application methods of generator or tablet, with and without recirculation. Some fumigations failed in some storages when the fumigation used phosphine solely. In some cases successful fumigation can be achieved by the combination of phosphine and dichlorvos, especially for the control of psocids, weevils, moths, red flour beetle and so on in. Some field trials are reported in this paper.

1 Materials and Methods

1.1 Warehouse

A flat storage was used for the trail that is 29.5 meters in length, 23.2 meters in width and 6 meters in height for wheat bulk. The capacity of the warehouse is 3 200 tons. There were 4 112 cubic meters in volume for bulk and 2 800 cubic meters for head space. There was a polyester foam sparkled on the inter surface of the roof. The half decreasing time from 500 Pa to 250 Pa was 180 seconds for the gastightness.

1.2 Establishment in the Warehouse

There was some ventilation duct on the floor that has 30 per cent holes. The ducts were distributed every 5 meters and used as a part of recirculation fumigation system for phosphine. There were also some pipes and a fan for fumigant recirculation when aluminium phosphide tablets were applied on the top of bulk. An electronic temperature monitoring system was set in the bulk with sensors arranged in five meter intervals horizontally and one and half meter intervals vertically.

1.3 Instruments

An electronic phosphine monitor was used for the concentration monitoring in the range 0 – 1 000 mL/m^3. An electronic phosphine alarm monitor was used for safety monitoring around the warehouse. Some plastic trays were laid in the ventilation duct for application of tablets.

1.4 Methods

Firstly, the whole grain was aerated to even temperature and moisture content.

Three to four plastic trays were put into the entrance of the ventilation duct for the application of tablets of aluminium phosphide. Most tablets of aluminium phosphide were applied on the top of grain in plastic trays. The total dosage of the phosphide was ten kilograms.

Six plastic bottle cages that contained insect and feed were placed on the top near the window to test efficacy. Ten insects of each species were set in the cages containing cracked and wheat seed that included *Cryptolestes ferugineus* (Stephens), *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* (Motschulsk and psocids.

Some empty bags were hung on the frame of the warehouse. DDVP emulsion in water was sprayed on the bags. No liquid was applied directly to the grain.

In the first and second day there was no recirculation. On third day the recirculation fan was started for the fumigant distribution through the bulk. The fan worked until the concentration ratio of lowest to highest was over 0.6, with the phosphine monitored daily. Thereafter the concentration was monitored every three days.

The temperature of grain was also moni-

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tored once a week. Some recirculation operations were carried out for uniformity of phosphine concentration. The fumigation began on 20th July, 2007.

The survival of insect in cages was checked at the first, second, and third weeks. The fumigation was ended after forty days.

2 Results and Analysis

2.1 Temperature Change

The average temperature change of grain during fumigation is shown in table1. Point 1 to point 5 were located on the four corners and the centre and were one meter underneath of bulk surface.

Table 1. Temperature (℃) of grain in the upper layer

Checking date	Point 1	Point 2	Point 3	Point 4	Point 5
2007.07.24	23.7	15.8	20.2	23.8	17.5
2007.07.31	22.8	16.5	20.8	22.9	17.1
2007.08.07	23.6	17	21.2	23.5	17.4
2007.08.14	24.8	17.4	21.6	24.6	18.1
2007.08.21	24.5	18	22.5	24.7	18.1
2007.08.28	25.2	18.4	23.2	26.1	18.5

Table1 indicates that the temperature in upper layer of grain did not increase. The fumigation had less effect on the grain temperature than the climate.

2.2 Phosphine Concentration

Changes in phosphine concentration during the fumigation are shown in table 2.

Table 2. Phosphine concentration changes (mL/m^3)

Checking date	Point 1	Point 2	Point 3	Point 4	Point 5	Head space
2007.07.23	260	309	316	275	240	232
2007.07.27	226	248	260	225	203	199
2007.07.31	190	192	207	195	180	162
2007.08.06	176	175	178	177	182	122
2007.08.10	125	172	182	185	180	150
2007.08.14	123	124	135	130	114	116
2007.08.20	90	108	100	95	90	102
2007.08.26	54	68	117	78	63	56
2007.08.31	45	50	80	65	52	46

A phosphine concentration over 100 mL/m^3 was maintained for more than twenty days (table2). The phosphine distribution in different monitored points was uniform. The recirculation system of the warehouse worked well.

2.3 Killing Effect on Insects

Table 3. Dead number of insect at different time

Checking date	Week 1	Week 2	Week 3	Week 3
Red flour beetle	30	30	30	30
weevil	30	30	30	30
Rust flat beetle	28	30	30	30
psocids	30	30	30	30

The result of mortality of insect in cages indicates that red flour beetle had the greatest tolerance to phosphine. The result that rust flat beetle was easy to kill does not means it has less tolerance to phosphine. It may be that adults died easily in the cages. A shorter time of fumigation had less killing effect on psocids,

3 Conclusions

Phosphine fumigation mixed with DDVP was more effective to insect killing than phosphine alone. A longer time is needed for phosphine fumigation. During this process some adults may produce new eggs that have higher tolerance to phosphine than adults. As DDVP can kill adults in a shorter time, there should be few eggs at the start of fumigation. Therefore phosphine plus DDVP fumigation is a more effective way of insect control than phosphine alone. DDVP does not easily penetrate bulk grains. The recirculation of the fumigation helps the distribution of DDVP. Control of psocide should be more effective by the addition of DDVP and recirculation.

Acknowledgements

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0425

ECO_2 Controlled Atmosphere Low – Oxygen Disinfestation of Post Harvest Commodities, Structures, Silos and (export/import) Containers

Nico Vroom* and Jacobien van Golen

Abstract: The ECO_2 Controlled Atmosphere (CA) treatment, based on low – oxygen is commercially used world – wide to control insects in post harvest commodities, structures, silos, and container cargo (imported and exported and treated according Quarantine and Pre – shipment regulations). CA treatments have gained industry and government acceptance as a non – toxic fumigant technology for a variety of applications.

Treatments are carried out by applying them in climate controlled rooms, silos, barges or containers with fixed or mobile installations. CA has shown to be effective in controlling eggs, larvae and pupae, present in different sorts of dried commodities.

CA treatments have many advantages over traditional fumigants, including no pest resistance, residue – free and safe. Installations equipped to carry out CA treatments are available in 14 countries serving a wide variety of industries.

Key words: Controlled Atmospheres, heat, disinfestations, stored product pest control, fumigation, quality preservation, insects.

Introduction

The use of Controlled Atmospheres (CA) to control insect in post – harvest durables is growing rapidly, replacing toxic chemicals such as Methyl Bromide and Phosphine. In the past, CA had some disadvantages in price, longer treatment times and availability but currently these constraints are reduced by the technical developments of the Dutch company, ECO_2.

The ECO_2 CA process is based on establishment of a low-oxygen environment which kills insects. The ECO_2 b. v. developed commercial application of CA to control all stages of insects, rats and mice in food, associated products, artifacts, silos, food (processing) facilities, airplanes and barges.

In this study, low oxygen CA are established by means of an oxygen burner system or a nitrogen generator. The low-oxygen atmospheres are applied in airtight environments which range from 1 m^3 to 1 000 m^3. Insects in all stages, present in the products treated, are eliminated (99.9 % lt) due to oxygen suffocation and dehydration. One unique effect of CA is that insects do not die inside the product. The insects try to escape the low – oxygen condition in the product by moving towards the walls of the chambers, thus moving out of the product.

Materials and Methods

Exposure time with CA

The up-to-date application of CA (= EcO_2 Rapid Treatment®) decreases treatment times for stored products pests to an acceptable level. This decrease in treatment time was managed because of mechanical developments in the technology and the machinery used to perform CA. Treatment times now vary between 3 to 5 days, depending on the type of product (density level) and type of insect (exposure level).

To decrease treatment time, improvements in airflow circulation in the airtight treatment chambers were developed, shortening the time to heat the products. Tests conducted in several EcO_2 service centers showed very positive results. The results of one test conducted with dried organic peaches from South Africa are shown in the Figures 1 and 2. Figure 1 visualises the rapid increase in temperature measured by 5 data loggers, placed in the dried peaches at different positions in the treatment chamber. The increase in temperature progresses relatively uniformly throughout the cargo treatment. Figure 2 shows the former situation when the improvements were not yet applied to the existing equipment.

Each insect species has different exposure

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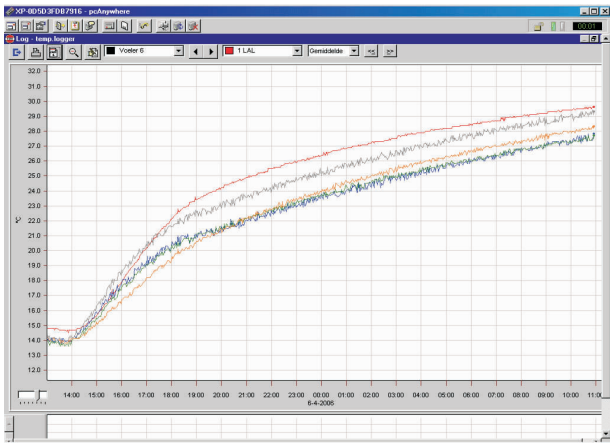

Fig. 1 Decreased heat – up times with the ECO_2 Rapid treatment®

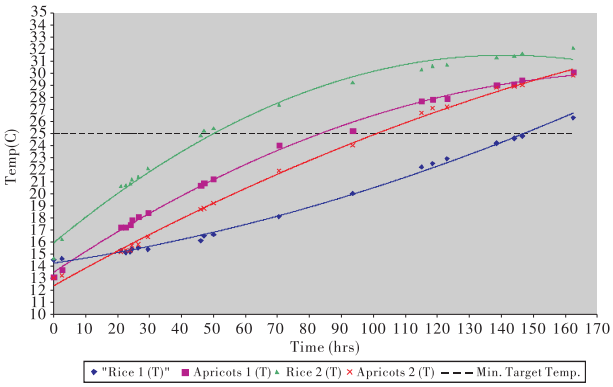

Fig. 2 Standard heat – up times using CA without the ECO_2 Rapid treatment®

Control of insects with CA

times to control each stage of development. When lower product temperatures are used, exposure times will increase. When higher product temperatures are used, exposure times will decrease. This effect is very import for the industry where time is money.

Given this valuable information, the ECO_2 Rapid Treatment® was developed to create an optimum environment for the insects to be dehydrated and suffocated from a lack of oxygen. During the development of the treatment time improvement, several different insects species where tested. All stages of the insects (adult, pupae, larvae, eggs) were tested. Treatment was carried out using the ECO_2 Controlled Atmosphere including Rapid Treatment®. Insects were treated according the parameters stated in Table 1.

Table 1. Insect species treated with ECO_2 Controlled Atmosphere incl. Rapid Treatment®

Insect Species	Life Stage	Treatment Type	Treatment Parameters
Carpoglyphus lactis	All stages	CA	CA, 38℃, 24hrs
Acarus spp.	All stages	CA	CA, 32℃, 24hrs
Carpophilus dimidiatus	All stages	CA	CA, 40℃, 16hrs
Ephestia elutella	All stages	CA	CA, 35℃, 10hrs
Ephestia Cautella	All stages	CA	CA, 35℃, 10hrs
Plodia interpunctella	All stages	CA	CA, 34℃, 16 hrs
Oryzaephilus mercator	All stages	CA	CA, 36℃, 16hrs
Oryzaephilus surinamensis	All stages	CA	CA, 30℃, 24hrs
Sitophilus oryzae	All stages	CA	CA, 35℃, 48hrs
Sitophilus granarius	All stages	CA	CA, 30℃, 4days
Stegobium paniceum	All stages	CA	CA, 32℃, 24hrs
Tribolium castaneum	All stages	CA	CA, 34℃, 24hrs
Bruchus ssp.	All stages	CA	CA, 32℃, 2days
Rhizopertha dominica	All stages	CA	CA, 32℃, 3days
Sitotroga cerealella	All stages	CA	CA, 30℃, 3days
Tribolium confusum	All stages	CA	CA, 30℃, 36hrs

Each insect species was controlled according the given parameters. Control samples of insects were prepared similarly but not subjected to treatment.

Test showed 100% effective control of all tested insect species according the given parameters.

Price and Availability Fumigation with CA

Toxic fumigants are still widely used but due to the phase out of Methyl Bromide consumers are pushed to use other fumigants or technologies. Phosphine is a world-wide fumigant and is affordable. However, the fumigation takes long exposure times to be effective and the product is meeting increased levels of pest resistance. Sulfuryl Fluoride is another fumigant which however cannot guarantee an effectiveness of 99.9% lt, and is not yet registered in every country to be used for insect control on food commodities. Phosphine and Sulfuryl Fluoride need investments in fumigation rooms and information technology to be applied on an acceptable level.

CA are most efficient in airtight climate rooms which are made of solid, impermeable panels. These rooms orten require a large investment and a large volume. ECO_2 managed to implement their converter based system in a 20ft shipping container which is moveable and connectable to different areas, as long as the proposed treatment area can be made gastight.

The system can be connected to a 40ft isolated shipping container. The smallest treatment unit is 24 m/ton per treatment. With the system designed and built as a transportable unit, prices of the treatments are at reasonable levels and affordable for use by small and medium sized companies. A company can purchase there own system, constructed turn-key at the desired location. Treatment prices range a few EUR per metric tonne (depending on yearly volume).

ECO_2 CA process installations and facilities are currently available in 14 countries in LatinAmerica, Asia, Africa, Middle East and Europe. These facilities consist of more than 105 treatment sites of which some have 12 rooms on one facility (Fig. 3 and 4). Information about the efficiency of treatments can be demonstrated by reports. The system is proven in commercial practice as an effective pest control option.

Fig. 3 CA facility in Greece (total 1029 m^3 treatment rooms)

Fig. 4 CA facility which fits 2 TEU in one treatment room

Usability of CA

Treatment of durable commodities with CA as developed by ECO_2 are carried out in gastight climate chambers, isolated containers, silo's, warehouses, barges, etc. The machinery is moveable and can even be placed outside and connected to the treatment area inside. Small rooms are available for yearly volumes of 1 300 metric tonnes and can be upgraded to rooms with a yearly volume of more than 20 000 metric tonnes. Computer software makes it possible to monitor and control the entire process on-line and trained operators at a central treatment location monitor each individual treatment to maintain a constant level of quality control. The entire process is easy to use and owners of such systems only have to take care of loading and unloading the rooms and closing the doors. The rest of the system is entirely automatic.

Products that are exposed to CA in the facilities as described are a variety of food commodities, harvested and transported world wide. Each individual facility is equipped to handle variable sorts and quantities of products depending on the requirements of the user. A wide variety of cereals, pulses, nuts, spices, dried fruits, seeds and others are treated every day.

CA is also highly effective in the control of insects in furniture, art, antique and library or museum items. The technology will not deteriorate the products and their value will not be damaged.

Conclusions

The use of Controlled Atmosphere is more competitive now than a few years ago. Barriers of treatment time, price, availability and usability have been lowered considerably. The growing trend in awareness and food safety is forcing commodity and wood product industries to implement better and safer fumigation technologies. The use of CA has several advantages compared to existing methods for chemical insect treatments on products:

Insects do not die inside the product. The insects try to escape the low oxygen atmosphere by moving out of the product toward the sides of the chamber.

There is no use of insecticides and thus no residues.

The method is environmental friendly.

The system can be used without waiting for a fumigator.

Each treatment is certified by an internationally recognized certificate of treatement.

No insect resistance is found with the use of Controlled Atmosphere.

There is very low danger for the working personnel.

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0426

Circulation Fumigation with Phosphine in a Large Warehouse

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Abstract: Good insecticidal results were achieved with a low dose of phosphine in re – circulatory fumigation in a large warehouse.

Key words: large warehouse, phosphine, re – circulatory fumigation

Introduction

Since 1998, the state has invested in the construction of a number of storages in the central grain reserves, where the most common type is a large warehouse. Compared with the general silo, with high grain lines, the characteristics of a large capacity warehouses make fumigation difficult: phosphine penetration is limited, it is not uniformly distributed and incomplete control may cause pest resistance. In light of this situation, we experimented with a low-dose re-circulatory phosphine fumigation in a large warehouse.

1 Material and Methods

1.1 Test Warehouse

The chosen warehouses were the new large ones: No. 18, 21, and 22, built in the year 2000. With their 48m length, 24m span and grain line 6m, 12000 m^3 volume warehouses, warehouse capacity of 5,000 tons; the layout is a group of four with unilateral ventilation. It is made waterproof (with Storage Mainland Ping and permanent Ping).

1.2 Condition of Tested Grain

No. 18, 21, 22 warehouses were loaded with the new maize from local farmlands in July 2002. Food and water impurities and other indicators were in line with the state average standards. Specific conditions are shown in Table 1.

1.3 Fumigation Operation and the Use of Equipment

1.3.1 Fumigation equipment

Table 1. Basic food situation

Bin NO.	variety	quantity	moisture	impurity	geimination	pest condition (T)	
			%	%	%	variety	density (No./Kg)
No. 18	maize	5006	12.4	0.8	84.4	maize weevil	3
No. 21	maize	5006	12.7	0.5	86.5	maize weevil	3
No. 22	maize	5002	12.5	0.6	85.9	maize weevil	2

1.3.2 Fumigation operation

Equipment Connection: Generator in accordance with the regulations, carbon dioxide intake and carbon dioxide cylinders (connecting five cylinder), and carbon dioxide mixed phosphine, circulation and circulation of inlet and good connections. The circulation fan is connected to power, and its output measured with a wire anemometer measurement. The circulation is regulated taking the wind speed into account. Use soapy water or cleaning agent in the pipeline connecting the smear to check for air bubbles; if they appear, take timely mending leakage measures, such as: a plastic glass or tape.

1.3.3 Confined fumigation

While the 22nd warehouse being closed for16 days, the 18th, the 21st Wharf were closed 22 days after the casual air ventilation, and use of phosphine. Alarm detection warehouses measured concentrations of phosphine. When the concentration of phosphine is below 0.2 mL/m^3 (ppm), the staff is allowed to carry out inspection.

Qinhuangdao Depot Directly Under Central Grain Reserves Economic Developing – area No. 4 Beijiing East road, Qinhuangdao, Hebei, 066206, P. R. China

1.4 Dosing and Gas Production

In a warehouse of 12 000m^3 volume and 0.3 g/m^3 administration, aim at 200 mL/m^3 concentration (ppm); the first dose is 15 kg. The phosphine gas generator quickly produces phosphine and the release of phosphine in carbon dioxide is used.

1.5 Measuring points and position of, insect cages

1.5.1 Gas measurement points

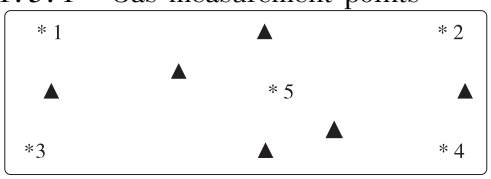

Fig. 1 Layout of concentration measuring points and pest cages(* measurement point, ▲testing pest cage)

As shown in Figure 1, five probes for phosphine concentration were deployed in the center of the warehouse at 1.40, and 80 cm depths. They stay in place and each has a plastic hose which leads to the warehouse.

1.5.2 Test insect cage layout

Pest species were taken directly from the warehouses, and also a laboratory culture. See Figure 1 for embedded location, at 30 cm under the grain surface.

1.5.3 Concentration Measurement

In the first application test for leakage with a phosphine Alarm Detection in the pipeline and warehouse doors and windows; measure phosphine every two hours in the warehouse; take readings at the five points in the warehouse every day in the morning, and afternoon to test for evenness of concentration.

1.6 Warehouse Hermetic Sealing and Testing Conditions

Seal with plastic Films, sealing tape and glass plastic.

All grain ago, the various positions were Empty storage air tightness test. See table 2.

Table 2. Measurement of warehouse air tightness

Bin NO.	storage situation	500Pa to 250Pa half life mean(s)
NO. 18	empty	60
NO. 21	empty	71
NO. 22	empty	41

2 Results and Discussion

2.1 Insecticidal Effect of Fumigation

After the fumigation, store inspection showed that the insects were all killed (Table 3). The caged insects were dead, whether those taken from or the or those taken from laboratory cultures. Because the samples stored a month in the laboratory on cultured food contained no insects, the fumigation was effective.

Table 3. After the fumigation grain warehouses

Bin NO.	moisture %	temperature (℃)	germination % grain plane	pest condition (No./kg) middle and lower layer	
NO. 18	12.4	25.1	84.1	0	0
NO. 21	12.7	25.2	85.6	0	0
NO. 22	12.5	25.0	86.3	0	0

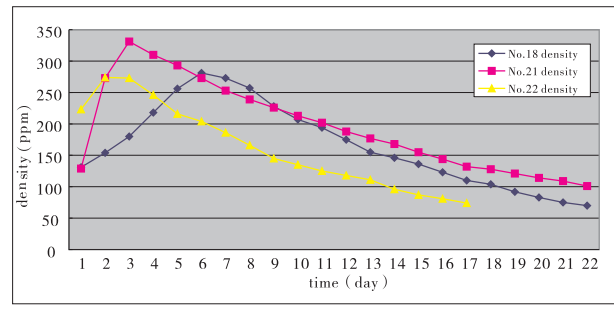

Fig. 2 Curve of average concentration in warehouses NO. 18, NO. 21 and NO. 22

2.2 Phosphine Gas Concentrations

Figure 2 shows the average concentrations in storages 18, 21 and 22. In No. 22 and 18 storages initial circulation warehouses lasted 36 hours (No. 18 did not meet this standard due to equipment failure). This was followed by four hours circulation every morning (total circulation 60 hours after shutdown). The No. 18 positions did not quickly achieve the highest value. In No. 21, there was consecutive circulation 72 hours and no further circulation. The highest concentration depended nota only on the time and continuous circulation, but on the air tightness of the warehouse. No. 22 storage had the worst air tightness and the lowest phosphine concentrations.

Phosphine gas concentrations began to increase and then peak and decline. After it had declined to approximately 150 mL/m^3 (ppm), the rate of decline decreased. This has the following main aspects: First, as the warehouse and the circulation pipe forms a closed circulation system, the increase in pressure from the introduction of gas will cause losses. This raises the question of when to stop recirculation. Secondly, because the new warehouse wall is not very dry, this has increased phosphine adsorp-

tion; Finally, because the food itself has a large number of phosphine gas adsorption sites, a few days after the fumigation phosphine declines.

2.3 Initial Dosage is Reasonable

Phosphine fumigation circulation Technical Specification (Trial): fumigation concentration, when the average temperature in the grain is more than 25℃ and resistant insect species are present, maintain phosphine concentration at a minimum of 70 mL/m^3 (ppm). Use more than when the average temperature in the grain is below 25℃. When insect-resistant strains are present, maintain phosphine concentration at a minimum 100 mL/m^3 (ppm). Considering the stored grain pest density, type and the air tightness of the warehouse, and other factors, the low – dose fumigation is set in the concentration of 200 mL/m^3 (ppm). But in No. 21, because its good gas – tightness maintains phosphine a long time (more than 200 mL/m^3 (ppm) for 10 days), the initial concentration can be lowered to not less than 100 mL/m^3 (ppm). If results in No. 21 continues to confirmed, the fumigation outcome will be better. Although No. 22 has poor air tightness, the concentration was maintained above 70 mL/m^3 (ppm). Given this situation, the dose can be reduced by proper improvements.

2.4 The hermetic Quality of the Warehouse

The hermetic quality and, to a certain extent, the impact of fumigation is the key to success and, at the same time, will impact on the development of resistance to pests and environmental pollution. From the data in Table 2 it can be seen that warehouses pressure decreased from 500 to 250 Pa exceeded the technical specifications 40 seconds half-life. From 2.2 it can be seen, that with better air tightness and warehouses, effective concentrations can be maintained over longer duration. The poorer the air tightness, the greater is the rate of decline of phospine.

$$\text{decline rate}\% = [(C_{max} - CN)/C_{max}] \times N \times 100\%$$

C_{max} of the highest average concentration; CN days for the fumigation of the average concentration of N, N the number of days for the fumigation

Table 4. Degressive ratio & air tightness

Bin NO.	decline rate(%)	half life(s)
NO. 18	16.5	60
NO. 21	15.3	71

2.5 Temperature, Moisture, Changes in Germination

The grain Heap surface temperature increased slightly, but the fumigation had little effect on temperature. After fumigant release there were some small local changes in moisture content, up or down, but no GRAIN partial condensation. The average moisture content did not change; moisture changes should be considered as normal water balance processes. Germination was slightly lower than before the fumigation. These results show that the test operating conditions and technology are feasible.

3 Conclusion

3.1 Low – dose circulation fumigation has provided a guarantee of success, if air tightness is good. Maintaining effective concentrations for a long time, inhibit the development of stored grain pests, and achieve100%

3.2 Stored Grain Pests generally occur in the hot and humid season, being influenced by warehouse temperature and humidity, but the experiment proved that, in this environment, circulation fumigation of food, has little effect on temperature and moisture, and will not lead to grain stack temperature and moisture changes.

3.3 Circulation for some time does not require daily circulation.

Acknowledgements

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0427

Test on Recirculation Fumigation under Plastic Sheet in Squat Silo

Wu Jiang, Lu Juncang, Yang Dong, Wang Zhe, Qiang Jingzhi and Zeng Xiaofan

Abstract: To improve the sealing performance of squat silos, an experiment on recirculation fumigation under plastic sheet in squat silo was made at Daminggong Grain Depot. Comparing with the common recirculation fumigation technologies, it has more advantages, such as the fumigant concentration balance being reached in shorter time, maintaining a high enough dosage for sufficient time, good cost – benefit and the chance of infesting insects again being reduced.

Key words: squat silo, recirculation fumigation under plastic sheet

Introduction

Silos with ratio of height to diameter of less than 1.5 have often been called "squat" silos. As squat silo has such advantages as large storage capability, small floor area, easy automatic implementation, good aeration and recirculation fumigation systems, ease of loading and unloading with several silos in-line, in the late 1990s, starting in 1998, when the project of construction twenty-five thousands million kilogram warehouse was put into practice, a number of squat silos were built for state grain reserve storage. To improve the sealing performance, grain mass surface should be sealed by plastic sheet, under the impulse of recirculation blower, the technology of recirculation fumigation under plastic sheet can make phosphine circulated through circle ducts and other ventilating ducts equipped in advance.

In recent years, along with this technology was put into production in some large warehouses and squat silos, it has created many difficulties, such as phosphine infiltrating slowly by gravity in high and large grain masses (like the new 30 × 60m warehouses and 30 m diameter squats), uneven gas distribution, resulting in poor insecticidal control effects, labors intensity and other negative issues. But in the new construction at state grain depots since 1998, recirculation fumigation has been frequently used. The technology had some choke points, such as strictly required warehouse sealing performance, high non – effective dosage rates in the procession of fumigation implementation, phosphine losing excessively, storages being quickly infested with insects again shortly after fumigation. In addition, in the process of implementation in squat silos, the silo roof ventilation hole was very difficult to seal. Therefore, to conquer some disadvantages in the procession of the common recirculation fumigation, the research on recirculation fumigation under plastic sheet in squat silo had been made at Daminggong Grain Depot State Grain Reserves, in Shaanxi province. Many good effects have been achieved by this experiment.

1 Materials and Methods

1.1 Materials

1.1.1 Testing warehouse

No. 11 squat silo was chosen as the testing warehouse. It was built in 1998, designed for a capacity of 8750 tons and whole volume of 12410.9 cubic meters. It has equipped with immobile recirculation fumigation system. The wheat for testing was loaded in the squat silo in 2005. The specific qualities and pests of storage grain are listed in Table 1.

Table 1. The specific qualities and pests of storage sweat at No. 11 squat silo

Amount (ton)	Grain moisture (%)	Average temperature of grain mass (℃)	Species of main pests	Pests density (No./kg)	Height of grain mass (m)	Volume of grain mass (m^3)	Type of recirculation fumigation device
7772.945	11.5	20.6	Rusty grain beetle	5	13.9	9820.35	Built – in piping with recir culation blower

State Grain Reserves Xi' an Daminggong Depot, Shaanxi, Xi' an, 710016

1.1.2 Chemicals and Its dose

Aluminium Phosphide (56%), carbon dioxide, application concentration of 1.22 g/m^3 with chemicals, with the total dose of 12 kg, at the volume ratio of carbon dioxide to phosphine of 98∶2. delivered from fixed phosphine generator.

1.1.3 Phosphine Recirculation Device

YYWF7122 type of recirculation fumigation fan: quantity of ventilation of 600 m^3/h, pressure of ventilation of 1000 Pa, power of 0.55 kW; moveable recirculation ducts under PVC plastic surface sheet: Polyvinyl Chloride (PVC) tube with diameter of 110 millimeters and 4 meters long; as well as recirculation fumigation ducts outside the silo.

1.1.4 Phosphine Provider

LM – KF3608 – V type of mobile fixed fumigation device outside of warehouse.

1.1.5 Device for Detection

DST – 01D type of phosphine monitor, DST – 01A type of phosphine alarm apparatus.

1.1.6 Ventilation Ducts

Underground conduit with pectinate ventilation ducts and six ventilation hole, equipped at both south and north sides of squat silo.

1.2 Methods

1.2.1 Equipment with Recirculation Fumigation Ducts under Sheet

Connect one port of the PVC flexible duct, Φ110 mm diameter and 4 m long, with the upper recirculation gas suction hole in squat silo, embed another port 10 cm under grain mass surface.

1.2.2 Make Grain Mass and Ducts Sealed

After completing equipping of recirculation fumigation ducts under sheet, cover grain mass with PVC film in squat silo, and joint the connection place of PVC film with bonding machine, then connect PVC film with thermometric cables and walls respectively by scotch tape, at the end, seal with scotch tape again at the edge of ventilation ducts.

1.2.3 Gas Tightness Test

After having sealed grain mass and ventilation holes, the half life of pressure, which it takes in rising the pressure of silo from – 500Pa to – 250Pa, would be tested as a piece of parameter of silo tightness.

1.2.4 Equip Devices

Connect recirculation ducts with the scavenge port of LM – KF3608 – V type of mobile out-warehouse fumigation device, and the high-pressure soft duct of CO_2 cylinder with the intake port of out-warehouse fumigation device.

1.2.5 Gas-leaking Test

By operating the recirculation fumigation fans and generator unit of mixed phosphine and carbon dioxide, check whether working smoothly, and check for gas-leakage by soap bubble test.

1.2.6 Application with Chemicals

Put 12 kg Aluminium Phosphide (AlP) prepared before into pot storage for chemicals, and then open the valve of CO_2 cylinder and make the gas pressure keep the level of 0.2 MPa. 5 minutes later, the recirculation ducts have been cleaned by CO_2, and provide chemicals at the rate of 45 g per minute.

1.2.7 Recirculation

At the early stage of providing chemicals, open the device for recirculation, it would not finish until the concentration of phosphine in both sides of south and north was in balance approximately (the minimum to maximum ⩾ 0.6).

1.2.8 Measurement for Phosphine Concentration

Since 4 hours after recirculation, phosphine concentration has been detected once by phosphine monitor everyday. It should not end until finished.

1.2.9 Inspection on Pests Control

After fumigation was over, the residue should continue to aerate properly. Inspection on pests control should not be carried on by grain sampling until chemicals scattered out.

2 Results

2.1 Airtight Quality of the Squat Silo

It has been shown that the half life of pressure of No 11 squat silo was 110 seconds by gas tightness test.

2.2 Phosphine Concentration Alteration

It has been shown from phosphine concentration determination that the phosphine concentration in grain mass would be in balance approximately 24 hours after fumigation, and it would reach the maximum value one hour later, and then began to decrease gradually, till about 30 hours after fumigation, the ratio of the minimum concentration to the maximum one has become 0.75, the uniformity of phosphine concentration in grain mass has kept the level of 80%. 28 days later, the average phosphine concentration in grain mass still could keep 70 mL/m^3 upwards. The total concentration-time product (CT) could be beyond 180mg. h/m^3.

2.3 Effects on Pests Control

After having cultured the sampling pests for 14 days, none of them was found to keep alive and the mortality was 100%.

3 Analyses and Discussion

As the same as the common recirculation fumigation, comparing with the common fumigation, recirculation fumigation under sheet have more advantages, including decreasing the application quantity of chemicals, being easy to phosphine distributing evenly as soon as possible, convenient to add chemicals and safeguarding workers healthy etc.. Therefore, emphasis on comparing with the common recirculation fumigation will be listed as follows.

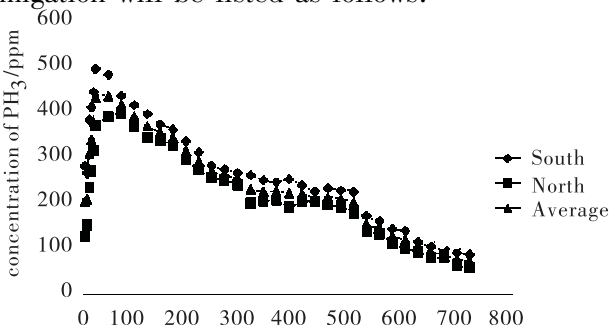

Fig. 1 Relationship between PH_3 concentration with fumigation hours in No. 11 squat silo

3.1 By recirculation fumigation under sheet, the effective fumigation concentration would be reached in shorter time around the whole silo, and a high enough dosage could be maintained for sufficient time. Thereby, the effect of fumigation would be improved

3.1.1 Its airtight quality be superior to one while the common recirculation fumigation

From the results of gas tightness test, its airtight quality was 110 seconds, however, while the same squat silo was carried on the common recirculation fumigation in 2000, its airtight quality measured was 75 seconds, obviously, its airtight quality while it carried on recirculation fumigation under sheet was superior to one while the common recirculation fumigation.

3.1.2 Phosphine uniformity in grain mass be superior to one while the common recirculation fumigation

As the gas volume circulated which recirculation fumigation under sheet needs was less than that the common recirculation fumigation needs, the times gas exchanged per second became more frequent, and it took less time for phosphine concentration coming into balance than the common recirculation fumigation, and less time for gas circulating. Moreover, at recirculation fumigation under sheet, after concentration into balance, the ratio of the minimum concentration to the maximum concentration always kept 0.7 upwards. From the figure on PH_3 concentration fumigation hours, it seems smooth and the phosphine concentration decreased on an even keel.

3.1.3 Maintain a high enough dosage for sufficient time by recirculation fumigation under sheet

In the first 30 days after fumigation, PH_3 concentration in No. 11 squat silo decreased at the rate of 2.87%. After being sealed, phosphine concentration inside still could keep 100 mL/m^3 upwards for 26 days to kill all life stages of the infesting insects. However, at the common recirculation fumigation, phosphine concentration decayed at much greater speed, according to the paper reported[1], 5.7 hours later the common recirculation fumigation, the maximum average concentration reached the level of 791.0 mL/m^3, however, 113 hours later, phosphine concentration in grain mass arrived at 102.5 mL/m^3, 140 hours later, it only left at 31.1 mL/m^3, therefore, in the first 6 days after fumigation completed, phosphine decreased at the rate of 16% everyday, which was 4 times than recirculation fumigation under sheet. In addition, at recirculation fumigation under sheet, It would took 14 days for phosphine concentration decreasing from 400 mL/m^3 to 100 mL/m^3, and at common recirculation fumigation, it would only took 4 days. Therefore, to reach a certain CT value, it would need to apply chemicals at the common recirculation fumigation with over 2 times than at recirculation fumigation under sheet. From these standpoints, during recirculation fumigation under sheet, it would not only save non – effect application quantity under the sheet, and also decrease the total chemicals application quantity.

3.2 Good Cost – benefits at Recirculation Fumigation Under Sheet

Comparing with the common recirculation fumigation, as no chemicals application in the empty space, the volume of fumigation became less, and less dosage would be provided, moreover, it took less time on recirculation, and needed less electrical consumption, so it had better economical benefits, ecological benefits and social benefits. While fumigation expenditure under sheet per ton grain in No 11 squat silo was 0.123 RMB, the one of common recirculation

fumigation was 0.317 RMB. So it could save 60% upwards once. In addition, as the small possibility of infested insects again after recirculation fumigation, grain could be stored safely by carrying on low oxygen and no chemicals, and then grain contamination and environment pollution also decreased further.

Table 2. expenditure table on recirculation fumigation under sheet

Aluminium Phosphide			Carbon Dioxide			Electrical Consumption by Fumigation Device		
Dosage (kg)	Price (RMB/kg)	total (RMB)	Dosage (kg)	Price (RMB/kg)	total (RMB)	Time(h)	Power(kW)	Fees(RMB)
12	30	360	200	2	400	3.5	5.2	12.7

Electrical Consumption by Recirculation Ventilator			Health Care for Fumigation Workers(RMB)	Expenditure Each Ton Grain(RMB/t)
Time(h)	Power(kW)	Fees(RMB)		
80	0.55	32.6	150	0.123

3.3 At Recirculation Fumigation under Sheet, Safety Should Be in Care

Though gas tightness quality was better at recirculation fumigation under sheet, still could little phosphine leak outside, so in case of checking inside in the implementation process of fumigation, workers should be careful and wear Full Face Canister Respirator well.

3.4 Prevent from Dewfall of Grain Mass in the Implementation Process of Fumigation

As the squat silo was higher, stack effect came into being easily. Under the promotion of stack effect and recirculation ventilator, the moisture in grain mass would go upward, if the moisture gone upward could not scatter out, it would be easy to produce dewfall on the surface of grain mass. So while carrying on fumigation under sheet, check and inspection should be taken frequently to ensure grain storage safety.

In a word, recirculation fumigation under sheet, with broad application prospects, will be the first alternative technology for grain storage fumigation in the future.

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0428

Application of Sealed Flexible Vacuum – Hermetic Storage System for Quality Preservation of Turkish Red Chili Pepper

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Abastract: In this study hermetic and vacuum storage methods were tested under small commercial scale by comparison with traditional storage methods in the warehouse, assessing the quality parameters of Turkish red chili peppers (RCP). One ton of crushed and mechanically dried RCP with maximum 10 1% of moisture content were stored for 7 months under a low pressure of 80 – 100 mm Hg, sealed hermetic conditions with traditional storage method (open piles in warehouses) used as control. Basic quality parameters related to moisture contents, color, microbial loads and aflatoxin, were determined before storage and after 7 months storage. Field scale trials indicated that the best quality red chili pepper resulted from vacuum storage with very low changes in quality parameters (pungency, colour, aflatoxin). Although hermetic storage resulted in high level losses of colour, microbial growth and aflatoxin contamination were prevented, and the pungency of red chili pepper was preserved. This study successfully demonstrated the feasibility of commercial application of hermetic and vacuum storage technology for long-term storage of red chili pepper for the first time in the world. Vacuum technology was proven to be an effective, chemical-free and economical method to disinfest commodities of insects, to inhibit the development of moulds, aflatoxin occurrence and to prevent quality damage of red chili pepper due to the oxidative and fermentative processes. In conclusion, this small scale commercial study indicates that sealed flexible vacuum-hermetic storage technology offered potentially significant advantages over traditional storage methods in ability to enhance preservation of quality parameters such as colour, pungency and control of aflatoxin of RCP for long-term storage

Key words: red chili pepper, aflatoxin, colour, *Capsaicin*, vacuum, hermetic storage

Introduction

The *Capsicum* species of red chili peppers are grown worldwide for fresh fruit and spices production. The main types of spices are powders that are derived from hot, red – coloured chili fruit or from mild, red – coloured paprika fruit. The resulting spice is referred to as chili or paprika spice. The red Chili pepper (*Capsicum annum* L.) is an extremely important crop for the Turkish food industry as well as world food sector. It is mainly produced in South and West Anatolia, and Marmara regions in Turkey. Almost all of the produced red pepper in South Anatolia region of Turkey is largely processed into spices, while the other regions produced it for fresh consumption and red pepper paste or sauce(ztekin et al. ,1999). Red chili peppers especially produced in Kahramanmaras, Gaziantep and anl urfa province of Turkey are well known for their pungent taste and flavours. Other red pepper spices produced in Kayseri, Bursa and Bilecik provinces are also famous for their sweetness. Although both the pungent and non-pungent red peppers are very important crops having high export potential for Turkey, main focus of this research is red chili pepper for spices production.

On the average, production of powdered and crushed RCP in Kahramanmara and surrounding areas is about 18 000 tones/year which constitutes 45% of Turkey's total red pepper spices production of around 40 000 tons/year (Anonymous, 2001). Although the final quality of processed RCP is assessed by a number of different parameters, colour and pungency levels are accepted as the most obvious parameters (Kim et al. ,2002). However, taste and flavour of non-pungent paprika powders are also important. In addition the spice trade may specify limits of impurity, levels of microbial counts of fungi, yeasts, *Salmonella* and coli-

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forms, particle size and moisture content. Red pepper is a very sensitive product for aflatoxin formation depending on suitable processing conditions (Coksoyler, 1999). Many survey showed the presence of xerophilic mould species, especially *Aspergillus fumigatus*, *A. flavus*, *A. niger*, *A. ochraceus*, in most pepper samples (El – Kady et al., 1995; Adego et al., 1996; Freire et al., 2000; Vrabcheva, 2000).

There are several major technical and economic problems in the processing and storage phases of Turkish red chili pepper (RCP) production. Fungal contamination greatly increases the risk of *aflatoxin* development on the chili peppers. Both the lack of a cleaning process of the freshly harvested chili pods and the traditional solar drying method in the open, increase the risk of fungal and subsequent mycotoxin development on the chili peppers. Once the chili peppers have been dried to 10% to 12% moisture content they are liable to reabsorb moisture by exposure to ambient humidity during their prolonged storage period. Then they become vulnerable to renewed fungal attacks, and possible development of mycotoxins, which is highly detrimental to their taste and aroma, and increases the risk of toxic residues. Stored product insects can also damage and contaminate the chili peppers during long-term storage by reducing their nutritive value, affecting their handling properties and contaminating them with body parts and excreta, all of which reduce their quality. Moreover, there is a serious problem of quality deterioration of chili peppers during storage due to the oxidative processes that result in changes in the color pigments (ztekin et al., 2006). These problems associated with the manufacturing and storage of chili peppers reduce the competitive power of Turkey on the international market and decrease Turkey's share of this lucrative export commodity.

Objective

The primary objective of this study is to store a high volume of red chili pepper under vacuum and hermetic conditions using sealed flexible PVC storage containers (Vacuum – Hermetic Fumigation (V – HF) technology). Storage of this commodity under vacuum and hermetic conditions was studied in comparison with their storage by traditional method (in open piles in warehouses) by evaluating some quality parameters including mould growth, aflatoxin occurrence, insect infestation and quality damage.

Materials and Methods

Experimental Set up of Vacuum – Hermetic Storage System

A small scale commercial trial was conducted in a red chili pepper processing company, BBERYUM Ltd in Gaziantep, Turkey. In this study we used two new transportable flexible storage units of 5 m^3 capacity each termed the "Volcani Cube™" or "GrainPro Cocoon" originally designed for hermetic storage. To adapt the hermetic cube system to work under low pressures, modifications were made on the cube and the pump:

a. Modifications of the cube

The connection between the vacuum cube and the pump is through a hard PVC 1.5″ tube located at the base of the cube. This hard tube is connected to flexible 1.5″ tubing through a one – way vacuum line valve by a quick release connection. The one – way valve is required to render the system modular, enabling the user to connect several cubes to the same vacuum pump or disconnect one of the cubes without changing the pressure in the other connected cubes. To enable accurate measurements of the pressure in the cube, a small outlet at the top of the cube was added, and a 6 mm tube was connected directly to the sensor of the vacuum pump transducer;

b. The modifications on the vacuum pump

The low pressure in the cubes is established using a rotary vane oil – lubricated vacuum pump. In order to minimize damage to the pump by dust particles and commodity vapors, two filters, a dust filter and a carbon filter, were added at the flexible 1.5″tube connection to the pump. The pressure is monitored by a control panel for on and off mode of the pump at a predetermined pressure and starting it again when the pressure in the cube rises above a desired pressure. To provide the control panel with the pressure data needed to monitor the pump, the control panel is connected to a pressure transducer, the sensor of which is connected via a 6 mm i. d. tube to the opening on top of the storage cube.

Description of Small – scale Commercial Trial

The trial was conducted in a warehouse of a red chili pepper processing company, BBERYUM Ltd in Gaziantep, Turkey. One ton of crushed and mechanically dried RCP with maximum 10% ± 1% moisture content were stored for 7 months under a low pressure of 80

-100 mm Hg, sealed hermetic conditions with traditional storage method (open piles in warehouses) used as control. The selected test site was a level concrete floor in a warehouse. The warehouse was 80 m in length by 70 m width by 8 m high. On the walls near the roof there was a web of brick size holes providing ventilation of the storage warehouse when the doors were closed. Each hermetic and vacuum cube contained 40 jute bags, each weighing 25 kg (total 1 000 kg per cube). The cubes were loaded manually and stacked to a height of three layers. A control stack consisting of 2 pallets, 20 jute bags per pallet, for a total of 40 jute bags was used without applying vacuum, leaving opened in piles in warehouses.

Three samples of 5 kg were collected from each hermetic and vacuum cubes, and from the control stack before and after 7 months storage. The samples were taken from the top, the middle and the bottom layer. Each sample of 5 kg was a combined sample of red chili pepper collected from sacks located at the 4 corners and center of the sampled layer. In each of the vacuum and hermetic cube, 14 sacks were sampled representing 35% of all sacks in each cube. The same method was used to sample the control stack, 14 bags were sampled representing 35 % of all sacks.

Two data loggers (HOBO Pro Series) were inserted in the hermetic and vacuum cubes and in the control stack, one at the bottom and one at the top to record the temperatures and r. h. during the trials. One data logger was placed outside the cube for 7 months vacuum and hermetic storage to record the ambient conditions in the storage area. Pressure was monitored using continuous reading transducers in the vacuum treatments. Oxygen (O_2) and carbon dioxide (CO_2) level in hermetic storage container were monitored by using hand - operated O_2/CO_2 analyzer (PBI Dansensor).

Quality Analysis

Basic quality parameters related to moisture contents, color, microbial loads, and aflatoxin were determined before storage and after 7 months storage.

Colour Measurement

Surface colour of chili pepper samples was measured before and after 7 months storage by using a colour meter (Minolta Co.; Model: Chroma cr - 100). The colour meter was calibrated against a standard calibration plate of a white surface and set to CIE Standard Illuminate C. The display was set to CIE $L^* a^* b^*$ colour coordinates. At least six random readings per sample were recorded and the average values of colour parameters (L^*, a^*, b^*, C^* and H) with standard deviation values and the colour difference (ΔE^*, ΔL^*, Δa^*, Δb^*, ΔC^* and ΔH^*) were reported. The colour brightness coordinate L^* measures the whiteness value of a colour and ranges from black at 0 to white at 100. The chromaticity coordination a^* and b^* have no specific numerical limits. The coordinate a^* represents red when positive and green when negative, while the coordinate b^* represents yellow when positive and blue when negative. The metric chroma C^* and metric hue angle H^* were derived from the values for CIE $L^* a^* b^*$ colour coordinates. ΔE^*, ΔL^*, Δa^*, Δb^*, ΔC^* and ΔH^* were used to describe the colour quality difference (Anonymous, 1996).

Microbiological Analysis

Representative 10 - g portions of red chili pepper from each sample were aseptically weighed and homogenized with 90 mL sterile peptone - physiological saline (0.1 % w/v neutral peptone, 0.85 % w/v sodium chloride, pH 7.2). Serial decimal dilutions were prepared with the same diluents, duplicate counting plates were prepared using appropriate dilutions. After incubation at appropriate temperatures, the colonies that appeared on the selected plates were counted as colony forming units (cfu) per gram weight sample. The surface spread method was used for total aerobic mesophylic bacteria (Nutrient Agar (NA) - Merck) and yeast/moulds (Potato Dextrose Agar (PDA) - Merck) at 30 ℃ for 48 - 72 h, 25 ℃ for 3 - 5 days, respectively (Borcakli et al., 1994; Temiz, 1996). *E. coli* and coliform were determined by the MPN method (3 tubes) on Florocoult Laurly Sulfate Broth (Merck) at 37 ℃ for 24 hours (Williams and Busta, 2000; Borcakli et al., 1994)

Aflatoxin Analysis

Samples were analyzed using the validated method of Association of Official Analytical Chemists International (AOAC, 2000). All samples were finely ground, thoroughly mixed, extracted and filtered. The immunoaffinity column was diluted with 60 mL PBS and applied to the conditioned column (2 - 3 mL/min). After that, the column was washed with 15 mL water (5mL/min) and dried by passing air through it. Finally, bound aflatoxins were eluted slowly with 2 mL methanol. All reagents were of recog-

nized analytical grade. The presence of aflatoxins was detected by high performance liquid chromatography (HPLC) using a post – column derivitisation electrochemically generated bromine (cobra cell) and a fluorescence detector.

Capsaicin Analysis

Capsaicin is quatitated using the isocratic HPLC method of Woodbury. (1980). Sample extracts was obtained by soxhleting 10g of ground peppers with 250 mL of HPLC grade acetone (Merck) for 5 hours. The extract was vacuum evaporated to 5 mL at room temperature. Bisphenol A (Aldrich), a common antioxidant was used as an internal standard. 1 g of oleoresin and 30 mg Bisphenol A were dissolved in 5 mL acetonitrile (Merck). A 2 mL aliquot was filtered through a Sep – pak C – 18 cartridge (Alltech). Ten microliters of this sample were injected directly to the HPLC system (Waters Associates Model ALC/GPC equipped with aM. 6000A pump, a U6Kinjection). The column was – Bondapak C – 18 column (300 × 4 mm, 5 m). Separation was accomplished via a variable wavelength UV detector (Water Associates) set at 280 nm. The isocratic mobile phase was methanol: water (60:40) with a

Data Analysis

Statistical analysis was performed by analysis variance (ANOVA) and Duncan's multiple range test to establish the actually differing applications. All these statistical studies were conducted using the SPSS software (SPSS Inc., version 11.0).

Results and Discussion

Daily temperature (C) and relative humidity changes (%) during (a) vacuum, (b) hermetic and (c) traditional storage of red chili pepper for a period of 7 months are given in Figure 1. Hermetic and vacuum storage effectively prevented moisture exchange between the surrounding air and the red chili pepper. There was only a slight increase in relative humidity of 2% and 5.2% in the hermetic and vacuum storage respectively. In contrast, relative humidity of control treatment (traditional storage) showed great fluctuations during the storage period, ranging from 32.5% to 82.1%. This result indicates that the moisture content of red chili pepper in open storage can be increased, mainly through moisture content exchange with the surrounding ambient air. It appears that there was not much difference in temperature changes in hermetic, vacuum and traditional storage. Similar results for other commodities such as barley, paddy rice and corn etc. have been reported by Varnava et al. (1995), Navarro et al. (1998) and Donahaye et al. (1999).

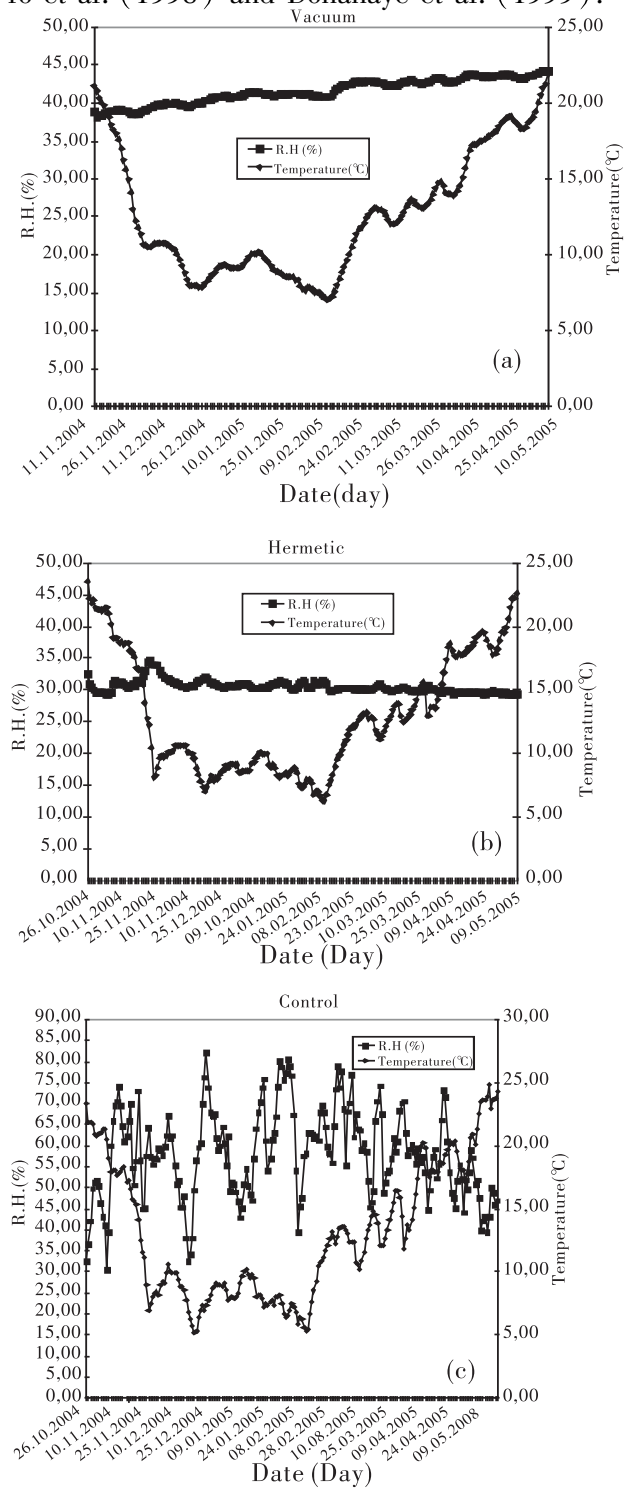

Fig. 1 Daily temperature (℃) and relative humidity changes (%) during (a) vacuum, (b) hermetic and (c) traditional storage of red chili pepper for a period of 7 months.

Colour stability of red chili pepper stored under different storage methods for a period of 7 months is given Table 1. Storage methods had a

significant influence on colour parameters of L^* (colour brightness), a^* (redness) and b^* (yellowness). After 7 months storage of RCP by traditional storage method, a considerable decrease in L^*, a^* and b^* values of RCP samples was found as compared to their initial average colour parameters. However, the L^* and a^* values after 7 months storage under hermetic condition indicated a considerable increasing tendency, while no significant difference in a^* values of RCP samples were found as compared to their initial average redness values. Contrary to these two storage methods, no significant change in all three colour parameters of RCP samples stored under vacuum for 7 months was found as compared to their initial average colour parameters. It appears that vacuum storage method gave the best results having retained initial reddish orange colour of RCP samples during 7 months storage. Similarly, Klieber (2000) reported that the most effective treatment was storing red chili pepper powder under nitrogen or vacuum; this reduced the weekly rate of colour loss to 3% – 5%. It was effective as oxygen that is needed for colour pigment autoxidation was excluded.

Table 1. Colour stability of red chili pepper stored under different storage methods for a period of 7 months

Storage method	Colour difference models				
	ΔL^*	Δa^*	Δb^*	ΔC^*	ΔH^*
Control	−4.128	−3.217	−7.500	−8.027	1.471
Hermetic	6.428	−0.333	7.539	6.324	4.117
Vacuum	−1.002	−2.761	−2.408	−3.534	0.966

Changes of microbiological parameters during vacuum, hermetic and traditional storage of red chili pepper for a period of 7 months are given in Table 2. Mould-yeast count in RCP samples stored by traditional storage method was increased significantly compared to their initial counts, whereas no significant change in mould-yeast count was found by vacuum and hermetic storage methods. As a result of this, there was an increase in Aflatoxin B1 and of total aflatoxin (B1 + B2 + G1 + G2) (g/kg) on RCP samples stored by traditional storage method for long-term storage, while no increase in Aflatoxin B1 and of total aflatoxin on RCP samples was found by vacuum and hermetic storage. Whereas there was a decrease in TAMM (total aerobic mesophyllic microorganism) count by vacuum and hermetic storage, significant increase in TAMM count was found by traditional storage methods compared with its initial count. The counts of coliform group bacteria and *E. coli* were found no significant change by all storage methods compared to their initial methods.

Table 2. Changes of microbiological parameters during vacuum, hermetic and traditional storage of red chili pepper for a period of 7 months

Parameters	Before storage	Control	Hermetic	Vacuum
Aflatoxin B1 (μg/kg)	<0.2	1.56	<0.2	<0.2
Total Aflatoxin (μg/kg)	<0.5	1.79	<0.5	<0.5
TAMB (cuf*/g)	2.1×10^5	5.2×10^6	3.9×10^3	1.1×10^4
Yeast – Mould (cuf/g)	3.3×10^4	3.6×10^5	3×10^4	2.7×10^3
Coliform (cuf/g)	<7	<7	<7	<7
E. coli (cuf/g)	<3	<3	<3	<3

* cuf: colony unit forming

Changes of capsaicin level and moisture content during vacuum, hermetic and traditional storage of red chili pepper for a period of 7 months are given Table 3. There was a significant reduction in capsaicin level of RCP samples stored by hermetic and traditional storage methods, compared to the initial levels before the storage. The highest reduction in capsaicin level of RCP samples stored by traditional methods was found and followed by hermetic storage method. Having no significant difference in capsaicin level of RCP samples stored by vacuum method compared to their initial levels, vacuum storage also indicated the best option of preserving the pungency levels of RCP during its prolonged storage period. Laboratory trials indicated that red chili pepper needed to be stored under vacuum, hermetic and high – CO_2 conditions to slow the loss of pungency during long-term storage (Isikber et al., 2006). Our result was in line with the results reported by Isikber et al. (2006). There was a significant increase in moisture content of RCP samples stored by traditional storage method for a period of 7 months, whereas no significant increase in their moisture contents was found by vacuum and hermetic storage. It indicates that RCP stored by traditional method are liable to reabsorb moisture by exposure to ambient humidity

during their prolonged storage period.

Table 3. Changes of capsaicin level and moisture content during vacuum, hermetic and traditional storage of red chili pepper for a period of 7 months

Parameters	Before storage	Control	Hermetic	Vacuum
Capsaicin level (mg/kg)	89.79 ± 0.38 A	83.01 ± 0.25 C	86.10 ± 0.31 B	88.35 ± 0.22 AB
Moisture content (%)	10.03 ± 0.25 A	12.38 ± 0.19 B	10.47 ± 0.28 A	9.68 ± 0.31 A

Different upper case letters indicate significant differences among means within a row for a particular activity (ANOVA followed by LSD, $\alpha = 0.01$).

Small scale commercial trials indicated that the best quality red chili pepper resulted from vacuum storage with very low changes in quality parameters (pungency, colour, aflatoxin). On the other hand, hermetic storage resulted in high level losses of colour, while microbial growth and aflatoxin contamination were prevented, and the pungency of red chili pepper was preserved. In conclusion, this small scale commercial study indicates that sealed flexible vacuum-hermetic storage technology offered potentially significant advantages over traditional storage methods in ability to enhance preservation of quality parameters such as colour, pungency and aflatoxin of RCP for long – term storage.

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0429

Research on Different Fumigation Methods for Controlling Booklice(Psocids)

Deng Shuhua and Chen Quling

Abstract: The authors performed warehouse experiments to evaluate four models: recirculation fumigation, routine fumigation, routine fumigation combined with protectant and single-purpose protectant to control booklice in High Flat Warehouses and Arched Slab Warehouses. The results show that routine fumigation combined with protectant can maintain the density of booklice below 50/kg over 12 months and is a relatively effective method to control booklice.

Key words: booklice, recirculation fumigation, boutine fumigation, protectant

Introduction

At present, a severe problem that grain stored faces in state warehouses is rampant booklice(psocids). Booklice not only result in the loss of grain storage, but also their remains and excrement give out a foul smell, which contaminates grain storage and environment. All along, people have been paying more attention to insect control of relatively bigger insects such as the grain moth, *Sitophilus zeamais* Motsch., ignoring booklice. However, because of short growing period and fast reproduction of booklice, it has become one of pervasive insects all over the country. Meanwhile, because of its small body, ease of escaping, parthenogenesis, PH_3 resistance etc., booklice control has become a spiny problem in the work of insect control of grain storage.

In 2006, in order to investigate new approaches to booklice control, we cooperated with Hunan Dong'an State Grain Reserve Warehouseto develop recirculation fumigation, routine fumigation, routine fumigation plus protectant and single-purpose protectant as four experimental methods to control booklice in High Flat Warehouses and Arched Slab Warehouses.

1 Materials and Methods

1.1 Facilities and Materials

1.1.1 Experimental warehouse type Arched Slab Warehouse and High Flat Warehouse.

1.1.2 Experimental seeds Early Indica Rice, Medium Indica Rice, Late Indica Rice.

1.1.3 Experimental facilities grain grading sifter, wooden rake, equipment for recirculation fumigation.

1.2 Experiment Handling

Basic situation of experimental warehouse, handling method, using dosage etc., (see Table 1 below)

Table 1. Experimental warehouse type, dosage and grain t

Type and No.	Arched slab warehouse(area 600m^2)			High flat warehouse(area 600m^2)			
	104-01	104-02	103-01	110-02	108-02	107-01	106-02
Handling agent	Shushiling + routine fumigation	Shushiling	Routine fumigation	Shushiling + routine fumigation	Routine fumigation	Shushiling	Recirculation fumigation
AlP dosage	30kg		50kg	20 kg	30 kg		20 kg
Protectant dosage	60 kg	60kg		40kg		40kg	
Grain storage types	Medium Indica Rice	Medium Indica Rice	Medium Indica Rice	Late Indica Rice	Late Indica Rice	Early Indica Rice	Late Indica Rice
Reserves(t)	1499	1505	1501	2130	2128	2137	2122
Year	2004	2005	2004	2004	2005	2005	2005
Water(%)	12.3	12.4	12.2	12.4	13.5	12.6	12.5

Hunan Cereals and Oils Science Research & Design Institute

Type and No.	Arched slab warehouse(area 600m^2)			High flat warehouse(area 600m^2)			
	104 – 01	104 – 02	103 – 01	110 – 02	108 – 02	107 – 01	106 – 02
mixture (%)	0.6	0.7	0.7	0.7	0.7	0.7	0.8

1.3 Experimental Agents

1.3.1 Shushiling powder(3%) made by Hunan Cereals and Oils Science Research & Design Institute

1.3.2 Aluminium phosphide(AlP) AlP tablet(56%) made in Shenyang.

1.4 Method for Using Agent

1.4.1 Shushiling powder(3%) handling

Investigating population density before using dosage and selecting D1.00mm grain grading sifter, the experimenters powder the surface of grain with 75% Shushiling powder. After powder falls on the surface of grain, drag wooden rake back and forth on the surface of grain so that the chemical agent can be turned into the grain layer 15cm to 30cm; then cover the surface of grain with 25% remaining powder and turn the surface over by using a wooden rate; More dosage should be used on the surface of grain near the gate of warehouse and 1.0m away from the wall.

1.4.2 Recirculation fumigation

Referring to *New Grain Storage Technology* to process recirculation fumigation of phosphine

1.4.3 Routine fumigation

Referring to *Standard of Cereals and Oils Storage Technology* to process fumigation by adopting the method of spraying dosage on the surface

1.5 Investigating Population Density

On April 20, based on the booklice situation in the previous year, the experimenters selected five sample places in the severe part of the booklice population. Sampling amount is not less than 2kg in each sample. Population density is checked by using a grain grading sifter. After applying pesticides, population density should be checked throughout the fixed period.

2 Results and Analysis

2.1 The Four Handling Methods for the Efficiency of Booklice control in High flat Warehouse

Table 2. The four handling methods for the efficiency of booklice control in high flat warehouse

investigating time	Warehouse No. and handling methods			
	107 – 01	108 – 02	106 – 02	110 – 02
	Shushiling	Routine fumigation	Recirculation fumigation	Shushiling + Routine fumigation
4.20	25	13	10	26
4.21	Application using dosage			Application using dosage
5.20	2	32	35	3
6.20	1	45	40	1
7.09	5	70	60	4
7.17	7	fumigation	fumigation	6
7.26	10			10
7.31	8	close	close	8
8.10	26			6
8.18	35	ventilation	ventilation	18
8.30	45	0	0	fumigation
9.07	40	8	2	close
9.14	65	17	5	
9.22	80	35	10	ventilation
9.29	200	50	15	0
10.07	300	90	47	0
10.23	innumerable	200	78	0
11.10	innumerable	innumerable	160	0
11.21	innumerable	innumerable	300	0

According to Table 2, after only using Shushiling powder (3%) in warehouse 107 – 01, booklice population density dramatically decreased from 25/kg on April 20 to 1/kg on June 20 and low-population density lasted for nearly three months. It didn't begin to increase until the early of August. By the end of September, because of high grain temperature and humidity, booklice reproduced very quickly. The experiment shows that Shushiling has sound efficiency for booklice control and that the control period amounts to five months, but it can't thoroughly disinfest booklice.

In experimental warehouses 108 – 02 and 106 – 02, protectant of grain storage was not used. With the increasingly high temperature and grain humidity, booklice production and reproduction became very fast, activity was frequent, which caused population density to become large. Therefore, on July 17 fumigation of phosphine had to proceed. After ventilating on August 18, without the efficient control of the protectant, booklice activity became rampant again in two experimental warehouses, and moreover the speed rapidly increased and surpassed the prevention and control level. Considering the cost of prevention and control and machinery ventilation to be processed, grain file handling was not implemented.

In warehouse 110 – 02, firstly we used Shushiling of grain storage protectant and then combined ALP routine fumigation to control booklice, whose population density basically kept below 50/kg throughout 10 months. The control indicator had nota been reached (control indicator decided internally by China grain reserves corporation).

Therefore, we concluded that routine fumigation plus protectant has quite outstanding efficiency for prevention and control of booklice in the high flat warehouse.

2.2 Three Handling Methods for the Efficiency of the Prevention and Control of Booklice in Arched slab Warehouse

According to Table 3, in experimental warehouse 104 – 02, on April 21 after using only Shushiling powder (3%), booklice were efficiently controlled. By the end of August booklice density was basically controlled under 50/kg. But in September population density dramatically increased and rapidly reached the range of prevention and control. Therefore, in order to efficiently control booklice to produce, 30 kg of Shushiling powder (3%) had to be added.

As for experimental warehouse 103 – 01, without using grain storage protectant and with the increasing temperature and grain temperature, booklice production and reproduction became fast, which caused population density to increase rapidly. On July 31, fumigation of phosphine was required to control and prevent booklice. After ventilating on August 18, without the efficient control of grain storage protectant booklice reproduced very quickly and rapidly reached the range of prevention and control. In addition to this, because grain moth, *Sitophilus zeamais* Motsch. and other main insects were rampant, a second fumigation was initiated on October 23.

Table 3. Three methods for the efficiency of the prevention and control of booklice in arched slab warehouse (unit: insect/kg)

Investigating time	Warehouse No. and handling methods		
	104 – 01	104 – 02	103 – 01
	Shushiling + Routine fumigation	Shushiling	Routine fumigation
4.20	19	15	11
4.21	using dosage	using dosage	
5.20	9	10	30
6.20	7	8	50
6.30	10	20	70
7.09	15	5	120
7.17	20	8	140
7.26	40	10	280
7.31	40	15	fumigation
8.10	48	30	sealed
8.18	fumigation	45	ventilation

Investigating time	Warehouse No. and handling methods		
	104 – 01	104 – 02	103 – 01
	Shushiling + Routine fumigation	Shushiling	Routine fumigation
8.30	close	40	10
9.07	ventilation	80	15
9.14	0	100	20
9.22	0	200	20
9.29	0	using dosage	25
10.7	0	35	70
10.23	0	14	fumigation
11.10	0	9	close
11.21	0	10	ventilation

In experimental warehouse 104 – 01, we adopted the method of fumigation combined with protectant so that booklice population density could be kept low throughout the storage period.

Therefore, we conclude that fumigation combined with protectant has good effects to control the production and reproduction of booklice in arched slab warehouse.

3 Conclusion and Discussion

3.1 Because PH_3 has sound penetrating ability, routine fumigation and recirculation fumigation can achieve a high one-off killing rate. Recirculatory fumigation is better than routine fumigation.

However, each fumigation method has the disadvantage of a short control period so that booklice recurs very quickly after ventilating.

3.2 Grain storage protectant of Shushiling, with a long control period over five months, is relatively effective for booklice control. Because it functions as a contact and stomach poison, booklice in deeper layer can not be killed or thoroughly destroyed.

3.3 Fumigation combined with protectant can give full play to the strong penetrating ability of PH_3, which thoroughly kills pests, coupled with the long control period feature of protectant. The result is that production and reproduction of booklice can be efficiently controlled to ensure booklice density, over a year, remains less than 50/kg a year (control indicator decided internally by China Grain Reserves Corporation).

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0430

Phosphine Recirculation Fumigation in Horizontal Storage in Low-temperature and Dry Region

Wu Lei, Ai Shaozi and Liu Ningquan

Abstract: The concentration change of phosphine was measured during recirculation fumigation in horizontal storage in low-temperature dry region. Aluminum phosphide was laid in the ventilation vents, the doseage of which was 0.7 g/m^3. After phosphine generated from aluminium phosphide fomulation reacted with vapor in air, all the stored pests such as *Sitophilus zeamais* Motschulsky, *Rhizopertha dominica* (Fabricius) was killed completely. Compared with the expense of the conventional fumigation, the one of recirculation above was to reduce 68.7%, and a comparative analysis of it had been carried on.

Key words: horizontal storage, aluminum phosphide, recirculation

Compared with general warehouses built earlier, new horizontal storage warehouses are larger overall with greater height. Limited by the penetrating ability of phosphine by gravity, pests were hard to kill with first fumigation, and often had to be fumigated again. The resistant of pests against PH_3 was increased, which causes the waste of manpower and resources. Phosphine recirculation fumigation technique is a management to control pests in this storages, In 1998, China began building new larger horizontal storage warehouses at state grain depots. These new 3 500 to 5 000 ton storages included recirculation fumigation systems incorporated with in-floor aeration ducts, which has played an important role in controlling stored pests and maintaining grain storage security. Recirculation fumigation systems were better designed to distribute phosphine gas evenly, providing higher grain security by killing insects much more effectively than the conventional fumigation.

Zhongning depot is situated at Ningxia in the northwest of china, which is in Low-temperature Dry Region all the year. During the winter, the average temperature is below 0 C for 5 months. The summer is short, a period lasting 3 months. According to climate characteristic, the depot staff took measures to improve grain security in order to reduce the number of fumigations and the dosage levels of phosphine, to save cost of storage and reduce the labor intensity.

Since 2002, Zhongning depot proposed new pest management practices to preserve grain, including such measures as laying aluminum phosphide in the ventilation duct chanels using phosphine generated from aluminium phosphide fomulation which reacts with vapor in air, aeration for cooling in the fall and winter, phosphine fumigation under plastic sheeting in the spring, and ventilation in the summer. Using all these grain management practicies all the pests were killed, which prevented insects from reproducing so much. After these measures, fumigation one time each year has provided obvious economic efficiency.

1 Materials

1.1 Horizontal Storage

The capacity of the #5 depot warehouse was 3 600 t. The length was 36.8m and width 24.0m. The total volume was 8 390 m^3, with the height of grain, 5.5 m. The volume of grain storage is 4 602 m^3 and the headspace volume above the grain is 3 788 m^3. The horizontal storage has four U - shaped perforated aeration duct channels. The polyvinyl chloride sheets were used to seal all the doors, windows, vents and aeration fan ducts.

1.2 Grain quality and environment

The kind of grain stored was wheat, with moisture of 12.5%, impurity of 0.8%. There were 3 721 t of wheat in the horizontal storage. The bulk temperature, highest grain temperature, and average grain temperature in the horizontal storage was 25℃, 25.8℃, and 16℃. The average ambient air humidity outside and air humidity in storage was 60% and 69%.

1.3 Pest Density

Pest density in the storage averaged 16 per kg: *Sitophilus zeamais* Motschulsky was 10 per kg, *Rhizopertha dominica* (Fabricius) was 3 per

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kg, *Sitotroga cerealella* (Olivier) was 3 per kg.

1.4 Fumigants

The 56% aluminium phosphide pills were made in Shenyang. The doseage of 0.7 g/m^3, provided a target concentration of PH_3 of 100 ppm required 6 kg of phosphine pills.

1.5 Equipment and Apparatus

Fixed recirculation fan and in – place suction piping from the warehouse headspace with pressure piping connected to the aeration in – floor ducts. .

Phosphine monitor: XL – 210G, Beijing Liangkemao CO., LTD, Range: 0.1 – 1000 ppm.

Alarm apparatus for phosphine personnel safety: XL – 200, , Beijing Liangkemao CO., LTD.

2 Methods

2.1 Confined Horizontal Storage

The windows and doors were sealed with polyvinyl chloride film sheets. In this way, the half – decay time for depot gastightness test was 1 minute and 20 seconds, which conformed to the state grain depot guideline request for horizontal storage.

2.2 Establishment of gas Sampling points

There are five sampling points within four corners and the central. The corner point is 1 meter away from the wall, and central point was intersected point of diagonal lines. All the gas tube sampling points were 1.5m below the grain surface. Each point had a return sample tubing line for monitoring PH_3.

2.3 Adjustment Wind Speed for Aeration

Operate aeration fans, then adjust the air volume distribution valves until air volume in each of the four aeration ducts achieved balance.

2.4 Determination of Grain Temperature

The grain temperature in the surface, the middle, the lower, and the base of the storage was monitored by using the microcomputer temperature measurement and data logging system.

2.5 Fumigation

Six kg of aluminium phosphide tablets were laid in four ventilation ducts, with 1.5 kg in each channel. Aluminium phosphide pills were in small plastic bags (0.50m, 0.10m), then phosphine was generated from aluminium phosphide fomulation reacted with vapor in air.

2.6 Start and Stop of the Recirculation Blowe

It takes a long time to produce PH_3 since phosphine from the time it starts to be generated. After 12h, the recirculation blower was started, The blower should be stopped as soon as PH_3 at all gas monitoring points reach an approximate balance, or uniform gas concentration.

2.7 Phosphine Concentration Test

Phosphine concentration was tested by a XL – 210 phosphine monitor starting when the recirculation fan started, 12h from start of fumigation and every 12h after that until the fumigation was completed.

2.8 Aeration in the Winter

The depot was located in the northwest of China, which was in low-temperature, dry region all the year. The winter lasted a long time below 0℃, and the relative humidity is below 65% all the year. All these environment factors were adopted to aerate for grain storage. The moisture did not reduce significantly from the low-power aeration, which provide a low volume airflow through the grain. In this way, the fan-hours of ventilation and the consumption of energy was low.

2.9 Heat Insulation in the Spring

Before the temperature in the spring rose, windows, doors and ventilation vents of the horizontal storage were sealed at the end of February, in order to maintain the low grain temperature after aeration, specially the temperature of the surface grain influenced by the horizontal storage headspace temperature. The grain surface was sealed with PVC sheeting, then was covered with a layer of bags of rice husk to insulate surface grain from warm headspace temperatures. .

2.10 Ventilation in Summer

In summer, the highest temperature reached above 38C. Because of the roof absorption and radiation of sunlight energy, elevation of the horizontal storage temperature and humidity was main problem causing the grain surface temperature to rise which caused stored pest to grow again. Opening windows and doors, and operation of the axial – flow fan reduced the temperature and humidity of the horizontal storage between midnight and 6 am. . This ventilation of the headspace lowered the roof radiation warming influence on the grain surface temperature and delayed the temperature rise.

3 Results and Discussion

3.1 Fumigation

The results indicated that the grain management strategy was effective to kill all the stored pest by maintaining the phosphine concentration for 552h(23 days), then to continue airtight for 7 days, and dispersing PH_3 for 2 days. After one month, no live insect was found at sampling points where the pest density is big before fumigation.

3.2 The Change of the Phosphine Concentration

The distribution of the phosphine concentration was shown as Table 1. From the result analysis, the ratio between the highest and the lowest concentration was 0.75 after recirculation for 24h, and the average phosphine concentration was 80ppm. After recirculation for 120h, the concentration distributed evenly, and the ratio was 0.97. The average phosphine concentration achieved the highest level 192 hours later(163 ppm at 312 h), then dropped slowly. The total time of fumigation was 30 days.

Table 1. The change of the phosphine concentration

Time (h)	phosphine concentration(ppm)						
	1	2	3	4	5	6	average
12	60	56	58	98	72	46	65
24	88	80	79	104	92	60	83
36	97	94	92	128	104	90	101
48	119	111	110	156	126	101	119
60	136	130	128	170	130	120	133
72	151	144	144	166	155	140	150
96	154	149	147	161	151	142	152
120	155	152	147	170	149	144	157
144	149	153	146	170	156	169	159
192	151	154	151	172	158	170	163
216	156	158	156	171	160	178	157
240	154	154	158	169	157	177	159
264	156	157	155	171	161	176	162
288	155	154	156	168	159	181	162
312	156	156	157	169	160	176	163
336	154	155	152	164	161	174	160
360	152	157	151	160	160	170	158
384	148	150	149	151	146	168	152
432	143	144	145	146	144	152	150
456	138	137	137	138	137	145	149
480	130	125	128	129	147	145	137
504	109	109	110	114	119	139	117
527	103	105	106	103	106	1128	109
552	97	94	98	96	96	103	97
576	82	70	84	84	80	86	83

3.3 Cost Analysis

The recirculation: The dose of Aluminum phosphide was 6 kg at the price of 37 yuan per kg, so the total expense was 222 yuan. The subsidy for Staff workers was 300 yuan, and the other cost(electric power, sealing materials and supplies) is 150 yuan. Finally, the sum total expense was 672 yuan.

The conventional fumigation: The dose of aluminum phosphide was 58 kg, so the total expense was 2 146 yuan, and the subsidy for Staffs was 300 yuan, the sum above is 2 446 yuan.

Compared with the expense of the conventional fumigation, The operating cost of Aluminum phosphide recirculation above was to reduce 1 774 yuan, and these was only 31.3% that of the conventional fumigation. Pest efficacy and grain quality was much better with recirculation.

3.4 The Change of Temperature for A Year

Table 2. The change of the temperature in 2002

date	outside	bulk	surface	middle	lower	base	warehouse
2002.1.29.	-11.9	-9.2	-1.1	-3	-2.1	0	-1.5
2002.2.26.	-1.4	2.7	-1.3	-2.2	-1.2	4.0	-0.2
2002.3.26.	16.7	6.5	1.5	-1.1	0	5.6	1.5
2002.4.29.	15.5	11.4	6.3	1.1	2.0	7.4	4.2
2002.5.28.	21.0	16.6	9.8	2.9	3.5	8.6	6.2
2002.6.25.	28.7	22.7	13.7	5.3	5.4	10.0	8.6
2002.7.29.	24.8	23.7	17.4	7.8	7.0	11.2	10.8
2002.8.26.	31.0	23.7	17.5	9.0	7.4	11.4	11.3
2002.9.29.	16.5	16.3	16.2	9.6	7.4	11.2	11.1
2002.10.28.	9.0	9.6	13.9	9.3	6.9	10.4	10.1
2002.11.25.	7.1	0.9	9.3	8.2	6.4	9.6	8.4
2002.12.23.	-11.9	1.3	5.2	1.8	1.2	2.3	2.6

The change of temperature for a year was shown as table 2. The average temperature of grain was 11℃ and it was benefit to use low temperature storage.

4 Conclusions

4.1 Aluminum phosphide was laid in the ventilation vents. The speed of the dynamic deliquescence was slow, but the phosphine distrib-

uted uniformly. Due to excellent sealing of the warehouse, the effective concentration was maintained for a long time, with good results of controlling pests.

4.2 The expense of the recirculation was only 31.3% that of the conventional fumigation.

4.3 Safe. Reduced the time contacting with the poison gas, and the labor intensity was small.

4.4 Low temperature storage combined with phosphine gas recirculation gave good security.

4.5 The average temperature of storage was below 15℃ all the year.

4.6 Recirculation fumigation in well sealed storage was effective to inhibit stored pests. Fumigation only one time in a year attached obvious economic efficiency and it reduced the times of fumigation and the pollution, saved the expense.

0431

QuickPHlo – R Formulation Fumigant Generators: a New Safer and Environmentally Friendly Phosphine Fumigation Process

Pushpaksen. P. Asher

Abstract: Phosphine is the most commonly used fumigation fumigant worldwide. Aluminium phosphide has been the primary choice of commercial fumigators for decades. The use of phosphine is increasing with Methyl Bromide phase out. Conventional formulations of Aluminium Phosphide and Magnesium Phosphide have been used for more than the past half century without improvement in the method of application or formulation. There are many limitations to conventional phosphine formulations; manufacturing and application safety have continually been major concerns.

The QuickPHlo – R aluminium phosphide formulation and the QuickPHlo – R phosphinegenerator series were developed to overcome the conventional phosphine safety, environmental and consumer food contamination limitations. The formulation is very safe to use. The QuickPHlo – R phosphine generator is very operator friendly and safe. This generator has a built – in deactivation system and scrubber to treat the residue of aluminium phosphide, drasticallyreducing levels of active ingredient to safe levels for disposal. This new innovative technology has many advantages which guarantee safety, quality and precision fumigations.

Presentation

Current Metal Phosphide formulation in use have an active ingredient of 56% – 66%, in the form of tablets/pellets/sachet/plates are used as a fumigant to kill stored grain insects. They are extensively used for fumigation of grains, cereals, dry fruits, ships, food, tobacco and other products/commodities. These formulations have been used since many years without much improvement in formulation or the method of application. Fumigators had no choice but to put up with the limitations of the product available.

Newer version is the Phosphine in cylinder with CO_2 as diluent or the pure form which dispense Phosphine gas with a device.

QuickPHlo – R™ Phosphine Generator & Granular Aluminium Phosphide Formulation

QuickPHlo – R™ Phosphine Generator is the latest device for fumigation with phosphine This is the safest, accurate and most economical method for fumigation with phosphine.

The Generator consists of a pot, deactivation tank, scrubber & a control panel All the equipment is mounted on a structure. The control panel performs all operations automatically. All that the fumigator has to do is press a button to start generation. The panel is very operator friendly and easy to operate.

A new granular formulation of Aluminium Phosphide having an active ingredient of 77.5% is used. The formulation is free from ammonia and dust. The formulation is put in the pot and reacted with water. The rate of generation of phosphine does not depend on ambient conditions like temperature and relative humidity.

Phosphine gas generated is circulated through the structure/commodity to be fumigated. This ensures uniform gas concentration. All the phosphine is generated and distributed in the structure in less than 1.5 – 2.5 hours. After reaction is complete in the pot, the active content in the formulation is about 1%. The deactivation system reduces the active content in the formulation to less than 0.1%.

The generator has back up power, in case of power failure. In case of an emergency, it is has an emergency shut down button.

The generator can be moved from one location/site to another.

QuickPHlo – R Phosphine Generators are made in different sizes referred by capcity to generate the quantity of gas

56 Gms
112 Gms
0.5 KGS
1.0 KGS
2.5 KGS
5.0 KGS
10.0 KGS
12.5 KGS

General Manager of Fumigation, United Phosphorus Limited (UPL), 3 – 11, GIDC, Vapi, India.
Email: asherpp@ uniphos. com

15.0 KGS
20.0 KGS
25.0 KGS

The generator is designed to suit individual customer requirements.

QuickPHlo – R™ formulation is available in different pack sizes conveniently packed in aluminium foil packs.

Pack Size GMS	Phosphine Generation GMS
125	250
550	250
2200	1000
4400	2000

Schematic Diagram of QuickPHlo R™ – Generator

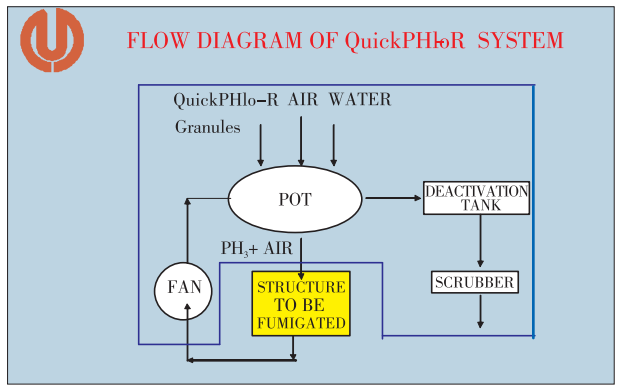

Table 1. Benefits of new formulation vs conventional formulations

Sr No	Activity	QuickPHlo – R™ Granules	Conventional formulation	Remarks
1	Active ingredient	77.5 %	56%	Less chemical used for generating the same quantity of Phosphine gas. The A I content is 40% higher. With less starting material, less residue is also generated
2	Operations	Automatic	All manual	The QuickPHlo – R generator has programmable Logic controller which controls all the operations, with built in check list. Where as in the conventional form, all operations are manual and the operator is exposed to dangerous working conditions.
3	Dust content in the product	0	0.5% –1%	Very fine dust is in the tablets and pellets. This flies when the product is applied. Operator needs dust protection for respiratory system and for hands.
4	Product Packing	Al. foil	Al flask	Flasks are very cumbersome and laborious to open. Aluminium foil packing can be cut easily with a scissor/knife
5	Product use	No ignition	Sometimes ignition can occur	Occasionally, it is observed that when the conventional formulation pack is opened, it ignites. The granules never have an ignition on opening the aluminium foil pack.
6	Phosphine concentration	0/very low	>2000	Phosphine concentration on opening the flask is very high. The same with the granules is zero/very low – which is very safe for the operator.
7				
8	Application of product	Into reaction pot	To be carried to the top of the silo/structure	on ground outside the structure. The granules are added to the reaction pot. This takes few seconds. The same with the conventional formulation, the product has to be carried to the top of the silo/storage structure and applied to the commodity manually. This takes much more time and is hazardous operation. Phosphine gas release starts immediately and concentration starts building up in the structure/commodity when the operator is still applying the product.

Sr No	Activity	QuickPHlo – R™ Granules	Conventional formulation	Remarks
8	Application of product			The commodity fumigated gets contaminated with the product residue in the conventional formulation, whereas with the granular formulation, it remains in the reaction pot, only Phosphine gas enters the structure/commodity
9	Ammonia in the formulation	NO	YES	Ammonia is additional pollutant which is eliminated from the new granular formulation
10	Gas generation	2 – 3 hrs	4 – 10 days	The gas generation is immediate in the QuickPHlo – R generator and the time for gas generation is independent of the ambient conditions like temperature and humidity. The rate of generation of Phosphine gas from the conventional formulation is totally dependent on moisture in the atmosphere and the temperature.
11	Gas concentration	Uniform in the entire commodity	Not uniform	The QuickPHlo – R™ generator has a fan which pushes the gas in the entire structure and the gas is re circulated. The conventional formulation has highest concentration around it and least away from it. Most of the times, the gas concentration is not uniform in the structure.
12	Hazards	Very limited	More chances	In the conventional form, since the Phosphine gas generation is happening in the structure, there is fire hazard. The same does not happen with QuickPHlo – R generator, since all the generation of gas is out side the structure. There have been instances of fire in the commodity, due water condensation/water leakage
13	A I content after decomposition	<1%	3% – 4%	QuickPHlo – R™ granules, after reaction with water have an A I content of less than 1% (which is further deactivated) after 2 hrs of reaction. Where as the conventional form has an A I content of 3 – 4 days in tropical climate/or higher when the temperatures are lower.
14	Residue	Liquid form	Fine dust	Fine dust handling is hazardous (still has high A I content)
15	Residue treatment	Part of the system	Separate infrastructure required	The slurry after reaction is taken to a deactivation tank, where the waste water is treated and the A I content of the product is further reduced. Gas generated during deactivation is scrubbed in a charcoal scrubber which is also part of the QuickPHlo – R™ generator. Where as the conventional formulation needs separate treatment facility and the higher A I (3% – 4%) is treated. What ever gas is generated is let into atmosphere.
16	A I content after deactivation	<0.1%	>1%	The residue in the conventional formulation needs 6 – 8 hours stirring to reach a level of >1%. Where as the QuickPHlo – R™ formulation has an AI of <0.1% after deactivation in 3 hours.

Sr No	Activity	QuickPHlo – R™ Granules	Conventional formulation	Remarks
17	Residue withdrawal	No	Yes	Since the QuickPHlo – R™ granules are not applied to the commodity, they donot have to be withdrawn. Where as the conventional methods, the residue has to be withdrawn/ gets mixed with the commodity.

There are more advantages and all cannot be listed.

Since most of us are familiar with the conventional form, the comparison is made with this form.

Table 2. Similarly, Phosphine cylinders can also be compared with the new technology

Sr No	Activity	QuickPHlo – RTM Granules	Phosphine cylinders	Remarks
1.	Logistics	Easy to handle	Very laborious	Cylinders are on steel racks
2.	Hazard Classification no	4.3	2.3	Safety requirements enhanced with phosphine cylinders
3.	UN No	1397	2199	More hazardous to handle
4.	Costs	No rentals	Pay Rentals	You pay rentals after certain time on retention of cylinders
5.	Phosphine	Generated when required	Always in the form of phosphine	Hazard increases with Phosphine. Where as the formulation is safe and generates phosphine only when required

Some Photograph of QuickPHIo – R generator:

0432

Studies on the Models of Electronic Supervision in Methyl Bromide Fumigation of Wood Packing Materials

Jin Guangyao, Tang Zheng, Wu Xinhua, Ma Jianhua and Huang Jiaping

Abstract: Methyl bromide fumigation is widely used for plant quarantine treatment. However, the traditional supervised approach always has problems of poor accuracy and veracity in dealing with the quarantine inspection data, and practical work can not meet the technical requirements of MB fumigation of AQSIQ (Administration of Quality Supervision, Inspection and Quarantine). After more than two years of system development and field testing, the project of "*studies on the models of electronic supervision in methyl bromide fumigation of wood packing materials*" has achieved the following three results: 1. we adopt infrared spectra absorption of methyl bromide sensor (SM95 – S2) as a systematic methyl bromide Detector; 2. the MB sensor will be placed far away from the dosed pipeline and as close as possible to the top; 3. we find by the field testing that the specificity, stability, reliability and accuracy especially supervision of MB concentration of this system accords with the related requirements of No. 69 and proclamation No. 105 by the general bureau and the national standard named《JJG693 – 2004 flammable gas detection alarm》 In this standard, the accuracy of fumigation system has reached to 5% scope. It has accomplished the long-distance electronic supervision of MB fumigation by CIQ supervised platform. The system accomplished long-distance automatic supervision of MB concentration and temperature in the fumigant warehouse during wood packing fumigation, recording time and concentration. It has also accomplished the automatic generation and print of the reports, such as Bill of Documents, and warrants of fumigant results. The data can not be modified by hand in the system. Several practical applications prove that this method can not only reduce burdens on enterprises, but also save inspection and quarantine departments a lot of human resources, and reduce work intensity and contradictions. Thus it achieves the goals of "accelerating, reducing burdens, increasing efficiency, and tightening supervision".

Key words: wood packing materials, methyl bromide, quarantine treatment, electronic supervision

Studies on the Models of Electronic Supervision in Methyl Bromide Fumigation of Wood Packing Materials

1 Preface

Wood packing materials are extensively used in international trade. As they have many advantages such as economy, convenience, security and invulnerability, in many cases wood packing materials cannot be replaced by other ones. Because wood packing materials can easily contain forest noxious creatures and transmit them through international trade, strict quarantine measures are established in many countries. Methyl bromide is a kind of fumigation medicine, which has the characteristics of deep penetrability, stable chemical properties and strong toxicity. Methyl bromide fumigation is one of the important methods of wood packing quarantine treatment. In the traditional supervised way, supervisors sample and test over time. However, this way has problems of poor accuracy and veracity in dealing with the quarantine inspection data; it wastes time, is highly hazardous, and has many hidden safety troubles and interference factors so that it cannot truly reflect the change state of MB concentration in the fumigant warehouse. To standardize the supervision of wood packing quarantine, according to No. 69 and proclamation No. 105 by the general bureau, quarantine institutions decided to supervise the process of pest and disease control. After studying survey meters for MB and temperature, designs of data collecting and data transmitting, supervised platforms for corporations and bureau, and systematic integration and accuracy, we successfully accomplished a system of *electronic supervision in methyl bromide fumigation of wood packing materials*. In field investigations, this electronic supervision system has the advantages of specificity, stabilization, reliability and integrity (because figures cannot be easily modified), These achievements conformed to the related requirements of No. 69 and proclamation No. 105 by the general bureau and the national standard named JJG693 – 2004 *flammable gas detection alarm.*

Changshu Exit – Entry Inspection and Quarantine Bureau, Jiangsu, China

2 Materials and Methods

2.1 Experimental Materials

Standard fumigant warehouse: It contains 25 m^3, conforming to the requirements of SN/T1143 – 2002 (simple fumigant warehouse regulations of plant quarantine);

Wood packing: pine/miscellaneous wooden block (specification: 110cm × 110cm);

MB: concentration: 98.5%, produced by Shandong Changyi Chemical Plant (authorized MB producing factory by the bureau);

Aluminium phosphide: concentration: 56%, Lianyungang Haitang jintiandi Chemical Plant;

Sulfuric fluoride: concentration: 99%, Shandong Longkou Chemical Plant;

Fumigation gas concentration survey meter:

①solid catalyzed burning type MB transducer (produced by American IST Co., full range 150g/m^3 (35 000ppm), permissive value ±5%, ie. ±7.5g/m^3);

② infra – red spectral absorption type MB transducer SM95 – S2 (produced by American IST Co., full range 150g/m^3 (35000ppm), permissive value ±5%, ie. ±7.5g/m^3

③heat conduction XK – Ⅲ MB survey meter;

④TM3 type XK – Ⅲ MB survey meter, produced by U.K;

⑤MINIRAE2000 VOC gas survey meter, produced by American RAE SYSTEM INC.

Temperature survey meter: PT100 type temperature transducer;

Data collecting reading instrument: JS05D intelligent electronic monitoring station for kiln;

Electronic platform instrument: TCS – A electronic platform instrument for both alternating and direct current (produced by Shanghai Youshenghanhengqi Co. ltd., measured by Changshu Measurement Bureau);

Data transmitting equipment: RS485, RS232;

Computer: CPU1.8G, 256K memory, hard disk 60G/7200 turn;

INTERNET: Telecom Broadband (broadband 2m).

2.2 Testing Method

2.2.1 Design Principle of the System Experiment

The electronic supervision system in this study consists of six functional modules: MB transducer, temperature transducer, intelligent supervised platform, RS485 communication equipment, supervised platforms for corporations and bureau. The design principle Fig. ture is shown in Fig. 1:

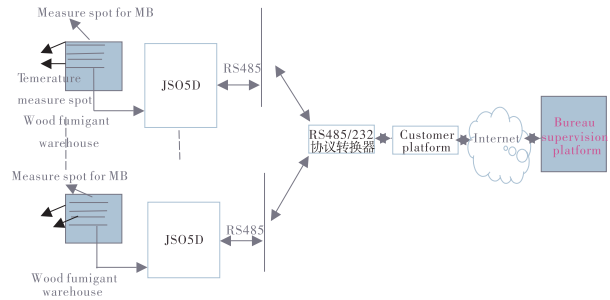

Fig. 1 principle of work

2.2.2 MB transducer filtration

Because the electronic supervision system in this study requires that all the data should be able to be collected and transmitted, the transducers must have data output ports. However, in this study, only 5 transducers have data output function: solid catalyzed burning type MB transducer, infra-red spectral absorption type MB transducer SM95 – S2 and MINI2000, etc. However, MINI2000 cannot be used since it has too short a measurement range (0ppm – 2000ppm), which is not suitable for high concentrations. The other two transducers are tested for their accuracy. The result shows that solid catalyzed burning type transducer cannot be used since it is easily poisoned, which can not reflect the concentration of MB in the fumigant warehouse (see Fig. 2); as infra-red spectral absorption type MB transducer (SM95 – S2) has the advantages of wide measure range, high sensitivity, good selectivity, long life and is not easily poisoned, it suits the study.

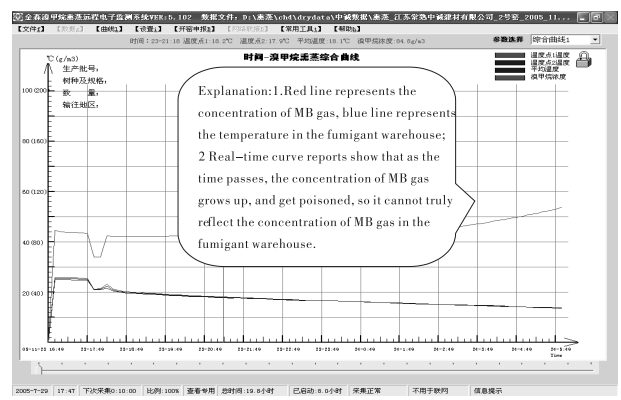

Fig. 2 poisoning symptom of solid catalyzed burning type transducer

2.2.3 Calibration Test of the Instruments

2.2.3.1 Measuring Instruments (except MB, temperature transducer)

Measuring instruments such as scales, mercury thermometer, and digital multimeter should be sent to the national legal measuring units for testing, and to obtain the corresponding certifications.

2.2.3.2 PT100 Temperature Transducer Calibration

Calibrate with the tested mercury thermometer the PT100 temperature transducer. From +14℃ to +29℃, there are 18 data spots. The permissive error of the data is ±0.5℃, the real calibration error is ±0.1 – 0.2℃, which conforms to the requirement. About the reports, see appendix 3.

2.2.3.3 On – the – site Calibration of Supervision System

On – the – site calibration on MB transducer requires:

a. Electrifying the MB transducer, warming up 12 hours before the fumigant, according to the service instruction, then warm up about 30 minutes before using.

b. Calculating the concentration of MB by the weighing method. Measure the volume of the warehouse V (m^3), weigh MB of weight M (kg), plunge MB after aerification through pipeline of medicine. Turn on circulating fan to distribute MB equally in the warehouse.

c. Calculate the concentration of MB with $C(g/m^3) = M(kg)/V(m^3)$; the permissive error should be in the range of operating error. (Generally, the range of the operating error should be about 5%).

d. Run supervising software and hardware system.

e. After MB was equally distributed, (about half an hour after application, the reading of MB transducer is stable), the supervising instrument shows the concentration of MB (C_1), and the computer software shows the data (C_2) ($C_1 = C_2$); calibrate the readings of MB with the instrument, to make C_1 equal C, which is calculated concentration of MB by the calculating method, i. e., $C_1 = C$.

f. After the installation of the system at the scene, the legal unit will measure the system with a MB standard. About the result, please see the report on the scene by Jiangsu Province Measure Testing Tech Institute.

2.2.4 Experiments and Steps

2.2.4.1 Testing Method of Accuracy of the indicative value of concentration of MB——Empty Warehouse Experiment

To validate the accuracy of the system testing, this experiment is conducted in an empty warehouse in advance of commercial fumigations. Put the MB transducer onto the top of the fumigant warehouse (the place of lowest MB concentration), compare the weighing (calculated) concentration to the indicative concentration in the system. On the condition of no wood packing, apply fumigant in the warehouse, then turn on the circulating fan in the warehouse, at the same time, switch on the electronic supervising system to measure and collect automatically the concentration of MB and temperature at certain times. After 24 hours, open the door of the warehouse to spread the poison. Analyze the data collected by the system (we will take the data collected at the point of 0.5 hour as the original data), validate the accuracy and reliability of the testing system; assess long – distance transmit, supervising long – distance, data analysis and automatic printing of the data and report.

2.2.4.2 Study on the specificity of the MB transducer

To study the specificity of the system, we fumigate with aluminium phosphide and sulfuryl fluoride at 11 – 25℃ in the empty warehouse for 24 hours, then obtain and record the readings of the MB sensor.

2.2.4.3 Testing Method of Accuracy of the Experiment——Full Warehouse Experiment

Put the MB transducer onto the top of the fumigant warehouse, place some wood packing into the warehouse and close the door of the warehouse. Apply a determined amount, turn on the circulating fan, switch on the automatic supervising system to test and collect the data on concentration of MB and temperature, then open the door of the warehouse to spread the poison after 24 hours. When the experiment is finished, compare the concentration of 2 hours, 4 hours, 12 hours and 24 hours required by the No. 105 with the actual concentration.

2.2.4.4 Test Experiment of Different Locations Where MB Transducer Is Put

As MB gas is heavier than the air, in the same closed container, lamination will happen, so in this experiment MB transducer will be put on the top, the middle and the bottom of the fumigant warehouse. Compare the calculated concentration with the indicative concentration in the system. Apply fumigant to the warehouse with the same quantity and volume of wood packing and turn on the circulating fan and electronic supervising system to measure and collect concentration of MB and temperature. Open

the door of the warehouse to spread the poison after 24 hours, and make a comparative analysis of the concentration of the theoretical data and collected data when the transducers are placed in the different locations.

3 Results and Analysis

Experiment of Accuracy of MB indicative value

In the warehouse of Xingfu and Huasen Co., the theoretical doses are 80g/m^3, 64g/m^3, 56g/m^3 and 48g/m^3 respectively. Test 3 times of the same group, to make sure the temperature in the warehouse conform to the requirement of the fumigation. The data is in the table 1.

Table 1. Table of Indicative Value Error

Experiment condition: Drug in the empty warehouse, and turn on the circulating fan.

No.	Experiment site	Theoretical value	CK(g/m^3)	Initial concentration	Test value (0.5h)	Data number	Average	Value error(%)
				0	76.6	2006-4-23-13-16ch4		
1	Xingfu	80g/m^3	0.1	0	76.0	2006-5-6-17-29ch4	75.6	-2.93
				0	74.2	2006-5-31-15-20ch4		
				-2	59.8	2006-11-30-15-28ch4		
2	Xingfu	64g/m^3	0.1	-2	59.3	2006-11-30-17-59ch4	61.0	-2
				-5.9	63.9	2006-12-1-16-9ch4		
				-5.8	55.3	2006-11-29-16-49ch4		
3	Huasen	56g/m^3	0.1	2	53.6	2006-12-1-17-18ch4	55.1	-0.6
				-4.5	56.4	2006-12-3-15-59ch4		
				10	46.5	2006-12-1-14-26ch4		
4	Huasen	48g/m^3	0.1	10	46.4	2006-12-1-15-32ch4	46.57	-0.93
				8	46.8	2006-12-1-16-23ch4		

Note: testing value = actual value - original value

According to the indicative error of the instrument ≤5FS% (FS is full range) of the regulation in JJG693 - 2004 *Combustible Gas Testing Annunciator*, the formula of the error should be:

$$\text{Indicative value error} = \frac{averageval\ ue - theoretice\ lvalue}{fullrange} \times 100\%$$

From the data in the table 1: indicative value of the four groups 80g/m^3, 64g/m^3, 56g/m^3, 48g/m^3 are 2.93%, 2%, 0.6% and 0.93%, the errors are all less than 5%. In the regulated range and conform to the related testing standard of the state.

3.2 Study on the Specificity of the MB Transducer

The experiment is made in Xingfu Wood Factory, we set 3 treatment experiments in the range of 11 - 25℃, i.e., blank test, aluminium phosphide and sulfuryl fluoride experiments. Each will be treated 3 times, blank contrastive experiments (24 hours), aluminium phosphide experiments (48 hours) and sulfuryl fluoride experiments (24 hours). Collect data of transducer after 2, 4, 12, 24 and 48 hours and make contrastive analysis (see table 2).

Table 2. Data Analysis of Study on the Specificity of MB Transducer

No.	Fumigant medicine type	quantity (g/m^3)	Time (h)	2h	4h	24h	48h
1			48	1.9	1.5	1.5	3.2
2	phosphine	7.5	48	-0.3	-0.4	1.5	1.5
3			48	0.9	0.8	1.2	1.4
4			24	-1.7	-2.0	-0.7	
5	sulfuryl fluoride	80	24	-0.6	-0.6	1.0	
6			24	0.5	0.6	0.7	
7			24	2.0	2.0	1.1	
8	contrast with no fumigant	0	24	1.5	1.9	2.7	
9			24	1.1	1.4	1.5	

According to the data in the blank experiment of aluminium phosphide and sulfuryl fluoride, MB transducer has no reaction to phosphine and sulfuryl fluoride. The transducer has good specificity and stability.

3.3 Validating Experiment of Accuracy of the System

According to requirements No. 60 and No. 105, integrated with the actual work, we classify the data of different concentration and compare them with the related MB fumigation technical requirements regulated by the bureau. The full warehouse MB data classification is in table 3; related technical requirements of MB fumigation treatment are in table 4.

Table 3. Data classification table of wood packing in the full warehouse

No.	Treatment site	Theoretical medicine concentration	System test value (g/m^3)						Data No.
			0.5h	2h	4h	12h	16h	24h	
1	Xingfu	$80g/m^3$	80.6	83.3	77.8	66.4	62.6	55.7	2006-7-22-18-7ch4
2	Xingfu	$80g/m^3$	86.9	77.7	70.3	55.5	51.8	45.1	2006-7-24-10-32 ch4
3	Xingfu	$80g/m^3$	82.3	80.3	75.6	65	60.1		2006-7-25-14-6 ch4
4	Xingfu	$80g/m^3$	87.8	82.2	72.8	60.1	56.8	49.9	2006-7-26-14-5 ch4
5	Huasen	$80g/m^3$	80.2	80.8	75.5	62	55.4	45.7	2006-8-30-18-53 ch4
6	Xingfu	$64g/m^3$	71.4	55.3	48.5	43.0	40.8		2006-9-4-16-26 ch4
7	Xingfu	$64g/m^3$	68.6	69.3	66.5	61.2	58.5	51.4	2006-9-9-15-2 ch4
8	Xingfu	$64g/m^3$	59.5	68.1	65.5	58.7	55.7	49.8	2006-9-10-17-14 ch4
9	Xingfu	$64g/m^3$	64	65.5	64	56.7	53.5	47.1	2006-9-11-18-22 ch4
10	Huasen	$64g/m^3$	64	66.2	62.9	56.0	54.1		2006-11-24-13-25ch4
11	Huasen	$48g/m^3$	45.1	46.3	45.4	39.1	33.5	30.5	2006-9-2-16-40 ch4
12	Xingfu	CKg/m^3	1.5	1.6	1.4	0.8	1.3	1.1	2006-12-12-10-36 ch4

Table 4. Related technical requirements of MB fumigation treatment

Reports	Temperature (℃)	Dosage (g/m^3)	Min concentration (g/m^3)					
			0.5h	2h	4h	12h	16h	24h
NO. 69	≥21	48	36	24	17		14	
	≥16	56	42	28	20		17	
	≥11	64	48	32	22		19	
No. 105 (from 2006.10.1)	≥21	48		36	31	28		24
	≥16	56		42	36	32		28
	≥11	64		48	42	36		32

The data compared with requirements of fumigation in No. 60 and No. 105 shows: in the concentration 80 g/m^3, 64g/m^3, 48g/m^3 and the time 0.5, 2, 4, 12, 16 and 24 hours, the results conforms to the requirement of the lowest concentration. "The automatic supervising system of MB fumigation of wood packing" has stable properties in the treatment process of MB fumigation in the full warehouse, and truly reflects and report the process of fumigation treatment. All the data in the fumigation process are true and efficient, conforming to the requirements of No. 60 and No. 105.

3.4 Test Experiment of Different Locations where MB Transducer Is Placed

In Xingfu Wood factory, MB transducer will be put on the top, the middle and the bottom of the fumigant warehouse. The theoretical concentration is 80g/m^3, and we collect the data in 0.5, 2, 3, 12 and 16 hours (see table 5). Then calculate the average of each group, and draw the graph (see Fig. 2).

Table 5. Selected Measuring Points Table of Transducers of MB Concentration

Experiment condition: circulating fan is turned on, fumigant is dropped into the empty warehouse;
Environment temperature ≥20℃, the same fumigant, the same weighing system; MB concentration measuring value(g/m^3) = testing indicative value(g/m^3) − original concentration(g/m^3)

Location of transducer	Standard value(g/m^3)	Test times	Corresponding curve	Concentration value of MB(g/m^3)				
				0.5h	2h	4h	12h	16h
top	80	1	2006 − 4 − 23 − 13 − 16ch4 (Xingfu)	76.6	73.7	71.8	66.3	
		2	2006 − 5 − 6 − 17 − 29ch4 (Xingfu)	76.1	73.9	71.5	63.8	59.2
		3	2006 − 5 − 31 − 15 − 20ch4 (Xingfu)	74.2	72.3	71.5	62.4	59.2
			Average	75.6	73.3	71.6	64.2	59.2
mid	80	1	2006 − 6 − 4 − 13 − 8ch4 (Xingfu)	76.6	76.6	73.3	64.6	61.0
		2	2006 − 6 − 2 − 15 − 59ch4 (Xingfu)	72.3	73.8	72	63.7	59.6
		3	2006 − 11 − 26 − 10 − 13ch4 (Xingfu)	84.3	84.5	78.3	63.8	58
			Average	77.7	78.3	74.5	64.0	59.5
bottom	80	1	2006 − 4 − 29 − 9 − 38. ch4 (Xingfu)	79.0	83.1	75.8		
		2	2006 − 5 − 27 − 15 − 13. ch4 (Xingfu)	81.7	80.7	72.3	68.3	65.7
		3	2006 − 5 − 28 − 16 − 0. ch4 (Xingfu)	82.5	84.9	81	70.5	55.4
			Average	81.1	82.9	76.4	69.4	60.6

The data in table 5 and curve in Fig. 3 show that concentration of MB in the fumigation warehouse on the top is relatively low, in the middle less, at the bottom highest. As the time passes, the difference gets less; after 16 hours, the top, middle and bottom concentrations are approaching the same value.

There are abundant studies on distributing MB in the fumigation warehouse at home. One summarises: about MB fumigation in the container, the gas in the container is not distributed equally. If the gas inside is not mixed transmit by an outside force, generally there will be 24 hours to reach balance. The dose quantity in empty or full container, top or bottom, single or multi locations to drug and the at the horizon level can be balanced more quickly but it is hard to get the gas to be distributed uprightly to reach balance[3]. The testing data shows when the transducer is put on the top, the concentration at the lowest point is reflected. Thus, the MB transducer should be placed far from the pipeline of drugging; the closer to the top place, the better. Secondly, we set the circulating fan in the warehouse, to make sure the fumigation gas in the warehouse is balanced; setting a transducer in the warehouse can reflect the concentration of MB in the warehouse.

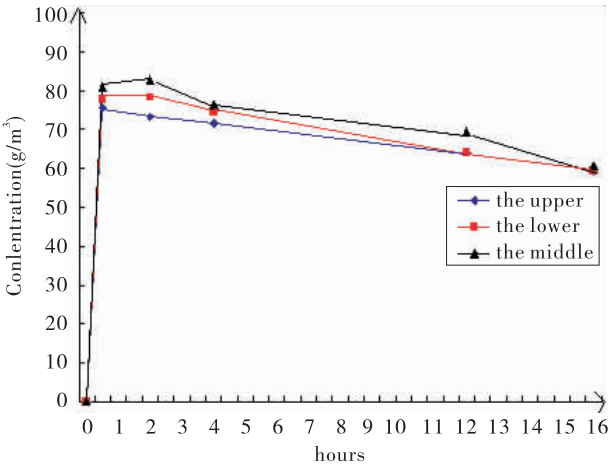

Fig. 3 **location of MB transducer**

4 Conclusion

"Studies on the models of electronic supervision in methyl bromide fumigation of wood packing materials" started in 2005. After studying and making experiments at the scene for over a year, the system accomplished long – distance automatic supervision of MB concentration and temperature in the fumigant warehouse during wood packing fumigation in given time and quantitative amount; and it has also accomplished the automatic generation and print of the reports and warrants of fumigant results. The data is very true and cannot be modified by hand in the system. Stability, reliability and accuracy especially supervision of MB concentration of this system accords with the related requirements of No. 69 and proclamation No. 105 by the general bureau and the national standard named JJG693 – 2004 *flammable gas detection alarm.*

Several practical application in commercial enterprises prove that this method can not only reduce burdens of enterprises, but also save a lot of human resources for the inspection and quarantine departments, and reduce work intensity and the contradictions, thus achieve the goals of " accelerating, reducing burdens, increasing efficiency, and tightening supervision". The study also accomplishes the long – distance electronic supervision in methyl bromide fumigation of wood packing materials for the inspection and quarantine departments, thus achieving the goals of " accelerating, reducing burdens, increasing efficiency, and tightening supervision", meanwhile, reducing burdens of enterprises.

Acknowledgements

We Dr Jim Desmarchelier for help with the manuscript.

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0433

Methyl Bromide Recapture Technology

Wil Grullemans

Abstract: Methyl Bromide recapture technology has been commercialised to address the environmental and health and safety problems which arise from the unfettered use of this gas. Regulations requiring recapture are spreading across a number of regions, driven by local air quality, worker and community health concerns as well as international obligations under the Montreal Protocol.

Key words: methyl bromide, recapture technology

Introduction

Global Methyl Bromide use has been reduced dramatically and is a credit to the success of the Montreal Protocol on Substances that Deplete the Ozone Layer. This has largely been achieved by substitutions in many applications in both post – harvest and stored product treatments [1].

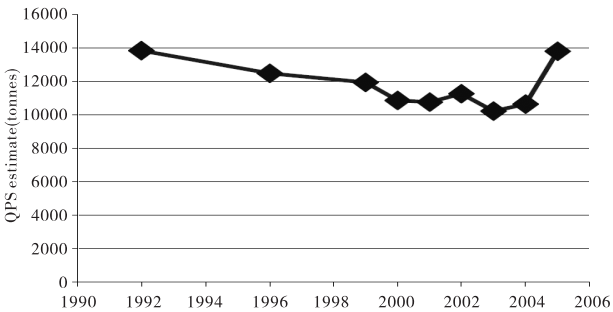

Fig. 1 Worldwide Production of Methyl Bromide for QPS Use (MBTOC and UNEP Data)

The main usage of Methyl Bromide in developed countries is now for Quarantine and Pre-Shipment applications, and there are signs that its usage has increased in certain areas [2]. It may be many years until an effective substitute is proven for all quarantine requirements, which are essential for biosecurity and international trade. For these applications the merits of applying recapture technologies is self-evident.

Benefits of Recapture

Methyl Bromide is a serious Ozone Depleting Substance and its impact is reported to be up to 50 times as destructive of the stratospheric ozone layer as chlorine from Chlorofluorocarbons [3]. The chemistry of ozone depletion is shown in Fig. 2 below:

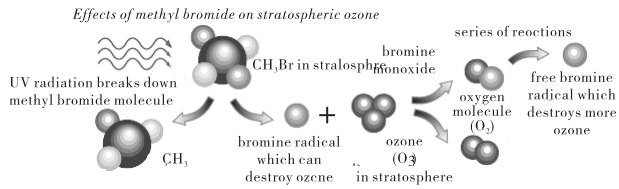

Fig. 2 Effects of Methyl Bromide on Stratospheric Ozone [4]

Local air quality can be compromised by emissions of this gas, which disperses in response to weather conditions, temperature, wind direction and other factors. An example of the spread of emissions is shown in Fig. 2. As the gas is odourless and colourless at unsafe concentrations, the impact on local workers and communities is obvious.

The contours on this graph (Fig. 3) were created after readings were taken from live fumigations at the Port depots, and results incorporated into computer models showing gas dispersions. Present fumigation methods result in risks categorised as ranging up to Moderate and Extreme, risks which can exceed current and proposed regulatory criteria [5]. Nordiko's systems reduce these risks by recapturing the highly toxic gases that are used in fumigation.

Technologies are under development for the recycling and reuse of Methyl Bromide after recapture. For larger volume applications, this can provide an economic benefit. However, this is hindered by the relatively low cost of this gas today.

Technologies Available

There are a number of technologies available which can be used for recapture purposes. These include recapture on filters such as activated carbon or zeolite, wet scrubbing in solu-

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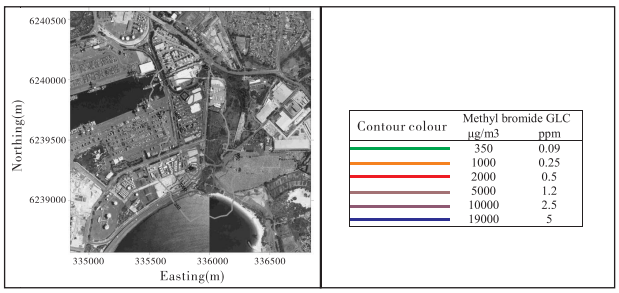

Fig. 3 Concentrations of Methyl Bromide afte Releaser from Operations in a Major Australian Port [6]

tions such as sodium thiosulphate or ammonium thiosulphate, condensation from fumigation atmospheres or even recycling from one fumigation enclosure to another.

The most widely used approach is recapture onto activated carbon filters. The gas can then be destroyed by chemical reaction, desorbed and incinerated at high temperature, carbon with gas degraded as landfill or (with appropriate equipment) be released from the filter and reused.

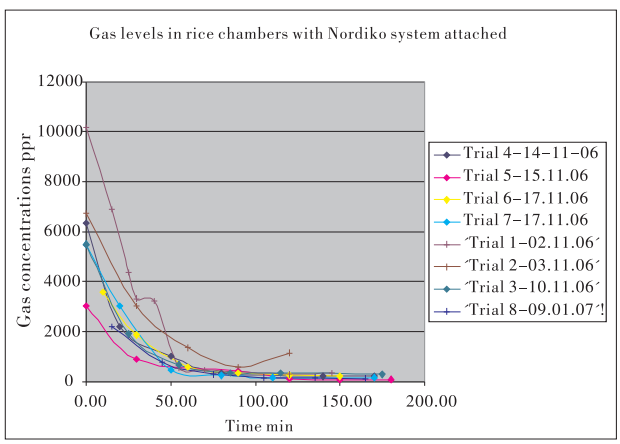

Fig. 4 Gas Levels in Rice Chambers with Nordiko System Attached [7]

Fig. 5 Large Scale Recapture Systems

One of the most important steps in the recapture process is the means by which the fumigant is evacuated from the fumigation enclosure and trapped on the filter medium. This has been the subject of considerable research and for the most widely used fumigation enclosure the shipping container is the subject of a number of patents.

Nordiko Systems

Nordiko is arguably the most commercially advanced company involved in fumigation, recapture and ventilation technology. It is an Australian based company that has developed and patented a number of systems.

These systems are now in use around the world in a range of locations and industries:

Australia, New Zealand, Malaysia, Belgium, USA, Mexico, Chile, India, Italy, Poland and the UK. Industries served include: ISPM 15 Timber, Fresh Produce, Container Depots, Airports, Warehouses, Grains and Customs etc.

Fig. 6 Nordiko Methyl Bromide recapture Systems in Antwerp Port, Belgium

Nordiko wants to develop the market in China for this type of technology in collaboration with government, institutions and private industry.

Safe Levels

The safe level of exposure to Methyl Bromide, or any fumigant, varies by country. Table 2 sets out TLV levels for a selection of countries:

Table 2. Comparative Maximum Exposure Standards to Residual Gases [8]

	Methyl Bromide	Ethylene Dibromide	Ethylene Oxide	Formal – dehyde	Hydrogen Cyanide	Phosphine	Sulphuryl Fluoride	Chloropicrin
Australia	5ppm	0.5ppm	1ppm	1ppm	10ppm	0.3ppm	5ppm	0.1ppm
New Zealand	5ppm	0.5ppm	1ppm	1ppm	10ppm	0.3ppm	5ppm	0.1ppm
USA	1ppm	1ppm	---	0.3ppm	4.7ppm	0.3ppm	5ppm	0.1ppm
India	5ppm	---	---	---	---	---	---	---
EU	0.5ppm			0.1ppm	10ppm	0.01ppm		0.1ppm

The safe level has also declined over time, as more experience has been gained of the deleterious effects of this gas on human beings and the natural environment.

Health effects of exposure to Methyl Bromide may include: headaches, throat and eye irritation, shortness of breath, chest pain, nausea, fatigue, dizziness, numbness, central nervous system and respiratory system failure, amongst other symptoms [9].

A recent example of community concerns over the risk of exposure to Methyl Bromide arose in Nelson, New Zealand an important log and timber exporting centre. An Environmental Court decision was recently reached which set strict controls on the nature and extent of fumigations and required recapture systems to be used in future[10].

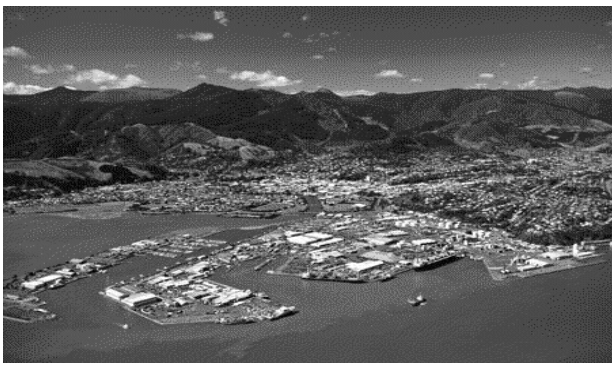

Fig. 7 Aerial Photo of Nelson Port [11]

Mandated Regions

A growing number of regions around the world are recognising that Methyl Bromide will not be replaced in the short term for many important quarantine and pre-shipment applications, and have taken the iniative to mandate recapture.

Nelson in New Zealand has been mentioned before and is a township that depends to a significant degree upon its log and timber trade as part of its export income. The first recapture systems are being installed in July 2008 for container fumigations, with bulk timber recapture system planned for the end of the year.

The Government of **Belgium** established a rigorous protocol which had to be met by accredited recapture systems and independently audited. A target has initially been set to recapture 80% of the gas available at the conclusion of the fumigation, with the expectation to increase this target over time. Recapture commenced on July 1, 2007 and has operated successfully for over a year by now.

In **Germany** the largest port where virtually all methyl bromide fumigations are performed is Hamburg. The port has adopted the protocol as established in Belgium, and has set September 1 2008 as the date from which adoption of an accredited recapture system must be made. It is noteworthy that the recapture directive, has been set more broadly, to encompass other toxic gases such as phosphine and sulfuryl fluoride as technically feasible.

The island state of **Tasmania** has some of the most stringent quarantine regulations in Australia and introduced a requirement to use "latest technology" during methyl bromide fumigations as early as 2006. This was interpreted at the time to mean adoption of recapture technology for methyl bromide, and it is now the state in Australia with the largest number of recapture systems installed.

Airports often have quarantine treatment facilities located in their environs, and at least two airports have required that recapture systems are used for Methyl Bromide fumigations. In Dallas-Fort Worth in the USA there is a large scale recapture plant, and at Perth International Airport in Australia fresh produce and timber fumigations must be performed using recapture systems.

The **USA** is going through the process of reregistering Methyl Bromide as a fumigant, and it is understood that consideration is being given to reduce the "**buffer zone**" surrounding fumigations, when recapture systems are in use. This is a sensible application of an economic and health and safety interpretation of the risks which arise during fumigations.

Inside the **European Union** Methyl Bromide was not put onto the list of approved chemicals and therefore may not be used for any applications from 2009/10, unless reregistered. It may be the case that the gas is reregistered for QPS usage only, and only on the basis that it is used with **accredited fumigation recapture technology**.

Residual Gas

Methyl Bromide is probably the most commonly encountered residual gas determined inside shipping containers. This poses a threat to those people unpacking the containers and in associated warehouse facilities.

Ventilation and recapture systems have been developed to effectively address the risks created by this phenomenon, allowing fast and safe turnaround of containers.

Residual gas can arise from other sources in addition to Methyl Bromide for example

Formaldehyde can arise from wood glues and products. It has recently been listed as a carcinogen in Europe.

Fig. 8 **Nordiko Residual Gas Extraction Unit Filter Model**

There are many other gases which have been found to arise inside shipping containers, these include: Dichloroethane, Phosphine, Sulfuryl Fluoride, Ethylene DiBromide, Hydrogen Cyanide etc. International experience is that between 1 in 3 and 1 in 5 containers can contain an unsafe level of gas.

Other Fumigants

Recapture technology is adaptable to other fumigants and gases beyond just Methyl Bromide. Many gases give rise to health and safety risks, therefore the benefits of ventilation and if applicable recapture of residual gases from inside shipping containers and other enclosures are very real.

Conclusion

Methyl Bromide recapture and ventilation technology has developed into an international business which is technically and commercially available and increasingly beneficial in many countries and regions.

Nordiko is one of a number of suppliers of equipment which can allow the benefits of recapture to be applied for the sake of the local and global environment and the health and safety of our workers and the general population.

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0434

Methyl Bromide (MeBr) as a Quarantine Treatment for Some Insects in Wood

Huang Qinglin*, Kang Fenfen, Lou Xuri and Liu Yongsheng

Abstract: With the risk of the spread of quarantine pests in the wood trade, an effective treatment for wood plays an important role in quarantine measures. Based on field experiments, confirmatory fumigations were carried out using methyl bromide (MeBr). For wood fumigation under tarpaulins, experiments were conducted at doses of ≥48g/m^3, with a 16 hour exposure time when treatment temperatures were above 15℃; and when treatment temperatures were above 27.5℃, the MeBr dose was adjusted to ≥32g/m^3 with a 24 hour exposure time. All larvas of beetles and insects (belonging to Scolytidae) were completely killed. In a fumigation chamber, the following MeBr doses were used; 80 g/m^3 (5 – 10℃), 64 g/m^3 (11 – 15℃) and 48 g/m^3 (≥16℃) in which all trial insects were completely killed.

Key words: methyl bromide, wood, fumigation, Cerambycidae, *Scolytidae*

Introduction

All logs imported into China, from various parts of the world, are covered with bark and are potential sources for quarantine pests being introduced into our country. Wood-boring beetles (*Cerambycidae*) and some insects (mainly belong to *Scolytidae*) are very damaging pests. Potentially, they are a serious threat to our forests, lumber, and the esthetic and dollar values of properties and to the diversity of tree species in the forest environment.

Currently, according to ISPM15, methyl bromide fumigation and heat treatment are the only two treatment methods allowed for regulated wood packaging material. As methyl bromide (MeBr) is permeable through timber, it is capable of the eradication of all beetles and wood-boring insects. Here we report the results of wood fumigation with MeBr, which included land tarpaulin fumigation, railway wagon fumigation, and shipboard container fumigation.

Materials and Methods

Imported timbers tested were larchwood, silver birchwood and sprucewood from Russia, cherrywood and beechwood from European Union countries. Within these timbers, larva, pupa and adults of the following insects are likely to be found; *Monochamus*, *Ips subelongatus* Motsch., *Ips typographus* Linnaeus., *Scolytus ratzeburgi* Jans and *Tetropium castaneum* Linnaeus. The moisture contents were in the range of 29.2% – 55.1%.

Materials: new polyethylene (PE) sheeting, thickness > 0.15mm; double – face glued tarpaulin, length16.5m, width 6.2m, thickness 0.3 – 0.4mm; railway wagons, length 12.4m, width 2.8m, high 2.0m; transport containers, 40 feet.

Fumigant: MeBr packed in 40kg pressurised steel cylinders, purity 98%, made by Jiangsu Lianyungang Dead Sea Bromine Compounds.

Fumigant concentration testing: the XK – III, thermal conductivity instrument of Chinese manufacture (CPQ Technology Company, Animal and Plant Quarantine Institute, Beijing, People's Republic of China); the American made Fumiscope (Key Chemical, Clearwater, FL); 10s50, portable gas chromatograph; MiniRAE 2000 VOC detector, made by RAE systems inc.

Method: The fumigation procedures were carried out according to two standards (SN/T1123 – 2002 and SN/T1124 – 2002) of China quarantine treatment code.

When fumigating wood stacks under tarpaulins or railway wagons they were sited on flat ground at a minimum distance of 50m from any habited area. The wood stacks were constructed in an orderly pile me with a measured volume less than 300m^3. The volume of the railway wagon was 120m^3. The moisture content of the wood in each stack was tested and recorded.

For every stack two appropriately marked sampling tubes were inserted. One was in the centre of the stack and the other was placed un-

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der the bark of an insect ridden log.

When sealing the log stacks a piece of canvas was placed on the corners of the stack to protect the PE sheeting against tearing. The system was sealed with the use of sandbags around the periphery of the stack. The transport and railway containers were checked for cracks and sealed with strong adhesive tape.

Fumigation: the fumigant doses were computed volumetrically by calculating the volume of the required grams of MeBr gas with the V = nRT/P relationship. When the gas application was finished, chamber recirculation fans were turned on for 0.5h to enhance the distribution of the MeBr though the fumigated area.

Fumigant Concentration measurement: Fumigant concentrations were monitored after 0.5, 2, 4, 8 (or 12), 16 and 24 hours by using a thermal conductivity (TC) instrument, the XK – III, which was functionally similar to the American made Fumiscope.

Aeration/Ventilation: After 24 hours, the covered sheets were removed or the container doors were opened and the fumigated area was aerated for 30 min.

Environmental measurements: During the introduction of the fumigant and during the fumigation and aeration period, a VOC detector monitored gas leak around the treatment areas and 1m, 10m, 30m and 50m down wind from the stack or container.

Insect mortality: All larva were counted and evaluated after fumigation. Larvae were considered dead if they were limp and showed no movement. Larvae that were turgid or had body movement were considered alive.

Results and Conclusion

Tarpaulin Fumigation

The results show that when the treatment temperature was above 15℃, MeBr fumigant dose ≥ 48g/m^3, and with an exposure time 16 hours, and a concentration ≥ 35.5g/m^3 before aeration, all larva of beetles and insects (belong to Scolytidae) can be completely killed (Table 1). When treatment temperature is above 27.5℃, a MeBr dose of ≥ 32g/m^3, with an exposure time 24 hours, and a concentration of ≥ 19g/m^3 before aeration, the mortality of insects was 100% (Table 1).

Table 1. Data of tarpaulin fumigation

Exposure time, h	Dose (g/m^3)	Stack number	Volumem^3	Concentration (g/m^3) 0.5	2	4	12	16	24	Exposure temperature, ℃/relative humidity	Mortality%
12	48	3	120	77	73	49	46	---	---	15 – 29℃	100
		12	170	60	47	44	41	---	---	42 – 80%	100
	64	5	225	75	69	61	42	---	---	15 – 29℃	100
		4	50	80	53	42	34	---	---	42 – 80%	100
	80	1	38	107	94	92	63	---	---	15 – 29℃	100
		2	115	106	104	85	70	---	---	42 – 80%	100
16	48	6	126	84	77	63	55	39	---	15 – 29℃	100
		7	115	67	50	49	44	32	---	42 – 80%	100
	64	10	430	85	70	64	63	54	---	15 – 29℃	100
		11	18	90	83	76	68	47	---	42 – 80%	100
	80	8	235	119	105	102	91	77	---	15 – 29℃	100
		9	207	122	114	83	78	70	---	42 – 80%	100
24	48	14	114	74	70	67	54	48	27	15 – 29℃	100
		13	600	87	83	71	65	56	40	42 – 80%	100
	64	17	225	114	108	97	93	76	35	15 – 29℃	100
		18	54	139	130	108	90	78	48	42 – 80%	100
	80	15	56	140	135	132	120	94	65	15 – 29℃	100
		16	85	122	120	102	97	88	57	42 – 80%	100
	48	1	888	87	74	71	68	56	34	27.5 – 43℃	100
		2	684	98	91	79	70	66	37	51 – 84%	100
	32	3	1440	70	69	55	50	34	21	27.5 – 43℃	100
		4	3490	78	76	62	55	41	17	51 – 84%	100

Environmental Air Testing

Testing of the environmental air during exposure and aeration period showed that there was some gas leakage. The result indicated that the greater the distance from the fumigation facility, the lower fumigant concentration. 30m from the fumigated stack can be regarded as a safe distance.

Container Fumigation

The container fumigation results showed that all the indicated MeBr dosages and exposure time options can completely kill the trial insects. Especially 5 – 10℃ & 80g/m^3, 11 – 15℃ & 64 g/m^3 and ≥16℃ & 48 g/m^3 MeBr can completely kil all beetles and insects (belonging to Scolytidae). The average MeBr concentrations were 49.3 g/m^3, 42.1 g/m^3 and 33.2 g/m^3 before aeration in above three dosages (Table 2).

Table 2. Data from container fumigation

Temperature (℃)	Dose (g/m^3)	Repeat	Concentration (g/m^3)						Insect samples	Mortality
			0.5h	2h	4h	8h	16h	24h		
5 – 10	80	1	102.0	90.7	79.2	69.3	57.7	––	17	100
		2	90.1	83.3	69.6	54.3	41.1	––	21	100
		3	110.0	93.0	82.1	70.7	––	49.2	34	100
		4	127.0	97.7	85.3	69.2	––	50.5	25	100
	64	1	94.7	84.3	69.2	56.5	45.9	––	27	100
		2	80.7	77.8	68.5	50.9	38.2	––	19	100
		3	105.2	86.2	72.1	55.7	––	37.2	28	100
		4	96.0	87.6	81.0	69.3	––	49.3	40	100
11 – 15	80	1	137.7	110.0	97.2	76.2	64.2	––	34	100
		2	129.7	108.3	87.8	67.4	56.7	––	30	100
		3	125.5	95.7	83.7	71.3	––	53.2	23	100
		4	105.5	92.1	82.3	73.3	––	48.3	19	100
	48	1	86.2	76.7	57.7	45.2	36.6	––	24	100
		2	80.2	69.2	50.1	38.1	29.8	––	34	100
		3	90.1	82.0	62.5	48.3	––	25.0	26	100
		4	87.7	79.1	64.2	50.7	––	27.2	31	100
16 – 20	64	1	97.6	88.7	76.6	57.0	44.0	––	29	100
		2	100.7	80.6	67.7	48.1	36.6	––	16	100
		3	101.2	86.0	72.2	51.5	––	32.5	44	100
		4	105.0	92.0	78.0	59.6	––	37.7	32	100
	80	1	119.3	101.6	81.1	70.6	59.7	––	22	100
		2	106.4	97.0	78.1	65.5	44.3	––	31	100
		3	117.0	97.5	82.7	69.3	––	55.4	34	100
		4	123.0	107.2	92.3	67.7	––	42.4	28	100
>21	48	1	93.7	76.5	63.3	48.6	40.8	––	29	100
		2	87.7	70.5	58.6	43.4	35.4	––	24	100
		3	97.3	80.0	67.3	50.2	––	38.2	22	100
		4	86.2	73.6	64.0	46.5	––	25.1	33	100
	64	1	115.0	92.7	80.2	72.5	48.6	––	30	100
		2	102.4	85.5	71.8	63.7	40.1	––	30	100
		3	100.0	87.0	72.3	65.5	––	39.0	19	100
		4	93.7	82.5	70.2	62.3	– –	35.5	19	100

Discussion

There is no doubt that proper MeBr dosage and enough exposure time can completely kill beetles and insects (belonging to Scolytidae) larva. But, there are some points we should notice. Ambient air and wood humidity was high. Difference in temperature between day and night at the colder temperatures resulted in the formation of large amounts of ice. Some pieces of wood released a noticeable quantity of CO_2. All above factors influence the readings on the gas detector, leading to data instability. After several trials, we found that the average moisture of the wood was > 20%, and the relative humidity in fumigation space was 42% – 84%. We used desiccant and CO_2 sorbent to make the data more uniform and the trial more reliable.

Although fumigant concentration is low in the fumigation area, care should be taken that fumigation team members operate environmental MeBr gas monitoring instruments and wear a full-face mask with the correct filter and clothing to cover exposed skin.

Currently MeBr is regulated internationally through acceptance of the Montreal Protocol of 1998. Much research has been directed toward MeBr alternatives and the reduction and recovery of MeBr.

Acknowledgement

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0435

Modified Atmosphere Applications in Museums

Mevlüt EMEKCI* and Ahmet Güray FERIZLI

Abstract: Historical Palaces in Istanbul (Turkey) are infested with the furniture beetle (*Anobium punctatum*) with the aid of favorable conditions such as high humidity and temperature.

Due to ban of Methyl bromide in Turkey, the furniture beetle disinfestations of wooden historical artifacts have been shifted to use of alternative applications such as modified atmospheres.

In this study, portable wooden artifacts, which have damage risks with the traditional fumigation procedures, were confined in PVC cubes of 30 m^3 volumes to apply with modified atmospheres composed of conditioned high nitrogen ((99%).

Nitrogen gas was obtained using a Nitrogen Gas Generator and a SCADA (supervisory control and data acquisition) system were also set to maintain N_2 concentrations in several PVC units at desired levels during the application period of 30 days.

Results showed that portable PVC modified atmosphere units were effective to retain N_2 concentrations at high levels, and the PLC SCADA system successfully controlled the N_2 levels in several PVC units, and that no insect survived after 30 days of exposure to high nitrogen.

Introduction

A variety of pests inhabit historical buildings & museums (Schrock, 1988) and can cause significant damage to valuable artifacts unless the necessary control measures applied (Reichmuth *et al.*, 1993). The nature of the historical artifacts imply that some traditional chemicals can not be applicable in the historical buildings & museums due to damage risks to the artifacts, and also to environment and to operators (Dawson, 1988; Florian, 1998).

Modified atmosphere applications, on the other hand, offer effective pest control approaches in various environments including museums, and do not pose the major risks mentioned above (Bailey and Banks, 1980; Daniel *et al.*, 1993; Gilberg, 1989; Navarro, 1978; Reichmuth *et al*, 1993, Rust and Kennedy, 1993; Zycherman and Schrock, 1988)

Turkey has a lot of historical palaces/pavilions which subject to damage by wood pests. Of these, 12 Ottoman palaces/pavilions in Istanbul serve as museums under the administration of Department of National Palaces of Turkish Grand National Assembly. Wooden structural parts and/or wooden artifacts of those palaces have been continuously damaged by *Anabium punctatum* De Geer in the lack of necessary control mesaures throughout the many decades.

The importance of the damage caused by the pests to Ottoman Palaces has led to formation a project which financially supported by SPO (Turkish Republic Prime Ministry State Planing Organization) in 2003. In the framework of that project transportable wooden artifacts of Ottoman Pavilions were fumigated on site by means of modified atmospheres applied in PVC envelopes (cubes). This communication gives information on the application of modified atmosphere in Ottoman palaces against wood – boring insects.

Materials and Methods

Test Insects

Despite our several attempts we could not provide live insects from different laboratories. Thus, wood pieces showing live *Anobium punctatum* larval activity taken from the restoration department were used as infested materials. The other test insects were some of the well known stored product insect (Table 1.) Test individuals are gently placed in special PVC vials of 10 – 20 mL volume whose lids fitted with very fine wire mesh of diameter of 1 cm to prevent the escape of insects and to ensure air passage.

PVC Cubes

Modified atmosphere applications were made in 18 PVC cubes of 30 m^3 volume each. These cubes were originally designed for toxic gases or carbon dioxide fumigations, especially in the food and agriculture industry. However we have tested and developed a protocol to maintain a low oxygen atmosphere of 1% in the

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cubes by the Scada system. The cube which also has an internal frame of galvanised pipe(id, 2.5 cm) were composed of a bottom floor and a top cover part which were joined together with a PVC tongue – & – groove zipper after filled with artifacts. For gas flushing, cubes were provided with inlet and outlets openings at opposite directions, which can be closed by means of gas tight screw lids.

Modified Atmosphere Treatments

Low oxygen atmospheres composed of high nitrogen gas were obtained using a nitrogen gas generator(On Site Gas Systems, Inc., USA) of 4 Nm^3/h outlet flow capacity. Nitrogen gas obtained from the gas generator was humidified at ambient Rh and conveyed to each of the 18 pieces of PVC cubes. A PLC Scada system was also set up to restore nitrogen levels in different cubes when the oxygen level increased above 1%. The cubes were initially flushed with a high flow rate(30 m^3/h) of nitrogen using liquid nitrogen dewars of 240 L capacity to shorten the gas pushing time and when the oxygen level inside the cubes dropped below 3%, nitrogen gas generator with the PLC Scada system was run to get a further reduction to 1%. The oxygen concentration, relative humidity, and temperature in the cubes were continuously monitored. Treatments were continued 30 days.

Table 1. Numbers and the ages of insect species according to the developmental stages used for modified atmosphere applications.

Insect species	Developmental stages			
	Eggs	Larvae	Pupae	Adult
Tribolium castaneum	1 – 3 d(50)*	Mature (25)	1 – 3 d (15)	5 – 10 d (25)
Tribolium confusum	1 – 3 d (50)	Mature (25)	1 – 3 d (15)	5 – 10 d (25)
Trogoderma granarium	1 – 3 d (50)	Mature (25)	1 – 3 d (15)	5 – 10 d (25)
Rhyzopertha dominica	1 – 3 d (50)	Mature (25)	1 – 3 d (15)	5 – 10 d (25)
Infested wood pieces	wood materials showing insect activity			

* Number of the test individuals

After the treatments, test insects in special enclosures were returned to laboratory and counts were made to check the mortality. Wood pieces showing insect activity were also kept at the laboratory in special PVC boxes to observe any insect activity.

Results and Disussion

Temperature and relative humidity data measured inside the cubes during 30 days of exposure were shown in Figure 2. Oxygen concentrations were between 1% – 1.5% throughout the treatments (Fig. 3). Mortality records showed that all the test insect were killed by the treatments of high nitrogen. Similarly, infested wood pieces with *Anobium punctatum* which separately kept in plexiglas containers after the treatment did not show any larval activity during 2 years after the treatments(Table 2).

Based on data presented by Frank(1991) and Reichmuth *et al.* (1991), Reichmuth *et al.* (1993) informed that some wood – boring pests including *Anobium punctatum*, *Hylotrupes bajulus* L., and *Lyctus brunneus* Stephens were completely killed for up to 35 days at the temperatures ranging from 16 to 35℃. They also report that at 16℃, adults (separate), eggs (in wood and separate), and larvae(in wood) of Anobium punctatum were completely killed in 35 days. Similar results were obtained with other wood – boring insects. Valentin(1990) showed that exposures to 1.0% O_2 atmospheres for 20 days killed deathwatch and powderpost beetles (Rust and Kennedy, 1993). Gilberg(1989) reported that, insect pests frequently encountered in museums, 7 day exposures at 30℃ and 65% – 70% RH to 0.421% O_2 in nitrogen killed webbing clothes moths, cigarette beetles, drugstore beetles, carpet beetles and powderpost beetles(Rust and Kennedy, 1993)

In conclusion, low oxygen atmosphere using high nitrogen in PVC cubes offer safe and efficient control of pests of wood in artifacts preserved in museums.

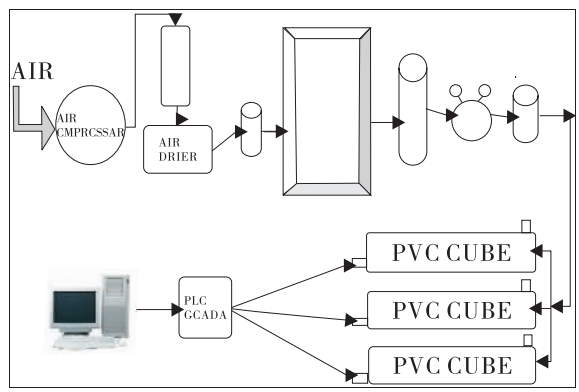

Fig. 1 Modified atmosphere fumigation system design

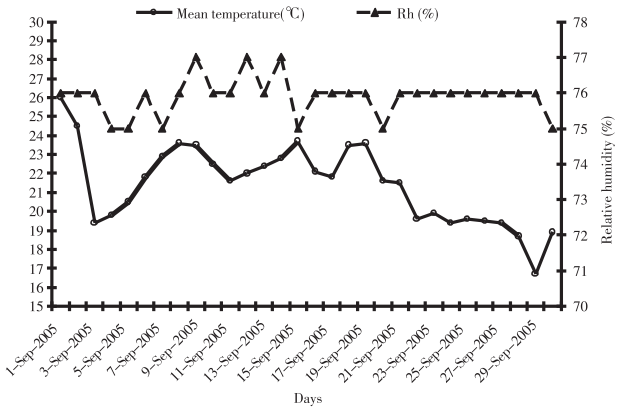

Fig. 2 Daily mean temperature(℃) and Rh(%) data recorded inside the cubes during the modified atmosphere treatments

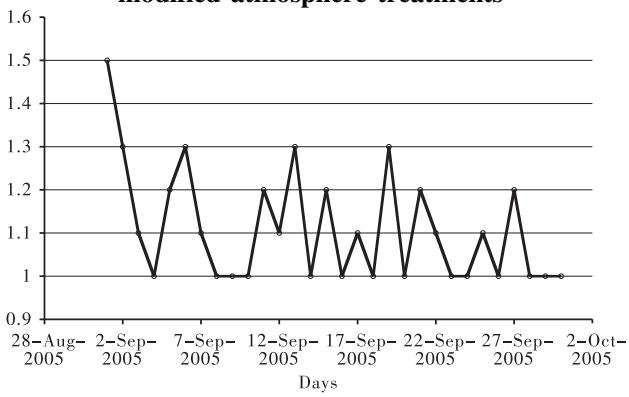

Fig. 3 Daily mean oxygen level(%) recorded inside the cubes during the modified atmosphere treatments.

Table 2. Mortality of the life stages of test insects during modified atmosphere treatment(%)

Insect species	Developmental stages			
	Eggs	Larvae	Pupae	Adult
Tribolium castaneum	100	100	100	100
Tribolium confusum	100	100	100	100
Trogoderma granarium	100	100	100	100
Rhyzopertha dominica	100	100	100	100
Wood pieces showing insect activity	No insect activity was observed during 2 years after the treatments			

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0436

Controlling Insects by Ozone in a Wheat Storehouse

Li Zhimin[1] and Cao Yuede[2]

Abstract: The test of controlling insects with ozone in 900t of high moisture wheat was conducted through mobile modular ventilation system and ozone generators. Recirculation fumigation in the grain bulk through mobile ventilation ducts with four to six ozone generator was tested on 4 species of stored grain insect pests: *Sitophilus oryzae* (Linnaeus), *Cryptolestes ferrugineus* (Stephens), *Rhyzopertha dominica* (Fabricius), *Tribolium castaneum* (Herbst). The stored grain insect density was 10 to 30 insects per kilogram grain. Stored grain insets were completely killed by ozone at 15 ppm concentration and 290 hours exposure.

Key words: ozone, stored grain insects control, mobile ventilation system

1 Preface

As an allotrope of oxygen and a strong oxidizing agent, ozone with a half-life in the atmosphere of 20 – 30 minutes was extensively applied in the medicine and the food professions. It was mainly used to destroy germs on surfaces, and in water and for space disinfections. Ozone as a fumigant could be also used for destroying stored grain insects. As it changes into oxygen, there is no pollution to the food and also no harm to the environment.

Many domestic and international experts have researched ozone's effects on insects. Erdman (1979) reported that *Tribolium. castaneum* (Herbst) and *T. confusum Jacquelin du Val* were all dead after exposure to 450 ppm ozone for 7 hours at 30℃. Li Changguo et. al. reported that ozone from an ozone generator at 3 – 30ppm applied intermittently for a long time in the storehouse space could effectively control the development of grain insects inside the wheat. Linda J. M used ozone of 10 – 50 ppm to fumigate adults of *Sitophilus. zeamais* Motschulsky, *T. castaneum* (Herbst), *T. confusum Jacquelin du Val* and larvae of *Sitotroga cerealella* (Olivier) in the laboratory. Cao Zhanggui used ozone at 15 – 120ppm to fumigate adults of *S. zeamais* Motschulsky, *Rhyzopertha dominica* (Fabricius) and *T. castaneum* (Herbst). The work established the lethal CT value of adults. Fan Shengliang concluded that greater than 10 ppm ozone could kill insects when it was introduced through an air duct into part of a warehouse that was sealed.

In the reports on ozone to control insects, Fan Shengliang's partial experiment was the only experiment in warehouses. In the experiments reported here, high concentration ozone produced by many ozone generators was introduced into the grain through ventilation pipes. It was evenly distributed in the whole grain storehouse, which had previously been sealed. The lethal time and CT value, as well as the moisture, were calculated by periodically measuring ozone concentrations and moisture content.

2 Materials and Methods

2.1 Experiment Storehouse and Time

The experiment was carried on in the Yaonan Heishui State Grain Reserves. The storehouse was 60 meter long, 24 meter wide and 6 meter high.

The wheat for experiment came from Henan province and the moisture content was 12.1%, while the height of wheat was 4.1 meter.

The storehouse had groups of three 4 – 72A type centrifugal blowers with 7.5kW power. The experiment started on 5 June 2007 and ended on 20 June.

2.2 Ozone

2.2.1 ozone generator and supporting facilities

a. 6 YLHD – LJC type ozone generators.

b. 5 BLZ – 11 – 3A type circulation fumigation machines.

c. 6 YBT – 1.1 type anti – explosion centrifugal fan

d. 6 sets of QG · JC · 12 type partial vent-

1. *Jilin Branch, State Grain Reserves* 130033
2. *State Grain Reserves Yaonan Heishui Depot*

ilators and 55 sets of ventilation ducts (two punched ventilation ducts and two porous ones)

e. 2 LDQ – 1400WI multi – function samplers

The equipment was provided from Qinpeng green equipment limited liability warehouse protection company.

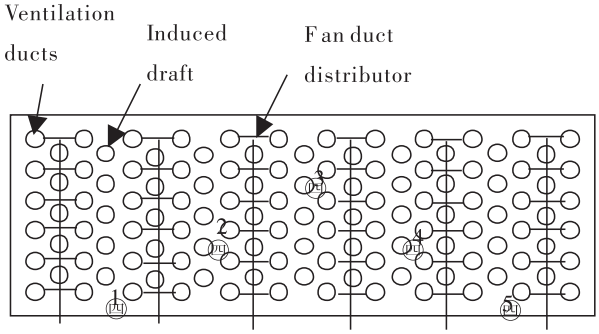

Fig. 1 **The plane arrangement of the ventilation ducts**

2.2.2 The connection of ozone generator and its supporting facilities

The ventilation ducts were put into bulk grain, each group of them consisted of alternate groups of two punched ventilation ducts and two porous ones(see chart 1). The branch ventilation duct of the distributor was connected with ventilation ducts, and then the anti – explosion centrifugal fan was connected with the distributor. The ozone generator was placed on the surface of the bulk grain. The ozone was sucked out by the circulation fumigation machines, then pressed into the bulk grain by the anti – explosion centrifugal fan and entered into the inside space of the storehouse through the guide ventilation ducts. The ozone flowed in a closed circulation system. When the ozone and produce had been bought into balance, the ozone concentration was determined. The connection of single distributor is shown in the chart 2.

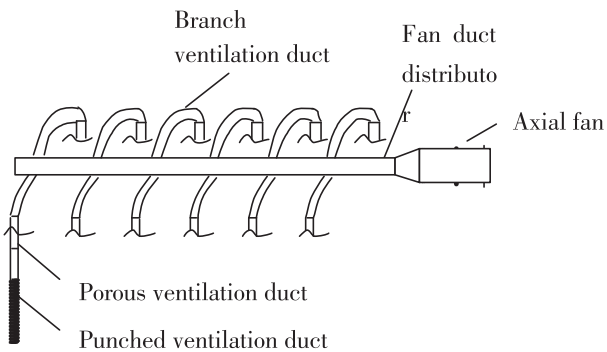

Fig. 2 **The connection of single ventilation duct**

2.2.3 measurement of the ozone concentration

2.2.3.1 detecting tube

The ozone density was measured by the detecting tube made by the labor department occupational safety bureau Beijing labor insurance. The detection range was 2 – 50ppm. The ozone concentration was calculated by the colour change after sucking 100mL through the tube.

2.2.3.2 Distribution of sampling points

Because the storehouse was enclosed, the ozone concentration in the grain surface and inside the grain became consistent after the ozone concentration had been brought into balance.

2.3 Stored Grain Insects

2.3.1 detection of stored grain insects

Five places inside the storehouse were chosen to detect insects.

2.3.2 Detecting the live or dead insects

The stored grain insects were assessed as live as long as their body could move even if their legs stretched. If the insects didn't move for a long time, they were determined to be dead.

2.4 Laboratory Apparatus and Equipments

2.4.1 grain statement detection system

Temperature measurement cables were made by State Grain Reserves Yaonan Heishui Depot and distributed uniformly in the whole grain bulk. Pocket thermodetector was made by Taizhou.

2.4.2 Detecting the moisture and quality

The measurement of moisture was standardized according to the Chinese Standard method. The measurement instrument of rapid moisture measurement was used to measure moisture. Some storage indicators such as Fatty acid, germination rate, viscosity and quality indicators such as percentage of flour and quality of flour were measured according to the Chinese Standard method.

2.4.3 microbioassay

Microorganism fast measurement instrument

2.5 Other Instruments LDQ – 1400W

2.6 Type Multi – function Samplers

1 LDS – ID type computer moisture measurement instrument

Some ozone detecting tubes

150mL volume suction tube

3 Results and Discussions

3.1 Ozone Concentration Distribution in the Experiment Storehouse

Because the ozone generator was placed inside the storehouse and ozone was sent into

the grain, the ozone concentration of the grain surface could represent the one inside the grain bulk. The ozone concentration depended on the number of ozone generators. The maximum ozone concentration was 30ppm and the minimum was 10ppm.

3.2 Effect of Ozone on Stored Grain Insects

The mortality of the stored grain insects caused by ozone is shown in table 1.

Table 1. the death of the stored grain insects caused by ozone

Time(h)	Interval of time(h)	Ozone density (ppm)	CT value (mg·h/L)	Total CT value (mg·h/L)	Number of insects(Number/kg)			Moisture (%)
					Live insects	Dead insects	Mortality (%)	
0	0	0	0	0	30	2	6	16.0
40	40	15	1.28	1.28	37	3	8	16.0
72	32	30	2.05	3.33	15	4	21	15.5
134	62	20	2.65	5.98	10	1	9	15.0
226	92	10	1.97	7.95	10	20	67	15.0
284	58	10	1.24	9.19	0	20	100	15.0

The change of the ozone concentration was large if it was detected by the detecting tube. The average ozone concentration was used to calculate CT values.

The stored grain insects were mainly *S. zeamais* Motschulsky and a few others, such as *R. dominica* (Fabricius), *T. castaneum* (Herbst) and *C. ferrugineus* (Stephens).

For complete mortality, the CT value should reach 9.19 mg·h/L(mg. hours/litre), the average ozone concentration 15ppm and the minimum should be bove 10ppm for approximately 12 days (table 1). These results were consistent with those of Linda J. M s(1998).

3.3 The change of Temperature, Moisture and Quality of the Grain

3.3.1 temperature change

The whole grain temperature rose up to 40℃, it was more 15℃ than the air temperature, after ozone aeration. The whole grain temperature decreased to a little more than the air temperature through the blower and the ventilation ducts in 1 day after the experiment was finished.

3.3.2 the change of the grain moisture

The grain moisture decreased 1% during the fumigation from an initial high value of 16% (table 1).

3.3.3 the change in grain quality

The experimental and control wheat samples were all examined by the Jilin Quality Detect Central (table 2).

Table 2. the wheat quality change

Detecting item	Before	After
Water absorption of gluten(%)	194	194
Falling number(s)	305	294
Fatty acid (mgKOH/100g dry basis)	14.5	20.9
viscosity(mm^2/s)	7.7	6.4
Steamed bread taste grade(grade)	77	80
Germination rate(%)	94	61
Percentage of flour(%)	67.6	67.6
Value of farinograph	45	47

After ozone fumigation, some quality indicators, such as fatty acid, viscosity, falling number and water absorption of gluten, were almost the same as before. Others such as steamed bread taste grade, percentage of flour, value of farinograph, were better than before. The germination rate decreased obviously. It is concluded that the ozone had no bad effect on wheat quality if it was not used as seed.

Table 3. contrast of the microorganism activity before and after ozone treatment

	Before treatment		After treatment	
	Sampling point	average	Sampling point	average
Microorganism activity(u)	541 668 591 608 531 627 549 619 592 620	595	638 639 635 666 645 626 602 625 628 642 639 659 638 596 645	635

3.4 Microorganism Activity

Microorganism activity was detected in some sampling points before and after treatment. The average microorganism activity was almost the same before and after treatment. It

was concluded that microorganism activity was restrained and not increased.

3.5 Expense and Efficiency

3.5.1 expense

The expense included equipment and operating costs such as energy change cost.

Equipment costs included 123 thousand yuan for 6 ozone generators, 156 thousand yuan for 6 sets of partial ventilators, 46.2 thousand yuan for some ventilation ducts.

Operating costs were mainly energy changes: the cost of 6 ozone generators with 16.8 kW and 6 axial fans with 6.6 kW. The experiment lasted about 294 hours and used 6880 kW power. 819t wheat was treated. Average cost of one ton grain was 8.4 kW or 7.56 yuan according to 0.9 yuan per kilowatt.

3.5.2 efficiency

The stored grain insects were all dead in the experiment and there was no residue, no harm to human, animals and environment, because the ozone dissociation product was oxygen. Pesticide residue in the grain was reduced because the ozone resolved the pesticide, while the grain quality remained unchanged. So it met pollution – free food requirements.

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0437

Fumigation Applications in Historical Buildings

Ahmet Güray FERIZLI* and Mevlüt EMEKCI

Abstract: Disinfestations of historical buildings by re – circulating of a gaseous fumigant through the building is a fumigation technique that was practiced. The building constructed from a mix of wood and stone is a historical palace, in Istanbul (Turkey), which is a volume of 40.000 m^3, composed of two floors and a basement. It contains extensive use of mother – of – pearl that covered almost all of its surfaces. There are also detailed painted landscapes on the ceiling. Valuable old sculptures of wood, paintings on wood or with wooden frames, as well as parquet floor material are all very susceptible to damage by wood boring insects. The main pest in that building was the furniture beetle. Damage was very serious on the wooden parts.

Methyl bromide was the only registered fumigant at 2005 in Turkey. So, fumigation was carried out using methyl bromide. Because of fire risk of fan electricity, re – circulation system was set up outside of the building and electricity was cut off during fumigation inside the building. Before fumigation, all sensitive and precious objects were kept in specially designed cube in the building to disinfest using modified atmosphere application.

The building was fumigated with methyl bromide at 20 – 32 g/m^3 for two days of exposure. Whenever gas concentration decreases, additional gases were added to keep desired concentration inside the building.

A piece of heavily infested wooden sample were kept inside the building during the fumigation. After aeration of the building, wooden sample were kept in glass cabinet for 3 months without any insect activity.

Key words: the furniture beetle, *Anobium punctatum*, wooden artifacts, historical buildings fumigation, methyl bromide

Introduction

There are many historical buildings including palaces in Istanbuln Turkey. Of these, 12 Ottoman palaces in Istanbul serve as museums under the administration of Department of National Palaces of Turkish Grand National Assembly. Artifacts made of wood and wood containing structures often suffer from extensive damage caused by wood-boring insects that over time can reduce large volumes of wood to hidden networks of sawdust-filled channels. The common furniture beetle, *Anobium punctatum* De Geer (Coleoptera: Anobiidae), is a widespread pest of wood in temperate regions, favored by high levels of relative humidity (Hansen and Jensen, 1998). Historical Palaces in Istanbul (Turkey) are infested with the furniture beetle (*Anobium punctatum*), because of the favorable conditions such as high humidity and temperature. Thus, it is a common pest of wooden objects and wooden parts of the Palaces. Precious old sculptures from wood, paintings with wooden frames as well as furs and skins in museums are very susceptible to damage by insects that are able to digest cellulose.

Wood-infesting beetles are difficult to control because their immature stages feed within wood, and usually remain undetected by conventional inspection methods. The larvae feed and grow within the wood creating a network of tunnels closely packed with frass (fine dust). The main sign of activity is fine dust of wood under the wooden objects, which was common in the palace.

Methyl bromide had been the most frequently used of the museum fumigats and many safe and effective treatments had been carried out all over the world (Bond, 1984). However, it is reactive and fumigation will produce chemical changes in objects. Objects such as wool and horsehair may give off a very strong smell after treatment. Methyl bromide has now been banned because it is an ozone-depleting gas. Thus, sulfuryl fluoride as an alternative to methyl bromide is now used for fumigating buildings.

The importance of the damage caused by the pests to Palaces has led to formation a project that financially supported by SPO (Turkish

Ankara University, Faculty of Agriculture, Department of Plant Protection 06110 *Ankara, Turkey*

Republic Prime Ministry State Planning Organization) in 2003. In the context of that project the structure of the Ottoman Pavilion was fumigated. Below given fumigation was the last fumigation in Turkey for the museum disinfestations using methyl bromide.

Application Technique

The building constructed from a mix of wood and stone is a historical palace, in Istanbul (Turkey), which is a volume of 40.000 m^3, composed of two floors and a basement. It contains extensive use of mother-of-pearl that covered almost all of its surfaces. There are also detailed painted landscapes on the ceiling. Valuable old sculptures of wood, paintings on wood or with wooden frames, as well as parquet floor material are all very susceptible to damage by wood boring insects (Reichmuth et al., 1993). The main pest in that building was the furniture beetle. Damage was very serious on the wooden parts.

Fumigation of the building was combined with modified atmosphere application. All wooden artifacts and textile materials were confined in PVC cubes of 30m^3 volumes to apply with modified atmospheres composed of conditioned high nitrogen ((98.5%) in the palace. Modified atmosphere using nitrogen gas generator controlled by SCADA (supervisory control and data acquisition) system. At the end of application system were shut off and gas vent out of the cubes were closed, then fumigation of the building was started. During modified atmosphere application, building was sealed to improve the structure's fumigant retention properties. Doors and windows were sealed with methyl bromide proof polyethylene sheeting and tape. Particular attention was devoted to sealing of the building.

Because of fire risk of fan electricity inside the building, re-circulation fans outside of the building were used to gas introducing, sucking, distributing and also aeration. Thus, electricity was cut off inside the building during fumigation. Re-circulation system was consists of four pump (each 2 500m^3 per hour) and gas evaporation chamber (3 m^3). System sucks inside air and introduces it into the evaporation chamber, then extends mixed air to inside the building using PVC ductwork. Methyl bromide was released into the evaporation chamber using copper pipe connections.

For the sucking air from the building and introducing air mixed Methyl bromide PVC flexible pipe (40 cm diameter) were used outside of the building. Inside the building, sucking pipes (40 cm diameter) extended to each floor including basement. Main introducing main pipes inside the building extended to each floor including roof except basement. For each level, main pipe extended to each room using appropriate connection and pipe (10 cm or 20 cm diameter). When the re-circulation system was started to work, air was sucked mainly from the center of the each floor and basement using pipes (40 cm diameter). Sucked air pushed to the first and second floor and also roof. In each room, gas introduction was measured and tried to equalize according to the volume using sealing tape to the open end of the pipe. Before fumigation, gas distribution to everywhere inside the building was secured. Fumigant gas did not introduced to the basement, because methyl bromide heavier than air. So, it is expected that gas was naturally goes into the basement. Though, there were only sucking pipe in the basement to prevent accumulation of the fumigant.

Before fumigation, gas-monitoring lines were extended to the outside from different locations of the building. For the effectiveness tests, heavily infected wood piece collected from exchanged parts in carpenter's workshop were placed different locations of the building. For laboratory reared test insects, eggs, larvae, pupae and adult stages of *Trogoderma granarium* Everts (Coleoptera: Dermestidae), *Rhyzopertha dominica* (F.) Coleoptera, Bostrichidae, *Tribolium castaneum* Herbst and *Tribolium confusum* (DuVal) (Coleoptera: Tenebrionidae) were used to evaluation of the effectiveness of the application (Table 1).

Table 1. Numbers and the ages of laboratory reared test insect species according to the developmental stages used for fumigation.

Insect species	Developmental stages			
	Eggs	Larvae	Pupae	Adult
Tribolium castaneum	1 – 3 d (50)*	Mature (25)	1 – 3 d (15)	5 – 10 d (25)
Tribolium confusum	1 – 3 d (50)	Mature (25)	1 – 3 d (15)	5 – 10 d (25)
Trogoderma granarium	1 – 3 d (50)	Mature (25)	1 – 3 d (15)	5 – 10 d (25)
Rhyzopertha dominica	1 – 3 d (50)	Mature (25)	1 – 3 d (15)	5 – 10 d (25)

Test individuals number

After preparation of the system, fumigation was started with methyl bromide at 25 – 32g/m^3 for two days of exposure. Whenever gas concen-

tration decrease to 25 g/m^3, additional gases which is necessary because of the inevitable losses of gas were added to keep desired concentration during the first day inside the building. For the second day, gas concentrations inside the building were tried to keep 20 g/m^3. Re-circulation system were kept working during gas introduction and equalization inside the building. When the concentration of fumigant inside the building equalize after introduction, system were kept switched off till to another gas introduction.

After 48 – hour exposure period, sucking pipes separated from re-circulation system. Though, air from outside was pushed to the building through the gas introducing pipes. System were kept working for 24 hours, then windows and door opened for the aeration. After 6 hours, building was checked and secured to reentry.

Results and Discussion

During the last decades Methyl bromide was used to eradicate pests in artifacts in museums. In some countries, sulfuryl fluoride is used for the control of wood boring insects mainly *Anobium punctatum* and *Ptilinus pectinicornis* (Bess and Ota, 1960; Meikle and Stewart, 1962; Binker, 1993). But, available fumigants on the market were only methyl bromide and phosphine during our study in Turkey. Thus, we used methyl bromide for the fumigation of structure of the historical building.

With passive application of methyl bromide, gas build – up starts slowly in structure, and then it reaches maximum level and then, decreases because of leakage. The rates of decrease will vary depending upon gas leakage for the structural fumigation. With forced air recirculation, gas introduction into the structure can be increased and there will not be big difference on concentration between each floor. In our study, we observed that gas release into the structure was very quick, and gas concentration between each level of height in the building was not high, because of unique recirculation. The temperature during fumigation was around 22℃. In the present study, recorded gas concentration values were plotted against the exposure period (Fig 1). Concentrations built up to a maximum in a 6 h of the treatment as gas continuously added (Figs 1). The decay in gas concentration in the structure was 25% between 6 to 8 h of the fumigation. This can be attributed predominantly to (a) gas circulation was not enough to evenly distribution at that time of exposure in the building, so recirculation system was kept working, and (b) leakage. In the present study because of our strategy to keep the gas concentration stable, gas addition to the building was repeated till to end of fumigation. Thus, we could not calculate half loss time.

Table 2. Mortality of the life stages of test insects after fumigation (%)

Insect species	Developmental stages			
	Eggs	Larvae	Pupae	Adult
Tribolium castaneum	100	100	100	100
Tribolium confusum	100	100	100	100
Trogoderma granarium	100	100	100	100
Rhyzopertha dominica	100	100	100	100

After fumigation, heavily infected wooden pieces were kept in a ventilated class enclosure for 3 moths. Visual investigation showed there was no fine dust of wood under the wooden pieces. Moreover, mortality was also determined by cutting the wood piece to find larvae. Both indications shoved that fumigation was successful. After fumigation, laboratory test insect samples were kept a week in controlled condition, and mortality were determined. It was found that mortality was complete (Table 2).

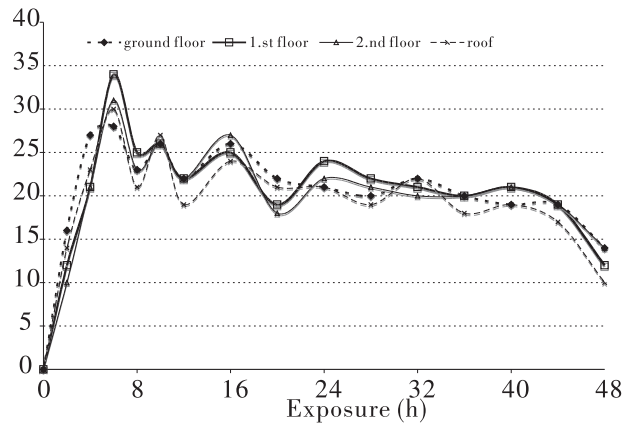

Fig 1. Methyl bromide concentrations (g/m^3) during fumigation of the historical building

Wood for construction, as artifacts and other purposes can be infested and attacked by insects being capable to digest wood. Our study showed that museums and palaces the aspect of keeping the structural stability and preservation of artifacts may be combined by using modified atmosphere and fumigation at the same time.

Acknowledgements

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0438

Applied Research on Controlling Stored-grain Insects with Nitrogen

Chen Mingshun[1], Xu Decun[1], Wan Chunmiao[1] and Song Wei[2]

Abstract: Under sealed conditions, oxygen was separated from the atmosphere by a homemade high – purity nitrogen generator for grain storage to generate high – purity nitrogen. High – purity nitrogen was dispersed and distributed uniformly in the bulk through re – circulation pipe under film. To control stored-grain insects, the concentration of nitrogen was maintained above 95% for at least 19 days, which made insects under long – term hypoxic circumstance and finally die for lack of the necessary amount of oxygen. In addition, oxygen concentration below 5% decreased the rate of wheat physiological metabolic activity, respiration, quality deterioration, and growth of grain – storage microbes. It increased wheat security in storage.

Key words: nitrogen, stored-grain Insects, controlled atmosphere storage(CA storage), applied research

Introduction

With the development of people's living standards, their concept of food consumption has changed from sufficent and good food to safe, nutritious and environment-friendly food, and Green Food is increasingly welcomed. Grain reserve is a state policy of guaranteeing social and economic development. The quality of stored-grain has directly affected the quality of people's life. Today's main technology of controlling insect in grain reserve is chemical fumigation. The pollution caused by this to grain can't be express quantitatively. In general, fumigation in grain is neither safe to grain or to operators.

As the disadvantage of chemical agents in controlling stored-grain insects is taken more seriously, some developed countries start to use alternative technology to replace chemical agents. Some of the economically developed countries, such as America, Japan, Russia and Australia, have gradually reduced the usage of chemical agents on stored-grain, and developed modern technologies of grain reserve towards integrated management including low temperature storage, CA storage, physical and biological storage, and made many studies on CO_2 CA storage. Canada, Japan and other countries in the world have also researched the technology controlling stored-grain insects with nitrogen.

Since 1998, the infrastructure facilities of grain reserve in China have made much headway. The central government invested much treasury bonds on construction of modern depots of State Grain with volume of 100 billions. It involves the application and extension of four new technologies for grain reverse, low temperature grain storage with grain cooler, computerized grain conditions control system, mechanical aeration and phosphine re-circulation fumigation, that promoted smooth development of research on new technology, equipment, techniques, instruments and method for oil and grain reserve.

At present, chemical fumigation technology, commonly used for stored-grain insects control, has advantages of killing insects quickly, thoroughly, economically, with labor-saving and so on, but also with its problems, increasingly resistance to phosphine, increasingly phosphine dosage, phase-out of methyl bromide, and limited use of dichlorovos. . In addition, chemical agents made chemical residues and pollution in stored grain, and damage people's health by long-term exposure.

The CO_2 CA storage could effectively control the stored-rain insects and decrease the rate of wheat physiological metabolic activity, respiration, quality deterioration, and growth of grain-storage microbes, but its cost was much higher than traditional fumigation, and four times higher than N_2 CA storage. Cost was the great obstacle that prevented its development and application.

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The Nanjing depot of state grain developed a new type of grain storage device, mobile high-purity nitrogen generator ZCBGPN$_2$9 – 30PSA, in 2004, and researched N_2 CA storage technology in horizontal warehouses. The research achieved good results, promoted technical progress in grain storage sector, and played an important role in protection of people's health.

1 Materials and Method

1.1 Test Depot

1.1.1 N_2 CA storage: No. 20, horizontal warehouse, specification, 30m × 21m, grain height, 6m.

1.1.2 CO_2 CA storage: No. 19, horizontal warehouse, specification, 30m × 21m, grain height, 6m.

1.1.3 Gas tightness measurement: Surface of grain bulk was sealed with two layers PE sheeting; Vent opening and door were sealed with two grooves method; Gas tightness, measured according to negative atmospheric pressure 300 Pa back to 150 Pa, was more than 2 minutes.

1.1.4 Ventilation system: over – ground ducting, one machine, two groups of three ducts, ventilation pipe network 30cm under grain surface. .

1.1.5 Equip with nitrogen and carbon dioxide detecting device and Grain monitoring system.

1.1.6 Insect monitoring device: Automatic insect monitor MCS – 180 with five transducers, five 400 scan lines, wireless transmission device with a transmit distance of more than 1000 meters.

1.2 Test Grain

Depot No.	Variety	Amount (tonne)	Insect and Number (Insects per square meter)	Water content (%)	Impurity (%)	Grain temperature (℃)			Garnered in time
						Upper layer	Middle layer	Under layer	
19	Middle – late hsien Rice	2169	10 *Sitophilus zeamais*	13.1	0.7	29	17.6	13.6	06.10
20	Middle – late hsien Rice	2120	10 *Sitophilus zeamais*	13.4	1	27.1	14.5	12.2	06.11

1.3 High-purity Nitrogen Generator

Nitrogen was separated from air by pressure swing adsorption. The generator had four parts, air purification, nitrogen generation, nitrogen output and nitrogen conveyor pipe in depot. It was a mobile device.

1.3.1 Air purification: To ensure the long – term stable operation of the N_2 generator, an adsorption tower as a part of the generator must be provided with clean and dry air. Thus it was necessary to remove water, dust and oil in air by certain filter before air entered the adsorption tower.

1.3.2 Nitrogen generation: Nitrogen of more than 99% purity was directly generated from adsorption tower by technology of pressure swing adsorption at speed of 30 – 60m^3/h. Adsorption material was high-performance carbon molecular sieve.

1.3.3 Nitrogen output: To ensure the effective N_2 concentration in the test depot, the adsorption tower was equipped with N_2 monitoring device at the N_2 duct exit, and a gas analyzer was provided at the vent opening of depot.

1.3.4 Nitrogen conveyor pipe in depot: Nitrogen was promoted to dispersed and distributed uniformly in the bulk through a re-circulation pipe network under film.

1.4 Insects bags and monitoring device

1.4.1 Test insects: three kinds of representative insects cultured in lab, *Sitophilus zeamais*, *Rhyzopertha dominica*, *Cryptolestes ferrugineus*.

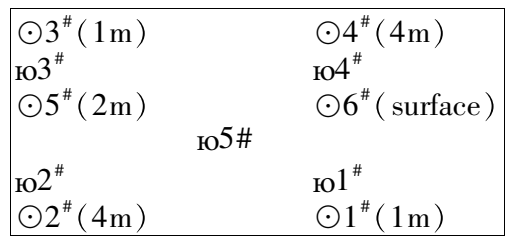


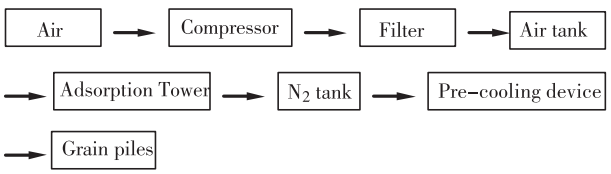
Figure. Drawing of distribution of insect bags and monitoring devices in No. 19, No. 20 depots Note: "⊙" and "ю" represent insect bag and monitoring device, respectively.

1.4.2 Test insect bags: each bag filled

with 10 *Sitophilus zeamais*, *Rhyzopertha dominica*, and *Cryptolestes ferrugineu*, sealed and prepared for use.

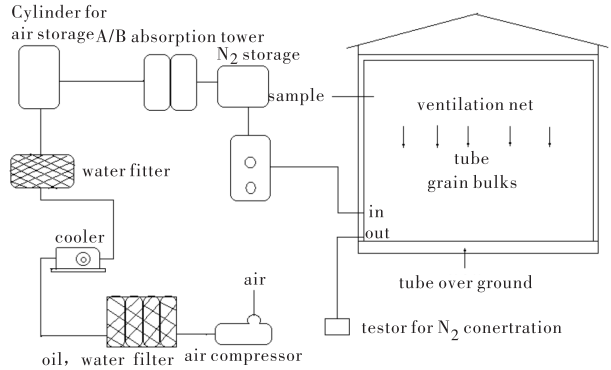
1.4.3 Insects bags and monitoring device were pre-buried as follows by in-depth slender

2 Test Procedure



Drawing of N_2 CA Storage Process

The N_2 CA storage device was tested then the high-purity nitrogen generator was started to generate and pump N_2 into test depot. The concentration of nitrogen was maintained above 95% for at least 19 days. The N_2 concentration was regularly measured. The high-purity nitrogen generator was started to add N_2 once the N_2 concentration fell below 95%.



3 Results

3.1 Observation of Insect Situation in Operation Interval of N_2 Supply System

See Table 1 for more details.

Table 1. N_2 concentration and insect behavior in No. 20 depot.

Time			umulative time of N_2 supply(h)	Change of N_2 content of different position in depot						Insect situation
Month	Day	Hour/minute		1	2	3	4	5	after Ventilation	
6	24	9:40	Start	/	/	/	/	/	/	Crept rapidly
6	24	20:40	17	82.1	82.5	83.7	82.5	85.2	85.4	/
6	25	19:40	37	89.8	88.4	89.3	89.4	90.4	90.3	/
6	26	23:40	62	95.6	95.8	96.6	96.4	96.6	96.9	Crept rapidly
6	27	20:40	71	96.3	96.5	96.6	96.7	97.2	97.8	/
6	28	9:40	71, pause	96.4	96.5	96.8	96.6	96.5	/	/
6	29	9:40	/	96.3	96.3	96.7	96.5	96.2	/	/
6	30	9:40	/	95.9	95.9	96.2	95.8	95.7	/	Crept rapidly
7	1	9:40	/	95.6	95.1	95.3	95.7	95.3	/	*Sitophilus zeamais slow down*; *other insect changed little*
7	2	9:40	/	95.1	94.8	95.0	95.2	94.9	/	
7	3	8:00	Continue	94.8	94.9	95.1	95.0	94.8	94.5	*Cryptolestes ferrugineus Crept rapidly*
7	4	8:00	95	95.9	95.6	96.1	95.8	96.7	97.6	
7	4	22:00	109, pause	96.8	96.4	96.5	96.7	97.3	97.6	*Rhyzopertha dominica move slowly*
7	5	8:00	/	96.5	96.4	96.3	96.8	96.6	/	*Sitophilus zeamais spot circumvolve*
7	6	8:00	/	96.3	96.4	95.8	96.3	96.0	/	*Rhyzopertha dominica roll around slowly*
7	7	8:00	/	95.8	95.8	95.7	95.4	95.8	/	*Cryptolestes ferrugineus slow down*
7	8	8:00	Continue	95.3	95.2	95.2	95.3	94.9	94.7	
7	9	8:00	133	95.8	95.7	96.2	95.7	96.4	97.1	*Sitophilus zeamais*、*Rhyzopertha dominica immobilized*, *Cryptolestes ferrugineus slightly move*

Time			umulative time of N_2 supply(h)	Change of N_2 content of different position in depot						Insect situation
Month	Day	Hour/minute		1	2	3	4	5	after Ventilation	
7	10	8:00	157, pause	96.8	96.9	96.4	96.7	97.2	97.8	
7	11	8:00	157	96.1	96.2	95.8	95.9	96.7	96.6	All insects immobilized
7	12	8:00	157	95.7	95.2	95.6	95.2	95.7	95.6	All insects immobilized
7	14	10:00	Open the depot, took out insects	94.8	95.1	94.9	94.5	94.7	/	No living insects

3.2 Control Test of CO_2 CA Storage

3.2.1 Checking Equipment

(1) Detected whether the CO_2 monitoring device and pneumatic valve on the detecting tube were in proper operation, and checked the CO_2 infra-red analytical instrument.

(2) Detected whether the pipeline for conveying CO_2 was in good operation and whether there was water in pipeline to ensure the security of stored-grain.

(3) Detected whether the valve, electromagnetic valve and electric valve on main pipeline worked normally.

(4) Detected whether the CO_2 tank, heating device for gas tank, pressure regulating device etc. can function normally

(5) Tested whether the half-life of gas tightness of test depot reached the standard requirement. If the half-life of gas tightness of test depot didn't meet the standard, the test depot was reconstructed

3.2.2 CO_2 CA storage

During the fumigation interval, control the temperature of CO_2 near the average level, maintain the amount of air flow at 200m^3/h, keep the inner pressure of pipeline steady, adjust oil scale of pressure balance pipe to make the inner pressure of depot around 100 Pa. When the CO_2 concentration of upper layer in depot reached above 70%, closed the valve and stop the fumigation.

Based on results of everyday detection, we found 10% CO_2 concentration attenuation everyday in the first five days, after five days, the CO_2 concentration attenuation everyday was reduced to 5%. After 10 days, the CO_2 concentration in the upper layer was reduced to about 45%, however, the CO_2 concentration of the lower layer was about 80%. Because CO_2 concentration must be at least 45% during fumigation, so we used re-circulation fans to balance and maintain the CO_2 concentration above 45%. See Table 2 for more details about CO_2 concentration of No. 19 depot.

Table 2. Automatic Record of CO_2 concentration of No. 19 depot

Time	Upper layer					Middle layer					Lower layer					Average	Note
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5		
2007-6-8 21:21	71.8	71.8	72.5	72.4	71.3	72.0	71.5	72.5	72.3	72.0	71.9	70.9	72.4	71.1	71.4	73.8	
2007-6-9 8:46	72.0	70.9	71.9	68.5	56.3	72.3	71.0	69.8	68.6	59.4	59.3	56.6	53.4	55.9	49.6	63.7	
2007-6-10 8:38	67.6	67.0	67.3	73.8	50.4	68.4	61.1	56.5	72.5	51.8	43.0	38.8	37.5	43.0	41.1	55.9	
2007-6-11 8:17	64.5	70.4	68.5	79.1	50.4	65.5	57.5	55.3	68.4	51.0	43.3	39.9	38.5	37.1	41.6	55.3	
2007-6-12 8:18	64.5	70.4	66.5	79.1	50.4	65.5	57.5	55.3	68.4	51.0	41.3	39.9	38.5	37.1	41.6	55.1	
2007-6-13 7:54	70.3	76.9	68.8	88.3	58.5	71.4	64.4	62.3	65.3	58.4	48.5	46.5	44.6	42.8	48.8	63.8	2 hours re-circulation
2007-6-14 8:03	67.0	74.1	65.2	74.3	49.3	68.0	62.6	60.6	63.0	49.1	41.1	45.8	44.1	42.3	41.1	56.5	
2007-6-15 7:37	74.4	93.1	89.7	91.4	63.1	75.9	81.3	78.4	80.5	63.5	54.1	61.9	59.3	56.6	54.4	73.8	Added one ton of N_2
2007-6-16 7:48	71.6	78.8	79.6	78.3	53.3	64.5	67.5	65.5	67.6	53.1	45.6	50.5	48.9	47.0	45.5	63.2	

Time	Upper layer					Middle layer					Lower layer					Average	Note
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5		
2007 – 6 – 17 8:15	65.8	72.5	71.2	80.4	55.0	67.0	62.0	67.8	70.1	55.4	47.6	46.3	51.3	49.4	47.5	68.6	
2007 – 6 – 18 8:16	74.6	81.0	70.5	80.1	63.0	76.3	70.1	67.6	70.0	55.3	55.0	53.3	51.6	49.5	48.0	64.4	
2007 – 6 – 19 8:24	72.3	79.6	70.3	79.0	61.3	74.3	68.4	66.1	68.6	54.1	53.6	51.3	50.5	48.6	47.0	63.6	
2007 – 6 – 20 8:40	70.8	76.5	65.7	76.1	58.1	72.1	65.6	63.3	66.4	58.5	51.1	49.8	48.3	46.4	45.0	68.9	
2007 – 6 – 21 7:50	68.6	68.6	62.8	68.3	50.5	70.6	58.6	56.1	59.1	51.3	50.6	47.2	41.8	40.4	39.3	55.6	
2007 – 6 – 22 8:01	78.5	84.6	59.2	82.9	56.6	68.7	73.3	69.8	73.0	57.3	50.0	54.8	53.4	51.6	49.8	64.2	2 hours re-circulation
2007 – 6 – 23 7:50	63.6	68.5	54.3	74.0	50.1	64.9	58.4	55.6	65.0	50.5	44.4	42.8	42.3	41.0	43.9	54.6	
2007 – 6 – 24 7:47	61.8	66.1	50.6	71.3	48.8	63.0	56.8	59.3	63.0	48.9	43.5	42.0	45.8	44.5	42.9	53.9	
2007 – 6 – 25 7:10	59.4	63.3	46.8	67.8	46.5	60.6	54.6	51.8	60.3	46.8	41.9	40.5	40.1	39.0	41.5	58.7	

4 Analysis

4.1 The results in Table 1 showed the insect bags pre-buried in No. 20 depot were taken out at July 14 and insects were reared at condition of RH75% ,28℃ for 15 d, and no living insects were found 15 days later. The results showed stored-grain insects could be effectively controlled by controlled atmosphere of N_2 at concentration above 95% for 19 d.

4.2 The results in Table 1 showed the death of stored-grain insects was a long process and different insects had different times to deaath. *Rhyzopertha dominica*, *Cryptolestes ferrugineus* and *Sitophilus zeamais* were relatively difficult to control, especially *Cryptolestes ferrugineus*, which survived more than 10 days, stared to die till the 13th day, and completely died from the 16th day, under condition of 96% N_2. To control the stored-grain insects completely, especially the insects in corner, the fumigation period was extended to 19 days. The results showed the N_2 CA storage in our study and traditional phosphine fumigation had the same fumigation period of 19 days.

4.3 Comparison of N_2 CA storage, CO_2 CA storage, and Phosphine recirculation fumigation under plastic sheeting (PRFPS). See Table 3 for more details.

Table 3. Comparison of N_2 CA storage, CO_2 CA storage, and PRFPS

Content \ Technology	N_2 CA storage	CO_2 CA storage	PRFPS
Principle	High concentration N_2 was generated by home-made high-purity nitrogen generator and then dispersed and distributed uniformly in the grain bulk through recirculation pipe under film. Long-term lack of oxygen causes the death of stored-grain insects.	Depot was sealed, and then filled with CO_2 by gas storage tank. CO_2 dispersed and distributed in the grain bulk. Long-term lack of oxygen causes the death of stored-grain insects.	To kill the stored-grain insects, the phosphine fumigant was forced to disperse and distribut uniformly in the grain bulk rapidly by recirculation fumigation device.
Operation evaluation	Simple operation, small in size, move easily, need no specific person to look after, relatively high requirement in depot gas tightness.	Complex operation, high requirement in depot gas tightness.	Simple operation, relatively low requirement in depot gas tightness.

Content \ Technology	N_2 CA storage	CO_2 CA storage	PRFPS
Cost	Middle-cost, Abundant in raw material, High power-consumption,	High-cost, expensive CO_2, high power-consumption,	Low-cost, inexpensive fumigant.
Pollution	Pollution-free, Residue-free, No toxic side effects	No pollution and residues to stored-grain, mass inhalation may result in acidosis, CO_2 is a kind of greenhouse effect substance	With pollution and residues to stored-grain, with pollution to air and water resources, long-term use may result in occupational disease.
Equipment corrosion	Stable, No corrosion	some corrosion, water-soluble	corrosion to mental equipment and grain monitoring-control system.
Comprehensive assessment	Small economic input, environment-friendly, good effect of insect control and freshness-Preservation	Relatively big one-off input, good effect of insect control and freshness-Preservation	With pollution and residues, good effect of insect control, no function of freshness-Preservation

4.4 Economic cost comparison among technologies different kind of stored-grain insects control

Table 4. Economic cost comparison of N_2 CA storage, CO_2 CA storage, and PRFPS

Content \ Technology	N_2 CA storage	CO_2 CA storage	PRFPS
Depreciation expense	20,000 RMB for 12 depots, according to depreciation period of 10 years, 2,000 RMB every year, 1667 RMB per depot every year	400,0000 RMB for 18 depots, according to depreciation period of 20 years, 20,0000 RMB every year, 11,111 RMB per depot every year	12,0000 RMB for 18 depots, according to 10 depreciation period of 10 years, 1,2000 RMB every year, 667 RMB per depot every year
Sealing expense	Design of horizontal warehouse sealing, 386 RMB/depot/year	500 RMB per depot, for construction of whole depot gas tightness	386 RMB per depot every year, for sealing
Chemical expense	None (Air as raw material)	4200 RMB, 6 tonnes of CO_2 for each depot, 700 RMB per tonne CO_2	400 RMB, 2 kilograms of Alp and 200 kilograms of CO_2 for each depot
Electricity expense	1900 RMB each depot	250 RMB each depot	Not counting
Other expense	No	No	Nutrition fee, 200 RMB
Cost on per tone grain every year	1.41	5.71	0.59
Total cost of each depot	1667 + 386 + 1900 = 3953	11111 + 500 + 4200 + 250 = 16061	667 + 386 + 400 + 200 = 1653

The result showed that the cost of N_2 CA storage was only a quarter of the cost of CO_2 CA storage.

5 Discussion

N_2 CA storage and CO_2 CA storage are each part of the development trend of green CA grain storage. N_2 CA storage was applied in our depot, Nanjing depot of state grain, in 2004 for the first time, and was being phased in our depot. N_2 CA storage had many obvious advantages at this stage. Compared to the CO_2 CA storage, the N_2 CA storage had the same ability to control the stored-grain insects, but its cost was only a quarter of the cost of CO_2 CA storage. In addition, clean N_2 won't create any pollution and corrosion, in contrast, it realized green grain storage and increased the value of product.

The N_2 CA storage developed by Nanjing depot of state grain, with advantages of small size high-purity nitrogen generator, easy opera-

tion, good insect control, low cost, was a new technology focused on the actual situation of the grain depot. This technology was applicable to full-bin application, especially for depots with a dispersion network or a re-circulation network, and was also applicable to poor air-tightness depot after low-cost air-tightness reform. The N_2 CA storage, having general practical applicability, was applicable to not only common flat warehouses, but also large granaries such as silos and silo bins and would have a bright future in the domain of green grain storage.

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SESSION 5

SEALING TECHNIQUES AND CA ENGINEERING

Chairpersons:
Digvir Jayas, Canada
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0501

Freight Containers——Are They Sufficiently Gastight for Quarantine & Pre-shipment Fumigation with Methyl Bromide in the 21st Century?

J. E. van Someren Graver[1] and H. J. Banks[2]

Abstract: Freight containers are used to transport virtually all non-bulk cargoes traded internationally, with a considerable proportion of this freight still subject to quarantine or pre-shipment fumigation with methyl bromide. Most of these fumigations are carried out under sheets. Some quarantine and other authorities allow fumigations to be carried out directly in the container, without sheeting or tarping, often with additional restrictions to ensure success of the treatments. Restrictions include specifying minimum levels of sealing or gastightness, such as measured by pressure test, or use of particular kinds of containers only, e. g. reefers. Where a container can be demonstrated to be gastight by pressure testing, it may be used directly as a 'chamber' or gastight enclosure.

This paper presents a summary of the mathematical background to pressure testing, information relating to gas loss from freight containers, and some practical observations on fumigant retention at different pressure test values. Both the theory and limited practical studies available support a minimum pressure test specification of 10 seconds pressure half life for containers to be used directly as enclosures, without sheeting. This applies to the container in the filling condition to be treated. Below this level of sealing, risk of fumigation failure increases.

Key words: fumigation, pressure testing, methyl bromide, emissions.

Introduction

It is well known that many fumigations carried out in tarpless (unsheeted) containers fail because the sealing level of the container is poor, and there is excessive leakage. This means the fumigant gas is not retained for long enough at a high enough concentration to kill all life stages of the target pests because the requisite Ct-product is not achieved.

Some quarantine authorities discourage or do not approve the use of tarpless in-container fumigation of goods in general purpose containers because of the uncertainty over the sealing level and the risk of treatment failure. It is often an official requirement that the container be fumigated with doors open and under gastight sheets or tarpaulins ('tarped')[1]. Tarping of containers is a labour-intensive, time consuming operation. It is increasingly being regarded as an unsafe work practice by occupational health and safety authorities, particularly where there is wind or the containers are stacked. Furthermore, treatment under tarps has, itself, a substantial risk of failure under windy conditions or where poor quality (i. e. leaky) or damaged sheets are used[2].

An alternative to fumigation under sheets is to use the container itself as the fumigation enclosure. Many modern general purpose containers, such as those used for transport of durable stored products (e. g. grains, pulses, animal feeds, wooden materials), are quite well sealed. This procedure has been widely adopted in many parts of the world wherever the contents of containers have to be fumigated.

One of the main reasons for its popularity, particularly in developing countries, has been the availability of cans containing 680 or 750g methyl bromide, which made fumigation with this fumigant "as easy and simple as fumigation with phosphine" generated from aluminium phosphide tablets or pellets. This convenience together with a range of novel but unorthodox application equipment allowed one person provided with a moped to carry out fumigation treatments "very easily and very cheaply". This casual approach to fumigation provides, at best, no quality assurance beyond the dosage of methyl bromide applied into the container[2].

However, in-container fumigation is used on the assumption that the fumigant can be retained adequately to achieve target disinifestation levels. For quarantine treatments, the target Ct-levels can be quite high, and Barak et al.[3] provide an example of some in potential treat-

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ments of Asian Longhorn Beetle to quarantine standards-in untarped containers.

An important aspect of quarantine and preshipment fumigations is that they are carried out to a standard where the risk of failure is acceptable. However, this in turn requires monitoring of fumigant concentrations within the container (fumigation enclosure) to assess whether an adequate Ct-product has been achieved. While there are measures that can be taken to compensate for poor gasholding to some extent (e. g. topping up'[1,4], slow release systems[5]), it is better in a fumigation to retain gas at adequate levels with minimal or no intervention, and better still to have an objective measure of the sealing level of the enclosure before adding gas, to give confidence that the fumigation will be successful.

Pressure testing is a simple and quick quality control process that can be applied on most fumigation enclosures or containers, some flimsy or flexible structures excepted.

It would appear that the existence of pressure testing as a tool for quality control has been overlooked by the fumigation community. Pressure testing plays an ongoing role in our everyday lives, for example, by ensuring the safety of the canned food we eat, the cylinders that supply LPG to our kitchens, as well as enclosures used for fumigation.

Navarro[6] described some features of pressure testing for grain storages. Two forms are used with containers the pressure decay test (Pt-test) and pressure/equilibrium flow test (PQ-test). The pressure decay test involves adding or withdrawing air from the enclosure to give a set pressure differential across the enclosure walls, shutting off the air addition or removal and timing the decay of pressure between two set values, typically giving a pressure half life. The pressure/equilibrium flow test involves adding or withdrawing a measured, set flow of air and observing the equilibrium pressure difference achieved.

PQ-tests are slower to carry out and require more complex measuring equipment than Pt-tests. The latter require only a simple pressure measuring device and a timer, while the former requires accurate measurement of both pressure and air flow. Thermal (refrigerated or reefer) containers are built to a pressure test standard specified by a PQ-test[7] and some of the research with gasholding of containers carried out in the 1970s (e. g. Banks et al. [8]) also used PQ-tests to specify level of sealing. The PQ-test and Pt – tests are correlated for a particular type of container construction, but the correlation is different for different types[9].

A question is "what is the minimum test value that gives a satisfactory level of confidence in a fumigation while being achievable in routine practice" The choice is between increased risk of failure and industrial feasibility. Fortunately, the level of sealing that can routinely be achieved in practice also affords a satisfactory management of risk, in otherwise well conducted fumigations.

Because Pt-tests can be carried out quickly and easily, they tend to be specified for determining level of seal in fumigations. The discussion below is largely related to Pt-tests, with data originally related to PQ-tests translated to Pt-test equivalents.

Mathematical Background to Pressure Testing

Formulae relevant to pressure tests in containers were summarised by Banks[5].

In a decay test, the decay of pressure from the initial level Δp_1 to final level Δp_2 over time t for a container of volume V filled with material of mass m_{bulk} and true density ρ_{bulk} is given by:

$$\Delta p_1^{\,1-n} - \Delta p_2^{\,1-n} = (1-n)\ b\ K\ t \qquad (1)$$

for $n \neq 1$, and

$$\ln \Delta p_1 - \ln \Delta p_2 = b\ K\ t \qquad (2)$$

for $n = 1$, where

$$K = \rho RT/(28\ (V - m_{bulk}/\rho_{bulk})) \qquad (3)$$

and b, n are constants in the equation

$$Q = b\Delta p^n, \qquad (4)$$

relating input air flow, Q, to equilibrium differential pressure Δp.

The mathematics implies that the equilibrium flow for a set pressure (e. g. 250 Pa) should be inversely proportional to the pressure decay time (e. g. time from 200 to 100 Pa). However, because of nonlinearities in the pressure part of the equations, the pressure halving time varies with the pressure values used.

Typically loss of concentration of fumigant, c, in a container follows pseudo first order decay kinetics after an initial rapid sorption with apparent concentration at zero time of c_0 and where k is the decay constant or ventilation rate constant. Thus:

$$c = c_0 e^{-kt}. \qquad (5)$$

The decay constant may be made up of several individual components related to factors causing gas loss.

Factors Causing Gas Loss from Containers

There is a range of different forces that cause gas loss from structures, including freight containers[8,10] Their individual contribution to observed loss of fumigant can be modelled and ranked according to size and susceptibility to sealing.

For containers, the main forces causing gas loss are:

- Thermal expansion of gases within the container
- Synoptic and tidal barometric atmospheric pressure reductions
- Wind effects and transport velocity
- Ascent and descent.

It is apparent that those forces that are cyclic and operating on long time scales, of the order of hours or days, are insensitive to levels of sealing where there is a pressure decay time of the order of seconds. Those forces that are not cyclic, i. e. wind and transport velocity are directly affected by level of sealing.

The loss rate constant caused by wind or transport velocity, k_w, has been found experimentally[11] to be directly proportional to the equilibrium flow, Q_5, for a 5 Pa pressure.

Thus: $k_w = -\alpha v Q_5/V$, (6)

where α is 0.012 s · m^{-1} and v is the wind or transport velocity (or a combination thereof).

Banks[5] gave estimates for the contributions of these forces for under some typical condition, both static and in transit. Table 1 provides a recalculation of these figures for various decay times for a well filled container.

Table 1. Calculated rate constants (seconds^{-1}) for gas loss from a standard filled container, caused by various factors

	Pressure decay value (200 – 100 Pa) in seconds				
Factor	1	2	5	10	20
10℃ cycle per day in headspace	0.024	0.024	0.024	0.024	0.024
1000 Pa barometric pressure variation	0.010	0.010	0.010	0.010	0.010
Wind at 2 m s^{-1}	0.242	0.121	0.048	0.024	0.012
Totals	0.276	0.155	0.082	0.058	0.046

Calculated for a 28m^3 container filled with 22t wheat at 25C with value of $n = 0.6$ and an estimated air volume content of 10.2 m^3.

It can be seen from Table 1 that it is predicted that the wind effect from a moderate, but continuous, wind becomes similar in magnitude to the combined predicted effects of temperature and barometric pressure variation at around 10s pressure halving time (200 – 100 Pa).

This model is based on a filled container in the state that would typically be fumigated. The load provides some damping of the thermal cycling in the container. These results relate to decay time only and are independent of fill of the system. Decay times at a given level of seal are a function of the contained air volume. Thus the container must be tested at the fill level at which it will be fumigated. If it is tested empty, but treated full, the pressure test result needed has to be corrected according to the change in contained gas volume. In the example above (Table 1), a 10 sec decay half life in a filled container corresponds to about 27 sec empty.

Sustained average wind speeds of 2 m · s^{-1} (7 kph) are common in areas used for container fumigation. The adverse effects of wind on gasholding of fumigation enclosures, including containers, are well-known, though poorly documented. Mulhearn et al[12]. collected some information on this. A modern analysis using the CFD approach for fumigant retention in flour mills is given by Cryer[13].

Practical Observations of Fumigant Holding for Various Pressure Test Values

There are very few published, experimental studies on the influence of pressure testing on retention of fumigant under commercial conditions. The few that are available support a pressure test standard of around 10 s pressure half life. This level of sealing is easily attainable in modern ply floored general purpose containers, and reefers, in good condition (intact and undamaged door seals, well-fitting doors, no holes in the sides and roof).

Using tracer gas tests Banks et al.[5,8] showed that pressure tests were a good measure of the gasholding in freight containers that was caused by factors other than cyclic changes in internal temperature and external barometric pressure.

Ball and van Graver[14] summarised work with fumigation of hay in containers. Fig. 1 shows results from commercial fumigation of 41 containers with methyl bromide under various conditions and pressure test values. Some further information on retention of phosphine and methyl bromide as a function of pressure test is given by De Lima et al.[15].

Banks[5] showed that a 10 s half life provided adequate retention of CO_2 in a study of 36 containers loaded with grain and supplied with a slow release box to maintain CO_2 levels over the target 10 day exposure.

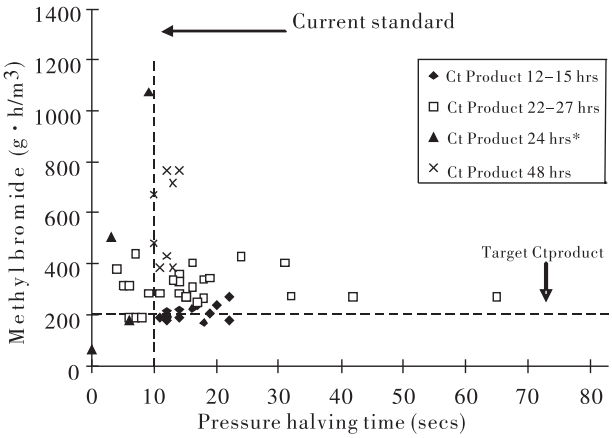

Fig. 1 Methyl bromide Ct products as a function of pressure decay testing of freight containers loaded with hay (redrawn from Ball and Graver[14])

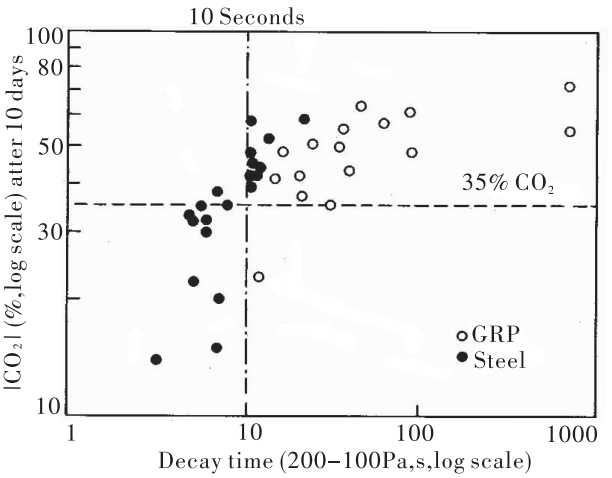

Fig. 2 CO_2 level present after 10 days as a function of pressure decay test in either static steel or glass reinforced plastic containers, load 18 t wheat, CO_2 added, 30 kg loose with 30 kg in a '10 day' box (redrawn from Sharp and Banks[10])

All these studies show good retention of fumigant at the 10 s half life or greater, with low risk of failure. Furthermore there were many successful fumigations at a half life between 5 – 10 s, but some indication of reduced reliability in this range. The data in Fig 2 indicate reduced gasholding in this range and substantial decrease below 5 s, while the data in Fig 1 shows 4 of the 8 fumigations to have been marginal with the target Ct-product of 200 ghm^{-3} not quite achieved at 24 h exposure. All containers that achieved > 10 s pressure test and were observed for > 22 h achieved > 200 ghm^{-3}.

While these data support the, 10 s decay standard of AQIS methyl bromide standard[1], their application to a 5 s half life decay time may, particularly under adverse weather conditions, be marginal.

Conclusions

The analysis above shows that under even moderate wind, e. g. 20kph (5. 5 m/s), sustained for a few hours, there is a substantial risk even with > 10 s decay time that the gasholding of methyl bromide may be insufficient for effective fumigation.

It is interesting that up to 24 February 2008, USDA APHIS had prudently forbidden in-container fumigation of containers (excepting reefers) with methyl bromide when severe winds, defined as sustained winds or gusts of 30 m. p. h. (about 13 m/s) or higher for any time period are forecast.

However, on 25 February 2008 the USDA APHIS[15] suspended tarpless container fumigations using methyl bromide due to concerns regarding their efficacy and instructed fumigators that all container fumigations must be conducted under tarpaulin. The notice continues by indicating that a study will be conducted to determine if the tarpless fumigation process would be efficacious if performed solely on containers that were pre-certified as leak-proof, based on pressure testing. The study will also determine the optimum dosage rate that is needed to maintain adequate gas concentrations of methyl bromide for an efficacious treatment.

The outcome of the proposed study will be of interest to fumigators who have deemed the AQIS pressure test requirement to be too limiting in its range, with calls to allow containers to be fumigated without enclosing them under fumigation sheets using a less stringent pressure test regime.

Will such compromises with the potential for both increased emissions of methyl bromide and higher risk of failure be accepted as best fumigation practice for quarantine purposes?

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0502

Effect of Airtightness Improvement in High Flat Warehouse on Recirculation Fumigation

Cui Dongyi and Wang Na

Abstract: Major gas leakage place in the tested warehouse after airtightness improvement were evaluated, and the pressure half-life ($t_{1/2}$) of the warehouse changed from 45s to 66s. Perform aluminum phosphide application experiment in the empty warehouse and perform timed test on changes of PH_3 concentration every day for 56 days continuously, the concentration half – life (reduces from 174mL/m^3 to 87 mL/m^3) is 31 days. After grain loading, perform PH_3 recirculation fumigation in it and the reference warehouse at the same time, under the same external conditions, perform timed tests on gas concentrations in the warehouse during fumigation process and collect, compare and analysis the obtained data to get the conclusion that the warehouse can keep the effective fumigation concentration for longer time through airtightness improvement.

Key words: flat warehouse, airtightness, recirculation fumigation, half-life

Fumigation is a process used for killing pest by chenicals in gas form. If the airtightness of fumigation environment is bad, it will cause leakage of fumigation gas and thus reduce the concentration of toxic gas and influence the efficacy of pest killing effect. For the past few years, recirculation fumigation technology has been used in various newly built warehouses generally. Since the recirculation fumigation technology use recirculation blower to force the fumigation gas to cycle and to be uniform in the fumigation environment during the fumigation process, if the airtightness of the fumigation environment can not meet the requirement, the leakage of toxic gas will happen at positive pressure section, and external gas will enter into negative pressure section[1].

Ct value shows that for most fumigation agents keeping lower concentration for longer time or higher concentration for shorter time results in same killing. However, based on practices and researches of many years, it has been proven that for PH_3 fumigation, the airtight time is more important than the concentration, and prolongation of the fumigation time of PH_3 is much better than the increasing of concentration[2]. It requires good airtightness of the fumigation warehouse to keep the effective concentration of the fumigation gas for adequate time. Therefore, when useing PH_3 fumigation, if the airtightness of the warehouse is not good, it will not be able to keep the time of effective concentration for pest killing, influence the fumigation effect seriously and result in failure of pest killing, or even result in vicious circle such as increasing of fumigation frequency, strengthening of resistance of pest, increasing of residual toxicity of grain, environment pollution, waste of human power and material resources and etc. Therefore, airtightness improvement of warehouse is the effective measure to keep the fumigation concentration and improve the fumigation effect.

The research of airtightness of fumigation warehouse is highly regarded by developed countries of the world. In Australia, this research has been started from the end of 1 970s and it performed airtightness improvement of warehouse and divided the warehouses into three classes by pressure decay method. It defined that if the time of reducing of original pressure from 2 500Pa to 1 500Pa is not less than 5 min, it will be defined as the first class warehouse; if the time of reducing of original pressure from 1 500Pa to 750Pa is not less than 5 min, it will be defined as the second class warehouse; if the time of reducing of original pressure from 500Pa to 250Pa is not less than 5 min, it will be defined as the third class warehouse [3]. In Holland, the fumigation warehouses also have been divided into three classes; the standard was the same as that of Australia basically. In Japan, it defined that after building of silo finished, increase the pressure of the empty warehouse to 4 900Pa, after 20 min., if the pressure is still more than 1 960 Pa, it will be defined as A class warehouse; if the pressure

Xinle Grain Depot, State Grain Reserves, Xinle, Hebei, 050700

is reduced to 980 Pa, it will be defined as B class warehouse. The airtightness standard of PH_3 fumigation of bagged grain of the Association of South East Asian Nations is that the time of reducing from 500Pa to 250 Pa should be more than 10 min[4].

Ever since a long time ago, grain storage technicians and related experts and scholars of our country also have done a large number of researches on airtightness of warehouse. In 1987, Tang Shungong suggested the standard of grain storage fumigation warehouse was that the time of reducing of pressure from 490 Pa to 250 Pa should be more than 30 seconds[5]; and after this Wu Zengqiang, Hu Dongsheng and Zhang Chengguang also performed determinations of airtightness of silos[6].

The test described in this article used advanced experiences at home and abroad for reference, and performed airtightness test in newly-built high flat warehouse by pressure decay method, then determined the changes of PH_3 concentrations in the empty warehouse and the reference warehouse separately after airtightness improvement is performed. The obtaining of these data provides some references for grain storage fumigation works in our company.

1 Airtightness Test and Concentration Decay Test of the Empty warehouse

1.1 Basic Content of the Test

Through test, we found that the major leakage places were door, window, mechanical ventilation outlet, axial-flow blower outlet, thermometric cable hole and wire hole, recirculation fumigation hole of the newly-built high flat warehouse and etc. Perform airtightness handling for above outlets and holes with suitable methods and thus improve the airtightness of the grain warehouse.

1.2 Materials of Test

1.2.1 Tested warehouse: No. 36 high flat warehouse, Xinle Grain Depot, State Grain Reserves, brick-concrete structure, top of arch bar; length: 53.74m, width: 23.15m, total volume: 13 434.06m^3, verified volume of the warehouse is 5 690 tones.

1.2.2 Silicone sealant Peeling strength of the material is 4N/mm, the maximum elongation is 35%, elastic recovery rate is 95%, movement capability is ± 25%, produced by Beijing Gutebang Material Technology Co., Ltd.

1.2.3 Centrifugal blower Power: 7.5 kW, air volume: 5712 – 10562m^3/h, wind pressure: 2554 – 1673 Pa, produced by Shijiazhuang Blower Factory.

1.2.4 PH_3 concentration tester Test range: 0 – 500mL/m^3, produced by German Drager Company.

1.2.5 Polystyrene foam plate Density: 20kg/m^3, thickness: 100mm. produced by Hebei Xinji.

1.2.6 Others Pressure gauge, gate valve, connecting cylinder, stopwatch and etc.

1.3 Test Process

1.3.1 Pressure decay method

1.3.1.1 Before handling of airtightness, performed normal sealing of holes such as door and window of the warehouse with plastic film, and kept one ventilation opening for installation of gate valve, connecting cylinder, centrifugal blower, and installed pressure gauge at the front – end of airtight gate valve. See figure 1.

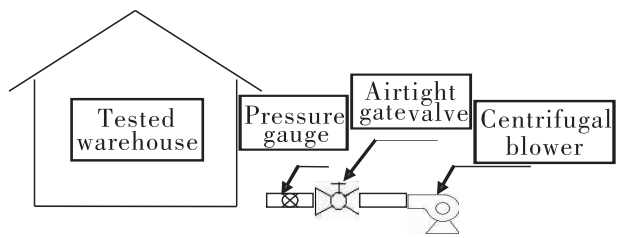

Fig. 1 Schematic diagram of airtightness test of warehouse

Performed pressurization through positive pressure ventilation by blower, when the pressure in the warehouse rose to 550Pa, closed the blower and airtight gate valve, and began to clock when the pressure reduced to 500Pa; the time of reducing to 250Pa i. e. the pressure half-life ($t_{1/2}$) was 45s. Performed observation and inspection for major places at which air leakage may happen at the same time, including door and window, thermometric cable hole, wire hole, recirculation fumigation hole and ventilation outlet, axial-flow blower outlet and etc. Determined if air leakage happened at the place which may had cracks by soap lye method. See table 1 for detailed status of air leakage.

Table 1. Record of air leakage inspection in the tested warehouse (pressure method)

Place	leakage (Y/N)	leakage degree
Door	Y	serious

Place	leakage(Y/N)	leakage degree
Thermometric cable hole	Y	general
Wire hole	Y	general
Recirculation fumigation hole	Y	general
Window	Y	general
Axial-flow blower outlet	Y	serious
Mechanical ventilation outlet	N	——

1.3.1.2 Handling measures of air tight

According to the test result of airtightness of the warehouse, performed airtightness improvements on related places, and the detailed measures are as follows:

Door: Installed one layer of polystyrene foam plate (hereinafter referred to as "polystyrene plate") near the inside of the external door. Joint seams between polystyrene plate and wall or polystyrene plate and polystyrene plate were sealed with silicone sealant. In order to enhance the strength of polystyrene plate, stick one layer of fiberboard on the polystyrene plate.

Window: Performed blocking with polystyrene plate, then smeared silicone sealant all around the joint seams between polystyrene plate and the window.

Axial-flow blower outlet: Performed sealing and blocking with polystyrene plate, and smeared silicone sealant all around the joint seams.

Recirculation fumigation hole: Smeared silicone sealant directly and filled up all around the pipelines and cracks of wall.

Mechanical ventilation outlet: In order to ensure the effect of test, performed blocking with polystyrene plate inside the outlet, then smeared silicone sealant all around the polystyrene plate.

1.3.1.3 After handling of air tight, performed pressure half-life test again; the method, steps and materials of pressurization was the same as

1.3.1.4 Through three times test, the pressure half-life was 66s. Performed observation and inspection on airtightness improvement places, and no air leakage was found.

1.3.2 Concentration decay method

After test with pressure method, charged 12kg of aluminum phosphide tablets into the tested warehouse and closed it immediately, tested for three times at east place and west place separately with PH_3 concentration tester on 8:30 every day (see table 2). After the airtight improvement, the half-life of PH_3 concentration (reduced from 174 mL/m^3 to 87mL/m^3) of the tested warehouse was 31 days.

Table 2. PH_3 concentration of the tested warehouse (mL/m^3)

Time (d)	1st time		2nd time		3rd time	
	East	West	East	West	East	West
1	23	19	18	18	21	18
2	67	63	67	60	69	67
3	108	106	109	110	110	110
4	143	140	146	143	147	147
5	150	148	154	150	154	158
6	161	162	161	163	162	160
7	168	169	170	168	171	169
8	174	173	172	178	175	174
9	170	168	164	166	165	167
10	159	160	157	159	160	167
11	150	156	151	156	160	160
12	157	152	157	152	157	154
13	149	149	149	147	151	147
14	147	144	147	146	147	146
15	143	139	143	139	144	140
16	142	138	142	138	142	138
17	135	132	135	132	135	132
18	132	129	131	129	131	129
19	127	129	127	126	127	126
20	124	125	124	125	124	125
21	124	116	124	116	124	116
22	116	113	115	113	114	113
23	112	109	111	109	110	109
24	109	106	109	106	109	106
25	107	106	107	106	107	106
26	105	102	104	102	103	103
27	104	103	103	102	102	101
28	100	99	101	99	100	99
29	99	98	98	97	99	98
30	96	95	96	95	96	95

Time (d)	1st time		2nd time		3rd time	
	East	West	East	West	East	West
31	94	92	94	93	94	92
32	92	92	92	92	92	92
33	93	92	92	92	92	92
34	92	91	91	92	91	90
35	91	90	90	89	89	87
36	89	89	88	94	87	86
37	86	86	88	92	93	88
38	87	85	88	89	86	87
39	80	82	88	81	83	82
40	78	79	78	79	77	80
41	76	76	76	76	76	76
42	74	73	74	73	74	73
43	73	72	72	72	73	72
44	71	70	70	70	70	70

1.4 Results and Analysis

1.4.1 Performed three times test of airtightness of the tested warehouse before and after airtightness improvement separately, and the pressure decay time of each time was the same basically and showed good reproducibility. Pressurization test of the empty warehouse showed that the pressure half-life $t_{1/2}$ after handling was prolonged from 45s to 66s, nearly prolonged for 1/3 time, and the improvement of airtightness was comparatively larger; see figure 2 for the airtightness curves before and after handling.

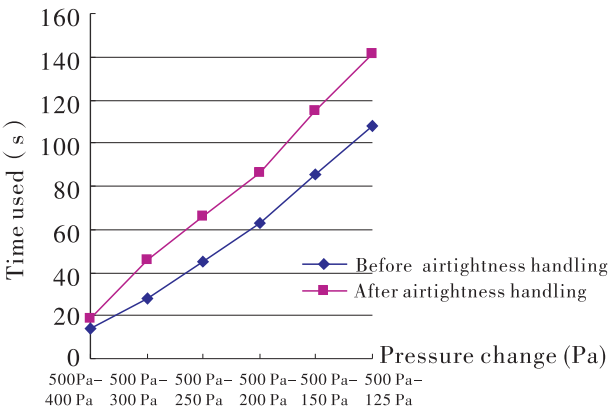

Fig. 2 curve graph of airtightness changes of the tested warehouse before and after handling

1.4.2 The major materials used for airtightness handling were polystyrene foam plate and silicone sealant. In which, polystyrene plate is the relatively ideal heat-proof, air-tight material, whose weight is light, and which is not easy to be distorted, no toxicity and heat proof. However, consider suitable density when select it and put it to use. Generally, choose the product whose density is around 20kg/m^3. Silicone sealant is a building material which is shapeless and can be pressed and used for filling of cracks, sealing and blocking of holes and make them airtight. It will not be softened excessively under the high temperature condition of North China, and brittle rupture of it will not happens under the low temperature in winter, the stickiness with joint seam is stable and it also has the function of water penetration resistance, its use is simple and it can be operated without professional personnel, and dismounting is convenience; polystyrene plate can be used repeatedly. These two materials are normal building materials and can be found everywhere in the market; they are cheap, practical and their performance-price ratio is higher.

1.4.3 When performing of airtightness improvement, one should not only pay attention to door, window, threading hole, ventilation opening, axial-flow blower outlet, but also showed pay attention to backfilling place of scaffolding during construction and cracks of wall.

1.4.4 The concentration half-life of this test was 31 days; theoretically, this air tight time is adequate to kill various pests in different stages. The set concentration of this test was 200 mL/m^3, and the actual measured maximum value was 178 mL/m^3; through analysis, the reason may be absorption and the leakage of some places which have not been inspected.

2 Field Test

2.1 Materials of Test

2.1.1 The tested warehouse was still No. 36 warehouse, in which, stored wheat was 5721t, impurity was 0.8%, water content was 11.2%, incomplete particles was 4.1%, grain loading time was August 2003; the reference warehouse was No. 48 warehouse of which structure was the same as that of No. 36 warehouse, its stored wheat was 5760t, impurity was 0.8%, water content was 12.1%, incomplete particles was 6.0%, grain loading time was July 2003, and the average grain temperature was 25.8 ℃. Both warehouses were bulk storage.

2.1.2 Aluminum phosphide tablet Purity 56%, Produced by Shandong Jining.

2.1.3 Centrifugal blower same as 1.2.3.

2.1.4 PH_3 concentration tester same as 1.2.4.

2.1.5 Others same as 1.2.6.

2.2 Test Process

Performed airtight handling for the tested warehouse according to the method of 1.3.1.2, and performed airtight of the door and window of the reference warehouse with PVC film only, then performed pressurization test separately; the results are shown in table 3 and table 4. The pressure half-life of the tested warehouse was 62s, and the pressure half-life of the reference warehouse was 38s.

Table 3. Record of field air tightness test of the tested warehouse (pressure method)

Changes of pressure (Pa)	Time used for determination of pressure decay(s)			
	1st time	2nd time	3rd time	Average value
500 – 400	16	17	17	16.7
500 – 300	42	42	43	42.3
500 – 250	60	63	64	62.3
500 – 200	79	80	81	80.0
500 – 150	108	110	110	109.3
500 – 125	132	133	132	132.3

Table 4. Record of field air tightness test of the reference warehouse (pressure method)

Changes of pressure (Pa)	Time used for determination of pressure decay(s)			
	1st time	2nd time	3rd time	Average value
500 – 400	12	12	13	12.3
500 – 300	26	27	27	26.7
500 – 250	38	39	38	38.3
500 – 200	59	58	57	58
500 – 150	79	80	79	79.3
500 – 125	100	100	100	100

From table 3 and 4, we can see that $t_{1/2}$ of the tested warehouse is higher than that of the reference warehouse, since $t_{1/2}$ represents the technical requirement of the warehouse airtightness for the PH_3 fumigation, and the higher $t_{1/2}$ represents the better airtightness of the warehouse.

Adult *Sitophilus zeamais* Motschulsky were found in both warehouses; performed recirculation fumigation with grain surface application. The concentration was set at 350 mL/m^3, dosage at 1.5g/m^3, and apply 20kg of 56% aluminum phosphide tablets separately, and set 120 application points. Set 10 test points of PH_3 concentration for each warehouse, and divided it into east area and west area, 5 points for each area. See figure 4 for details.

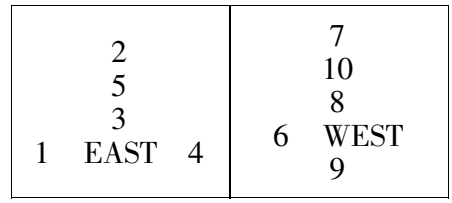

Fig. 3 Map of test points of PH_3 concentration

Test probes were buried at 50cm under the grain surface. Began to do fumigation from August 19, and performed circulation work 6 hours after application for 24 hours continuously. From the morning of the next day, tested the PH_3 concentration at 9:00 am every day. (table 5, table 6). Performed suitable recirculation according to the status of changing of the concentration; it should be not less than 12 hours for each time.

2.3 Result and Analysis

2.3.1 Pest killing effect: after the test finished, took 1kg of sample from both warehouses separately and cultured them in incubator of the laboratory under the conditions of 25℃ and 70% RH. Two weeks later, no live pest was found, and the pest killing ratios for both warehouses were 100%. Perform analysis from fumigation effect, and it is found that when the application dosages for the tested warehouse and the reference warehouse are higher than that specified in "Technical standards for PH_3 recirculation fumigation", the goal of complete pest killing can be achieved.

Table 5. Record of field PH_3 concentration of the tested warehouse unit: mL/m^3, d

Time	Test point 1		Test point 2		Test point 3		Test point 4		Test point 5	
	East	West	East	West	East	West	East	West	East	West
1	140	143	145	150	145	140	141	145	145	140
2	170	172	168	178	180	170	170	170	182	186
3	198	195	190	210	199	200	188	191	202	200
4	232	230	234	234	230	231	229	231	230	232
5	246	244	244	246	244	240	236	238	242	240
6	265	270	268	282	271	269	270	268	272	270
7	272	278	280	286	289	285	278	278	286	290
8	280	283	290	295	301	302	300	298	299	310
9	299	301	276	280	320	318	300	311	302	303
10	291	292	270	272	330	332	321	335	310	326
11	295	294	272	270	321	325	316	320	305	320

Time	Test point 1		Test point 2		Test point 3		Test point 4		Test point 5	
	East	West	East	West	East	West	East	West	East	West
12	300	290	275	270	315	321	305	306	300	311
13	291	288	270	272	295	300	289	295	290	298
14	283	283	268	270	292	293	270	275	280	279
15	282	276	254	248	280	262	248	276	253	288
16	289	282	267	260	290	270	240	265	250	275
17	274	270	230	256	289	278	240	266	240	280
18	268	260	220	248	270	270	220	257	230	263
19	246	245	208	230	263	260	200	250	210	261
20	252	241	208	232	260	253	192	244	203	261
21	243	230	218	224	270	246	188	230	212	248
22	237	222	210	210	268	232	179	211	216	233
23	222	208	204	191	253	222	172	200	204	231
24	210	198	200	178	230	200	169	191	200	223
25	202	184	194	168	219	185	168	177	198	206
26	199	170	189	150	200	168	170	159	175	192
27	196	169	188	143	200	162	158	157	189	189
28	184	150	171	132	189	151	150	138	173	159
29	162	139	154	120	173	138	140	129	163	140
30	154	119	140	101	161	102	123	103	139	120
31	141	96	130	88	151	87	113	80	127	92
32	132	90	118	84	146	83	102	78	120	90
33	114	90	108	80	132	80	94	79	113	88
34	102	89	100	82	128	79	90	78	101	86
35	105	88	95	78	112	82	87	70	93	71
36	90	75	84	72	105	68	81	70	82	76

Table 6. Record of field PH_3 concentration of the reference warehouse (unit: mL/m^3 d)

Time	Test point 1		Test point 2		Test point 3		Test point 4		Test point 5	
	East	West	East	West	East	West	East	West	East	West
1	132	125	130	128	135	140	138	158	120	125
2	160	155	160	150	161	165	165	172	150	154
3	192	188	172	177	175	172	178	189	167	160
4	212	200	190	198	190	189	202	216	200	200
5	240	232	213	215	230	226	220	231	218	223
6	266	260	235	236	256	257	255	250	248	256
7	273	270	275	270	298	289	275	279	262	273
8	290	288	278	278	315	320	288	290	278	295
9	265	263	282	280	290	289	291	282	280	284
10	248	250	260	271	275	270	273	274	262	250
11	230	249	235	264	260	259	262	254	255	245

Time	Test point 1		Test point 2		Test point 3		Test point 4		Test point 5	
	East	West	East	West	East	West	East	West	East	West
12	228	248	230	242	238	252	229	238	242	236
13	218	218	218	218	221	218	220	217	217	216
14	220	212	224	220	222	224	220	222	224	224
15	204	210	210	212	212	206	210	204	210	208
16	198	199	200	201	200	199	201	190	203	196
17	189	190	180	180	190	180	188	178	192	189
18	188	190	165	190	192	170	192	170	170	175
19	172	178	146	171	179	161	170	159	152	161
20	153	162	131	152	156	149	162	148	141	149
21	140	150	125	125	140	140	150	130	130	140
22	117	116	124	117	122	120	126	124	119	120
23	98	100	112	102	110	105	106	100	106	106
24	88	99	103	103	81	104	90	104	96	101
25	75	77	93	90	70	85	75	85	75	88
26	72	73	76	83	68	67	64	63	65	66

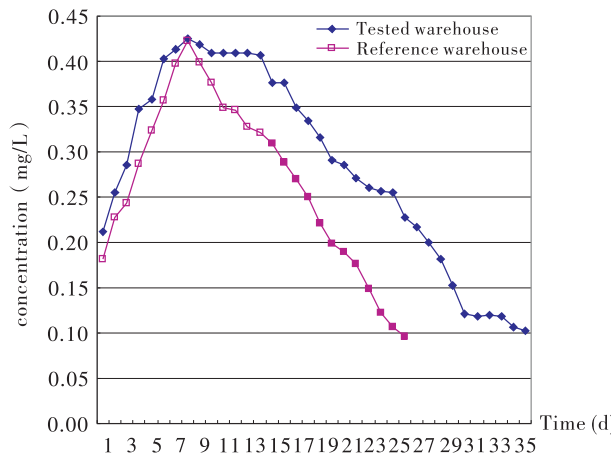

Fig. 4 Changes of PH_3 average concentration of the tested warehouse and the reference warehouse

2.3.2 Comparison of fumigation effects: Ct value of the tested warehouse was 224.5 mg h/L, the holding time of the effective concentration was 30 days; Ct value of the reference warehouse was 158.4mg h/L, the holding time of the effective concentration was 22 days. The Ct values and the holding times of the effective concentration for both warehouses were the same as the $t_{1/2}$ of both warehouses, i. e. $t_{1/2}$ of the tested warehouse was higher than that of the reference warehouse, and its Ct value and the holding time of the effective concentration were higher than those of the reference warehouse.

2.3.3 Changes of the concentration: after the concentration became to be uniform basically through recirculation, the minimum PH_3

concentration tested from each point was the fumigation concentration. From table 5, table 6 and figure 4, we can see that the trend of changing of the PH_3 fumigation concentration in the tested warehouse is relatively gentle; from the 8^{th} day to the 14^{th} day, the concentration kept in the higher range and concentration decay was slow. On the 15th day, the concentration reduced suddenly; through analysis, the reason may be the rate of ventilation was not enough since the recirculation time was shortened or because of slight leakage of individual place. In summary, the holding status of the PH_3 fumigation concentration of the tested warehouse was better than that of the reference warehouse. For the time of PH_3 fumigation concentration decaying to 100 mL/m^3, the tested warehouse was 30 days and the reference warehouse was 22 days. According to the effective concentration stated in "Technical standards for PH_3 recirculation fumigation", the tested warehouse can reduce the unit dosage and thus reduce total application dosage, it also can obtain good pest killing effect.

Test result showed that, the better airtightness of the warehouse, the longer holding time of the effective concentration, the higher Ct value, the better fumigation effect.

3 Conclusion

The test showed that, through the airtightness improvement of the warehouse, it can improve airtightness of the warehouse, prolong the pressure half-life, not only can improve the pest killing effect of fumigation and reduce fumigation cost, but also can reduce exchange of wet heat gas in and out of warehouse, reduce generation of entomomycete and thus reduce fumigation frequency, improve grain storage environment, delay aging of the stored grain and keep quality of the stored grain, realize energy saving and discharging reducing and create good social benefits and economic benefits.

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0503

Application Research on Sealing Technologies of CO_2 MA Granary and MA Grain Storage Technologies

Ma Zhongping, Ma Honglin, He Qile and Liao Guiyong

Abstract: The tendency of "green, ecology, harmony", which is consistent with development of grain storage technologies in the world, was the goal of Chinese grain storage industries development. Such no - chemical grain storage technologies as Modified Atmosphere (MA), low temperature were accord with development of grain storage technologies. Based on lots of investigation and argumentation on CO_2 MA grain storage, Chinese government attached great importance on grain storage with no chemicals, and successfully constructed the first CO_2 MA grain storage demonstration depot in Mianyang on December 2007. During its construction, the conventional type of "Through-wall Joint-frame" had been rectified to the type of "double - frame", which there were no scaffold holes in warehouse wall. Special gastight insulated doors and windows, axial fan windows and gastight insulated ventilation tube had been embedded and installed. Excellent gastight materials had been chosen to seal such places as warehouse walls, peak, the connection between ground with walls, craft holes, pipelines and so on, which influenced gastight performance of granary. Upon completion, inspection on MA granary settlement was done and it met with demands of construction qualities. Gastight performance was determined MA empty and field granaries by PT experimental method, and showed that all gastight performance met the demands of CO_2 MA granary, which the maximum half-life of empty granary was 490, and the minimum was 326, the maximum half-life of field granary was 497, and the minimum was 308. We could make a conclusion that the insulation performance of MA granary was better than ordinary granary by inspection on insulation performance of granary.

During MA granaries application, polyurethane and acrylic had been chosen as gastight materials with excellent performance by comparison carefully in Mianyang grain depot under state grain reserves, furthermore, the test method on gastight had been improved. Since 2002, Mianyang grain depot had made experiment on CO_2 MA grain storage for more than 2 years. Comparing with conventional grain storage, some researches had been carried out seriously, which focused on MA fumigation for pests control, grain qualities maintenance and so on. It had shown by experiment on CO_2 MA grain storage that fumigation with CO_2, at the concentration of 70% - 35% for 15 days, had the same effect with the conventional fumigation with phosphine, which could control and prevent pests growth completely. As being far away from pesticide resistance, it kept accordance with demands of grain storage with no-chemicals. Because CO_2 MA grain storage could postpone quality keeping period, alternation cycle and decrease the time for grain alteration, it would save lots of alteration fees and produce significant economic returns. Meanwhile, by preventing storemen far away from chemicals for grain storage, CO_2 MA grain storage protect physical and mental health of storemen. Moreover, it also protected environment from chemicals contamination, and had excellent social benefits.

key words: carbon dioxide, MA granary, grain storage

1 Sealing Technologies of CO_2 CA Granary

1.1 Special Treatments on Some Key Positions

1.1.1 Epoxy polyamide dope was used as sealing materials, and sprayed and coated with different concentration by different ways. It adhered onto granary wall without air bubble homogenously. After drying to film, there was no crack and holes.

1.1.2 Sealing treatment on the connection of granary walls and tip, ground and walls was carried out mainly by polyurethane coatings, Polyester Fiber cosmos or Bolivian filament cloth. Before sealing and coating, wider

Table 1. Test results on gastight of MA empty granary and field granary

No	pressure half-life of empty granary (seconds)	pressure half-life of empty granary (seconds)
12	336	333
13	340	338
14	338	316
15	340	351

Mianyang Grain Depot, State Grain Reserves 621000

gap was filled with polyester foam or caulking compound. To meet the sealing requirement, equipping and coating should be taken again and again for sealing, and donet onto cracks, air bubble and holes.

1.1.3 Airtight attemperator doors and windows, axial fan and airtight attemperator ventilation were made especially by professional factories. And during equipment, its frame was sealed tightly with the connection of walls by covering underground.

1.1.4 Enhancing airtight seam gum and sealant. There was gap of 1 cm wide and 18 cm deep among each vaulted board in MA granary. As reinforced concrete distortion is normal, it happened to alter. All kinds of operation holes also influenced on airtightness of granary. So, sealing treatment was done. To prevent granary from gas leak, which was caused by house distortion, cement mortar meeting relative expansion requirement was filled into crack in every vaulted boards, and then PH-T elastic epoxy resin seam gum was poured from top leakage for excellent sealing effect. Open steam sealant was used for sealing at entrance and exit, axial fan hole, manhole, supplying CO_2 hole and electric pipeline for grain inspection.

Table 2. Test results on heat insulation of MA and ordinary granary

Type of granary	MA granary(℃)	Control granary(℃)	Remarks
Temperature at 1 meter above granary surface	36.0	36.0	
Outter temperature at top of granary	45.0	45.0	Thermometer reached the top of granary
Temperature at superior string of vaulted board	30.5	41.0	Thermometer reached the superior string
Temperature in vaulted peak	30.5	34.0	
Temperature at inferior string of vaulted board	30.5	34.0	Thermometer reached the inferior string
Temperature at brim wall towards the sun	36.0	36.0	Thermometer reached the brim wall
Temperature at brim wall against the sun	32.5	32.5	Thermometer reached the brim wall
Environmental temperature	32.5	32.5	
Temperature in granary	29.8	31.8	Data on inspection grain condition

Type of granary		MA granary(℃)	Control granary(℃)	Remarks
K value	Below 2.1 meters	0.41	0.42	$W/m^2.K$
	Beyond 2.1 meters	0.47	0.48	
	The peak of house	0.22	0.51	

2 Sealing Technologies Research on MA Granary

During CO_2 MA granary application for 5 years, as the phenomena of expansion and contraction, which happened due to natural sedimentation and temperature alteration, some cracks at the gap in granary vaulted boards and manhole were found, meanwhile, aging and deformation appeared in gum at doors and windows. These factors influenced on airtightness performance of MA granary, and its gastight could not reach the requirement. So our depots have carried out extensive and deep researches on how to choose excellent airtight materials and apply with advanced gastight technologies. After such series of treatment having been taken, as carrying necessary improvement on granary, gastight performance had been reached to requirement. And MA technologies had been made sure for effective application and active generalization.

2.1 Enhancing Gastight of Board Gap and Manhole

Such sealing materials as ordinary concrete, foreign concrete, epoxy resin, polyurethane and acrylate were used for filling up board cracks and manhole in our depot for the past few years. It has been found that whether ordinary concrete or foreign concrete, all of them had some characteristics on hardness in winter and tenacity in summer. There were some cracks after hardness in winter, and granary began to leak gas. After tenacity in summer, some concrete fell onto pavement or grain mass. So it could have some bad influence on sanitation in granary, even make some pollution in grain. In winter, epoxy resin became harder and brittle, and then some cracks appeared and granary gastightness was influence.

Polyurethane was gave better gastightness and extension quality than acrylate, however, the price of polyurethane was 2 – 3 times more than acrylate. If only application with polyurethane, the cost of grain storage would be raised. So combination of polyurethane with acrylate was applied in our depot. Meanwhile, for assuring the qualities of treatment, after consultation with

several companies, finally Chengdu Haizhinian Waterproof Science Reserve Co. Ltd., which embraced strong technologies and excellent service qualities, was chosen. Ten MA granary board cracks and manholes had been taken some treatment during 2004 – 2006. After gastightness test, gastightness of MA empty and filled granary all were beyond 4 minutes, and met the requirement gastightness.

2.2 Reconstructing Electric Pipeline in MA Granary

Electric pipeline in ten MA granaries was done in 2005, 2006. At the same time, package sealing has been carried on for electric pipeline, including changing equipment lamp on vaulted board gap to granary walls surrounding, adjusting incandescent bulb to fluorescent lamp, and filled lamp socket out, so gastight qualities in granary had been intensified.

2.3 Replacing Sealant of MA Granary Doors and Windows

In 2007, some sealants with good sealing qualities and excellent texture were chosen to replace others sealants aged and deformed in ten MA doors and windows. So the gastight of granary doors and windows had been intensified and met the requirement.

2.4 Reconstructing Windows in MA-Granary

Stainless steel plates had been used in the doors and windows of CO_2 MA granary designed in the first period. Tightening and sealing gudgeon should be taken to complete by high-altitude operation. It was not only difficult to take in practice, but also having hidden danger in safes. So under the premises of not influence on gastight of doors and windows, windows have taken some reconstruction in MA granary during the first period. To reconstruction from plate type to connecting rod pushing, it made sure for operation in safe, and decreasing storemen works intension.

3 Test on Grain Storage with CO_2 MA

3.1 Experimental Granary

3.1.1 No. 10 ordinary granary: It was 48 meters long, 24 meters wide, and 6 meters high, containing 3 895 t of long-grain nonglutinous rice, harvested in 2001, filled in depots on February, 2002. Grade of paddy was the third grade, moisture content was 12.7%, impurity rate 0.5%, so the paddy was regarded as being suitable for storage. Gastight performance of empty granary was 45, of filled granary was 41.

3.1.2 No. 12 MA granary It was 48 meters long, 24 meters wide, and 6 meters high, containing 4173 t of long-grain nonglutinous rice, harvested in 2001, filled on March, 2002. Grade of paddy was the third grade, moisture content was 12.3%, impurity rate 0.6%, so the paddy was regarded as being suitable for storage. Gastight performance of empty granary was 336, of filled granary was 333.

3.2 Effect on Pests Control by Fumigation

No. 12 MA granary was treatment granary, meanwhile ordinary granary was regarded as control group. Ten group test pests sample, including pesticide sensitive strain maize beetle, grain borer and red flour beetle, pesticide resistance strain rice weevil, grain borer and red flour beetle, its resistance index was 196 times, 204 times and 8 times, respectively than sensitive. Six group test pests had been equipped in No. 10 ordinary granary, which pest varieties was the same with No. 12.

No. 12 MA granary was filled with 10 t CO_2 of on May 11th, 2002. Its concentration decreased as Figure 1. Fumigation with AlP was done for pests in No. 10 ordinary granary. (by the way of intermission mixture fumigation at twice, AlP quantities of 21 kg, CO_2 quantities of 21 kg, sealed for 28 days). All test pests samples were taken outside from two granaries after 30 days. Lethal ratios of test adult pests was determined, and sent them to The pests control center of Chengdu grain storage research institute for inspection their lethal ratios again, meanwhile, lethal ratios of their eggs, pupae and larva have been inspected after culturing for 42 days by experimental methods. It has been shown from experimental inspection that all adult grain storage pests and insects at every period (egg, pupae and larvae) have been controlled in No. 12 and 10 granaries. The results indicated that the way, with 70% – 35% CO_2 concentration for 15 days in large storage house, could prevent and control these grain storage pests completely. It had the same pests control effects with fumigation with phosphine.

3.3 Influence CO_2 MA Grain Storage on Grain Qualities

Long-grain nonglutinous rice was loaded into No. 12 MA granary and No. 10 ordinary granary on March, 2002 and February, 2002 respectively. After filling the granary up, samples have been taken from different locations for inspection grain qualities. During grain storage,

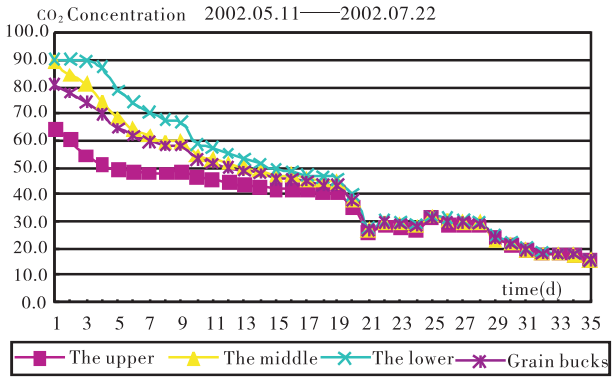

Fig. 1 CO_2 decrease tendency of concentration in MA granary

their qualities was determined periodically, and some comparison analyses were carried out. The results of inspection are given in Table 1 and Table 2.

From data in Table 1 and Table 2, we could see that grain qualities in No. 12 were the same with in No. 10 at the time of entrance granary. And as grain storage time increased, there was low difference in such physical indexes as moisture content, impurity and so on, but large difference in such chemical indexes as fatty acid value, viscosity and germination ratios. After paddy stored in No. 10 ordinary granary for 10 months, its fatty acid value raised from 19.1mgKOH/100g to 23.9 mgKOH/100g, viscosity value decreased from 14.8 mm^2/s to 10.6 mm^2/s, germination ratio decreased from 77% to 34%, taste panel scores decreased from 81 to 78. Meanwhile, in No. 12 MA granary, paddy's fatty acid value raised from 19.7mgKOH/100g to 21.8 mgKOH/100g, viscosity value decreased from 14.6 mm^2/s to 10.9mm^2/s, germination ratio decreased from 80% to 52%, taste panel scores decreased from 81 to 78. After paddy stored in No. 10 ordinary granary for 20 months, its fatty acid value raised from 19.1 mgKOH/100g to 26.1 mgKOH/100g, viscosity value decreased from 14.8 mm^2/s to 8.7 mm^2/s, germination ratio decreased from 77% to 3%, taste panel scores decreased from 81 to 73. Meanwhile, in No. 12 MA granary, paddy's fatty acid value raised from 19.7 mgKOH/100g to 22.4 mgKOH/100g, viscosity value decreased from 14.6 mm^2/s to 8.6 mm^2/s, germination ratio decreased from 80% to 36%, taste panel scores decreased from 81 to 75. After paddy stored in No. 10 ordinary granary for 28 months, its fatty acid value raised from 19.1 mgKOH/100g to 32.0 mgKOH/100g, its qualities was not fit for storage continuingly, and needed be alternated to grain market. Meanwhile, in No. 12 MA granary, paddy's fatty acid value raised from 19.7 mgKOH/100g to 27.3 mgKOH/100g, it still could be able to be in storage.

From data above, it has been shown that such paddy qualities alteration velocity in MA granary was slower than in conventional storage, as fatty acid value raising, viscosity decreasing, germination decreasing and taste panel scores decreasing. At the same conditions, paddy's storage time for proper qualities could be postpone appropriatly. So MA could postpone paddy storage period relatively, decrease the time of alternating into market. It would save lots of alternation fee for our country, and had great economic returns.

4 Application with MA Grain Storage in Our Depot

Since experiments on CO_2 MA grain storage began in 2002, check&accept successfully in 2004, so far all ten MA granaries constructed in our depot applied CO_2 MA technologies for grain storage. During these technologies application for more than 5 years, by lots of summarizing and explorations, we has comprehended deeply that CO_2 MA technologies for grain storage had better advantages than conventional grain storage technologies.

4.1 During conventional grain storage, when AlP has been applied for pests control, there will be phosphine residue in grain and environments contamination. However, with CO_2 MA for grain storage, it could protect mankind health from chemical residue in grain and environments contamination effectively.

4.2 As single application with AlP to control pests for many years in conventional grain storage, and chemicals application in no standard sometimes, pesticide resistance of pests has being increasing, and it became more and more difficult to control and prevent insects pests. However, CO_2 had stronger ability to control such grain storage pests with high pesticide resistance as lice and rusty grain beetle as well. It provided with new pests control technologies and practice ways for inhibition pesticide resistance in large house grain storage

4.3 As a kind of grain storage technologies with no contamination and nuisanceless, MA CO_2 grain storage technologies kept accordance with requirement for green foods from the publics and tendency of grain markets.

4.4 As innovation further of grain distribution system and alteration of relation between

supply with need in grain market, grain qualities kept in no stabilization, such as large impurities, high moisture content and pests contamination. Because of excellent gastight of MA granary, pests would be very difficult to enter granary. Meanwhile, at certain CO_2 concentration in MA granary, hiding performance of pests made them be MA granary away. So MA grain storage technologies provided promise for grain reserved in safe at state grain storage depots, where could arrived at the index of four-free during whole year.

4.5 As CO_2 application with MA grain storage, there would be lots of time for MA granary in sealing stage every year. If computer for inspecting grain storage condition kept normal, storemen would not need to enter granary for inspecting grain condition frequently. So the work qualities and intensification has been lowered relatively. Meanwhile, far away from chemicals for grain storage, it was helpful for storemen health.

4.6 Successful application with CO_2 MA for grian storage had such advantages for enhancing international competitive strength, as raising competitive strength in market for grain alternation, increasing economic returns, intensifying suitability of Chinese grain export to international market.

5 Prospects

MA with CO_2 has no social effects of pollution for grain storage, which could control pests effectively, inhibit mould growth and postpone grain aging. Moreover, it has avoided effects of chemicals harmful to storemen, contamination to grain and environment. As fumigation with PH_3, it would take eroding on equipments in granary (especially inspection system on grain condition). Contrary with fumigation with PH_3, it could prevent devices from eroding to save the fee for treatment materials eroded by PH_3, meanwhile, it also could avoid from others factors such as pesticide resistance raising of grain storage pests. Since being in consistent with the tendency of demands for green foods from the publics and development of grain markets, there was large and potential social values and economic returns. Being fit for the demands of grain storage development tendency of "high quality, high benefit, rich nutrition, low spoilage, low contamination, low cost", these ways would be extended and spread further as scientific development and economical advancement.

Table 1. Inspection results on paddy's qualities in No. 10 granary (ordinary granary)

Data	Brown rice rate(%)	Head-Ricerate (%)	Moisture content (%)	Impurity (%)	Fatty acid value (KOHmg/100g)	Viscosity (mm^2/s)	Taste panel scores (mark)	Germination rate (%)	Whether fit for storage or not	Color and luster
2002.5	76.8	53.6	12.3	0.7	19.1	14.8	81	77	yes	normal
2002.10	76.9	55.2	12.4	0.5	22.3	13.2	79	58	yes	normal
2003.3	76.1	53.0	12.6	0.6	23.9	10.6	78	34	yes	normal
2003.9	75.8	52.6	12.8	0.7	27.0	8.8	73	17	yes	normal
2004.3	76.0	51.5	12.9	0.6	26.1	8.7	73	3	yes	normal
2004.9	75.8	50.0	13.0	0.6	32.0		71	0	yes	normal

Table 2. Inspection results on paddy's qualities in No. 12 granary (MA granary)

Data	Brown rice rate(%)	Head-Rice rate (%)	Moisture content (%)	Impurity (%)	Fatty acid value (KOHmg/100g)	Viscosity (mm^2/s)	Taste panel scores (mark)	Germination rate (%)	Whether fit for storage or not	Color and luster
2002.4	76.9	54.0	12.7	0.7	19.7	14.6	81	80	yes	normal
2002.10	76.7	54.3	12.3	0.7	20.9	12.3	79	63	yes	normal
2003.3	77.0	53.5	12.7	0.8	21.8	10.9	78	52	yes	normal
2003.9	77.0	53.0	12.8	0.6	24.5	8.8	77	41	yes	normal
2004.3	76.5	52.8	12.9	0.6	22.4	8.6	75	36	yes	normal
2004.9	76.2	50.0	12.9	0.7	27.8	8.6	75	24	yes	normal
2005.3	76.9	52.0	12.8	1.0	26.4	/	75	/	yes	normal
2005.9	76.8	51.4	12.5	0.8	27.3	/	75	/	yes	normal

Table 3. Inspection results on paddy's qualities in No 10 granary (ordinary granary)

Data	Brown rice rate(%)	Head-Ricerate (%)	Moisture content (%)	Impurity (%)	Fatty acid value (KOHmg/100g)	Viscosity (mm^2/s)	Taste panel scores (mark)	Germination rate (%)	Whether fit for storage or not	Color and luster
2002.05	76.8	53.6	12.3	0.7	19.1	14.8	81	77	yes	normal
2002.10	76.9	55.2	12.4	0.5	22.3	13.2	79	58	yes	normal
2003.03	76.1	53.0	12.6	0.6	23.9	10.6	78	34	yes	normal
2003.09	75.8	52.6	12.8	0.7	27.0	8.8	73	17	yes	normal
2004.03	76.0	51.5	12.9	0.6	26.1	8.7	73	3	yes	normal
2004.09	75.8	50.0	13.0	0.6	32.0	/	71	0	yes	normal

Table 4. Inspection results on paddy's qualities in No 12 granary (MA granary)

Data	Brown rice rate(%)	Head-Ricerate (%)	Moisture content (%)	Impurity (%)	Fatty acid value (KOHmg/100g)	Viscosity (mm^2/s)	Taste panel scores (mark)	Germination rate (%)	Whether fit for storage or not	Color and luster
2002.04	76.9	54.0	12.7	0.7	19.7	14.6	81	80	yes	normal
2002.10	76.7	54.3	12.3	0.7	20.9	12.3	79	63	yes	normal
2003.03	77.0	53.5	12.7	0.8	21.8	10.9	78	52	yes	normal
2003.09	77.0	53.0	12.8	0.6	24.5	8.8	77	41	yes	normal
2004.03	76.5	52.8	12.9	0.6	22.4	8.6	75	36	yes	normal
2004.09	76.2	50.0	12.9	0.7	27.8	8.6	75	24	yes	normal
2005.03	76.9	52.0	12.8	1.0	26.4	/	75	/	yes	normal
2005.09	76.8	51.4	12.5	0.8	27.3	/	75	/	yes	normal

6 Acknowledgements

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0504

Contrast Trial about the Relationship between Gas Tightness and Fumigation Effect

Zou Jiancheng[1], Ou Guoqing[1], Jiang Chungui[1], Wang Yaowu[1] and Liu Yuchen[2]

Abstract: The contrast trials about the relationship between gas tightness and fumigation effect were carried out. The results showed that, the better gas tightness of the storehouses have, the longer time the effective concentration can be maintained, and the better fumigation effect can be achieved.

Key words: half – life; fumigation; gas tightness

Introduction

State Grain Reserves Ningxiang Depot has 17 large storehouses and 4 squat silos, 16 and 4 of which are started to set up in the end of 1999 and 2001, and to load grain for the first time in the beginning of 2000 and 2002, respectively. For having been used for 4 – 6 years, all storehouses, especially the doors, windows and vents, show the aging phenomenon to some certain extent, which affects gas tightness of the storehouses, mostly shown as the gradual increase of the phosphine concentration decline speed and the dose of fumigation to rise year by year. In this trial, 5 representative storehouses were chosen to test the gas tightness and the relationship between gas tightness and fumigation effect was introduced.

1 Materials and Methods

1.1 Materials

1.1.1 4 – 72 – 6c type Centrifugal Fan; U type manometer; stopwatch

1.1.2 Experimental Storehouses (table 1)

Table 1. Basic Circs of the Experimental Storehouses

No.	Shape	Year to set up	Year to get use	Gas tightness Measures	Full or Empty
10	large storehouse	1999	2000	door and windows: Sealed completely and heat preservation	full
15	squat silo	1999	2000	routine measures	full
16	squat silo	1999	2000	routine measures	full
19	large storehouse	2001	2002	windows: Sealed completely and heat preservation; door: sealed by film	full
20	large storehouse	2001	2002	windows: Sealed completely and heat preservation; door: sealed by film	full

1.2 Experimental Methods

1.2.1 storehouses Gas tightness Test

(1) Experimental storehouses gas tightness were tested and strengthened according to the request.

(2) U type manometer was connected with the phosphine check-conduit through tube; two or three experimenters went inside of storehouses and prepared to check the air leaks; Centrifugal fan was used to add pressure into storehouses through air-conduits. when the pressure fell to 700 – 800Pa, air – conduits were closed, and when the pressure decreased to 500Pa, stopwatch was turned on, when the pressure fell to 250Pa, stopwatch was turned off and the reading was noted.

1.2.2 Fumigation

LS/T 1 201 – 2 002 Aluminium Phosphide Recirculation Fumigation

2 Results and Discussions

2.1 Results of Storehouses Gas Tightness Test

The gas tightness tests carried out from March 28 to 29 in 2006 indicated that gas tightness of the most storehouses did not meet the technical specification request (table 2).

1. *State Grain Reserves Ningxiang Depot, Ningxiang, Hunan*, 410600
2. *Henan Technology University, Zhengzhou, Henan*, 450052

Table 2. Results of Storehouses Gas tightness Test

NO.	Item	First	Second	Third	Expectation of Half – life	Standard	Crucial Air Leak Location
10	Max pressure(Pa)	400	400	400	–	≥40 〞	14 of 16 windows leaked air seriously, resulting in the pressure too low to test the half – life
	Time pressure(Pa)	–	–	–			
	End pressure(Pa)	–	–	–			
	Half-life(s)	–	–	–			
15	Max pressure(Pa)	700	700	700	68 〞 17	≥60 〞	1) the grain subway exits; 2) the boundary edge of electric cable to measure temperature and the roof of storehouse; 3) the manholes and the entrance of illuminate electric wire; 4) the grain exit of scraping transport machine;
	Time pressure(Pa)	500	500	500			
	End pressure(Pa)	250	250	250			
	Half-life(s)	45 〞 65	44 〞 32	44 〞 02			
16	Max pressure(Pa)	800	800	800	75 〞 57	≥60 〞	1) the grain subway exits; 2) two holes of centrifugal fan; 3) the manholes
	Time pressure(Pa)	500	500	500			
	End pressure(Pa)	250	250	250			
	Half-life(s)	80 〞 95	77 〞 47	68 〞 28			
19	Max pressure(Pa)	800	800	800	44 〞 66	≥40 〞	1) 12 of the windows; 2) the door
	Time pressure(Pa)	500	500	500			
	End pressure(Pa)	250	250	250			
	Half-life(s)	66 〞 30	70 〞 00	68 〞 20			
20	Max pressure(Pa)	800	800	800	30 〞 03	≥40 〞	All of the windows
	Time pressure(Pa)	500	500	500			
	End pressure(Pa)	250	250	250			
	Half-life(s)	31 〞 84	30 〞 64	30 〞 61			

2.2 Fumigation before Gastight Strengthen

Table 3. Luminium Phosphide Recirculation Fumigation in 2005

Gas – Tightness Grade	NO.	Amount of grain, t	Breed	vol., m^3	Times	Dosage of Aluminium Phosphide				
						First Dosage, kg	Dosage Compensation		Total Dosage, kg	Dose, g/m^3
							Times	Dosage, kg		
1	10	4606	indica	10527	1	51	2	24	76	7
2	20	5315	indica	15065	1	60	2	36	96	6
3	19	5326	indica	15065	1	60	1	18	78	5
4	15	5157	wheat	8900	1	36	0	0	36	4
5	16	4217	indica	8900	1	36	0	0	36	4

2.3 Effects of Gas Tightness on Fumigation

According to the fumigation of five experimental warehouses, Gastightness is a critical key to a successful fumigation. The relationship between half-life, valid phosphine concentration and dose is shown in figure 1, the change of phosphine concentration is shown in figure 2.

Figure 1 and figure 2 show the effects of gas tightness on fumigation: 1) The better gas tightness of the warehouse has, the much lag in the decay of PH_3 concentration;

2) the longer pressure decay half life is, the longer time the effective concentration can be maintained;

3) the shorter pressure decay half life is,

the larger the dose should be;

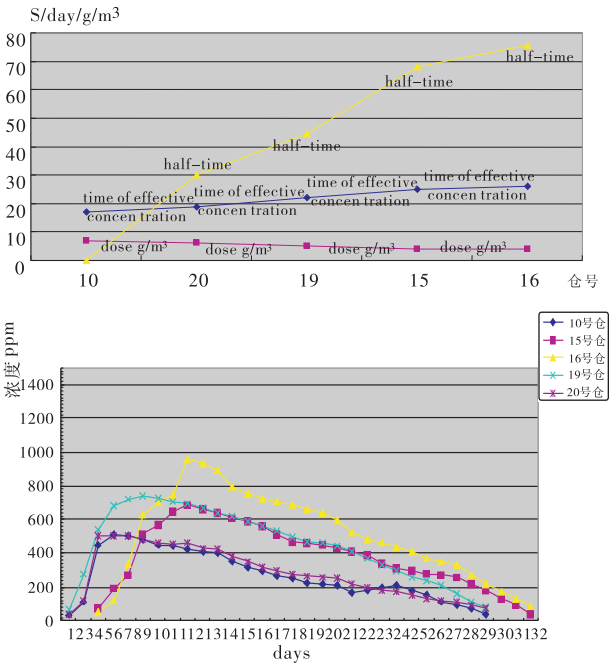

Fig. 1 and Fig. 2 show the effects of gas tightness on fumigation.

4) The time of effective phosphine concentration is not directly proportional to dose, which indicates that even as more aluminium phosphide is used, the maintainance time of effective concentration is not guaranteed.

2.4 Reasons of bad gas tightness

In the gas tightness tests, the air leak positions are mostly the windows of storehouses, as well as the conjunctions , various exits , entrances of the facilities and wall body of storehouses. The main reasons that result in the bad gas tightness of storehouses are as follows:

1) Aging of the Seal Materials.

Confined to existing seal material service life, seal materials of the doors and windows are gradually aging for long-term use, mainly appeared as the deformation, the flexibility decrease, and that can not be sealed completely.

2) The manufacture technologies is weak

After large storehouses were treated with sealed and heat-keeping doors and windows, with the increase of use time the structure begin to deform because of manufacture technologies, and then storehouses couldn't be sealed completely; the craft design of grain exits of scraping transport machines and grain subway exits of squat silos roof is not good enough.

3) The air leak positions are concealed

The air leak positions such as the boundary of various pipeline and storehouses body etc. , have always been ignored at check.

4) Other Reasons

Ground subside, heat expansion and cold contract results in the partial crack of the wall.

2.5 Measures of Gastight Treatment

1) Windows sealed by metal should be reconstructed. Materials with long service life and strong flexibility were chosen to replace those used in doors and windows now. And the deformed windows should be repaired. A system of periodical check and replace should be formed.

2) All parts that were connected with wall body of storehouses should be checked and treated by proper gastight methods

3) All of cracks of storehouses should be sealed completely with special materials.

4) Seal technologies should be improved and seal materials should be added for holes of grain exits and entrances of squat silos.

2.6 Fumigation after Gastight Strengthen

The situations of fumigation after taking the above-mentioned measures is in the table 4.

Table 4 Situations of fumigation after Gastight Strengthen in 2006

No.	Half – life	Time of 250ppm, day	Times of Fumigation	Dosage of Aluminium Phosphide				
				First Dosage, kg	Dosage Compensation		Total Dosage, kg	Dose, g/m^3
					Times	Dosage, kg		
10	50 ″ 02	18	1	60	0	0	60	5.7
19	60 ″ 23	15	1	60	0	0	60	4
20	58 ″ 40	15	1	60	0	0	60	4

The gas tightness performance of large storehouses No. 10, 19 and 20, which were treated by gastight, can attain more than 40 seconds, and the dosages of aluminium phosphide decreased by 21% ,23% ,38% respectively, the time of effective phosphine concentration met the requirement to kill pests thoroughly, avoiding dosage compensation and reducing the labor intensity of the personnel.

2.7 Conclusions

1) It is very necessary to carry out gas

tightness tests and take measures according to test results for better fumigation effects and lower fumigation expenditure.

2) Gas tightness is a critical key to the successful fumigation. The better gas tightness of the storehouse have, the longer time the effective concentration can be maintained, and the better fumigation effect can be achieved.

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Evaluation of Large, Modern Warehouse Storages Designed and Constructed for Application of Carbon Dioxide

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Abstract: The updated warehouses of controlled CO_2 storage with total capacity of 45 000 t were constructed at Mianyang, in which field experiments were successfully carried out. This paper introducs the construction techniques, CO_2 supply and distribute system, auto-monitor system, security and guarantee facilities, sealing materials, airtight techniques and field experiments by the techniques of CA grain storage couplied with natural low temperature grain storage. The results showed that design and construction techniques were reasonable. The pressure decay half-life reached 12 min from 500 pa declined to 250 Pa, and thus effectively ensured the concentration of CO_2. Auto supply and distribution for CO_2 as well as monitoring were realized during the whole process. The field experiment proved that both susceptible strains and resistant strains of *Sitophilus zeamais*, *Rhizopertha dominica* and *Tribolium castanuem* were 100% controlled with exposure for more than 14 d to CO_2 which concentration ranged from 35% – 75%. After incubating the mixed samples at different culture-conditions for 42 d, no alive adults appeared. There were also no significant changes in the quantity of carried microorganism in the grain stored with CO_2. Compared with conventional storage, after 40 months storage with CA grain storage couplied with natural low temperature grain storage for the new-harvest grain with safe moisture content and good quality, the grain quality was better and could be steady after unsealing.

Key words: CO_2 CA storage, natural low temperature grain storage, grain depot construction, field experiments

Introduction

With the continuous progress of technology and advance of life quality, people care much about the environment, and require of "green food", which are of high quality with no-chemical contamination. Current grain storage technology is developing towards an integrated way to reducing use of chemical pesticides, which including low-temperature storage, controlled atmosphere (CA) techniques and biological techniques. The controlled CO_2 techniques means inflating CO_2 into well-sealed warehouse so as to change the ecological storage environment, inhibite molds and respiration of grain, control pests, and delay grain aging.

Since 2000, the first modern warehouses with controlled CO_2 were constructed at Mianyang, China, with a total capacity of 45 000 t and consist of several chambers with a capacity of more than 5000 t for each. After the first-period construction, experiments were carried out compared with conventional storage.

1 Construction of the CA Warehouses

1.1 Construction Techniques

1.1.1 Condition of the warehouses

Warehouses with Controlled CO_2: 5 horizontal warehouses consist of 2 chambers, 24m width 96m length × 7.8m height, 6m height of grain bulk, 45 000 tons of total capacity, 5 000 tons capacity of each chamber.

Control Warehouses 1 horizontal warehouse with the same condition of the above, which adopts conventional storage as control.

1.1.2 Sealing Material

Based on the requirements of controlled CO_2 warehouse, several materials were screened out by comparation, which were flexible, tractile, resistant to extreme temperature and erosion, ultra-radiation-proof, non-contaminant or non-toxic, durable, easy to use, of low air-permeability, good adhesive ability, and with reasonable price.

1.1.3 The Position and Methods for

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2. *Mianyang Grain Storage Directly under Central Grain Reserves, China*

Sealing

According to the original design, the following steps were conducted:

Equipped the windows and venting pipes with air-tight and heat – preservation facilities as well as axial-flow fans to the windows → Brushed the interior wall of the warehouse with Coating A ↓ Sealing the wall, top, and floor of warehouse, made each bonding point into arch camber of 150 mm thickness ↓ Filled high elastic materials into the gaps of the camber ← Sealed all the openings of the warehouse, including the inlet hole, outlet hole, vent opening, axial-flow opening, check opening, CO_2 supply opening, and bonding point of lines etc.

1.1.4 Air-tightness of the Warehouses with CO_2

The pressure decay half-life of the warehouse with CO_2 reached more than 12 min from 500 pa declined to 250 pa, while the control only reached nearly 40 s. Through inspection, it was proved that the concentration of CO_2 could be effectively ensured by one-time introduction of CO_2.

1.2 Assembly of Assorted Facilities for Warehouses with CO_2

The key point for CO_2 storage techniques was assembling assorted equipments. In the construction, large-scale CO_2 supply and distribute system was conducted at the first time, which realized central supplying and distribution of CO_2. At the same time, auto-monitor system and security and guarantee facilities for CO_2 were developed and applied. Figure 1 shows the technical flow chart:

1.2.1 CO_2 supply and distribute system

The CO_2 supply and distribute system is used to store liquid CO_2 safely, vaporize and send gas CO_2 when necessary.

1.2.2 Auto-monitor system for CO_2 concentration

The system consist of gas sampling pipe network, gas control pipeline, infrared CO_2 detection facility, data traffic device, CO_2 supply control device, inspection computer, inspection software, which are shown in figure 2.

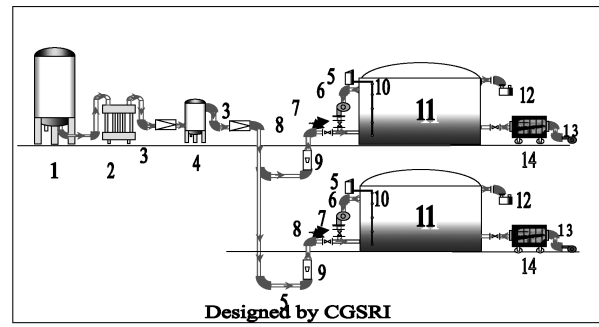

Fig. 1 Technical flow chart of CO_2 storage of grain designed by Chengdu Grain Storage Research Institute (CSR)

1. CO_2 cylinder, 2. evaporator, 3. decompression facility, 4. gas balance cylinder, 5. CO_2 supply pipeline, 6. circulation pipeline, 7. circulation fan, 8. switch valve, 9. flow meter, 10. auto-monitor system for CO_2 concentration, 11. warehouse storage with CO_2, 12. pressure balance facility, 13. fan, 14. intelligent control system

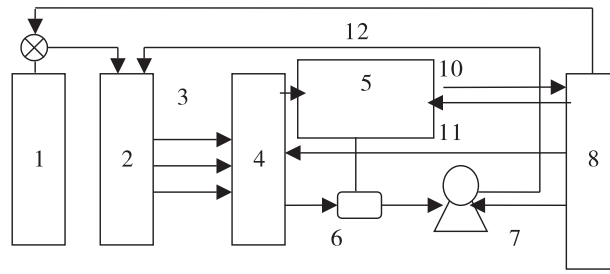

Fig. 2 The principle of auto-monitor system for CO_2 concentration

1. CO_2 supply source, 2. warehouse storage with CO_2, 3. sampling from multiple lines, 4. multicenter control device, 5. infrared CO_2 detect facility, 6. buffer, 7. pump, 8. inspection computer for telecontrol, 9. CO_2 supply control device, 10. data output, 11. on-off control, 12. signal return

The system could determine and report the CO_2 concentration of several points in the warehouse automatically, and can be run on MS Windows as well as be friendly for user to operate and update. Due to its excellent performance, the CO_2 concentration could be detected on-line well and truly, thus also lead to utilizing the CO_2 resource reasonably effectively and economically.

The system was based on the detection standard of China Measurement Technology Institute. The detection limit was 0% – 100%, basic error $\leqslant$ 1.0% (F · S), repeatability $\leqslant$ 0.5% (F · S); drift of zero and span < 1.0% (F · S)/48h.

1.2.3 Security and Guarantee Facilities

In order to avoid the troubles due to pressure difference between inside-warehouse and out-side warehouse, 2 sets of pressure balance facilities were assembled with each chamber of

the warehouse. The adjust scope for pressure ranged from 2 000Pa with detect precision of 20Pa. At the same time, 4 set of tote oxygen – breath devices were equipped and a relevant standard regulation was established, which all ensured the safety during operation.

2 The Application of Grain Storage with CO_2

After accomplished the first-period construction, field experiments were carried out for one year. During the first period of CO_2 storage, the CO_2 concentration ranged from 70% to 35% maintained more than 14day, while during the second period, effective concentration of CO_2 was maintained.

2.1 Effect Against the Insect Pests

2.1.1 Materials and Methods

Test Insects: Susceptible and resistant strains to phosphine of two weeks adult insects of *S. zeamais*, *R. dominica* and *T. castanuem* were tested. The resistant factors were 196, 204 and 8. Each test contains 20 adults and mixed-stage insects (eggs, larvae, pupae) of each above test insects. Control test was prepared the same way except adopt conventional storage. Each test was done in 10 replicates. After 1 month exposure, the mortality rate was recorded, and all the insects were sieved out and kept in incubator of 25(1 ± ℃, 70% ±5%; 30 ± 1℃, 70% ± 5% respectively to count the number of the next progeny. The emergence of progenies was observed 56d later after treatment.

Treatment of the test Insects: The test groups 1 – 6 were laid at the four corners of the warehouse at different heights. The test groups 7 – 10 were laid by the vent openings and check doors.

2.1.2 Results and Analysis

2.1.2.1 The Results of Test in Wheat Warehouse Storage with CO_2: All test adults of 3 insects of 2 strains were 100% killed. No next progenies of the mixed-stage cultured insects emerged after 56 days treatment.

2.1.2.2 The Results of Test in Paddy Warehouse Storage with CO_2: All test adults of 3 insects of 2 strains were 100% killed. No next progenies of the mixed-stage cultured insects emerged after 56 days treatment.

2.1.2.3 The Original Insects Existed in the Warehouse Before Treatment: It was found that the insect density of NO. 13 wheat warehouse with CO_2, was about 15 insects/kg wheat, which mainly consist of *S. zeamais* and *S. cerealella*. While after treatment with controlled CO_2, there were no insects emerging in the next half year. The same condition was with the NO. 14 wheat warehouse with CO_2, which had found booklice before while after treatment no booklice was alive in the warehouse.

2.1.3 Conclusion

From the result, it was clear that the treatment of 70% – 35% CO_2 for 14 days was very effective against each stage of the tested insects (susceptible and resistant). And no residue will be lead to at the same time.

The field experiment also showed that the techniques of controlled CO_2 was effective against booklice, which could be an alternative for control of booklice.

2.2 Inhibition Effect Against Molds

2.2.1 Materials and Methods

Warehouses and Test Grain

Warehouses: The new warehouse No. 12, 13, 14 and 15 were chosen as the test warehouses for CO_2 storage, each test warehouse contained with 3 000 – 5 000 t of grain;

Test Grain: Newly-harvested paddy and wheat from Sichuan and Hunan, with moisture content of 11.6% – 12.3%;

Sampling: Sampled every 3 or 4 months to analysis mycoflora in the grain, which had been stored for 1 year. The preparation of samples was conducted by the Chinese standard method GB4789 – 1 – 94.

2.2.2 Experimental Result and Analysis

2.2.2.1 Result

Through 370 days' CO_2 CA grain storage in the field, the examination result of grain fungi's germ quantity can be seen from the table 1, the examination of bacteriallogzaph is omitted.

Table 1. Examination result of grain fungi's germ quantity of the grain stored by CO_2 CA storage and by the normal storage of the exemplary facility (unit: entry per gram)

Warehouse number and type	Grain storage category	First	185 days	370 days
No. 10 normal warehouse	Paddy	5.4×103		2.5×103
No. 11 normal warehouse	Wheat	5.6×102	8.95×103	1.5×103
No. 12 CA warehouse	Paddy	2.7×104		2.5×103
No. 13 CA warehouse	Wheat	3.7×102	8.5×102	4.7×103

Warehouse number and type	Grain storage category	First	185 days	370 days
No. 14 CA warehouse	Wheat	7.8 × 102	8.2 × 102	1.9 × 103
No. 15 CA warehouse	Wheat	6.1 × 102		5.7 × 102

2.2.2.2 Analysis

We can see from the examination datum in the field germ quantity and bacteriallogzaph: grain fungi's germ quantity of the wheat and paddy which are stored by CO_2 CA in one year changes a little during the whole storage period. But when we examine and analyze the grain mildew bacteriallogzaph, the result has no obvious variety. Because of being limited by CA sampling, we take little specimen . But general speaking being handled by CO_2, the field fungi representative which can reflect grain's freshness degree—*hypnocyst* and *Fusarium avenaceum* are reducing gradually. Though the test-out rate which is represented by the *Aspergillus glaucus link*、*Aspergillus flavous link*、*Aspergillus candidus link* and so on is high, it was stable on the whole. The test-out fungi categories reduce gradually with the extension of storage time. Therefore adopting the CO_2 CA storage grain fungi are regardless on the quantity and the category for the complete moisture grain.

2.3 Quality Influent Effect

2.3.1 Experimental Material and Method

2.3.1.1 Experimental method

Choose there CA warehouses (No. 12 paddy warehouse, No. 13 wheat warehouse) which are newly set up by exemplary facility and two normal warehouses which are set up synchronically (No. 10 paddy warehouse and No. 11 wheat warehouse) as experimental warehouse. Conduct grain storage contrastive experiment of CO_2 CA and normal storage (mostly points PH_3 recirculation fumigation to kill pests). Measure grain's quality before this experiment, in October continuously and in May and April of the second year.

2.3.1.2 The method of quality judgment

The method of long-grain nonglutinous rice and wheat 's quality judgment is in line with the international standards. The moisture, the value of fatty acid and the conglutination degree were judged by the 105 ℃ constant weight method of GB5497 – 85, the cereal fatty acid measurement method of GB/T15684 – 1995 and capillary movement conglutination degree measurement method of GB5516 – 85.

2.3.1.3 The method of quality judgment

The quality index measured by this experiment is on the basis of the long-grain nonglutinous rice and wheat's storage quality controlling index of "The quality judgment standards of grain and oil storage" (try out) , unitedly distributed by the National Grain Reservation Bureau and the National Quality Technology Supervision Bureau in 1999 as well as according to the grain classing standards of " Paddy" of the international standards GB1350 – 1999 and "Wheat" of the GB1351 – 1999 to class grain.

2.3.2 Experimental result and analysis

2.3.2.1 The quality effect of long-grain nonglutinous rice

The experimental result can be seen from table 2.

Table 2. The long – grain nonglutinous rice's quality measurement result of CO_2 CA grain storage and the normal grain storage of the exemplary facility

Samping time (year. month)	Fatty acid value KOHmg/100g dry samle		Degree of viscosity mm^2/s		Germination percentage %		Tasting valuation score		Color & scent	
	No. 12	No. 10	No. 12	No. 10	No. 12	No. 10	No. 12	No. 10	No. 12	No. 10
2002.04	21.9	21.1	13.5	16.0	77	70	81	82	normal	normal
2002.09	20.2	21.6	13.5	16.0	65	68	–	80	normal	normal
2003.03	21.6	22.7	9.0	9.4	52	16	77	73	normal	normal
2003.09	22.4	26.1	8.8	8.8	41	17	77	73	normal	normal
2004.03	24.5	27.0	8.6	9.1	41	3	77	73	normal	normal
2004.09	26.4	32.0	8.6	–	24	–	–	77	normal	normal
2005.03	27.8	worked off	–	worked off	–	worked off	75	worked off	normal	worked off
2005.09	27.3	worked off	–	worked off	–	worked off	75	worked off	normal	worked off

No. 10: normal warehouse; No. 12: CA warehouse; – : no determine;

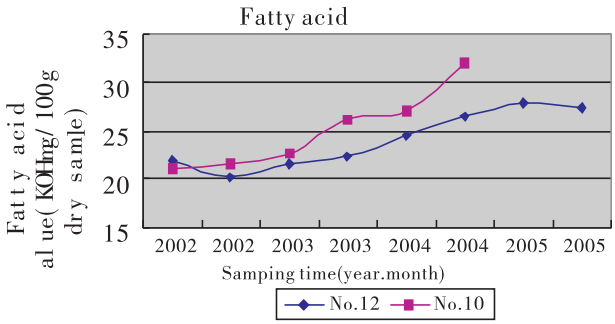

Fig. 3 The changes of Fatty acid value way and normal way

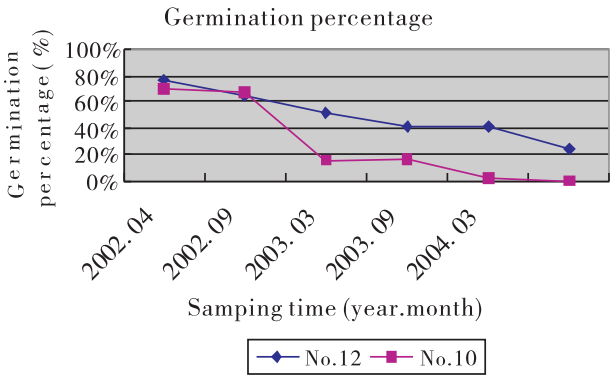

Fig. 4 The changes of Germination percentage stored by CA stored by CA way and normal way

We can see from the table 2, fig. 3 and fig. 4: After 40 months storage with CA storage coupling with natural low temperature storage for the long-grain nonglutinous rice with safe moisture content and good quality, the grain quality stored by CA was better than that of stored by normal grain storage. And it also explains that CO_2 CA storage changes slowly than normal storage. If reserved continuously the difference between them will enlarge and the advantage of CO_2 CA storage will be revealed further especially under high-hot and high-wet abominable circumstance.

2.3.2.2 Wheat's quality effect

Table 3 suggests the experimental result:

Table 3. Wheat's quality tested result of CO_2 CA grain storage and the normal grain storage of the exemplary facility

Samping time	Degree of viscosity mm^2/s		Flour muscle absorption%		Tasting valuation fen	
	No. 11	No. 13	No. 11	No. 13	No. 11	No. 13
2002.05	8.2	7.0	187	197	75	75
2002.10	9.3	8.2	194	197	77	77
2003.04	8.3	8.1	206	201	77	77
2004.04	8.1	8.1	202	204	74	74

No. 11: normal warehouse; No. 13: CA warehouse;

We can see from table 3: After 24 months wheat's quality has some improvement with CO_2 CA storage and normal storage. Furthermore, the difference between them is not obvious. Wheat's physiological late maturity and technologic late maturity give rise to wheat's quality improvement. Because wheat's capacity of bearing storage is good, the advantage of CO_2 CA storage over normal storage require a long time to reveal.

2.4 Quality Variety of CO_2 CA Storage after Unsealing

During the very day when they are unsealed to 50 days after disclosing, measure periodicallylong-grain nonglutinous rice's variety of CO_2 CA storage in No. 12 CA facility, which was taken sample every 10 days to moisture content, fatty acid, acidity, conglutination degree, sprouting rate, tasting value, color scent and scent. During the same period measure Broken rice yield and whole releas and analyze speed of the quality variety.

Experimental result can be seen from table 4

Table 4. Long-grain nonglutinous rice's quality variety of CO_2 CA storage in No. 12 facility after unsealing

Days of unsealing	Storage quality controlling index of wheat						acidity KOHmL/10g sampler
	Fatty acid value KOHmg/100g dry – sample	Degree of viscosity mm^2/s	Germination percentage %	Regress value	Tasting valuation score	Color & scent	
0	21.8	10.9	52	87.4	78	normal	1.3
10	22.3	11.1	51	87.6	78	normal	1.3
20	22.6	10.4	50	86.3	78	normal	1.3
30	22.6	10.3	52	86.2	78	normal	1.3
40	22.6	10.3	52	86.2	78	normal	1.4
50	22.6	10.3	52	86.2	78	normal	1.3

We can see from table 4: After unsealing quality index has no obvious change, and stor-

age quality controlling index changes slowly. It suggests that safe-moisture, good-quality grain which is dealt with rational CO_2 CA storage technology will not be badly changed after unsealing.

2.5 Experimental Result and Analysis

We can make conclusion from the above datum analysis:

2.5.1 Experimental Result

2.5.2 The usage expense of CO_2 CA grain storage per ton in a year is that paddy and wheat are less than 3.0 yuan, 2.5 yuan, respectively. If we adopt atmosphere source which are in line with the food-class liquid CO_2 standards (GB10621 – 89) of our country, then the price will reduce from 960 yuan per ton at present to 600 yuan, the usage expense of paddy and wheat will be under 2.3 yuan , 1.8 yuan per ton in a year respectively, direct materials cost also will be under 1.0 yuan per ton in a year.

2.5.3 CO_2 CA grain storage is character of green grain storage. The grain being stored for two years with CA storage after being plunged into the market expects to increase above 40 yuan per ton in a year according to the principle that superior quality should has higher price.

2.5.4 CA grain storage can kill pests effectively, prohibit bacteria, prolong grain storage's change worse rapidly, avoid chemical's danger for human, grain and environment. It can't erode the relevant establishment of grain warehouse and endanger PH_3 materials in PH_3 fumigation. Furthermore, it can avoid factor which can't be exactly numerated like the strength of grain storage pests' fastness. It answers for the demand of green food and current of foodstuff market. This latent social and economic benefit can't directly be accounted by money, so its comprehensive benefit, economic benefit is higher than that of normal grain storage pattern.

3 Demonstration Effect and Prospect

3.1 Demonstration Effect

Succeeding in construction of exemplary facility with CO_2 CA grain storage and applying in the field suggest:

3.1.1 Rebuild properly the tall bungalow warehouses which is constructed in our country at present, then the warehouse's airtightness can attain 500 Pa pressure half life 12 min, which can entirely fulfill the demand of CO_2 CA grain storage technology for the warehouse's air-tightness.

3.1.2 Our country's technique and equipment can completely realize the mode of centralized air feed at present and automatic supervise function of CO_2 concentration inside of the warehouse.

3.1.3 Reasonable C. A techniques can effectively prevent and control grain storage pests and completely avoid using chemistry medicament. Therefore, no social effects of pollution and no pollution green-storage will come true.

3.1.4 If we use CO_2 CA storage, then the foodstuff epiphyte will have no evident change not only quantity but also species for complete moisture foodstuff.

3.1.5 With CO_2 CA storage in 10 month, long-grain no glutinous rice's quality is superior to that of the normal storage. The wheat's contrastive effect is not obvious, because of its late ripeness and endurance. The quality of grain which is reserved rationally with CO_2 CA techniques will not become bad swiftly after unsealing.

3.1.6 If we use CO_2 CA storage, the grain storage comprehensive economic benefit and social benefit will excel to normal storage and it's developing direction will accord with the trend of the demand of green food and grain market.

3.2 Prospect

3.2.1 CO_2 CA storage technology is feasible for our construction and application as a sort of advanced technology of green storage.

3.2.2 The success that four CO_2 CA enlargement experimental grain facilities have constructed in China in 2002 further suggests the popularity and application of this technology in our country have been mature.

3.2.3 This technology is suited for the direction of diversification, high quality, high benefit, high nutrition, low waste, low pollution and low cost. It will further enlarge and popularize with the consummation of our country's economic development and grain's market system.

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0506

Experiment on Grain Storage by Controlled Atmosphere with Carbon Dioxide

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Abstract: Controlled atmosphere technologies with carbon dioxide for grain storage, which were safe, simple, effective and hygienical, could not only effectively control grain storage pests, but also keep chemicals from harming person, polluting grain, corroding grain storage devices and destroying environment compared to chemicals such as phosphine. "No chemicals emission" has been coming true and is required for green food and protecting environment for the mankind, meanwhile, it has provided a new way for inhibiting pests' pesticide resistance growth.

Key words: carbon dioxide, controlled atmosphere, fumigation

With the social development and progress, making such achievements as excellent qualities, fresh, environmental protection and pollution – free, minimizing the loss of quality and quantity of stored grain, would be inevitable for grain industries joining in competition and improving benefits. It should be developing grain storage technologies gradually which are pollution-free such as controlled atmosphere, low temperature, controlling by combination of physical and biological methods.

With technical guidance from superior technical administration office and ChengDu grain storage research institute, the experiment on controlling pests by controlled atmosphere with carbon dioxide firstly was carried on by JiuJiang National Grain Depot, China Grain & Oil Group Science & Technology Corp. in 2005, and lots of success achieved. Since 2005, comparing with the control granary with conventional grain storage technologies, the research about grain storage with carbon dioxide controlled atmosphere influence on grain qualities has been carried out.

1 Fumigation Principles of Controlled Atmosphere with Carbon Dioxide

Using large gas supplying system outside, matching with automatic system for inspection carbon dioxide in granaries, steering system for recirculation ventilation and devices for regulating pressure of granaries, carbon dioxide would be concentrated to input CA granaries with excellent sealing performance, and meanwhile, the concentration of carbon dioxide would be well-distributed because of forced recirculation system, and the continuous monitoring of the concentration. By altering gas composition in granaries, the ecological environment of pests and mould is destroyed, respiration of grain inhibited, deterioration of grain quality postponed and grain pests controlled.

2 Main Technologies Indexes of Experiments on Fumigation by Controlled Atmosphere with Carbon Dioxide in Filled Granaries

2.1 Indexes of gas tightness: the half time, in which pressure of 500 Pa decreased to 250 Pa, should over 240 s.

2.2 In normal condition, the consumption of carbon dioxide should be below 3 kg for one tonne of grain every year.

2.3 Insect mortality with carbon dioxide should reach 100%.

2.4 The cost should be below 4 Yuan for one tonne of grain every year.

3 System Configuration for Controlled Atmosphere with Carbon Dioxide

3.1 Gas Distribution System Outsides

It consisted of such devices as liquid carbon dioxide storage tank, evaporator, decompressor, gas balancing tank and airfeeding pipe. The gas distribution devices and craft flow-sheet was as follows. (Figure 1)

3.2 Automatic Monitoring System for Carbon Dioxide Concentration

Carbon dioxide automatic monitoring system consisted of many devices to achieve auto-

1. *Storage Office of JiangXi Branch, State Grain Reserves* 332000
2. *JiuJiang National Grain Depot, China Grain & Oil Group Science & Technology Corp.*
3. *GongQing Grain Depot, State Grain Reserves* 330300

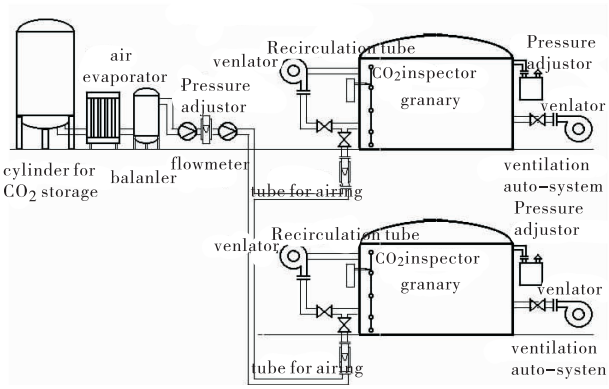

Fig. 1 Photograph of CA system with carbon dioxide

matic transportation and management of carbon dioxide, such as carbon dioxide gathering nets, administration system of gas pipe, metering equipment for carbon dioxide concentration, data communication system, computer and software for monitoring.

4 Granaries and Pests for Testing

4.1 Elementary Information of Experimental Granaries

4.1.1 NO.6 CA granary was large granary (30 meters long, 21 meters wide and 7.8 meters high). Grain was filled to 6.3 meters high. The index of gas tightness was about 7.3 min for empty granary, about 6.3 min for full granary. Granary was filled with 2170 t of late long-grain nonglutinous rice, which was produced in 2004 and stored in July, 2005. Furthermore, the average moisture was 13.5%, the surface, upper, middle and lower grain temperatures were 27℃, 25℃, 25℃, 24℃ respectively, and the average temperature was 25℃.

4.1.2 NO.3 ordinary granary, was 30 meters long, 21 meters wide, 7.8 meters high filled to 6.5 meters high. The index of gas tightness was about 0.7 min for empty granary, about 0.7 min for full granary. Granary was filled with 2291 t of late long-grain nonglutinous rice, which produced in 2004 and stored in July, 2005. Furthermore, the average moisture was 13.5%, the surface, upper, middle and lower grain temperature were 28℃, 26℃, 24℃, 24℃ respectively, and the average temperature was 25℃.

4.2 Pests Tested

There were three kinds of main grain storage pests species in No.6 CA granary (*Sitophilus zeamais*, *Rhizopertha dominica*, long-horned flour beetle), at a density of 17 insects per kilogram of grain. Same three insect species were also present in No.3 granary at s density of 9 insects per kilogram of grain.

4.2.1 Species of pests tested

There were three kinds of main grain storage pests with phosphine sensitivity (*Sitophilus zeamais*, *Rhizopertha dominica* and *Tribolium castaneum*). There were three kinds of main grain storage pests with phosphine resistance (*Sitophilus zeamais*, *Rhizopertha dominica* and *Tribolium castaneum*). Their phosphine resistance indexes were 196 times, 204 times and 8 times, respectively.

Six kinds of grain storage pests with all life stage (egg, larva, pupa and adult), were divided into 10 groups, each of which had 20 adult and other life stage.

Moreover, one control group was set up, each of which had 20 adults and other life stage. Their mortality was determined after 1 month.

4.2.2 Experimental method: Figure 2 shows the location of insect placement in the granary and depth, for the six groups of pests tested. The insects were placed into experimental granary by sampler. Four corner location were 2 m from walls. The seventh and eighth group of pests tested were put near the ventilation ports, the ninth and tenth were hanged 1 meter over the surface of grain bulks. All insects were located before sealing granaries. After expiration of experiment and exhausting gas, and mortality was assessed.

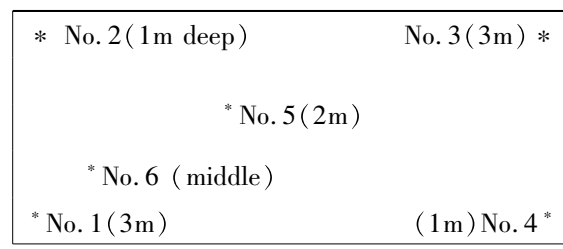

Fig. 2 Burying points of all pests tested and their depth

5 Experimental Inspection Objects

5.1 Carbon Dioxide Concentration Inspection in No.6 CA Granary

5.1.1 Figure 3 and 4 show the locations of CO_2 sampling.

5.1.2 Inspection methods

Carbon dioxide concentration was recorded automatically by carbon dioxide automatic monitoring system every day for 15 days. Meanwhile, to assure carbon dioxide distribution well, recirculation fans were started after aerating for 12 h, and were 24 h for recirculation upper.

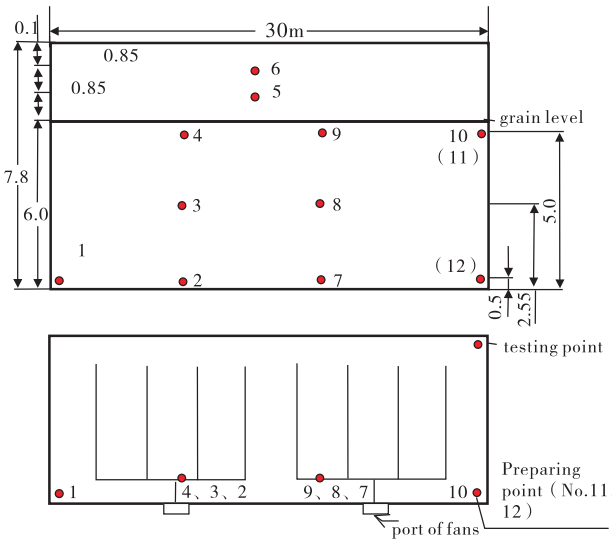

Fig. 3 and 4 Ichnography of locations of CO_2 sampling

5.2 Inspection Pests

After fumigation for 15 days by CA with carbon dioxide in No. 6, 18 days in No. 3 ordinary granary, tested pests in grain bulks were checked respectively and the mortality of pests were determined. In addition, the tested pests were kept at 25℃ and 75% RH for 30 days. We assured whether living pests occurring or not after inspection.

5.3 Analyses on Quantities Alteration

Quantities of paddy were sampled for inspection in No. 6 CA granary and No. 3 ordinary granary, periodic inspection and comparing analysis was taken during grain storage.

6 Results

6.1 Test Procedures

Starting at 17:00 p. m. on Sept. 22, 2005, carbon dioxide was filled in No. 6 CA granary, until 23:00 p. m. It took 6 h. According to the predicted proposal, such conditions were desired as temperature of carbon dioxide keeping about 22℃, 50 – 150Pa of pressure, flow at rate of 500 cubic meters each hour, 5.6 tons of carbon dioxide consumption and 2.58 kilograms of each ton grain gas consumption. Recirculation fumigation with AIP carried on in No. 5 ordinary granary on Sep. 2nd, 2005, which was at 13.5 kilograms of Aluminum phosphate consumption and 300 mL/m^3 of setting concentration.

Table 1. Concentration altering – time on CA experiment with CO_2 in No. 6

Date	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10
Sep. 22	88.95	81.75	73.02	68.07	31.24	64.48	85.83	75.86	67.62	65.19
Sep. 23	78.86	68.68	69.68	65.07	47.10	63.33	78.28	70.94	66.33	64.74
Sep. 24	60.79	61.32	62.67	62.34	53.40	59.34	63.17	63.13	62.55	59.35
Sep. 25	60.34	59.01	58.61	59.01	54.61	58.03	59.00	60.19	59.13	58.15
Sep. 26	55.49	55.44	55.15	55.78	52.07	55.02	55.15	57.06	56.51	56.08
Sep. 27	54.50	56.35	57.54	57.25	49.86	54.87	58.42	59.08	59.18	58.16
Sep. 28	52.97	53.66	55.47	55.50	48.63	53.27	55.18	54.66	54.43	54.41
Sep. 29	51.51	52.41	54.17	54.24	47.87	52.28	53.86	53.19	52.86	52.68
Sep. 30	50.48	51.40	53.10	53.07	47.27	51.31	54.16	51.57	51.33	51.16
Oct. 1	51.00	51.22	52.75	52.95	47.04	51.07	52.66	51.69	51.29	51.53
Oct. 2	50.64	50.78	52.34	52.43	46.64	50.56	52.29	53.94	54.89	54.74
Oct. 3	48.05	49.26	50.02	50.10	45.05	48.70	50.32	48.15	48.35	47.27
Oct. 4	46.74	48.20	49.71	49.61	44.34	47.92	49.59	45.13	43.10	44.88
Oct. 5	46.30	45.39	44.98	45.93	43.37	45.62	45.83	47.76	46.64	46.83
Oct. 6	46.83	45.08	44.19	44.78	42.41	44.51	44.82	46.59	45.53	46.12
Oct . 7	45.38	43.97	43.29	44.09	41.60	43.61	43.97	45.81	44.77	45.34
Oct. 8	44.78	43.25	42.24	43.09	40.44	42.96	43.27	45.22	44.24	44.54
Oct. 10	37.06	38.60	36.94	38.23	36.97	38.41	37.39	40.14	38.55	39.68
Oct. 12	34.49	36.80	35.26	36.56	36.13	36.88	35.73	38.05	36.36	37.42

6.2 Carbon Dioxide Concentration Altering during CA Fumigation in Grain Bulks (see fig. 1 and table 1 in details)

Carbon dioxide concentration altering during CA fumigation in No. 6 figure 1.

It has been shown from table 1 that the original average concentration was 58.2% on Sep 23rd after gasing and the whole average concentration still reached 37.8% 18 days later. It proved by gasing experiments that the required 35% of carbon dioxide concentration was maintained for over 15 days.

6.3 The Effect on Controlling Pests

According to this experimental proposal, the effect of controlling pests in No 6 CA granary and No. 3 ordinary granary was 100%. After taking 10 groups of sample pests tested buried in No 6 granary, no pests alive were found. The control group of pests tested were put into conditions with certain temperature and humidity after taking out, and then observed for one month, no living pests were found, the morality ratios reached 100%. Till Sep. 2006, there were 5 heads each kilogram of grain storage pests in No 3 ordinary granary, the main pests spices of which were *Sitophilus zeamais* and *Cryptolestes ferrugineus*. Recirculation fumigation with 10 kilograms of AlP was carried on again on 20th Sep..

However, since fumigation with carbon dioxide controlled atmosphere on Sep. 2005, there were no grain storage pests so far in No 6 CA granary. It showed that fumigation with carbon dioxide controlled atmosphere had the same effect as conventional fumigation with AlP in short term, however, here could keep longer term for no pests by fumigation with carbon dioxide controlled atmosphere, and there was more excellent fumigation effect than conventional fumigation with AlP.

6.4 Inspection and Analysis on Grain Qualities

The condition of qualities altering during paddy storage in No. 6 CA granary and No. 3 conventional granary were as follows. See table 2 and table 3 in details.

Table 2. Results on paddy qualities inspection in No. 6

Data (a-m)	moisture (%)	impurity (%)	Roughness rate (%)	Head rice (%)	Fatty acid value (mgKOH/100g)	Taste panel (scores)	Smell and color	Whether fit for storage or not
2005-7-31	13.5	0.9	76.3	50.5	19.7	87	normal	yes
2005-9-6	13.5	0.8	77.0	51.3	21.5	85	normal	yes
2006-3-7	13.8	1.0	77.0	55.5	22.8	84	normal	yes
2006-9-6	13.5	1.0	77.2	54.7	22.9	84	normal	yes
2007-3-7	13.5	1.0	77.1	54.5	24.5	82	normal	yes

Table 2. Results on paddy qualities inspection in No. 3

Data (a-m)	moisture (%)	impurity (%)	Roughness rate (%)	Head rice (%)	Fatty acid value (mgKOH/100g)	Taste panel (scores)	Smell and color	Whether fit for storage or not
2005-7-31	13.5	0.9	76.6	53.6	20.3	88	normal	yes
2005-9-6	13.5	0.8	77.0	52.2	20.4	85	normal	yes
2006-3-7	13.0	0.9	77.2	54.8	22.4	84	normal	yes
2006-9-6	13.2	0.9	77.1	54.8	24.2	83	normal	yes
2007-3-7	13.3	1.0	77.5	54.5	25.6	82	normal	yes

From table 2 and table 3, the qualities of late long-grain nonglutinous rice in No. 6 CA granary had the same as No. 3. There were rather little alteration in such indexes as moisture, impureness and roughness ratios etc. during grain storage. The fatty acid value of pay in No. 6 CA granary has become 24.5 mgKOH/100g till March 2006, here raising about 4.8 mgKOH/100g, and the taste panel went to 82 scores, decreasing about 5 scores. The fatty acid value of pay in No 3 ordinary granary has become 25.6 mgKOH/100g till March 2006, here raising about 5.3 mgKOH/100g, and the taste panel went to 82 scores, decreasing about 6

scores. It showed from analyses that the altering of the fatty acid value and taste panel scores in both No. 6 CA granary had the same as No. 3 ordinary granary. By comprehensive judgment, paddy qualities in both granaries were fit for storage.

7 Analyses on Economic Benefits

There were analyses between running fees for grain storage in No. 6 CA granary with No. 3 ordinary granary during the period of from July 2005 to March 2007. Except of such common fees as administration costs and staff salaries, the analyses costs only included objects between both storage ways during experiment.

Table 4. Comparative analyses economical benefits between CA grain storage with conventional fumigation

No.	Capability (t)	Quantity of grain (t)	Height of grain bulks (m)	Fumigation times	Fumigation costs (Yuan)			Coating film	Nutrition support	Consumption of power	Repairing fee for gas tightness	The total cost
					The main cost		Assistant cost					
					CO_2	AIP	CO_2					
6	2722	2140	6.3	1	4480			35		120	440	5075
3	2722	2291	6.5	2	630	850	800	400	60	240	2980	

From the comparative analyses in table 4, it showed that 2.37 Yuan each ton grain of preserving fees in No 6 CA granary was higher than 1.3 Yuan in No 3 ordinary granary, however, it only leaded small proportion in the whole preserving fees.

8 Analyses and Discussion

8.1 Analyses on Comprehensive Benefits

8.1.1 Though there was lower cost during conventional paddy storage, for the application of fumigation with phosphine, it would be inevitable to lead residual accumulation of grain increasingly after phosphine fumigation for a long term, there was much pollution on grain and environment to some extent. In addition, it would lead corrosion to such ancillary equipments as grain condition monitoring system, and raise pesticide resistance increasingly for grain storage. However, CA with carbon dioxide could not only be effective to control pests for grain storage safely, but also protect human, grain and environment from harming, contaminating and destroying by chemicals. It was consistent with requirement of people for green food and had excellent social benefits, meanwhile, it also applied a new way to inhibit pests resistance growth.

8.1.2 CA technology for grain storage is currently one of the most advanced technologies in the world. With the condition of grain storage industries in China, the approach for grain storage with carbon dioxide is one of most simple and effective technology among such three CA grain storage technologies as carbon dioxide filling. CA grain storage could make breakthrough from the situation which Chinese grain storage industries relied on phosphine to control pests in the past. If the achievement of "no emission of chemicals" in grain storage industries, it would be favor of prevention chemicals from human and animals pollution, ecologic destroy and making pollution-free green grain storage coming true.

At the same time, to decrease the use of chemicals to control pests, and then reduce pollution on grain, it would be favor of enterprises breaking green barriers to establish green and ecological imagine and competitive strength the domestic and international markets.

8.2 Gas tightness of Granary Was the Key to Application with CA Technologies with Carbon Dioxide

To achieve all technical indexes requirement of CA granary, the gas tightness performance of granary was essential. The concentration can be maintained during fumigation with carbon dioxide in excellent gas tightness granary. With gas consumption decreasing and costs lowering, the superiority of CA grain storage could be reflected.

Gas tightness inspection should be focus on the sealing effect of walls inside in CA granary, connecting between walls with grounds and so on. Airtight plastic joints, polyurethane and acrylic 885, 991, and other materials could be chosen to fill up cracks among boards and seal granaries at the top of granary (after filling with plastic joints at wider gapes, the treatment of "3

coating and 2 clothing" would take with fiberglass cloth and acrylic again).

To assure the sealing and insulation effect, gas tightness insulating windows and doors, windows axial fans, gas-tight insulation ventilation tube would be chosen, high weathering airtight plastic sealing would be applied. Leakage checking and mending was taken into practice by such methods as observation, listening and so on. After checking gas tightness performance carefully before fumigation, according to the proposal of gas tightness inspection by PT positive pressure, the test on gas tightness was carried on to ensure requires of gas tightness performance.

9 Prospect of Application

To ensuring grain storage in safe and no pollution, controlled atmosphere with carbon dioxide could effectively control grain storage pests and keep grain with safe moisture storage with no chemicals usage. It showed that application of these technologies have gone mature. Their successful application in JiuJiang National Grain Depot, China Grain & Oil Group Science & Technology Corp. and others districts approved that modern grain storage administration has centered on such goals as food quality, high-nutrition, high efficiency and low pollution and low-cost, and developed along the direction of intensive development. With the development and promotion of national economy furthermore, there would be broad application prospect.

10 Acknowledgements

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0507

Air-Tightness Treatment and Performance Analysis of Grain Warehouse in Northeast Region

Liu Changsheng, Cao Yi, Zhou Gangxia, Zhao Xu, Gao Shucheng and Deng Huichao

Abstract: This paper introduces the geographical location and climate characteristics, warehouse type and quantity of the new stored-grain warehouse since 1998 in northeast region, introduced air-tightness treatment situation, the methods of air-tightness test of the large warehouse and survey of the shallow silo in detail, finally, analyzed air-tightness data, put forward suggestions on warehouse building, air-tightness treatment and method of air-tightness test according to analysis results of air-tightness data.

Key words: warehouse; air-tightness; treatment; test

1 Regional Characteristics

The northeast area of China, includes Liaoning, Jilin and Heilongjiang provinces. This region is situated between 120° – 135° east longitude and 40° – 50° northern latitude, which belongs to the northeast cold and wet ecosystem area in the Chinese stored-grain regions. Its climatic characteristic is that air temperature varies to great extent in spring and autumn, sometimes the change exceeds 10℃ each day. In summer, it is very hot in this area with a long sunshine time and plentiful precipitation. The average air temperature is above 15℃. In winter, it is freezing in this area with a short sunshine time. The average air temperature is below 0℃.

2 Grain Warehouse Construction and Air-tightness Treatment

2.1 Grain Warehouse Construction

Northeast region is the major grain-producing area in China, and is known as "Grain Corridor". Therefore, in the 1 990s, there were 61 construction projects in the northeastern region among the world bank's projects in our country, the storage capacity came up to 2.369 billion tonnes, , which accounted for more than 82 % of the total storage capacity of the world bank's project between 1998 to the end of 2002 in the projects of the Central Storing Grain Depot construction, there were 480 construction projects in the northeastern region, the storage capacity came up to 8.418 billion tonnes, which accounted for more than 16. 6% of the total storage capacity. In these projects, there are 60 shallow silo items, capacity of storage was 3.89 billion tonnes, which accounted for the storage capacity of 78% of the total shallow silos. Main warehouse types are large warehouse and shallow silo, the main specifications are showed in Table 1.

Table 1.

warehouse type	Specifications (m)	Height of eave (m)
shallow silo	f30、f25	14.5, 17
large warehouse	60 × 27, 60 × 24	7.8

2.2 Air-tightness Treatment

Main air leakage location, number, air-tight facilities and air-tight materials are shown in Table 2.

Table 2.

Main air leakage location	Number		Main air-tight facilities and air-tight materials
	shallow silo	large warehouse	
doors	1	plastic package slot, plastic film	plastic package slot, plastic film
windows	0	lastic package slot, plastic film	lastic package slot, plastic film
mechanical ventilation window	6	blind plate, rubber pad, bolt	blind plate, rubber pad, bolt
axial fan window	4	air-tight brake gate, plastic package slot, plastic film	air-tight brake gate or plastic package slot, plastic film
circulation fumigation suction pipe	2	air-tight valve	air-tight valve
grain exit	5	air-tight gate	air-tight gate

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Main air leakage location	Number		Main air-tight facilities and air-tight materials
	shallow silo	large warehouse	
grain entrance	1	air-tight gate	air-tight gate
entrance above the warehouse	1	blind plate, rubber pad, bolt	blind plate, rubber pad, bolt
natural ventilation window	4	blind plate, rubber pad, bolt	blind plate, rubber pad, bolt
thermometric cable hole	29 – 31	glass putty, fusion of paraffin and river sand	glass putty or fusion of paraffin and river sand
other punch holes		Concrete, glass putty	Concrete or glass putty

3 Test and Survey on Air-tightness of Warehouse

Air-tightness, which is an important indicator for measuring the level of tightness of grain warehouse under airtight condition, either good or bad, can greatly affect grain storage at a low temperature and pest fumigation effect, it plays an important part in grain safety, so before using new grain warehouse, it's required to achieve the standard named hydrogen phosphide circulation fumigation technical regulations, which says that "the pressure of warehouse should decrease from 500Pa to 250Pa half-life, the time of flat warehouse is no less than 40s, the time of shallow silos and silos is no less than 1 min". We had tested the air-tightness of 105 local large warehouses from October 17th to December 31th in 2001.

Before testing, all of windows and doors were sealed by plastic films and seal layers along the seal groove inside warehouse, plastic films were loose enough to close to doors and windows or inside of anti-bird nets. The doors and windows were then closed; close recirculation fumigation suction pipe and valve of phosphine concentration measuring pipe; according to table2, all that should be sealed includes mechanical ventilation window, temperature measuring cable pipe, lighting cable pipe and local holes of connection between wall and top of warehouse.

According to air-tightness testing technological picture (Fig. 1), make sure that connecting equipment, instrument and air-tight gate, connection should be sealed and kept not leaking. Start Fans until the pressure inside warehouse up to more than 500 Pa, then quickly close fan and gate, record with a stopwatch when the Manometer reading is at 500 Pa, stop

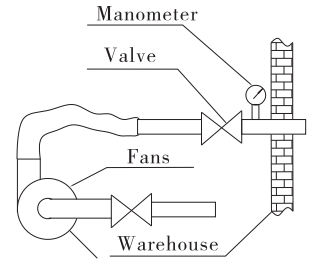

Fig. 1

when the reading is at 250 Pa, repeat the test for three times, record the results separately. During testing, notice that the pressure should not be more than 550Pa, because too much pressure may destroy warehouse; during pressurization, someone should be assigned to observe status of top of the warehouse, if local parts bulge at the top of warehouse, fans and the gate should be immediately closed, and pressurization stopped is required. During pressurization, anyone is prohibited standing outside the doors and windows in order to keep people safe in case that the windows and doors suddenly open.

Early in 2007, our institute gave a survey on the air-tightness of 18 shallow silos in northeast region, treatment method before air-tightness test is shown in table 2, testing methods is the same as that of large warehouse.

4 Results of Test and Survey

Classified statistics is given according to air-tightness data of tested and survey, The results are as follows: the average air-tightness of shallow silos (decrease from 500Pa to 250Pa half-life) are 65 s, the average air-tightness of large warehouse are 48 s.

5 Influencing Factors of Warehouse Air-tightness

5.1 Volume

Through the classified statistics of testing data, the result showed that in the same conditions, generally speaking, air-tightness of large warehouse is better than that of small one. Because to the same height, larger warehouse has larger volume, according to the ideal gas equation $pV = nRT$, it can be deduced that the leakage of air $\Delta n = 250V/RT$ during the pressure decreased from 500Pa to 250Pa, the RT is a fixed number under the same temperature, it is that the air leakage Δn is in direct proportion to the warehouse volume V, the more the volume, the more air leakage during pressure decreases from 500Pa to 250Pa, however, the number of leakage point of the large warehouse is the same

as that of the small one, namely, their air-leakage rate is basically the same, according to time of leakage half-life is equal to the quantities of air leakage divided by air leakage rate, so the half-life of large volume warehouse is longer than that of small volume warehouse.

5.2 Environmental Temperature

During testing, air-tightness of warehouse under high environmental temperature was better than that under low environmental temperature. The reason is that warehouse is usually constructed in summer, the temperature is a little high, when the temperature decreases, leak points can be found uneasily among all parts made up of warehouse, because construction materials expend with heat and contract with cool; at the same time, the environmental temperature, either high or low, impact on the plastic package quality of windows and doors, low temperature leads to poor plastic package quality of the doors and windows.

5.3 Pressure

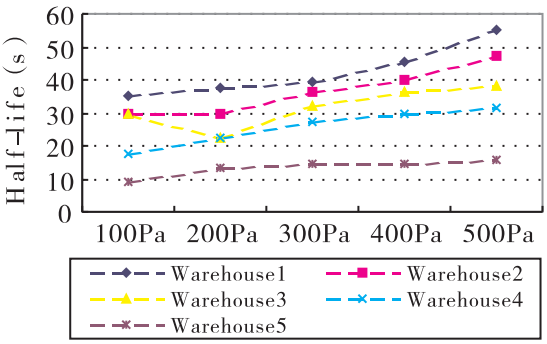

Fig. 2

Test 5 pressure attenuation data of different air-tightness warehouse, the method is that from 500Pa recording the time of 400Pa, 300Pa, 250Pa, 200Pa, 100Pa, 50Pa, until less than 50Pa, then the test data is processed to Fig. 2, Fig. 2. says that for the same warehouse it is showed by one curve, half-life increases as the increase of pressure; for different warehouse, half-life increases as the improve of air-tightness. Half-life of the same pressure of different warehouse is comparable with each other. Local intersect that presents on the curve describes that a few warehouse can bear different pressure in the range of specific pressure, the air in the warehouse leak more or less.

6 Proposal

The structure of all parts of warehouse's top should be constructed according to drawing strictly, especially the sealing construction of connection between panel gap and wall. If treating during late construction or after construction, it needs making facilities for working high above ground, construction will be affected by low temperature, which is hard to treat and will waste lots of human resources and material resources. Therefore, it is hard to achieve the ideal effect.

During the process of constructing warehouse, all kinds of pipes and holes should be sealed anytime. The warehouse is made up of many parts, for example, doors and windows, recirculation fumigation system, mechanical ventilation system, temperature testing system, electrical power distribution system and so on. Each part has different construction units, so it is inevitable to form holes on the body of warehouse. Therefore, each construction unit should be supervised to link up their work and in time plug holes formed after construction. Each units is responsible for itself, workload is small, sealing effect is good.

In order to prevent air leak by tighten too much at blanking plate, the thickness of blanking plate, flanges, pads should not less than 5mm. For reducing weight, when blanking plate is big, the thickness of middle part can be 3mm.

Because of lots of holes on the shallow silo, it is hard to seal, a proposal for adding plastic package slot.

For the warehouse which can not bear 500Pa, it is proposed to test its air-tightness by low pressure half-life, but the standard of air-tightness should less than 500Pa half-life.

Acknowledgements

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0508

Effect of Sealing Treatment for Warehouse and Grain Surface in High Flat Warehouse on Natural Oxygen Reducing

Gu Wenyi[1], Zou Wei[2], Liu Hongyan[3], Mo Dailiang[2], Chen Jibin[2], Niu Quan[2] and Cao Yang[4]

Abstract: The tested warehouse was the No. 8 warehouse of Beihai Grain Depot, State Grain Reserves, the reference warehouse was the No. 6 warehouse of Beihai Grain Depot, State Grain Reserves, the walls of the warehouses were brick-concrete structure and the thickness was 0.7m, the roof of the warehouse was steel roof truss structure, the length of warehouse was 56m, the width was 21 m, the height of grain pile was 6m; the warehouses have been equipped with grain temperature detection system and external fixed recirculation fumigation system. The sealing treatment process for warehouse and grain surface was as follows: performed repairing for cracks on terrace, wall surface and at wall corners with "two cloth and three times coating" method before warehouse-entry of corns; filled and sealed the gaps between vents of ventilating ducts and walls and crevices on wall-passing openings of cables; soft rubber gaskets were changed in the vents of ventilating ducts; after grain loading, performed sealing between the door of the warehouse and grain blocking plate and sealing for grain surface with "double-slot and double-film" and "double-slot and one-film" separately, the grain film was five-layer nylon co-extrusion film. After sealing, performed field airtightness test by negative pressure method with airtightness tester, the average 300Pa half-life was 275s. The average 280Pa half-life of the reference warehouse was 53.7s; it showed that the airtightness of the grain pile after treatment has been improved obviously. Sealed the grain pile of the tested warehouse for 45 days, and the concentration of oxygen in the grain pile was reduced from 20.5% to 9.7%, while the concentration of carbon dioxide increased to 4%. However, for the reference warehouse which has been also sealed for 45 days, the concentration of oxygen was only reduced to 16.1%. It showed that under the conditions of higher grain temperature and better airtightness, the corns produced that year and a higher pest density (23pcs/kg), the respirations of various organisms in the grain pile could reduce the concentration of oxygen in the grain pile and increased the concentration of carbon dioxide obviously. Although it could not kill the pests and microorganisms completely, it could have certain inhibition for growth of entomomycete. This research provides reference for application of low oxygen pest controlling and killing technology.

Key words: grain storage, corn, sealing, airtightness, low oxygen

Preface

With the extended applications of the green grain storage technologies such as gas adjustment and temperature controlling, the airtightness improving technologies for warehouse and grain piles which can ensure that former technologies achieve the best effect and the new technologies of heat insulation have been highly concerned. The existing researches show that ensuring the airtightness of the warehouse is the important condition for ensuring the safety and success of grain storage by gas adjustment[1].

Generally, the airtightness of the warehouse is tested by the pressure decay experiment (Pt experiment) at home and abroad, the "pressure half-life" tested under certain pressure in the Pt experiment can present the airtightness of the warehouse or grain pile; generally, in the same conditions, the longer pressure half-life, the better airtightness of the warehouse[2]. And then set the standards for pest killing by fumigation and gas adjustment according to the pressure half-life tested by the Pt experiment. As specified in the national "Technical procedure for PH_3 recirculation fumigation (trial implementation)", test the airtightness of the warehouse by positive pressure method, the pressure half-life reducing from 500Pa to 250Pa in the empty warehouse should be not less than 40s, and the pressure half-life of the low round warehouse under the same conditions should be not less than 60s[3]. In Australia, it is specified that for the first class warehouse, the time for

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pressure reducing from 2500 Pa to 1500 Pa in the empty warehouse should be not less than 5 min[4].

In order to obtain the better effects of fumigation and gas adjustment, the grain depots in many areas of our country have performed a large number of researches and tests for airtightness improving technology of warehouse and grain pile. For the status that the effects of doors and windows in horizontal warehouses and the effects of grain loading inlets, manholes, vents, ports of temperature measuring cables, terraces and walls in low round warehouses and silos on the integral airtightness of the warehouse were bigger, some grain depots has performed airtightness improving on these places with clear target[5-13]. The CBH company in Australia performed airtightness improvements in the "gas adjustment research" project (CAP), it covered the grain piles with air tight films, sealed the vents with slots and performed spray coating of polyurethane foaming materials on cracks of doors, windows and terraces and crevices of vents in the tested silos, then performed Pt experiment after improvements, the pressure half life reducing from 200Pa to 100Pa was more than 12 min[14].

The standard of airtightness for PH_3 recirculation fumigation of our country has been established to ensure the application effects of chemical fumigation medicines in grain warehouses [15] and provide basic conditions for the effect of fumigation and prevention and killing of drug-resistance pests; however, in aspect of prevention and killing of pests by gas adjustment, the requirement for airtightness of warehouse and grain pile is much higher; for example, the standard of airtightness for pest killing by CO_2 gas adjustment is that the 500Pa half-life should be more than 300s[16]. In order to perform the application demonstration of low oxygen pest killing and controlling technology in Beihai Grain Depot, State Grain Reserves, according to the actual status of grain warehouse in the depot, we performed airtightness improvement for the tested warehouse (No. 8 high flat warehouse), and tested the airtightness of grain pile after sealing, thus creating good conditions for performing the demonstration experiment. We report the process and result of the airtightness improving herein to provide reference for other depots.

1 Materials and Method

1.1 Materials

1.1.1 Tested grains and warehouses

We chose the No. 8 warehouse (corn) of Beihai Grain Depot, State Grain Reserves as the tested warehouse and No. 6 warehouse (corn) of Beihai Grain Depot, State Grain Reserves as the reference warehouse. See table 1 below for the status of the grains.

The tested warehouse was the high flat warehouse built in 1997 of which length was 56m, width was 21m and the designed height of pile was 6m; the walls of the warehouse were brick-concrete structure, with thickness of 0.7 m; the roof of the warehouse was steel roof truss structure and heat insulating materials was used on the surface of the warehouse. The ventilating system was three-set geosyncline ventilating net (one machine and three ways) which has been equipped with three 11kW centrifugal blowers; there were four axial flow fans of which powers were 0.55kW has been equipped on gable walls and bilateral walls; and it was equipped with complete test system for status of grain, and external fixed recirculation pipe network was installed on south side of walls and heat insulation treatment has been performed on these recirculation pipes.

When the warehouses were empty, we could see several obvious cracks on the walls and terraces of the warehouses: the cracks on the walls were relatively small and many of them were caused by empty plump of the rendering layers; there was one bigger crack of which width was 10mm and length was 2 – 4m in the middle of terrace, on the wall surface and at the junction of terrace separately; these cracks were caused by settlement of the ground; In the original design, the vent used rubber mat as the sealing material mostly, and its airtightness could meet the requirement at the beginning basically, but with the prolongation of the using time and increasing of the dismounting times of the cover boards during the ventilating process, the rubber mat became aging, damaged and no elasticity. The filling materials in the joint seams between vents and walls exposed to air for long time, so there were efflorescence and cracking-off phenomenon; and the sealing slots on the grain surface and doors had aging and damaged phenomenon.

1.1.2 Airtightness improving and sealing material for grain surface

Slots, plastic films, sealing plates and seal

Table 1. The status of grain in the tested warehouse

Bin №	kind	Quantity (t)	Stored time	Moirsture (%)	Impurity (%)
6	Yellow	4960	2006.4	13.3	1.0
8	corn	4938	2007.8	13.4	0.9

ants used for airtightness improving of the warehouse

Film sealing slot: made by rigid PVC; specification: 6 – 9mm; produced by Xinliang Storage Equipment Factory, Luqiao, Taizhou, Zhejiang.

Film sealing pipe: made by PVC of which size can match with the slot. Produced by Xinliang Storage Equipment Factory, Luqiao, Taizhou, Zhejiang.

Plastic film: five-layer nylon co-extrusion film; width: 2 – 16m; thickness: 0.08 – 0.12mm; specific gravity: 0.95g/cm^3; oxygen transmissibility: 56mL/m2 · 24h; manufacturer: Hengchang Plastic Factory, Suzhou, Anhui.

Gas sampling tube: PVC soft tube of which inner diameter is 5 × 7 mm; manufacturer: Guangdong Lianjiang Building Materials Factory.

Seam filling materials: sealant, concrete, lime powder, glue and etc. Mainly used for treating various joint seams to ensure the airtightness.

1.1.3 Instruments and equipments for test

Test instrument for airtightness: produced by Henan Future Mechanism & Electron Co., Ltd.; type: CQMY; rated voltage: 380V; air volume: 2 670 – 5 270 m^3/h; total pressure: 990 – 1 580 Pa; main shaft speed: 2900 r/min; power of motor: 3kw; other instruments: U-pressure gauge, stop watch and etc.

Tester for oxygen concentration: PGM – 2000 oxygen concentration tester, range: oxygen concentration 0 – 25%, used for test of the oxygen concentration in the warehouse. Manufacturer: RAE Systems (Shanghai) Inc.

Orsat gas analyzer[17]; QF190 Orsat gas analyzer. Manufacturer: Shanghai Yatai Glass Apparatus Co., Ltd. Used for adjustment of instruments and test of CO_2 concentration.

One set of other assistant tools such as stopwatch.

1.2 Method

1.2.1 Test method for pressure half-life:

Connect CQMY airtightness tester with U-pressure gauge and vent, start the equipment, and when the pressure in the tested warehouse reaches to – 350pa, stop the equipment and close the butterfly valves; begin to time when the pressure is – 300pa and record the time from – 300pa to – 150pa, repeat for 3 times and calculate the average pressure half-life.

1.2.2 Arrangement of gas sampling points

Perform blocking prevention treatment on one end of the gas sampling tube, then fix it on the iron rod which has screw threads on both head and end, and insert it into related depth of the grain pile. Another end of the gas sampling tube is introduced to the external gas tester. Seal the mouth of pipe with rubber. The locations of the gas sampling tubes in the grain pile are shown in figure 1 below; there are three depths for each gas sampling point, i. e. 1m, 3m and 5m away from the grain surface. There are totally 15 test points for gas concentration.

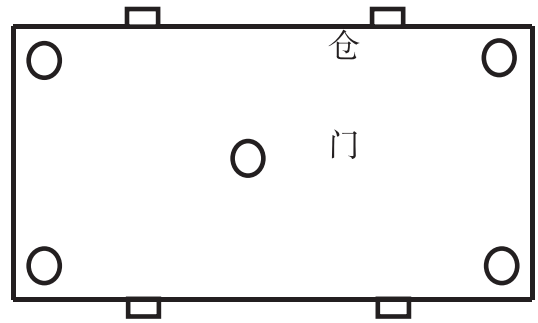

Fig. 1 Arrangement of the gas sampling points in the grain pile

1.2.3 Test method for oxygen concentration

Connect the PGM – 2000 oxygen concentration tester with the gas sampling tube in the gas testing box, start and run the tester until there is no change of concentration, test three times for each point and record the results.

1.2.4 Test method of CO_2

Take the gas from the gas sampling tube with 50mL of 3-way injector, do not take the first ten times, then inject it into Orsat gas analyzer from the 11th time and test the CO_2 concentration in the sample, perform sampling for three times for each sampling tube and take the average value.

1.2.5 Data treatment method

Use Microsoft Excel to perform the date analysis.

2 Result and Analysis

2.1 Technical Process of the Airtightness Improving for the Warehouse

2.1.1 Treatment for seams of terraces,

walls, wall corners and junctions.

First, cleaned the extend the cracks of terraces, walls, wall corners and junctions, then poured concrete and perform rendering and leveling with cement mortar. After drying of mortar, performed repairing with "two cloth and three times coating" method, i. e. brushed one layer of waterproof coating, stuck one layer of fiber cloth, after drying, brushed one layer of waterproof coating and stuck one layer of fiber cloth again, finally, brushed one layer of waterproof coating on the surface of the fiber cloth, the width of fiber cloth was about 500mm. The treatment process was the same as that reported by Chen Yuanzhu basically. [12]

2.1.2 Treatment of the vent

Performed de-scaling treatment for about 20cm on inner wall of the external end of the ventilating duct vent with sand paper to let it to be smooth. Then applied the adhesion agent on the places which have been de-scaled on the inner wall of ventilating duct vent; the width was about 3cm. Stuck silicon rubber mats which have been cut well onto them and compacted them uniformly, and performed curing for more than 24h.

2.1.3 Sealing treatment for grain surface

After grain loading, performed leveling of grain surface and then pressed glued board (arc shape at the wall corners) of which width was 10cm and thickness was 5mm into the wall which was 10cm above the grain storage line of 6m (the original slot was at 2cm above the grain storage line), then fixed the slot in the center of glued board with nails, finally, added the glue into lime powder and mixed them to mash, then performed filling of seams with the mash; the thickness which can just cover the glued board was better and made its surface to be smooth and flat. Performed curing for more than 48h. When performed sealing after complete curing, pressed the five-layer nylon co-extrusion film of which thickness was 0.12mm and the plastic pipe matched with slot into the slot. This formed the sealing of "double-slot and one film" on the grain surface.

2.1.4 Treatment for doorway

Dug a slot of which depth was 3cm and width was 5cm with tools at 5cm near the grain blocking plate on offside of the grain blocking plate and on the wall which was 5cm near the door separately, installed the sealing slots into them and sealed the mouths with lime and cement. The film pressing method was as above. These two slots and two films formed the sealing of "double-slot and double-film". In order to let the films stick to the plate tightly under the positive and negative pressure to relieve the pressure, installed wood plate between two layers of plastic films to fix them.

2.2 The Airtightness of the Tested Warehouse and the Reference Warehouse

Through a series of sealing treatments for walls, doors, vents and inlets of cables in the No. 8 warehouse, performed field test of airtightness by negative pressure method with airtightness tester; see table 2 for the results. The first tested pressure half-life was only 82s, at that time, the voice of air leakage could be heard at the door and the vent obviously, therefore, the intensive problem need to be resolved in the first stage was the sealing treatments for the door and vent. Tested the pressure half-life after improvements of door and vent, the result reached to nearly 200s, 116s higher than that of the first time, however, the airtightness was still not ideal at that time, then changed to use positive pressure, and inspected air leakage places with the soapy water coating method; during the process, we found small part of sealing pipes scaled off from the slot and we organized storekeepers to perform slot pressing treatment for sealing pipes; performed checking and repairing of leaks for sealing slots and sealing films of grain surface, after that, tested the pressure half-life for the third time and the half-life increased to 275s, 3.3 times bigger than that of the first time (82s).

The door and grain surface of the No. 6 warehouse were sealed with single plastic film. Since the airtightness of the installed sealing slots and hoses were not good at the building period of the warehouse and some parts were aged and damaged, when performed negative pressure test with the airtightness tester, the max. negative pressure only could reach to 280Pa (see table 2), and the time for pressure reducing to 140Pa was 53.7s.

The airtightness of the tested warehouse was better than that of the reference warehouse obviously.

Table 2. Test results of the airtightness

No	Date	Range (Pa)	Half-life (s)	Average half-life (s)	Remark
8	10.13	−300 – −150	90 79 77	82.0 ±4.0	Major air leakage places were doors and vents
8	10.15	−300 – −150	202 196 198	198.7 ±1.8	Cushion of the vent was replaced to soft rubber; used sealing of "double-film and double-slot" for doors.
8	10.16	+200 – +100	95	–	Inspected leaks with the soapy water coating method and found that the joint of airtightness tester and vent was not good, then treated with sealant
8	10.19	+200 – +100	43	–	Sealing films for grain surface and doors scaled off from slots
8	10.22	−300 – −150	277 276 273	275.3 ±1.2	Sealed and pressed slots for sealing films for grain surface and doors again, repaired leaks of films for grain surface
6	10.18	−280 – −140	56 53 52	53.7 ±1.2	As to reference warehouse, the increasing time of the pressure was very short and the max. Pressure only could reach to 280Pa, and the airtightness was bad

2.3 Natural Oxygen Reducing Result after the Sealing of Grain Pile

From table 3, we can see that after 45 days sealing of grain pile for the No. 8 warehouse, the oxygen concentration in the grain pile was reduced from 20.6% to 9.7% and the CO_2 concentration increased from 1.0% to 4.0%. Through analysis, the reasons may be:

1) Relatively higher density of pests. In the air tight environment, the breath of pests will consume oxygen gradually and accumulate CO_2 at the same time. From the sampling and testing, we found that the density of pests reached to 23pcs/kg, in which, *Cryptolestes pusillus* (Schonherr): 7; *Sitophilus zeamais* Motschulsky: 1; *Tribolium castaneum* (Herbst): 2; *Cryptolestes ferrugineus* (Stephens): 10; larva of *Plodia interpunctella* (Hubner): 3.

2) There was certain quantity of microorganisms in the corns obtained that year, and through the breath, microorganisms could reduce the oxygen in sealed grain pile. Some researches show that, microorganisms have excellent oxygen reducing effect[18].

3) The corns obtained that year had relatively stronger breath effect. Since the characteristics such as bigger embryo of corn, both new grain and old grain had very strong oxygen reducing effect[18].

We can see that good airtightness of the grain pile can reduce the oxygen concentration obviously.

Table 3. Changes of concentrations of oxygen and CO_2 in the tested warehouse under the air tight condition

Date[1]	AOC[2] (%)	ACO_2C[3] (%)	TW[4] (℃)	ATW[5] (℃)	T[6] (℃)
Sep. 15, 2007	20.6	0.5	27.5	18.0	27.6
Oct. 15, 2007	14.8	1.0	26.2	21.2	24.0
Oct. 22, 2007	12.3	2.9	25.7	21.8	22.8
Oct. 29, 2007	10.2	3.6	27.1	22.4	22.7
Oct. 30, 2007	9.7	4.0	25.1	22.6	17.6

Note: 1 Test time on 9:00 am; 2 Average oxygen concentration; 3 Average CO_2 concentration; 4 Temperature of warehouse; 5 Average temperature of warehouse; 6 Temperature

From table 4, the No. 6 reference warehouse was also sealed for 45 days and the oxygen concentration in the grain pile was reduced form 20.6% to 16.1% which was 6.4% higher than that of the tested warehouse; it showed that the oxygen concentration was reduced, but the range of reducing was smaller than that of the tested warehouse. Through analysis, the rea-

sons may be:

The airtightness of the reference warehouse was much worse than that of the tested warehouse, the diffusion of the gas concentration could reduce the oxygen concentration in the warehouse;

1) The density of pests in the reference warehouse was lower. Through sampling and testing of the grain pile, the density of pests was 2/kg;

2) Through low temperature storage of the stored grain in the reference warehouse for a period of time, it inhibited growth of partial microorganisms.

Table 4. Change of oxygen concentration in the reference warehouse (No. 6 warehouse) under the air tight condition

Test time (9:00am)	Average oxygen concentration (%)
May 21, 2007	20.6
May 31, 2007	19.9
June 10, 2007	18.6
June 20, 2007	17.2
June 30, 2007	16.5
July 5, 2007	16.1

3 Discussion

Although the origin of film sealing technology for grain pile is relatively earlier and it has been used extensively, the reports of airtightness testing after sealing and reducing degree of oxygen are few, and it is lack of strong support for this film sealing technology, however, this technology can ensure the effective implementation for green low oxygen stored grain, it is the precondition for effective inhibiting of growth and reproduction of pests and microorganisms under low oxygen condition. Luotian depot in Hubei has performed related research[18]; for the wheat of which water content was 12.2%, performed natural oxygen reducing for 55 days under the status that there were pests detected (24/kg), and the oxygen concentration was reduced to 12% around. In 1970s, Xiamen, Fujian performed test on paddy with low water content, performed five-face sealing for 1 million kgs of early paddy of which water content was 12.3%, after storage for a period of time, the content of oxygen in the grain pile was stable at 2% -6%; through testing, we found that the quantity of pests in the warehouse was inhibited. At the same period, Taicang, Jiangsu and other areas performed oxygen-reducing storage test over summer for late japonica rice, after sealing with PVC films on grain pile for a period of time, the oxygen content in grain pile was stable at 2% - 5%, and there was no pest through test[18]. These results were compatible with this research basically, except they did not test the airtightness of the grain pile and thus detailed comparison between the result of this research and that of them could not be performed. We suggest that in future researches and experiments, it would be better if each experimental organization can test the airtightness of the grain pile and warehouse after sealing to obtain a large number of data which can be used for reference and comparison, and establish the basis for gas adjustment and fumigation technologies.

This test also shows that through natural oxygen reducing, the oxygen concentration in the grain pile of which airtightness is relatively good also can be reduced to less than 10%. Although pests and microorganisms can not be killed completely, it has certain inhibition for growth of entomomycete, and provides better conditions for applications of pest controlling and killing by gas adjustment and fumigation technologies and establishes base for the next manual and mechanical oxygen reducing experiment. However, the further experiments and researches are still needed for the inhibiting effect of natural oxygen reducing on stored grain pests and mold.

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SESSION 6

EFFECTS ON INSECT CONTROL AND ECONOMIC THRESHOLDS (ET)

Chairpersons:
Wang Dianxuan, China
Greg Daglish, Australia

0601

Combining the Benefits of Cooling and Phosphine Fumigation to Meet the Biosecurity Challenge Posed by Grain Insects

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Abstract: Two of the best options for Australian farmers to manage insects in stored grain are aeration cooling and phosphine (PH_3) fumigation. Farmers are being encouraged to use aeration to preserve grain quality and to slow insect population growth. However, insects are surprisingly difficult to kill with cold and the temperatures achieved using aeration in Australia are unlikely to result in adequate control. Therefore, the best strategy would be to combine aeration and fumigation to meet market demands for grain free of live insects. This paper summarises laboratory and field research addressing the question of whether Australian farmers can successfully fumigate cool grain. Published data show that there are minimal differences between the most PH_3 – resistant Australian strains of the rice weevil (*Sitophilus oryzae*) and lesser grain borer (*Rhyzopertha dominica*) at 25℃, but we found that resistant rice weevils were much harder to control in cool grain. Loss of gaseous PH_3 through sorption into the grain kernels can reduce the amount of PH_3 to which insects are exposed, particularly in a highly sorptive grain such as sorghum. We found that sorption was lower in cool sorghum grain resulting in higher average concentrations. Sorghum was less sorptive the longer it was stored before being fumigated for the first time, also resulting in higher average concentrations. These trends were observed to a lesser extent in wheat. These laboratory results suggest that farmers would achieve the best results by cooling the grain first and fumigating later. Field trials have been conducted in silos of up to 158 m^3 capacity in three states, indicating that fumigating cool grain is a useful option for farmers who have sealable silos.

Key words: Phosphine, fumigation, resistance, temperature, sorption, silos

Introduction

Stored grain insects represent a biosecurity threat in Australia, because a zero tolerance for live insects exists for export grain and this standard often applies to grain being sold within Australia. Two of the best options for Australian farmers to manage insects in stored grain are aeration cooling and phosphine (PH_3) fumigation. Farmers are being encouraged to use aeration to preserve grain quality during storage and to slow insect population growth. In addition, natural cooling may result when grain is stored into the cooler months. However, insects are surprisingly difficult to kill with cold[1], and the temperatures achieved by Australian farmers using aeration are unlikely to result in adequate insect control. Therefore, the best strategy would be a combination of aeration and fumigation to meet market demands for grain free of live insects. A significant effect of lowering temperature, however, is that PH_3 efficacy is lower at lower temperatures[2]. Sorption, however, is likely to be lower cooler grain resulting in higher average PH_3 concentrations[3]. A study was undertaken, therefore, addressing the question of whether Australian farmers can successfully fu migate cool grain. This paper summarises the key findings from laboratory and field research undertaken during this study.

Materials and Methods

Efficacy Experiments

Efficacy experiments were conducted based on published methods[4,5]. Essentially, wheat containing mixed-age cultures (i. e. eggs, larvae, pupae and adults) were exposed to constant PH_3 concentrations at 15℃. Samples were taken at intervals during each fumigation, any live adults were recorded, and if no live adults were found the samples were incubated at 25℃ for 10 wk to allow any surviving eggs, larvae or

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pupae to complete development. The two strains chosen were a strong resistant strain of *Rhyzopertha dominica* (F.) and a weak resistant strain of *Sitophilus oryzae* (L.). The resistance factors based on adult mortality were about 600 times at 48 h for *R. dominica*)[6], and about 10 times at 24 h for *S. oryzae*[4].

Sorption Experiments

The general methods used in the sorption experiments were based on those of Daglish and Pavic[7]. Glass flasks(2 L capacity) were filled to 95% of volumetric capacity were injected with PH_3 at a dose of 1.5 mg/L based on the volume of the empty flasks. This dose equates to an application rate of 1.5 tablets of aluminium phosphide(AlP) per cubic metre of empty silo volume. Phosphine concentration was measured at intervals during storage beginning 2 h after injection and ending 11 days after injection. Grain was collected either at harvest or after a period of storage in farm silos, and generally stored frozen until shortly before the experiments began.

Farm Silo Trials

The approach used in the fumigation trials was based on that of Newman et al[8]. Essentially, silos containing grain were pressure-tested and then fumigated following the Australian label for AlP, i.e. 1.5 tablets(= 1.5 g PH_3) per cubic metre of silo capacity, and PH_3 concentrations were measured within the grain bulk during the fumigations. In all fumigations, concentrations were measured at approximately 3 – 5 points long the vertical axis of the silo, and in some cases concentrations were measured at other locations around silo. Concentrations were measured by attaching nylon sample tubing to Canary™ Silo Chek' monitors, and temperatures measured using a thermocouple or Tinytag™ data-logger. Although a total of 19 trials have been in three states (Queensland, New South Wales and Western Australia), only the results of one fumigation trial in New South Wales are reported in detail in this paper.

Two silos each of 58 m^3 capacity were used. One was 50% full with barley(11% mc) and served as the control silo, and the one that was fumigated was 40% full with wheat(11% mc) and the pressure halving time for this silo was 300 seconds. The grain in each silo had cooled naturally and had not been aerated. Mixed-age cultures (eggs, larvae, pupae and adults) of each insect species were placed into separate cages. A strong resistant strain of *R. dominica* and a susceptible strain of *S. oryzae* were used. Each cage was probed into grain in a sealed silo to a depth of 1 – 2 m. The wheat was then fumigated, by applying 100 tablets spread out on a tray in the headspace, and PH_3 concentrations and grain temperatures monitored daily from 15 – 27 August 2007. On completion of the fumigation, insect cages were removed and the grain within checked for live adults. All adults (live and dead) were removed and the remainder of the grain placed in jars, with some culture medium, were stored for 10 wk at 25℃, 65% rh. At this time the grain was sieved and checked for live adult insects to determine whether eggs, larvae or pupae survived the fumigation. To ensure that insects did not die from cold alone, cages of insects were placed into the control silo and treated exactly the same as the fumigated insects except that they were not fumigated.

Results and Discussion

Efficacy Experiments

We believe that the strong resistant *R. dominica* strain and the weak resistant *S. oryzae* strain used in this study reflect the strongest PH_3 resistances present in these species in Australia. Published data show that there are minimal differences between these two strains when they are fumigated at 25℃[4,5], but we found that the weak resistant strain of *S. oryzae* was much harder to control at 15℃. This shows that the relevant importance of resistant strains from different species depends on temperature.

Table 1. Results of phosphine fumigation of mixed-age populations of *Rhyzopertha*

Days elapsed	ive adults(Mean ± SD, n = 2) recovered from wheat after 8 wk incubation at 25℃.		
	R. dominica(Strong resistant)	*S. oryzae*(Weak resistant)	*C. ferrugineus*(Susceptible)
Concentration = 0.3 mg/L(210 ppm)			
0	783.0 ± 335.2a	4016.0 ± 340.8a	280.5 ± 87.0a
6	6.0 ± 1.4b	652.0 ± 5.7b	5.0 ± 0.0b
7	0.5 ± 0.7c	351.5 ± 2.1b	1.0 ± 1.4c
8	0.0 ± 0.0c	311.5 ± 94.0b	0.0 ± 0.0c

Days elapsed	ive adults (Mean ± SD, n = 2) recovered from wheat after 8 wk incubation at 25℃.		
	R. dominica (Strong resistant)	*S. oryzae* (Weak resistant)	*C. ferrugineus* (Susceptible)
9	0.0 ± 0.0c	96.0 ± 87.7c	0.0 ± 0.0c
10	0.0 ± 0.0c	97.5 ± 41.7c	0.0 ± 0.0c
11	0.0 ± 0.0c	7.5 ± 3.5d	0.0 ± 0.0c
Concentration = 1 mg/L (700 ppm)			
0	1048.0 ± 306.9a	4325.0 ± 312.5a	317.0 ± 142.8
8	82.0 ± 5.7b	618.0 ± 281.4a	0.0 ± 0.0
9	1.0 ± 1.4c	456.0 ± 176.8ab	0.0 ± 0.0
10	0.0 ± 0.0c	14.5 ± 2.1bc	0.0 ± 0.0
11	0.0 ± 0.0c	70.0 ± 97.6bc	0.0 ± 0.0
12	0.0 ± 0.0c	47.0 ± 63.6bc	0.0 ± 0.0
13	0.0 ± 0.0c	0.5 ± 0.7c	0.0 ± 0.0

Within each dose and species, means in columns followed by different letters are significantly different ($P < 0.05$) based on analysis of transformed data.

Sorption Experiments

Sorption by grain can reduce the amount of PH_3 to which insects are exposed. Table 2 shows the results of some of the sorption fumigations completed during the study. Percentage daily sorption was lower at 15℃ than at 25℃ in wheat and sorghum, meaning that cooler grain will have higher average concentrations than warmer grain, countering to some extent the problem of lower PH_3 efficacy at cooler temperature. Percentage daily sorption tended to decrease with age of grain meaning that delaying fumigation of grain may yield higher average concentrations. Table 2 shows that sorghum was much more sorptive than wheat, but the sorghum was also moister than wheat and rate of sorption is related to moisture content[3]. However, sorghum which had been stored for 3.5 months in a farm silo and was 12% mc was still more sorptive than wheat.

Table 2. Effect of storage at two temperatures on sorption in wheat and sorghum fumigated at 1.5 mg/L of flask volume. Mean PH_3 concentration after 2 h was 2.77 (SD = 0.05) mg/L for wheat and 2.49 (SD = 0.08) mg/L for sorghum.

Grain	Temperature (℃)	Approximate age of grain* (m)	Moisture content (%)	Daily sorption (%)
Wheat	15	0.5	12	4.2
		1	12	3.5
		2	12	3.9
		4	12	4.4
	25	0.5	12	9.4
		1	12	6.9
		2	12	6.4
		4	12	5.2
Sorghum	15	0.5	15	22.4
		1	15	15.6
		2	15	13.3
		4	14	11.9
	25	0.5	15	37.5
		1	15	27.9
		2	14	20.7
		4	14	16.1

* Ignoring time stored at −15℃

Farm Silo Trials

Nineteen trials were completed in sealable farm silos and the results cannot be given in detail here. As with earlier research on farm silos of this size (≤158 m^3 capacity)[7], we found that PH_3 concentrations tended to be lower deeper in the grain mass. In most cases we have assessed the fumigation success by comparing concentration x time profiles achieved in silos with the known responses of resistant strains of *R. dominica* and *S. oryzae* to PH_3 under laboratory conditions. However, information on efficacy against such strains is only available down to 15℃ and grain temperature in some of our trials was < 15℃. In the fumigation described in the Materials and Methods, cages of mixed − age cultures were actually inserted into the grain mass to confirm fumigation success in grain at < 15℃. Mean grain temperature measured at 2

m from the grain surface was very stable with 10.1℃ (SD = 0.3). The corresponding result for the control silo was 11.0℃ (SD = 0.1). Moisture content of the grain in both of these silos was 11.4%. Concentrations measured at three depths along the central axis are shown in Table 2. The lowest readings were measured at 5 m depth and these ranged from 35 to 888 ppm. There were no live adults of either the strong resistant strain of *R. dominica* or the susceptible strain of *S. oryzae* in the assessment made 10 wk after termination of the fumigation, even though there were on average 60.5 (SD = 17.7) and 38.5 (SD = 13.4) live adults were recovered from the corresponding controls.

Table 3. Phosphine concentrations (ppm) during fumigation of a 58 m^3 silo 40% filled with wheat (11.4% mc).

Days elapsed	Depth from top of silo (m)			
	5.0	3.0	1.0 (headspace)	Mean ± SD
1	35	125	128	96 ± 53
2	107	278	277	221 ± 98
3	208	413	408	343 ± 117
4	312	530	520	454 ± 123
5	436	669	658	588 ± 132
6	564	755	745	688 ± 108
7	634	803	796	744 ± 96
8	710	864	850	808 ± 85
9	789	918	900	869 ± 70
10	853	946	927	909 ± 49
11	836	943	926	902 ± 58
12	888	945	927	920 ± 29

Conclusion

We conclude that PH_3 fumigation of cool grain, i. e. grain has cooled naturally or has been cooled using aeration, is a useful option for Australian farmers who have sealable silos. Insect population growth will be zero or negligible in the cool grain, and fumigation can be used to control whatever insects are present at low densities.

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Survey and Analysis of Economic Thresholds for Insect Pest Control in Grain Storage in the Fujian Area of China

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Abstract: With reference to flour beetles and boring beetles, grain type, and fumigation temperatures, and so on, the basic relationship between stored grain insect control and economic thresholds were investigated by analyzing benefit and cost of the control practices in seven depots of State Grain Reserves (159 warehouses holding 891,700 t of grain) in Fujian province. The following main conclusions could be drawn. First, the economic threshold for boring beetles in stored grain was lower than that for flour beetles, and the reduced economic loss was more than control cost for boring beetles at the same condition. Second, the economic threshold for wheat and maize was lower than that for paddy rice, and treatment cost for controlling the same insects in wheat and maize was lower than the reduced economic loss at the same condition. Third, the economic threshold was lower when the grain temperature above 20℃, and higher when the grain temperature was below 15℃.

Key words: Fujian province, insect control, economic threshold

Introduction

The optimal environmental conditions for common stored grain insects are over 20℃ and 70% relative humidity. The high temperature and humidity climate of Fujian province is therefore very favorable for such insects. About 3.5 million t of stored grain requires insect control every year in Fujian. For insect control, factors such as insect species, grain type, grain temperature are relevant to economic thresholds. Most grain storage units implement phosphine fumigation when finding insects, without considering economic thresholds. This results in 3 – 4 fumigations on average for each batch of grain in storage, with significant social, economic and environmental impacts. These include increased insect resistance and associated difficulty in controlling insects, accelerated grain spoilage and contamination, shortened storage time, and decreased selling price. Therefore, direct and indirect economic value is very high when considering if insect control should be taken in Fujian province. Providing a relationship between insect control and economic threshold in a high temperature and humidity area based on insect species, grain type, control opportunity, and so on, to guide insect control practices in grass – root depots, will have great social, economic and environmental benefits.

Survey of Integrated Stored Grain Insect Pest Control in Fujian

Climatic Characteristics and Common Stored Grain Insects

Fujian province is located on the southeast coast of China. Most of Fujian experiences high temperature and humidity typical of sub-tropical coastal China, with warm winters, hot summers, and high rainfall. Average annual temperature is 17 – 21℃, with temperatures sometimes exceeding 40℃ for extended periods, and average annual relative humidity is 76 – 84%, so conditions are favorable for insect population growth. It was found that the frequency occurrence of stored grain insects, was over 50% by investigating stored grain insects species, damage in Xiamen, Sanming, Shaowu, Fuzhou, Putian, Zhangzhou, Quanzhou, from April to May in 2004[1], 2005, and 2006, and from June to September, 2007. Pest species included *Rhyzopertha dominica* (Fabricius), *Sitophilus zeamais* (Motschulsky), *Sitophilus oryzae* (Linnaeus), *Alphitobius diaperinus* Panzer, *Sitotroga cerealella* Oliver, *Tenebroides mauritanicus* (Linnaeus), *Tribolium castaneum* (Herbst), *Tribolium confusum* Duval and *Cryptolestes ferrugineus* (Stephens). of these species, *R. dominica*, *S. zeamais*, *S*, *cerealella*, *T. confusum* and *C. ferrugineus* were the most common, which greatly increased insect control difficulty and cost of depots of State Grain Reserves in this region of high temperature and humidity.

Investigation of Storage Equipment in Depots

1. *Fujian Branch of China Grain Reserves Corporation*, *Fuzhou* 350001
2. *Xiamen Depot State Grain Reserves*, *Xiamen* 361012
3. *Shaowu Depot State Grain Reserves*, *Xiamen* 354000

Depots in Fujian province are distributed very widely, and the storage equipment in the various warehouse differ greatly from each other. Most of the large warehouses, squat silos and other silos were built after 1998, and account for 28.2% of storages in Fujian. They have high levels of airtightness and heat insulation, and they are equipped with mechanical ventilation, recirculation fumigation, electronic temperature monitoring system, etc., which offered excellent hardware equipments for insect control. Most of warehouses, horizontal warehouse of Soviet, arch plate depots were built after the 1980s, accounting for 71.8% of storages in Fujian. They have low standards of airtightness and heat insulation, and most do not have mechanical ventilation, recirculation fumigation, or an electronic temperature monitoring system. Thus insect control is difficult and very costly.

The Present Insect Control Condition of Grass-root Depots

In the early stages of the establishment of the state grain reserves management system, the basic requirement of state and grain administration departments for grass-root depots was for "Four No's depots", i.e. no insects, no mould, no rodents or birds, and no accidents. In order to realize the requirement of no insects, most of grass-root depots took a "saturation management" strategy, i.e. immediately implementing fumigation once insects appeared. After the establishment of the Fujian vertical administration system of State Grain Reserves, insect control strategy was adjusted to an integrated pest management (IPM) strategy. Provided no influencing on safety grain storage and grain quality, integrated insect management (IPM) was positively popularized in Fujian. The amount of green grain storage without fumigation increased from 0% in 2003 to over 20% in 2008. Some warehouses achieved excellent results, with only one fumigation in 2 or even 3 years. Thus, over 20% stored grain in depots directly under State Grain Reserves are not fumigated.

Discussion on Application of Economic Threshold theory during Insect Control

Primary Principle of Economic Threshold

The primary principle of economic threshold of insect control is that control cost should be less than or equal to reduced economic loss. Sheng Cheng-fa [2] put forward the newest definition by integrating different definitions expounded by entomologists and agro-economists based on different understanding, i.e. the control measures should be taken when insect density reaches a definite density, otherwise, the insect will bring an expected loss which equals the expected cost of the control measures. Thus, economic threshold can be understood as degree tolerance before insect control. The higher the economic threshold, the higher degree of tolerance to the insect. Conversely, the lower the economic threshold, the lower degree of tolerance to the insect.

The impact factors of economic threshold are various, including the relationship between environmental factors and insect survival, development and reproduction, type and degree of damage caused by different insects, population density and injury level, suitable control opportunities, the availability and cost of control measures, selling value of the grain, and so on. The general model of economic threshold is the following equation [3].

$$ET = CF/EYHDS$$

Where ET is economic threshold, C is control cost, F is social adjustment factor, E is control effect, Y is yield when no insects, H is production price, D is yield loss damaged by per unit insect, and S is insect population natural livability. For insect control, Y should be grain natural storage quantity. Therefore, economic threshold is not invariable, and it will change with environmental conditions, insect species and its damage potential, production value, etc. The essence of economic threshold put forward by Sheng Cheng-fa [2] is a multidimensional, dynamic, random economic-ecology parameter, its theory value can not be known. The economic threshold should be estimated in relation to relevant major factors in storage practice.

The Relationship Between Stored Grain Insect Control and Economic Threshold in Fujian

The Relationship Between Different Kinds of Stored Grain Insect and Economic Threshold

The damage of boring insects in stored grain

In practice, the major stored grain boring insects are *R. dominica*, *S. zeamais*, *S. cerealella*, and their frequency of occurrence was about 85%. By trace-back investigation of 18 warehouses with the records of no fumigation and no stored grain at present time, it was found that boring insects occurred and were controlled with IPM measures in 13 warehouses, and the cost of

control was 0.10 – 0.15 yuan/t. As a result of taking timely and suitable control measures, the warehouses implemented only one time during 3 years storage, 2 times for 4 years storage, not only saving control cost 0.55 – 1.15 yuan/t, but also delaying grain spoilage and improving grain quality. The grain selling price was 20 – 40 yuan/t higher than that of grain in other warehouses not being timely controlled at the same condition.

By analyzing fumigation data in past years in Fujian depots directly under central grain reserves, it was found that if control measures were undertaken when average grain temperature is over 20℃ or over 25℃ in sites of insect occurrence in the grain mass, or the density of boring insects (e. g. *R. dominica* and *S. zeamais*) is over 5 – 10/kg, then control was relatively good and control cost relatively low.

The damage of flour insects in stored grain flour

The most common major flour insects are *C. ferrugineus* and *T. confusum*, and their frequency of occurrence is about 80%. By investigating 18 warehouses with the records of no fumigation and no stored grain at present time, it was found that fumigation was not immediately taken when flour insects occurred in five warehouses. They implemented IPM measures, i. e. taking some low cost control measures, such as trapping, and mechanical and physical treatment. The cost of control was 0.05 – 0.10 yuan/t. As a result of taking timely and suitable control measures, these warehouses implemented only one time for 3 years storage period, two times for 4 years storage period, not only saving control cost about 1.5 yuan/t, but also delaying grain spoilage. Their grain selling price was 20 – 30 yuan/t higher than that of grain in other warehouses not being timely controlled at the same condition.

It should comprehensively consider control cost when flour insects occurring. It is not necessary take chemical control, and IPM measures should be taken when no or a few boring insects occurring, and local *C. ferrugineus* and *T. confusum* density below 15 – 25/kg.

The Relationship between Stored Grain Kinds and Economic Threshold

By investigating the insect resistance in the three major grains (wheat, corn and paddy rice), it was found that the insect resistance in wheat and corn was comparatively weak, being susceptible to infestation by stored grain insects. The unsound kernels universally increased in infected wheat and corn, average increasing by about 1%, and more infected kernels, accordingly more kernels, by investigating the insect resistance of wheat stored in eight warehouses holding 20 000 t for two years in Xiamen grain purchasing and storage company, and the insect resistance of corn stored in eight warehouses holding 47 500 t for one year in Fuzhou, Putian and Xiamen depots directly under central grain reserves. It is obvious that insects give birth to more negative effect on wheat and corn quality, and less effect on paddy rice quality at the same condition.

Therefore, insects will bring more production loss of wheat and corn at favourable environmental condition. Meanwhile, since the absorption capability of paddy rice is stronger than that of wheat and corn, the dosage in paddy rice is often higher than that in wheat and corn during fumigation. According to practical experiences of depots directly under central grain reserves in recent several years, the dosage per tonne of paddy rice is often about 1.2 g higher than that of wheat and corn during fumigation under the same conditions, and its control cost is higher than that of wheat and corn. Therefore, IPM measures should be first taken when insects occurring in paddy rice, trying to reduce chemical fumigation.

The Relationship between Stored Grain Temperature and Economic Threshold

By tracing investigation on insect occurring and developing condition in 54 warehouses fumigated in the same year, it was found that the grain temperature in 42 warehouses was over 20℃, accounting for 78%, and the grain temperature in 12 warehouses was 20 – 15℃ during fumigation, accounting for 22% among the warehouses keeping no insect occurring for over 12 months next year after fumigation. The grain temperature in 18 warehouses was over 20℃, accounting for 78%, and the grain temperature in 5 warehouses was 20 – 15℃ during fumigation, accounting for 22% among the 23 warehouses keeping no insect occurring for over 18 months next year after fumigation. According to general model of economic threshold ET = CF/EYHDS, Svalue (insect population natural livability) increased, Evalue (control effect) also increased, and then ET value decreased.

Therefore, the insect control economic threshold is comparatively small when the grain temperature is over 20℃. Because the growth, development and reproduction of most of stored grain insects are inhibited when the grain tem-

perature is below 15℃, insect population natural livability is very low, control effect is bad, insect resistance is easily be induced, economic threshold also accordingly increases, thus trying to avoiding chemical fumigation.

The Relationship between Insect Control Measures and Economic Threshold

When insect density reaches the unendurable level in practice, phosphine fumigation is the most economic, convenient and effective measure. One time fumigation cost was about 0.6 yuan/t in depots directly under central grain reserves in Fujian based on investigation, including PH_3 quantity average 0.2 yuan/t, fumigation nutrition subsidization about average 0.25 yuan/t, labour wage, electricity expenses, and depreciation expenses of fumigation machine, plastic film and other fixed assets about average 0.15 yuan/t. But the fatty acid value of grain after fumigation will increase 2% – 3% in high temperature seasons, which accelerates grain spoilage, shortens storage time, increases grain alternation times with increasing cost about 50 yuan/t. In addition, the grain color and odor also is changed after fumigation, directly influencing grain selling price. The selling price of grain after several times fumigation is about 20 – 40 yuan/t lower than that of grain with no fumigation.

Discussion and Conclusion

Though the direct cost of chemical fumigation is comparatively small, it brought indirect huge economic loss. Hence, the control principle "prevent first, integrated control" should be carried out during grain storage, taking endurance philosophy to manage insects. Positively take integrated measures of non-chemical control to inhibit insects occurring, try to reduce chemical fumigation, especially avoid chemical fumigation in whole warehouse.

The damage of boring insects is more than that of flour insects. According to general model of economic threshold ET = CF/EYHDS, D value, i. e. yield loss damaged by per unit boring insects is more than that of per unit flour insects. Furthermore, for the most common flour insect, *C. ferrugineus*, due to having the highest resistance, control cost is higher than that of most of boring insects in Fujian area. Therefore, if the other condition is invariable, the economic threshold of boring insects is lower than that of flour insects in Fujian area, i. e. boring insects should be taken chemical fumigation when its density is very low, and flour insects may be taken chemical fumigation when its density is very high. However, the relationship is not absolute. Because of different insect resistance, the control cost is a little difference.

The economic threshold of wheat and corn is lower than that of paddy rice, i. e. for wheat and corn, control measures should be taken when insect density is very low, and for paddy rice, control measures may be taken when insect density is very high. But insect infection probability is very different even at the same condition for the same stored grain species. Dry, clean and sound grain kernel is hard to be infected, contrarily, it is easy to be infected.

The economic threshold of insect control is very low when the grain temperature is over 20℃. When the grain temperature is below 15℃ which can inhibit most of stored grain insects growth, development and reproduction, insect population natural livability is very low, control effect is relatively bad, insect resistance is easy to be induced, and the economic threshold is accordingly increasing. Thus, it need try to take integrated control measures and reduce chemical fumigation.

All in all, in order to reduce the insect control economic threshold, various factors should be integratively considered to select suitable control opportunity and measures. The following are the detailed suggestions:

1 Fumigation should be timely taken when average grain temperature is over 20℃ or insects occurring sites temperature is over 25℃ in wheat and corn warehouses, and when *R. dominica*, *S. zeamais*, and other boring insect population density reaches over 5 – 10/kg more than 3 parts of the grain mass, here control cost very low.

2 Temperature should be first decreased and fumigation done when average grain temperature is over 30℃ for corn and paddy rice, and over 35℃ for wheat. Not doing this will result in accelerated grain spoilage, a shorter storage period and lower selling price.

3 The effectiveness of fumigation is very good when average grain temperature is 20 – 30℃. When average grain temperature is below 15℃, chemical fumigation should be avoided and non-chemical control measures should be taken, or else insect resistance may be induced.

4 Controlled temperature grain storage, behaviour control, physical and mechanical control, biological control, local fumigation control, and other techniques should be positively taken to inhibit insect development, to reduce fumiga-

tion and reduce insect control economic threshold, when no insect or less insects existing, and when *C. ferrugineus* , *T. confusum* and other flour insects density is below 15 – 25/kg.

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Fumigant Effect of Essential Oils of Several Species of Plants on *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae)

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Abstract: Fumigant effects of essential oil vapours from 14 species were studied on adults of *Sitophilus zeamais* (Motschulsky). The oils came from *Mentha haplocalyx*, *M. spicata*, *Illicium verum*, *Myristica fragrans*, *Alpinia officinarum*, *Cinnamomum parthenoxylon*, *Acorus tatarinowii*, *Brassica juncea*, *Capsicum annuum*, *Litsea cubeb*, *Curcuma longa*, *Artemisia princeps*, *Pogostemon cablin* and *Cymbopogon citratus* was tested. Eight essential oils had fumigant activity on *S. zeamais* adults and oils from *M. haplocalyx* and *M. spicata* were the strongest. The LC_{50} values for oil from *M. haplocalyx* under the exposure periods of 24, 48 and 72 h were respectively 11.53, 9.49 and 7.93 μL/L. For *M. spicata* oil, LC_{50} values for 24, 48 and 72 h were respectively 13.43, 11.36 and 9.20 μL/L.

Key words: plant essential oils, *Sitophilus zeamais* (Motschulsky), fumigant effect, *Mentha haplocalyx*, *Mentha spicata*l

Introduction

Sitophilus zeamais (Motschulsky) (Coleoptera: Curculionidae) is an important primary pest of stored products around world. *Sitophilus zeamais* seriously affects stored products, including rice, wheat, corn, potatoes and their process products, as well as some special local products and Chinese medicinal materials[1]. The three-months loss rate of infested foodstuff can be 11.25%, increasing to 35.12% after six months. Fumigants and chemical repellents are mainly used to control *S. zeamais* in the national and local grain depots in China[2]. But because of the misuse of chemicals, *S. zeamais* has developed resistance to some insecticides and phosphine[3,4,5]. The resistance factor of *S. zeamais* to phosphine can reach 116 times[6].

Essential oils are volatile secondary metabolites produced by plants for their own needs other than nutrition. In general, they are complex mixtures of organic compounds that give characteristic odour and flavour to the plants. Studies found that they have various activities against insects, such as fumigant toxicity, contact toxicity, stomach toxicity, repellency and developmental retardation. Furthermore, there is no evidence of pests having resistance to essential oils[7,8,9,10,11].

In this report, we present results of a study on the fumigant effect of 14 species of plant essential oils on *S. zeamais*, aiming at providing a theoretical basis of developing insecticides using economical and safe plant materials against storage pests.

Materials and Methods

Insects and Rearing Conditions

Sitophilus zeamais were reared in our laboratory at the Institute of Urban Pest Control in Huazhong Agricultural University (China). The temperature in rearing room was kept at 27 ± 1℃, while the relative humidity was maintained at 70% ± 5%. Glass jars of 500 mL capacity, covered with calico, were used to contain whole wheat with a moisture content of 13 ± 1%. Wheat was washed in tap water, dried and heated at 80℃ for 2 h to prevent pre-infestation and then stored at the above laboratory conditions. When the second generation adults were 2 – 3 weeks old, they were used in the bioassays.

Essential oil Species

14 species of essential oils were tested. 8 essential oils were distilled in the laboratory and 6 were purchased in Jiangxi (Table 1).

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Table 1. List of 14 species of essential oils

Scientific name	Family	Chinese name	Extract from	Place
Mentha haplocalyx	Labiatae	Bohe	Leaf and stem	HZAU
Illicium verum	Illiciaceae	Bajiaohuixiang	Seed	HZAU
Myristica fragrans	Myristicaceae	Roudoukou	Seed	HZAU
Alpinia officinarum	Zingiberaceae	Gaoliangjiang	Rhizome	HZAU
Curcuma longa	Zingiberaceae	Jianghuang	Root	HZAU
Acorus gramineus	Araceae	Shichangpu	Rhizome	HZAU
Brassic juncea	Brassicaceae	Jiecai	Seed	HZAU
Capsicum annuum	Solanaceae	Lajiao	Fruit	HZAU
Mentha spicata	Labiatae	Liulanxiang	Leaf	Jiangxi
Cinnamomum parthenoxylon	Lauraceae	Huangzhang	Root	Jiangxi
Litsea cubeba	Lauraceae	Shancangzi	Fruit	Jiangxi
Artemisi princeps	Compositae	Aihao	Leaf	Jiangxi
Cymbopogon citratus	Gramineae	Xiangmao	Leaf	Jiangxi
Pogostemon cablin	Labiatae	Huoxiang	Leaf	Jiangxi

Extraction of Essential Oils

The plant materials were dried in the oven at 40℃, crushed using a vegetation disintegrator, and then they were filtered through a 40 mesh screen. Dry plant powders (30 g) were subjected to steam distillation to get the oil water mixture. All mixtures were collected and extracted by the petroleum ether. The petroleum ether extract was concentrated in the rotary evaporation machine to reach the maximum yield. The essential oils were collected in sealed brown bottles and refrigerated in the dark at 0 -4℃ until their use.

Fumigant bioassays of 14 Essential Oils

The sealed conical flask fumigant method used by Deng et al.[8] was adopted. Filter paper was cut to strips (1 cm wide 4 cm long) and we passed a thread through each strip. Then the thread was stuck to the middle of a plastic film. Thirty *S. zeamais* adults were introduced into a 250 mL conical flask, and 14.7 μL/L essential oil was dropped on the filter strip. The flask was sealed using plastic film and the strip hung in the center of the flask. Experiments were repeated four times for each essential oil. Control flasks contained no essential oil. All treatments were kept in the dark insect bioassay room at 27 ±1℃ and 70% ±5% relative humidity. The number of the dead insects was observed in terms of treatment time, and mortality was corrected for the control mortality. After comparing the fumigant results of all 14 essential oils, two were selected for further bioassays.

Fumigant Bioassay of Selected Essential Oils

The sealed conical flask fumigant method was adopted (see above). There were three exposure periods of each treatment (24, 48 and 72 h). Seven concentrations in the range of 2 - 32 μL/L were used for each exposure time and the Lethal Concentration 50 (LC_{50}) was determined. Each experiment was repeated fourtimes.

Statistical Analysis

Mortality = (Number dead/Total number) ×100%

Abbott's formula was used to correct the mortality:

Corrected mortality = (Treatment mortality - Control mortality)/(1 - Control mortality) × 100%

Mortality data were subjected to analysis of variance (ANOVA) and Fisher's Protected LSD was used to compare effects among treatments (SPSS 14.0 for Windows). LC_{50}, LC_{95} values were calculated using to probit analysis.

Results and Discussion

Results of Extraction of Essential Oils

After using steam distillation and evaporating the petroleum ether solvent, the extract rate was obtained for each of the eight species (Table 2). The resulting extract rates presented important differences. The extract rates of only two species reached 1%, i.e. *I. verum* with 1.87% and *C. longa* with 1.16%. The extract rates of the other six species were all under 1%, with a minimum of 0.08% for *C. annuum*.

Fumigant Effect of 14 Essential Oils

The experiment against *S. zeamais* was conducted at 27 ± 1℃ and 70% ± 5% relative humidity with a fumigant concentration of 14.7 μL/L. The corrected mortality of *S. zeamais* exposed for 24 and 48 h is shown in Table 3. It is obvious that the fumigant effect of plant essential oils against *S. zeamais* varies with the exposure time and species. As expected, mortality was significantly higher after 48 h exposure ($P < 0.05$). The species effect was significant too ($P < 0.05$). At 24 h, eight oils were not significantly different from the control. At 48 h, six oils among these eight had again no effect. The two other oils (*A. tatarinowii* and *B. juncea*) presented weak effects on *S. zeamais* mortality after 48 h exposure. *M. haplocalyx* oil and *M. spicata* oil presented significantly the most important fumigant effect after both 24 and 48 h exposure time. After 24 h, *M. spicata* oil and *M. haplocalyx* oil caused 65.83 and 86.67% mortality respectively, indicating the rapid availability of these two oils for *S. zeamais*. At 48 h, mortality from *M. haplocalyx* oil had reached 100%, and *M. spicata* oil values reached 79.17%. Compared with the 24 h exposure, corrected mortality from *I. verum* oil, *A. officinarum* oil, *M. haplocalyx* oil, *M. spicata* oil and *M. fragrans* oil increased more than 10%. The increase of toxicity of *I. verum* oil was especially obvious with over 30% mortality.

Table 2. Extract rate of essential oil of plants

Plant	Quantity of dry powder (g)	Quantity of essential oil (g)	Extraction rate (%)
I. verum	280	5.24	1.87
C. longa	360	4.17	1.16
M. fragrans	360	3.58	0.99
A. tatarinowii	220	1.38	0.63
A. aofficinarum	680	2.66	0.39
B. juncea	320	0.89	0.28
M. haplocalyx	720	2.02	0.28
C. annuum	600	0.48	0.08

Extract rate = Quantity of essential oil/Quantity of dry plant powder 100%.

Table 3. Toxicity to adults of *S. zeamais* of different species of essential oil vapours *

Treatment	Concentration (μL/L)	Corrected mortality after different treated periods (%) (Mean ± SE)	
		24h	48h
M. haplocalyx oil	14.7	86.67 ± 1.36 a	100.00 ± 0.00 a**
M. spicata oil	14.7	65.83 ± 0.83 b	79.17 ± 0.83 b
I. verum oil	14.7	21.67 ± 0.96 c	58.33 ± 3.19 c
M. fragrans oil	14.7	29.17 ± 2.10 d	42.50 ± 0.83 d
A. officinarum oil	14.7	18.33 ± 0.96 d	33.33 ± 1.36 e
C. parthenoxylon oil	14.7	11.67 ± 6.45 e	20.83 ± 2.50 f
A. tatarinowii oil	14.7	4.17 ± 0.83 f	5.00 ± 0.96 g
B. juncea oil	14.7	3.33 ± 1.36 f	5.00 ± 1.67 g
C. annuum oil	14.7	0.83 ± 0.83 f	4.17 ± 1.60 gh
L. cubeb oil	14.7	0.00 ± 0.00 f	3.33 ± 1.36 gh
C. longa oil	14.7	2.50 ± 0.83 f	2.50 ± 0.83 gh
A. princeps oil	14.7	0.00 ± 0.00 f	2.50 ± 1.60 gh
P. cablin oil	14.7	0.00 ± 0.00 f	1.67 ± 0.96 gh
C. citratus oil	14.7	0.00 ± 0.00 f	1.67 ± 0.96 gh
Control	14.7	0.00 ± 0.00 f	0.00 ± 0.00 h

* Each datum represents mean of four replicates.

** Means followed with different letters within the same column are significantly different at 5% level ($P < 0.05$) by Fisher's Protected LSD.

Toxicity of *M. haplocalyx* oil and *M. spicata* Oil at Different Exposure Periods

The fumigant toxicity experiment was conducted at 27 ± 1℃ and of 70% ± 5% relative humidity. In relation to exposure period and concentration, the toxicity of *M. haplocalyx* oil and *M. spicata* oil to adult *S. zeamais* is illustrated in Tables 4 and 5. The linear regression equation between probit mortality (Y) and the logarithm of concentration (x) is shown in Ta-

bles 6 and 7. The longer of the exposure period, the lower the LC_{50} values for *M. haplocalyx* oil and *M. spicata* oil. Values of LC_{50} for *M. haplocalyx* oil against *S. zeamais* were respectively 11.53, 9.49 and 7.93 μL/L after 24, 48 and 72 h. The LC_{50} value for 72 h exposure was 1.45 times lower than the LC_{50} value for 24 h, and range of decrease range was small. The linear regression equation between LC_{50} value (y) and exposure period (t) is: $y = 13.250 - 0.075t$; $r = 0.997$; $df = 1, 1$; $F = 168.750$; $P < 0.05$. In the fumigant experiment using *M. spicata* oil, the LC_{50} for 24 h exposure was 13.43 μL/L, decreasing to 11.36 μL/L after 48 h exposure. The LC_{50} value for 72 h exposure was 9.20 μL/L, which was 1.45 times lower than the LC_{50} value for 24 h exposure, and range of decrease was small. The linear regression equation between LC_{50} value (y) and exposure period (t) is: $y = 15.560 - 0.088t$; $r = 1.000$; $df = 1, 1$; $F = 6627.000$; $P < 0.05$. LC_{95} reached 16.54 μL/L and 21.40 μL/L after 72 h exposure time for *M. haplocalyx* oil and *M. spicata* oil. These results show the greater efficacy of *M. haplocalyx* oil compared to *M. spicata* oil also observed in the previous experiment.

Table 4. Toxicity to adults of *S. zeamais* of *M. haplocalyx* oil vapour*

24 h		48 h		72 h	
Concentration (μL/L)	Corrected mortality (%)	Concentration (μL/L)	Corrected mortality (%)	Concentration (μL/L)	Corrected mortality (%)
18	99.17	16	100.00	16	100.00
16	88.33	14	85.83	14	93.33
14	64.17	12	65.83	12	78.33
12	49.17	10	45.00	10	64.17
10	28.33	8	27.50	8	47.50
8	17.50	6	12.50	6	18.33
6	2.50	4	6.67	4	14.17
CK	0.00	CK	0.00	CK	0.00

* Each datum represents mean of four replicates.

Table 5. Toxicity to adults of *S. zeamais* of *M. spicata* oil vapour

24 h		48 h		72 h	
Concentration (μL/L)	Corrected mortality (%)	Concentration (μL/L)	Corrected mortality (%)	Concentration (μL/L)	Corrected mortality (%)
32	99.17	28	100.00	24	100.00
28	97.50	24	100.00	20	94.17
24	97.50	20	89.17	16	84.17
20	83.33	16	75.00	12	76.67
16	60.83	12	65.00	8	23.33
12	51.67	8	12.50	4	5.83
8	3.33	4	1.67	2	2.50
CK	0.00	CK	0.00	CK	0.00

Table 6. Toxicity to adults of *S. zeamais* of *M. haplocalyx* oil vapour

Exposure period (h)	Regression equation	LC_{50} (μL/L) (95% Confidence limits)	LC_{95} (μL/L)	DF	X^2
24	$Y = -7.9148 + 7.4528x$	11.53 (10.52 ~ 12.59)	19.17	5	20.444*
48	$Y = -5.5443 + 5.6724x$	9.49 (7.91 ~ 11.23)	18.51	5	41.384*
72	$Y = -4.6311 + 5.1503x$	7.93 (6.63 ~ 9.18)	16.54	5	29.507*

Table 7. Toxicity to adults of *S. zeamais* of *M. spicata* oil vapour

Exposure period(h)	Regression equation	LC_{50} (μL/L) (95% Confidence limits)	LC_{95} (μL/L)	DF	X^2
24	Y = −7.2037 +6.3864x	13.43(11.68 −14.99)	24.29	5	19.444*
48	Y = −6.2449 +5.9164x	11.36(9.57 −12.98)	21.55	5	22.313*
72	Y = −4.3236 +4.4861x	9.20(5.95 −12.38)	21.40	5	64.935*

Conclusion

This research indicates that eight of 14 essential oils had fumigation activity on the adult of *S. zeamais*. These were the oils from *M. haplocalyx*, *M. spicata*, *I. verum*, *M. fragrans*, *A. officinarum*, *C. parthenoxylon*, *A. tatarinowii* and *B. juncea*. The oils of *M. haplocalyx* and *M. spicata* were better than the others, especially *M. haplocalyx* oil. Further research on the toxicity of *M. haplocalyx* and *M. spicata* oils against adult *S. zeamais* during different exposure period and concentration showed that LC_{50} values decreased with increase of exposure period, which showed that these two essential oils had longer persistence. When the concentration of the essential oil is persistent, the fumigant effect is better at longer of exposure periods. Therefore prolonging the exposure period can reduce the quantity of the essential oil required.

There have been several reports describing research on control of *S. zeamais* with essential oils. Huang *et al.* [12] tested the effect of *Elletaria cardamomum* oil against *S. zeamais* and *Tribolium castaneum*, and the result indicated that the sensitivity of *S. zeamais* to *E. cardamomum* oil was double than the sensitivity of *T. castaneum*. Hou and Zhang [7] studied the fumigant effect and population inhibiting activity of 24 essential oils against *S. zeamais*, and found that *M. spicata* oil and *I. verum* oil had high fumigant activity. The research of Deng *et al.* [8] on the fumigant effect of nine essential oils against adult *S. zeamais* showed that *C. parthenoxylon* oil, *Melaleuca alternifolia* oil, *Citrus limonum* oil, *M. spicata* oil and *Pinus tabulaeformis* oil had the best fumigant effect, especially *C. parthenoxylon* oil. In the current study, the fumigant efficacy of *I. verum* oil and *C. parthenoxylon* oil were not very good, possibly for the reason that the temperature and the fumigation method are different in the two studies. Also, the toxicity data collected in this study were obtained in flasks without grain, and the data are likely to vary with the species and the quantity of grain. Thus the influence of temperature and grain on the fumigant efficacy of essential oils needs more study.

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0604

Effect of Different Quantities of Wheat on the Effectiveness of the Essential Oil Cineole against Stored Grain Insect Pests

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Abstract: These investigations aimed to determine the effect of wheat grain mass on the effectiveness of the essential oil cineole against adults of *Tribolium castaneumn* (Herbst.), *Rhyzopertha dominica* (F.) and *Cryptolestes ferrugineus* (L.) in an empty space, 50% and 95% spaces occupied with wheat. Concentration of cineole of 50g/m^3 in empty space induced 100% mortality in all three tested insect species. However, fumigation in space 50% occupied with wheat was absolutely effective against *C. ferrugineus*, with 89.5% efficacy against *R. dominica*, and only 11% against *T. castaneum*. In space 95% occupied with wheat mortality of *C. ferrugineus* was 88%, *R. dominica* 64% and *T. castaneum* 4.5% only. The price of natural cineole may be a significant barrier to adoption as a grain fumigant.

Key words: fumigation, cineole, *Tribolium castaneumn*, *Rhyzopertha dominica*, *Cryptolestes ferrugineus*

Introduction

The primary cause of food contamination and environmental pollution arising from agriculture are chemical pesticides[1]. Also, the pesticide residues in grain arising from postharvest treatments[2] come from their non-selective and uncritical application causing the toxic effects in the food and contamination of the environment[3]. With the growing evidence regarding detrimental effects of many of the conventional pesticides on health and environment, require for safer means of pest management has become very crucial[4].

The use of botanical pesticides is now emerging as one of the safer and prime means to protect crops and their products[5,6]. Among botanicals the plant volatile essential oils (EO) are the most frequently studied as pesticides for pests and diseases management [6,7,8]. However, EO, besides needing a large scale demonstration of their efficacy and penetration, need a lot of research in order to determine their toxicological and safety data prior to the registration[9]. Also, as with other groups of insecticides, the potential use of the natural EO in stored grain insect pest management depends on many factors. Some of the factors that may greatly prevent the adoption and use of the natural EO in stored grain fumigation are their relatively high concentrations needed for the effective protection of stored grain against insect pests[8,10], a great difference in the sensitivity of various insect species[10] and current prices of natural essential oils on the market (Korunic and Rozman, unpublished manuscript).

One of effective and safe EO is cineole, the active component of many natural EO such as eucalyptus. It is a cyclic ether with empirical formula $C_{10}H_{18}O$ and systematic name 1,3,3 – trimethyl – 2 – oxabicyclo[2,2,2]octane. Sometimes is traded commercially as "eucalyptol". It is readily biodegradable, un-reactive and relatively non-toxic[11]. Also, it inhibits the enzyme acetylcholinesterase[12], it interferes with sonic communication and mating in leafhoppers[13], and it is a mosquito feeding and ovipositional repellent[14].

Several researchers determined a good fumigant activity of cineole against stored-product insects[15,16,17,8,18,19,20]. Significant effect of grain on the effectiveness of cineole and other EO as well, has been determined by Shaaya et al.[21], Lee et al.[8] and Rozman et al.[22]. They found out that cineole was significantly less effective in a space occupied with wheat grain in the comparison with the effectiveness in an empty space.

The main objective of this research was a determination of the effectiveness of EO cineole in fumigation vessels filled with wheat to 0, 50 and 95% of capacity, against against adults of three stored grain insects. The species tested were the rusty grain beetle, *Cryptolestes ferrugineus* (L.), the lesser grain borer, *Rhyzopertha dominica* (F.), and the red flour beetle, *Tribolium castaneum* (Herbst).

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Materials and Methods

The essential oil used in the experiment was 99% cineole ($C_{10}H_{18}O$) purchased from "Sigma-Aldrich" (Export Division Gr nwalder Weg 30 D – 82041 Deisenhofen, Germany, EC No: 207 – 431 – 5).

Cultures of *C. ferrugineus*, *R. dominica* and *T. castaneum* were reared in the laboratory under controlled conditions (30 ± 1℃, 70% ± 5% r. h.) in darkness. *C. ferrugineus* and *R. dominica* were reared on whole wheat grain and *T. castaneum* on wheat flour containing 10% broken wheat kernels and 5% un-activated yeast.

- The commodity in the experiment was Canadian Western Hard red wheat, clean, with 14% m. c.
- The following combinations had been set up.
- Empty jars 450 mL in volume with 0.5 g of wheat flour and 10 wheat kernels at the bottom with introduced test insects (control).
- Uninfested grains 200 g in 450 mL jars (50% full) with introduced test insects (control).
- Uninfested grain 360 g in a 450mL jar (95% full) with introduced test insects (control).
- Empty jars with 0.5 g of flour and 10 wheat kernels at the bottom with introduced test insects. A piece of treated filter paper with 0.05 g of cineole (50 g/m^3) was put on the bottom of the jar. The jar was tightly closed with metal lid.
- Uninfested 200 g of grain in a 450 mL jar (50% full) with introduced test insects. A piece of treated filter paper with 0.05 g of cineole (50 g/m^3) was put on the surface of grain. The jar was tightly closed with metal lid.
- Uninfested 360 g of grain in a 450 mL jar (95% full) with introduced test insects. A piece of treated filter paper with 0.05 g of cineole (50 g/m^3) was put on the surface of grain. The jar was tightly closed with metal lid.

Each combination was repeated four times. One hundred unsexed adults (2 – 4 wk old) were introduced into each a replicate and each insect species was run separately.

The experiment was carried out under controlled laboratory conditions (30 ± 1℃, 70% ± 5% r. h.) in darkness. The results of the experiment were assessed after 2 days exposure.

All data were subjected to one-way analysis of variance (ANOVA) according to the GLM (general linear model) and LSD test entered in the table. Data processing was conducted by the SAS System for Windows 98. The figures that represent mean values were made by Microsoft Excel 2003.

Results and Discussion

In comparison to the control, fumigation with cineole at the dose of 50 g/m^3 proved to be absolutely effective in empty space with achieved mortality of 100% in all three insect species. Fumigation in space 50% occupied with grain was absolutely effective against *C. ferrugineus*, with obtained mortality for *R. dominica* of 89.50%, and for *T. castaneum* 11% only. *C. ferrugineus* had very good response to cineole fumigation in 95% occupied space (88% mortality), *R. dominica* showed mortality of 64%, whilst application to *T. castaneum* proved to be ineffective (4.5%) (Tables 1 – 3).

Table 1. Mortality (%) of *Cryptolestes ferrugineus* adults after 48 h exposure to an application of 50 g/m^3 cineole.

Space	Mortality (%) *			
	Control		Cineole 50 g/m^3	
	Mean	SD.	Mean	SD.
Space empty	0.50^c	0.57	100.00a	0.00
Space 50 % full with wheat grain	1.50^c	1.73	100.00a	0.00
Space 95 % full with wheat grain	1.50^c	1.73	88.00b	4.96

Table 2. Mortality (%) of *Rhyzopertha dominica* adults after 48 h exposure to an application of 50 g/m^3 cineole.

Space	Mortality (%) *			
	Control		Cineole 50 g/m^3	
	Mean	SD.	Mean	SD.
Space empty	1.00d	0.81	100.00^a	0.00
Space 50 % full with wheat grain	2.50^d	1.29	89.50^b	3.69
Space 95 % full with wheat grain	0.25^d	0.50	64.75^c	4.50

* means followed by the same letters are not significantly ($P > 0.05$) different as determined by the LSD – test.

Shaaya et al. [21] assessed the fumigant activities of a large number of essential oils extracted from various spices and herb plants against *T. castaneum*, *Sitophilus oryzae* (L.), *R. dominica* and *Oryzaephilus surinamensis* (L.). The most active was *Labiatae* sp. oil ZP51, at a concentration of

Table 3. Mortality(%) of *Tribolium castaneum* adults after 48 h exposure to an application of 50 g/m³ cineole.

Space	Mortality(%) *			
	Control		Cineole 50 g/m³	
	Mean	SD.	Mean	SD.
Space empty	0.00d	0.00	100.00a	0.00
Space 50 % full with wheat grain	0.00d	0.00	11.00b	1.41
Space 95 % full with wheat grain	0.00d	0.00	4.50c	1.29

* means followed by the same letters are not significantly ($P > 0.05$) different as determined by the LSD – test.

1.4 – 4.5 μL/L air(1.4 – 4.5 g/m³) and exposure time of 24 h causing 90% kill of all insects in space tests. However, in columns 70% filled with wheat, a concentration of 50 μL/L and 7 d exposure were needed to obtain 94 100% kill of the insects.

Lee et al.[8] studied the fumigant toxicity of 42 essential oils and found that six of them extracted from *Eucalyptus nicholi* (Maiden & Blakely), *E. codonocarpa* (Blakely & McKie), *E. blakelyi* (Maiden), Callistemon sieberi (F. Muell.), *Melaleuca fulgens* (R. Br.) and *M. armillary* (R. Br.) were toxic to *S. oryzae*, *R. dominica* and *T. castaneum*. The fumigant toxicity of five oils in the space 50% filled up with wheat was 3 – 5 times lower and in a case of EO extracted from *E. codonocarpa*, nine times lower than in an empty space.

Rozman et al.[22] determined the bioactivity of cineole essential oil against *S. oryzae* on stored wheat in spaces with or without wheat (empty space, 50 and 95% full). A concentration of 50 g/m³ cineole in empty space induced nearly 100% mortality of *S. oryzae*. However, with the fumigation in space 50% filled with wheat there was 57.5% mortality and in space 95% filled with wheat mortality was 34% only.

Our results are in a good agreement with the results of Shaaya et al.[21], Lee et al.[8] and Rozman et al.[22].

Although, we didn't study the reasons for such significant effect of wheat grain on the effectiveness, we believe that the probable cause is a considerable sorption of cineole in wheat grains and poor permeability of cineole vapours into seed inter – space which largely decreased the fumigation effect. According to Korunic and Rozman[10] to gain as similar results as obtained with phosphine and methyl – bromide cineole concentrations should range from 200 – 250 g · m³. Champ and Dyte[23] analyzed the concentrations of phosphine and methyl bromide and phosphine dose of 0.03 g/m³ and methyl – bromide dose of 1 g/m³, if applied in airtight space, were found to be enough to gain LD_{95} for *S oryzae*, while Lee et al.[8] reported required cineole dose of 42 g/m³ to gain LD_{95} for *S. oryzae* in the space 50% full up with the grain. The cost of 1 kg of phosphine pellets is approximately US $41.00, whilst 1 kg of cineole in packages of 100 g reaches about US $236.00. When the highest dosage of phosphine pellets is applied(30 pellets/t) with 1 kg of phosphine it is possible to fumigate approximately 55 t of grain. It means the cost of phosphine to fumigate 1 t of grain is about US $0.74. With 1 kg of 1,8 – cineole it is possible to fumigate 4 tons[10] to about 10 tons of grain[8]. It means the cost of 1,8 – cineole to fumigate 1 t of grain is US $23.60 to US $59.00. Such a considerable effect of grain on the effectiveness of cineole and relatively high price of cineole and other EO (Korunic and Rozman, unpublished manuscript) may greatly increase the cost of the grain fumigation with cineole and other natural EO and make them to expensive to be adopted for wider use.

Conclusions

At the applied dose of 50 g/m³ cineole proved to have varying fumigant effect against adults of the three species tested. It was most effective against *C. ferrugineus*, effective to a lesser degree against *R. dominica*, and least effective against *T. castaneum*. Very good results were gained in a fumigation of an empty space, but results of fumigation of a space occupied with wheat(50 or 95%) tended to be less successful or acceptable. Probable cause could be found in considerable sorption of cineole in wheat grains and poor permeability of cineole vapours into seed interspace and into grains, which largely lessen fumigation effect.

The effect of grain on significant decreasing of the effectiveness of cineole and relatively high price of cineole and other natural EO make them too expensive to be adopted for wider use.

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0605

Study on the Control Flat Grain Beetle (*Cryptolestes ferrugineus*. Stephens) Effectively with Multi-Fumigation Technology and Multi-Pesticide

Wang Yanan[1], Li Jiahai[2] and He Feng[3]

Abstract: Taking into account pest biology and pesticide characteristics, a study was done to evaluate (1) phoshine (PH_3) fumigation with recirculation, and (2) intermittent fumigation with recirculation using PH_3 and dichlorvos (DDVP), to design the new treatment protocols for controlling *Cryptolestes ferrugineus* Stephens and *Liposcelis bostrychophila* L. The aim was not only to maximize the evenness of PH_3 distribution in the warehouse, but exploiting differences in sensitivity of the two pests to PH_3 and DDVP, thus overcoming *C. ferrugineus* and *L. bostrychophila* with strong resistance to PH_3. The results indicated that an initial PH_3 concentration of 700 mL/m^3 usually means that PH_3 concentration is maintained at 300 – 500 mL/m^3 for 16 – 25 days with resulting in an insect disinfestation rate of was 100%, with no live insects detected for 1 year afterwards. Once PH_3 concentration falls to 200 mL/m^3, PH_3 fumigation fails to control *C. ferrugineus*.

Key words: Alp, DDVP, intermittent recirculation fumigation

Introduction

Population growth of *Cryptolestes ferrugineus* Stephens can be great during grain storage, causing extreme damage and threatening stored grain security and quality of stored grain. Because phosphine fumigation was used alone for such a long time, *C. ferrugineus* has developed a strong resistance to phosphine, and it has become common and difficult to control[1,2,3]. We concluded based on our experience of stored grain pest control, that the high temperature and humidity of the southern climatic zone, and the challenge of fumigating grain bulks more than 5 m high, that recirculation fumigation, mixed fumigation, intermittent fumigation, and other methods needed to be investigated to find out more effective prevention and treatment methods. Experiments were conducted in large warehouse with (1) an intermittent application of aluminum phosphide with recirculation, and (2) fumigation a combination of aluminum phosphide (AlP) and dichlorvos (DDVP) with recirculation. With intermittent fumigation the pesticide was applied twice during the one fumigation, and the decision about when to make the second application was based on the estimated developmental stages of pests and the concentration of fumigant in the grain bulk. By splitting the application of AlP during a single fumigation, we aimed to maintain effective concentrations for long enough to disinfest grain stored under sealed conditions, by mixing phosphine fumigation with release of the volatile DDVP we aimed to exploit different degrees of sensitivity of pests to phosphine and DDVP, and by using recirculation we aimed to distribute the phosphine and DDVP evenly through the grain bulk which is usually about 5 m deep.

Experiment on the Aluminium Phosphide Recirculation Fumigation Alone

It was found in the course of fumigation in 2006, that *C. ferrugineus* was difficult to control, and common in the hot and humid areas. In April 2006, we found *C. ferrugineus* and book lice or psocids (*Liposcelis bostrychophila* L) when screening the grain bulk in one of the warehouses, so it was fumigated on the April 30 of 2006. The warehouse was made gastight when the gates, windows and van openings were sealed with PVC film. Aluminium phosphide was placed in 280 spots evenly distributed on the surface of the grain, with 1.5 m between the spots. In addition, there were 42 chinaware utensils for release of DDVP. There was 2 655 t of wheat with an average grain temperature of 21 C, and grain moisture content of 13.6%. The density of *C. ferrugineus* was 15 – 18 adults/kg and there were many *L. bostrychophila*. The aluminium phosphide (AlP) was in the form of pellets produced by Shenyang Pesticide Factory (56% purity, 1.5 kg/bottle). A total of

1. *Sichuan Province Grain Bureau, Chengdu* 610012, *P. R. China*
2. *Sichuan Province Yi Bin City Grain Bureau, Yi Bin* 644000, *P. R. China*
3. *San Dao Guai State – owned Grain Depots, Yi Bin City* 621000, *P. R. China*

31 kg of AlP was used which was equivalent to a dose of 10g/m^3. Recirculation fumigation system was used and this consisted of two single – valve air blowers(0.75 kilowatt each), a phosphine monitor, and grain thermometer.

The fumigation began on 30 April 2006 at 2:30 pm when the air temperature was 20 C. Recirculation operated at the fixed times of 8:00 – 10:00 am and 1:00 – 2:00 pm for 7 days. On 15 May 2006, the phosphine concentrations were 42, 38, 44, 21 and 30 ppm after opening the warehouse for ventillation. The density of*C. ferrugineus* was 3 – 5 adults kg and few *L. bostrychophilas* were found after sample screening. Because the AlP reaction rate was quick using recirculation fumigation, high phosphine concentrations were achieved in a short time, but effective concentrations were not maintained for long in the grain. Therefore, the level of control was bad especially in relation to eggs and the larval. This experiment showed that AlP fumigation with recirculation, is not ideal for controlling *C. ferrugineus* or *L. bostrychophilas*.

Experiment on AlP + DDVP Intermittent Mixtures and Recirculation Fumigation

Cryptolestes ferrugineus was distributed broadly but because of its feeding habits it harms only broken grain. It occurred together with the saw-toothed grain beetle (*Oryzaephilus surinamensis* (*L.*)) and Cryptolestes turcicus (Grouville). At temperatures of 32 – 35℃, its life cycle is approximately 32 days and the adults are long-lived, and these insects can reproduce deep in the grain bulks. The eggs are quite tolerant to phosphine but the adults quite sensitive to phosphine. Also, psocids are quite sensitive to DDVP. This experiment investigated control of *T. castaneum* and *L. bostrychophila* using a combination of two continuous applications of phosphine at a low dose and DDVP.

The horizontal warehouse space volume was big meaning that gas-tightness was not good. We used the intermittent and recirculation fumigation technology to make up for the insufficient gas-tightness, and maintain the concentrations long enough to be lethal to insects. The first dose aimed kill the sensitive insect stages (larvae and adults), and the second dose after 6 – 10 days, aimed to kill larvae and adults that had developed from eggs and pupae that survived the first dose.

The experiment was conducted in the same storehouse as the first experiment. The storehouse was a horizontal warehouse constructed in 1988 (35 m long × 17.5 m wide) and the grain bulk was 5.3 m high with a volume of 3 246 m^3. There was 2 655 t of wheat with an average grain temperature 20.3 – 25.3℃ and moisture content of 13.6%. Under these conditions the luster and smell of the wheat is normal, and there is no condensation, molding or spoilage. The density of *C. ferrugineus* was 20 – 30 insects/kg. storage. The AlP was in the form of pellets produced by Shenyang Pesticide Factory (56% purity, 1.5 kg/bottle). In addition, DDVP and malathion were used. The recirculation fumigation system included a PVC gas pipeline (0.14 mm thickness), organized like the Chinese word"非" tape, and connected with the fumigation dead angle of grain bulks. The main pipe was 80 cm diameter, and the branches were 55 cm diameter. There were two single-valve air blowers (0.75 kilowatts each), a phosphine gas monitor and grain thermometer. The Alp dose was 9 g/m^3 or a total of 18 kg, plus a total of 20 bottles of DDVP (330 g each) and four bottles of malathion (1 kg each) for space disinfestations.

The first fumigation started on 24 October 2006, and the Alp dose was 6 g/m^3 or a total of 12 kg, and two insect sample bags had been installed on the grain surface and 30 cm bellow the grain surface. Each bag contained 36 *C. ferrugineus* and 12 maize weevils (*Sitophilus zeamais* (Motschulsky)). Fumigation method: The Alp was uniformly distributed over the grain surface in 294 spots securely in cloth bags, in air pipes in the grain bulk. (The idea was for the DDVP and phosphine to be mixed through the recirculation fumigation, therefore increasing the poisonous effect of the treatment.) Because the volatility of DDVP is high, the stomach poisonous function was obvious, but longevity of the residue is short, therefore it was sprayed to gunny bags directly, with the aim of maintaining a high concentration for a short time. Recirculation began the next day for the fixed periods of 8:00 – 10:00 am and 1:00 – 2:00 pm each day. Based on the phosphine concentrations measured, and the evenness of concentrations through the grain bulk, the recirculation was stopped after 7 days.

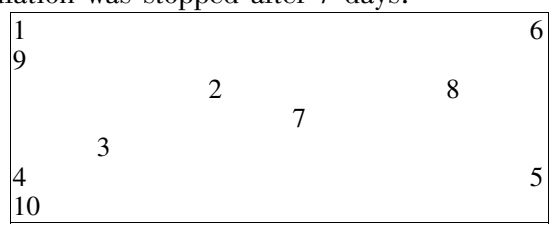

Fig. 1 **Gas cxamination site plan**

PH_3 Density examination method: On latter 12 hours after puts the insecticide, then start to examine the PH_3 density, afterward every day examines 1 time before starting the circulating fan, until the density drops to below 100ppm, then stop the examination.

On 7 November 2006 the warehouse was opened for pest inspection, and there were no live insects outside the grain bulks, but in the grain bulks there were 2 insects/kg. The second time fumigation started on the morning of 8 November 2006, with a dose of AlP of 3 g/m^3 or a total of 6 kg, together with 8 bottles of DDVP using the same methods used in the first fumigation. The phosphine gas concentration changed during the fumigation at the different sampling points (Table 1).

Table 1. Phosphine concentrations measured during fumigation.

Point Detection Date	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10
10.25	1 210	880	270	1 209	460	520	440	510	740	1 207
10.26	1 211	930	1 207	728	1 206	1 145	1 208	1 206	914	696
10.27	1 267	1 212	1 264	1 074	1 264	1 265	1 266	1 265	1 266	680
10.28	1 266	600	1 264	984	1 266	1 266	1 266	1 266	1 224	680
10.29	1 103	617	1 263	1 100	1 265	1 269	1 263	1 264	1 265	847
10.30	1 043	354	1 266	926	1 266	1 268	1 266	1 266	822	960
10.31	1 051	365	1 266	931	1 266	1 268	1 266	1 266	1 266	915
11.1	900	143	1263	960	1 266	1 266	1 266	1 266	1 056	804
11.2	689	118	950	750	986	1 037	1 055	889	883	721
11.3	432	56	428	396	523	453	432	406	382	343
11.4	121	41	96	108	56	38	77	81	80	38
Second time										
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10
11.11	895	905	876	964	1010	692	986	892	932	896
11.12	895	935	915	976	982	715	895	826	930	896
11.13	580	425	500	550	530	413	440	800	520	502
11.14	570	401	408	551	520	409	410	460	501	502
11.15	508	402	395	500	505	481	396	390	407	501
11.16	440	231	860	366	493	771	456	560	400	405
11.17	263	180	303	181	296	283	181	203	109	181

Note: In the grain bulks has not buried the PH_3 examination drive pipe, measured that the density is the grain bulks the surface layer (only to reference) the unit: ppm

The warehouse was opened on 17 November 2006 for ventilation and pest screening on 20 November 2006 confirmed 100% disinfestation. Routine weekly inspection for 1 year failed to discover any live pests, confirming control of phosphine resistant T. castaneum and other pests.

Analysis and Discussion

1. The use of intermittent applications together with recirculation may be a way to obtain better levels of control of *C. ferrugineus* and *L. bostrychophila*.
2. In order to control grain pests, instead of simply increasing the AlP dose, the level of gastightness should be increased as much as possible, to guarantee effective concentrations for longer. If the phosphine concentration is excessively high then it may cause *C. ferrugineus* to enter a protective stupor, making the achievement of ideal control difficult.
3. When controlling *C. ferrugineus*, the goal should be to achieve an initial concentration in the grain bulks of 700 mL/m^3, to maintain concentrations above 300 – 500 mL/m^3 for generally 16 – 25 days. Only then is it possible to achieve the ideal fumigation against *C. ferrugineus*. When the concentration drops below 200 mL/m^3 the fumigation will fail.

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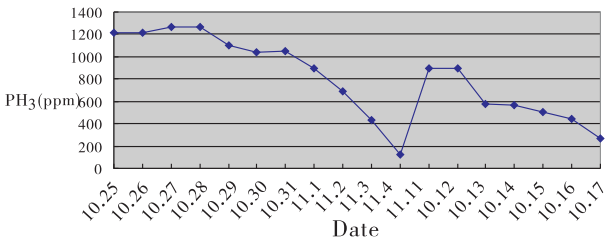
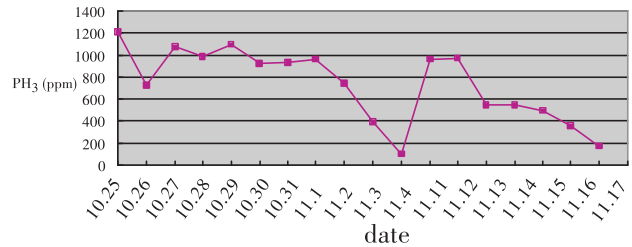
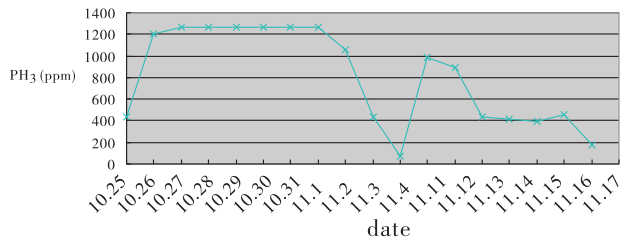


No. 2. PH_3 density change table



No. 3. PH_3 density change table



No. 4. PH_3 density change table

0606

The Potential Use of Natural Essential Oils in the Fumigation of Stored Agricultural Products

Zlatko Korunic[1*], Vlatka Rozman[2] and Irma Kalinovic[2]

Abstract: The authors give an overview of the concentrations of essential oils to control insect pests of stored grain, analyze the current prices of essential oils on the market and the cost of the fumigation, and discuss the potential of the introduction and the use of essential oil to fumigate stored grain. As with other groups of insecticides, the potential use of the natural essential oils (EO) in stored grain insect pest management depends on many barriers. Some of the barriers that may greatly prevent the adoption and use of the natural EO in stored grain fumigation are their relatively high concentrations needed for the effective protection of stored grain, a great difference in the sensitivity of various insect species, significant effect of different quantity of grain on the effectiveness and the current prices of natural essential oils on the market. Very high prices of essential oils, considering other characteristics (scent, sorption, penetration, aeration, etc.), may be really a very serious limiting factor for the application of natural essential oils in practice. There are two possible solutions to overcome the mentioned limiting factor; significant reduction of the prices of natural EO, or the production of the active components of natural EO synthetically.

Key words: essential oils, fumigation, cost price, stored agricultural products

Introduction

During the past few decades application of synthetic pesticides to control agricultural pests has been a standard practice. However, with the growing evidence regarding detrimental effects of many of the conventional pesticides on health and environment, require for safer means of pest management has become very crucial[1]. Despite of the numerous and ongoing research that have been conducted with new grain protectants, synthetic and natural ones, only a few have been adopted to be use as grain protectants (Daglish, 2006)[2].

The restrictions on the use of fumigants have posse new global challenges to food and chemical industry and have resulted in effort to develop and register new fumigants as an alternative, primary to methyl bromide[3,4]. There are several new developed fumigants or newer fumigant formulations such as sulfuryl fluoride[5,6,7], carbonyl sulphide[3,4], propylene oxide[8,9,10], methyl iodide[11], ozone[12], ethyl formate[13], cyanogen[14] and ethanDiNitrile[3,4].

The use of botanical pesticides has been emerging as one of prime means to protect crops and their products and the environment from pesticide pollution, which is a global problem[16,17]. When extracted from plants, these chemicals are referred to collectively as "botanicals". Since most of them generally degrade within a few days, and sometimes within a few hours, these insecticides must be applied more often. More frequent application, plus higher costs of production usually makes botanicals more expensive to use than synthetic insecticides[16]. Among botanicals the plant volatile essential oils (EO) are the most frequently studied as pesticides for pest and diseases management[18,17,19,20,21,22].

However, the essential oils, beside a large scale demonstration of their efficacy and penetration, need a lot of research in order to determine their toxicological and safety data prior to the re-registration[2]. Also, as with other groups of insecticides, the potential use of the natural EO in stored grain insect pest management depends on many factors. Isman (1997)[23] believed, in spite of mostly favourable toxicology and minimal environmental impact and the efficacy, botanicals and other natural insecticides need to fulfil many other considerations for the successful commercialization and use and this group of insecticides may find a place in applications where there is a greater tolerance for the presence of insects and a focus is placed on environmental safety.

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According to Rajendran and Sriranjini (2008)[24], although in laboratory tests with adult insects some of the plant extracts have shown significant insect toxicity, their physical properties such as high boiling point, high molecular weight and very low vapour pressure are barriers for application in large-scale fumigations. The authors believe that plant products have the potential for small-scale treatments and space fumigations. Still there is lack of data for single or multiple components of essential oils on sorption, tainting and residues in food commodities. Also, the requirements for the registration of plant products may be another barrier[24].

We believe that the other of factors that may greatly prevent the adaptation and use of the natural EO in stored grain fumigation are their relatively high concentrations needed for the effective protection of stored grain against insect pests, a great difference in the sensitivity of various insect species and the current prices of natural essential oils on the market.

The objectives of this review paper are:

(a) to give an overview of the concentrations of essential oils to control insect pests of stored grain,

(b) to analyze the current prices of essential oils on the market and the cost of the fumigation, and

(c) to discuss the potential of the introduction and the use of essential oil to fumigate stored grain.

Overview of Concentrations of Essential Oils to Control Stored Grain Insect

The concentrations of natural EO and its active components needed for effective fumigation have been studied by many researchers. In order to enable the comparison of toxicity data we analyzed only the reports that presented the doses of EO in the volume, mostly in μg/L or μL/L, published during the last 10 years.

Shaaya et al. (1997)[18] were assessed the fumigant activities of a large number of essential oils extracted from various spices and herb plants against *Tribolium castaneum* (Herbst) *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.) and *Oryzaephilus surinamensis* (L.). The highly active *Labiatae* sp. oil ZP51, in a concentration of 1.4 – 4.5 μL/L air and exposure time of 24 h caused 90% kill of all the insects in space tests. However, in columns 70% filled with wheat, a concentration of 50 μL/L and 7 d exposure were needed to obtain 94% – 100% kill of the insects.

Liu and Ho (1999)[25] evaluated the fumigant activities of the essential oil extracted from *Evodia rutaecarpa* Hook f. et Thomas, against *Sitophilus zeamais* (Motsch.) adults and *T. castaneum* larvae and adults. *S. zeamais* LC_{50} was 41 μg/L air and *T. castaneum* LC_{50} was 11.7 μg/L air.

Rahman and Schmidt (1999)[26] examined the toxic effects of vapors of essential oils of *Acorus calamus* (L.) rhizomes obtained from three countries; India, Russia, and Former Yugoslavia on the adults and eggs of *Callosobruchus phaseoli* (Gyllenhal) reared on seeds of *Lablab purpureus* (Medik.). Significant reduction of oviposition was found in oils vapors at 5 and 10 μL oil per 400 mL jar (12.5 to 25 μL oil per 1000 ml jar) after 24 h exposure. Newly-laid eggs were more susceptible than older ones.

Tun et al. (2000)[27] tested the ovicidal activity of essential oil vapors distilled from anise *Pimpinella anisum* (L.), cumin *Cuminum cyminum* (L.), eucalyptus *Eucalyptus camaldulensis* (Dehnh.), oregano *Origanum syriacum* (L.) var. *bevanii* and rosemary *Rosmarinus officinalis* (L.) against the confused flour beetle, *Tribolium confusum* (du Val.), and the Mediterranean flour moth, *Ephestia kuehniella* (Zeller). The exposure to vapours of essential oils from anise and cumin resulted in 100% mortality of the eggs. At a concentration of 98.5 μL/L of anise essential oil the LT_{99} values were 60.9 and 253.0 hours for *E. kuehniella* and *T. confusum*, respectively. For the same concentration of the essential oil of cumin, the LT_{99} value for *E. kuehniella* was 127.0 h.

Sánchez-Ramos and Castañera (2000)[28] found out that the vapor of natural monoterpenes pulegone, eucalyptol, linalool, fenchone, menthone, α – terpinene and γ – terpinene at the concentration of 14 μL/L or below generated 90% mortality of mobile stages of *Tyrophagus putrescentiae* (Schrank).

Lee et al. (2001)[29] examined the fumigant toxicity of different essential oils towards the rice weevil, *S. oryzae*. The essential oil from eucalyptus contained 1,8 – cineole (81.1%), limonene (7.6%) and α – pinene (4.0%). The oil generated LD_{50} = 28.9 L/L air. 1,8 – cineole was more active (LD_{50} = 23.5 μL/L air) than limenone and α – pinene. Benzaldehyde (LD_{50} = 8.65 μL/L air) occurring in peach and almond kernels had also a potent fumigant toxicity towards the rice weevils.

Papachristos and Stamopoulos (2002)[30] assessed the toxicity of vapours of the essential oils from *Lavandula hybrida* (Reverch.), *R. officinalis* and *Eucalyptus globulus* (Lab.) against the larvae and pupae of *Acanthoscelides obtectus* (Say.). The essential oil vapours were toxic to all immature stages tested with LC_{50} values ranging between 0.6 and 76 μL/L air, depending on oil and development stages.

Lee et al. (2003)[20] evaluated the fumigant toxicity of twenty naturally occurring monoterpenoids against *S. oryzae*, *T. castaneum*, *O. surinamensis*, the house fly, *Musca domestica* L., and the German cockroach, *Blattella germanica* L. Cineole, *l* – fenchone, and pulegone at 50μg/mL air caused 100% mortality in all five species tested.

Lee et al. (2004)[21] studied the potent fumigant toxicity of 42 essential oils and found out that six of them extracted form *Eucalyptus nicholi* (Maiden & Blakely), *E. codonocarpa* (Blakely & McKie), *E. blakely* (Maiden), *Callistemon sieberi* (F. Muell.), *Melaleuca fulgens* (R. Br.) and *M. armillary* (R. Br.) were toxic to *S. oryzae*, *R. dominica* and *T. castaneum*. The LD 50 and LD 95 against the adults of *S. oryzae* were between 19.0 to 30.6 and 43.6 to 56.0 μg/mL air, respectively. The LD95 of 1,8 – cineole was for *S. oryzae* 47.9, for *R. dominica* 30.4 and for *T. castaneum* 21.0 μg/mL air. The fumigant toxicity of five oils in the space 50% filled up with wheat was 3 to 5 times lower in 50% filled up the space than in an empty space and in a case of EO extracted from *E. codonocarpa* in 50% filled up the space with wheat, even 9 times less toxic.

Prajapati et al. (2005)[31] were evaluated the insecticidal, repellent and oviposition – deterrent activity of essential oils extracted from 10 medicinal plants against *Anopheles stephensi* (Liston), *Aedes aegypti* (L.) and *Culex quinquefasciatus* (Say.). The essential oil of *Pimpinella anisum* (L.) showed toxicity against 4th instar larvae of *A. stephensi* and *A. aegypti* with equivalent LD_{95} values of 115.7μg/mL, whereas it was 149.7μg/mL against *C. quinquefasciatus* larvae. Essential oils of *Zingiber officinale* and *Rosmarinus officinalis* were found to be ovicidal and repellent, respectively towards the three mosquito species.

Ketoh et al. (2005)[32] studied the effectiveness of the essential oil extracted from *Cymbopogon schoenanthus* (L.) against all development studies of *Callosobruchus maculatus* (Fab.). At the highest concentration tested (33.3 μL/L) all adults of *C. maculatus* were killed within 24 h of exposure to the oil and the development of newly laid eggs and neonate larvae was also inhibited.

Ketoh et al. (2006)[33] assessed the insecticidal activity of crude essential oil extracted from *Cymbopogon schoenanthus* (L.) and of its main constituent, piperitone, on different developmental stages of *C. maculatus*. Piperitone was more toxic to adults with a LC_{50} value of 1.6μL/L vs. 2.7 μL/L obtained with the crude extract.

Tapondjou et al. (2005)[34] investigated the toxicity of cymol and essential oils of *Cupressus sempervirens* (L.) and *Eucalyptus saligna* (Sm.) against *S. zeamais* and *T. confusum*. *Eucalyptus* oil was more toxic than *Cupressus* oil to both insect species (LD_{50} = 0.36μL/cm^2 for *S. zeamais* and 0.48μL/cm^2 for *T. confusum*) on filter paper discs, and was more toxic to *S. zeamais* on maize (LD_{50} = 38.05μL per 40g grain).

Wang et al. (2006)[35] investigated repellent and fumigant activity of essential oil from mugwort *Artemisia vulgaris* (L.) to *T. castaneum*. At 8.0μL/mL, mortality of adults reached 100%, but with 12 –, 14 – and 16 – day larvae, mortalities were 49%, 53% and 52%, respectively. At dosages of 10, 15 and 20μL/L air and a 96 h exposure period, mortality of eggs reached 100%. No larvae, pupae and adults were observed following a 60 L/L dosage.

Choi Won-Sik et al. (2006)[36] determined the toxicity of volatile components of thyme, sage, eucalyptus, and clove bud against the mushroom sciarid, *Lycoriella mali* (Fitch.) α – Pinene was the most toxic fumigant compound found in thyme essential oil (LD_{50} = 9.85 μL/L air) followed by β – pinene (LD_{50} = 11.85μL/L air) and linalool (LD_{50} = 21.15μL/L air). The mixture of α – and β – pinene exhibited stronger fumigant toxicity than α – or β – pinene itself against the mushroom fly adults.

Negahban et al. (2007)[37] determined the content of essential oil extracted form *Artemisia sieberi* (Besser). The oil contained camphor (54.7%), camphene (11.7%), 1,8 – cineol (9.9%), β – thujone (5.6%) and α – pinene (2.5%). The mortality of 7 days old adults of *C. maculatus*, *S. oryzae*, and *T. castaneum* increased with concentration from 37 to 926 μL/L and with exposure time from 3 to 24 h. A concentration of 37 μL/L and an exposure time of

24 h were sufficient to obtain 100% kill of the insects. *C. maculatus* was significantly more susceptible than *S. oryzae* and *T. castaneum*.

Rozman et al. (2007)[22] investigated the toxicity of 1,8 – cineole, camphor, eugenol, linalool, carvacrol, thymol, borneol, bornyl acetate and linalyl acetate against adults of *S. oryzae*, *R. dominica* and *T. castaneum*. The most sensitive species was *S. oryzae*, followed by *R. dominica*. *T. castaneum* was highly tolerant of the tested compounds. 1,8 – cineole, borneol and thymol were highly effective against *S. oryzae* when applied for 24 h at the lowest dose (0.14 μL/L). For *R. dominica* camphor and linalool were highly effective and produced 100% mortality in the same conditions. Against *T. castaneum* no oil compounds achieved more than 20% mortality after exposure for 24 h, even with the highest dose (139 μL/L). However, after 7 days exposure, 1,8 – cineole produced 92.5% mortality, followed by camphor (77.5%) and linalool (70.0%).

Stamopoulos et al. (2007)[38] were tested vapor form of monoterpenoids terpinen – 4 – ol, 1,8 – cineole, linalool, *R* – (+) – limonene and geraniol against different stages of *T. confusum*. The LC_{50} values ranging between 1.1 and 109.4 μL/L air) for terpinen – 4 – 01, from 4 and 278 μL/L air for (*R*) – (+) – limonene (with LC_{50} and from 1,8 – cineole 3.5 and 466 μL/L air were the most toxic to all stages tested, followed by linalool (with LC_{50} values ranging between 8.6 and 183.5 μL/L air) while the least toxic monoterpenoid tested was geraniol with LC_{50} values ranging between 607 and 1627 μL/L air.

Korunic and Rozman (2008)[39] carried out three different experiments with 1,8 – cineole and found out that the space occupied with different quantity of grain had a significant effect on the effectiveness of cineole against *S. oryzae*, *R. dominica*, *T. castaneum* and *Cryptolestes ferrugineus* (Steph.). The space occupied with more grain significantly reduced the efficacy of cineole against test insects.

The results of Shaaya et al. (1997)[25], Lee et al. (2004)[21] and Korunic and Rozman (2008)[39] demonstrated the significant effect of different quantity of wheat grain in the same volume on the effectiveness of EO against stored grain insect pests. In a space filled with grains for the successful control several times higher concentrations has to be applied in the comparison with concentrations applied in an empty space. This may be one of a very important limited factor for wider use of EO in grain fumigation.

Current Price of Essential Oils on the Market and the Cost of the Fumigation

Currently, EO are sold in different packages containing 5 ml, 14.75 g (1/2 oz) up to 907.2 g (32 oz) and 3780 ml (US gallon). The prices depend on the type of the essential oil, technology of the extraction, the size of the package and on producers, as well. The prices of EO sold by different producers, generally speaking, may be significantly different (Table 1). The size of the package greatly affects the cost of EO. One gram of Citronella EO in the package of 14.175 g (1/2 oz) costs US $ 0.49 but in a gallon (3789 ml) 1 mL. costs US $0.065; 1g of Lavandin organic EO in the package of 14.175g costs US $ 0.69 costs but in a gallon 1 mL costs US $0.16; 1 g of Lavender Provence – Organic EO in a package of 14.175 g costs US $1.28 but in a gallon 1 mL costs US $0.46, etc. Also, the prices of various EO are significantly different. For example, in the package of 14.175 g (1/2 oz) 1 g of different oils costs from US $ 0.49 (Citronella) to US $1.3 (Juniperus Berry). In the package of 907.2 g (32 oz) 1 g costs from US $0.32 (myrtle) to US $5.54 (Jasmine Absolute). In the package of 3789 ml (US gallon) 1 mL of different essential oils costs from US $0.064 (Citronella) to US $0.47 (Oregano) (the producer Dreaming Earth Botanicals, LLC, Ashenwill, NC, USA).

Potential of the Introduction and the Use of Essential Oil to Fumigate Stored Grain

Analyzing the prices of EO produced by numerous producers by searching data available on internet, by direct contact with the producers and by analyzing the results of the effectiveness of EO published by numerous authors, it is obvious that the prices may be the limited factor for the adoption and its wider use (Table 2). It is a great difference in approximate concentrations of phosphine, methyl bromide and EO 1.8 – cineole to give 95% and higher mortality of *S. oryzae* with 24 h exposure. According to Champ and Dyte (1976)[40] and re – calculated from Ct based on 20 h exposure, the approximate concentration of phosphine is 0.03 g/m^3; the approximate concentration of methyl bromide, from Ct based on 5 h exposure, is 1 g/m^3. Lee et al. (2004)[21] determined the concentration of 42 g/m^3 of 1,8 – cineole to give

95% mortality of *S. oryzae*. Korunic and Rozman(2008)[39] determined that 50 g/m^3 of cineole in an empty space and 48 h exposure caused 100% mortality of *S. oryzae*, in a space 50% filled with wheat grain the mortality was 57% and in a space filled up 95% with wheat grain the mortality was 34% only. Shaaya et al. (1997)[18] found out that the highly active *Labiatae* sp. oil ZP51, in a concentration of 1.4 – 4.5 μL/L air and exposure time of 24 h caused 90% killed *T. castaneum*, *S. oryzae*, *R. dominica* and *O. surinamensis*. However, in columns 70% filled up with wheat, a concentration of 50 μL/L and 7 d exposure were needed to obtain 94 100% kill of the insects. Lee et al. (2004)[21] found out that EO extracted form *Eucalyptus nicholii*, *E. codonocarpa*, *E. blakelyi*, *Callistemon sieberi*, *Malaleuca fulgens* and *M. armillary* were 3 to 5 times less toxic to *S. oryzae*, *R. dominica* and *T. castaneum* in a space 50% filled up with wheat in comparison with the toxicity in an empty space.

Table 1. The approximate prices of essential oils(EO)

Essential oil	Producer	Size of package *	Cost of package (US $)	Cost of 1 g or 1 mL (US $)
Lavender Provence Oganic(France)	Dreaming earth botanicals, LLC, Ashenville, NC, USA	3 780 mL (US galloon)	173.00	0.045
Bulgarian 0Lavender	Snowdrift Farm, Inc.	2268g(80 oz)	285.00	0.125
Lavandin Organic	Dreaming earth botanicals, LLC, Ashenville, NC, USA	3780 mL (US galloon)	623.00	0.164
Rosemary(Maroccan)	Snowdrift Farm, Inc.	2268g(80 oz)	154.95	0.068
Rosemary(Spanish)	Dreaming earth botanicals, LLC, Ashenville, NC, USA	3780 mL (US gallon)	528.00	0.139
Thyme linalool	Dreaming earth botanicals, LLC, Ashenville, NC, USA	907.2g(32 oz)	408.00	0.449
Bay(Laurus nobilis)	Dreaming earth botanicals, LLC, Ashenville, NC, USA	3789 mL (US gallon)	1214.00	0.320
Pepper, black	Dreaming earth botanicals, LLC, Ashenville, NC, SA	3789 mL (US gallon)	752.00	0.198
Bergamot	Dreaming earth botanicals, LLC, Ashenville, NC, USA	907.2g(32 oz)	338.00	0.372
Patchouli	Dreaming earth botanicals, LLC, Ashenville, NC, USA	3789 mL (US gallon)	731.00	0.192
Jasmin Obsolute	Dreaming earth botanicals, LLC, Ashenville, NC, USA	850.5g(30 oz)	4717.00	5.546
Basil	Dreaming earth botanicals, LLC, Ashenville, NC, USA	3789 mL (US gallon)	647.00	0.170
Oregano	Dreaming earth botanicals, LLC, Ashenville, NC, USA	3789 mL (US gallon)	1806.00	0.476

* The largest package available; smaller packages are significantly more expensive.

One(1)kg of phosphine pellets costs about US $41.00 US, whilst 1 kg of cineole in packages of 100g reaches about US $236.00. When the highest dosage of phosphine pellets is applied(30 pellets t^{-1}) with 1 kg of phosphine it is possible to fumigate approximately 55 tons of grain. It means the cost of phosphine to fumigate one tone of grain is about US $0.74. With 1 kg of 1,8 – cineole it is possible to fumigate 4 tons (Korunic and Rozman, 2008[39]; 95% space filled up with wheat) to about 23 tons of grain(Lee et al. 2004[21]; 50% space filled up with wheat). It means the cost of 1,8 – cineole to fumigate one ton of grain is from approximately US $10.00 to US $59.00. The great effect of grain on the reduction of the effectiveness of EO may greatly increase the cost of the grain fumigation with EO and make them too expensive to be adopted for grain fumigation use.

Conclusion

Besides of different barriers under the process of the registration, we find such a high price for cineole, and for other essential oils as well, considering other characteristics (scent, sorption, penetration, aeration etc.), as a serious limiting factor for the application of natural essential oils in practice. We believe that there are two solutions to overcome the mentioned

Table 2. The approximate prices of essential oils(EO) and approximate cost of fumigation of cubic meter

Essential oil	Producer	Size of package *	Cost of package (US $)	Cost of 1 g or 1 mL (US $)	Approximative concentration; reference	Approxi – mate cost(US $) to fumigate 1 m^3 **
Eucalyptus globulus(the usual eucalyptus)	Dreaming earth botanicals,LLC,Asheville,NC,USA	3780 mL (US gallon)	557.00	0.147	LD_{50} = 28.9 μL/L against S. oryzae [29]	Much more than [4.2]
1,8 – cineole	Acros organic	100 g	23.60	0.236	LD_{50} = 23.5 μL/L against *S. oryzae*[29]	Much more than 5.
					LC_{100} = 50μL/L against *S. oryzae*, *T. castaneum*, *O. surinamensis*, *Musca domestica*, *Blatella germanica*[20]	11.8
					LD_{95} for S. oryzae was 47.9 μL/L, for *R. dominca* was 30.4 and for *T. castaneum* 21 μL/L, in an empty space[21]	4.95 to 11.3
					LD_{50} = from 3.5 to 3.5 to 466g μL/L against *T. confusum* all stages[38]	Much more that 0.82 to109.9
					92.5% mortality of *T. castaneum* after 7 days of exposure to 138.8 μL/L[22]	32.7
					In an empty space LC_{100} = 50g/m^3 against *S. oryzae*, *R. dominica* and *T. castaneum*[39]	11.8
						11.8(*C. ferrugineus*)
					In a space 50% filled up with grain, LC_{100} = 50g/m^3 for *C. ferrugineus*, 150g/m^3 for *R. dominica*, and 250g/m^3 for *S. oryzae* and *T. castaneum*[39]	5.5(*R. dominica*) 59.0(*S. oryzae*, *T. castaneum*)
					In a space 95% fill up with grain 50g/m^3 caused mortality of 88% (*C. ferrugineus*), 64% (*R. dominica*) and 4.5% (*T. castaneum*)[39]	Much more than 11.8
Camphor	Aldrich	100g	74.45	0.744	77% mortality of T. castaneum after 7 days of exposure to 139 μL/L[22]	Much more than 74.4
Linalool	Aldrich	100 g	25.93	0.259	70% mortality of T. castaneum after 7 days of exposure to 139 μL/L[22]	Much more than25.9 to 44.9
					LD_{50} = from 8.6 to 183.5 μL/L against *T. confusum* all stages[38]	Much more than 2.2 to 47.5
					LD_{90} = 14 μL/L against *Tyrophagus putrescentiae*[28]	More than3.6

Essential oil	Producer	Size of package *	Cost of package (US $)	Cost of 1 g or 1 mL (US $)	Approximative concentration; reference	Approxi – mate cost (US $) to fumigate 1 m^3 **
Thyme linalool	Dreaming earth botanicals, LLC, Ashenville, NC, USA	907.2g (32 oz)	408.00	0.449	LD_{50} = 21.5 μL/L against *Lycoriella mali*[36]	Much more than 5.5 to 9.6
Aniseed (Anise seed)	Dreaming earth botanicals, LLC, Ashenville, NC, USA	3 789 mL (US gallon)	375.00	0.098	Anise essential oil LC_{99} = 98.5 μL/L against *E. kuehniella* and *T. castaneum*[27]	9.65

* the largest package available; smaller packages are significantly more expensive.

** μL/L is equal to ml in cubic meter; close to g in cubic meter depending on the density of EO (for example density of cineole is 0.9225 g/cm^3, linalool 0.858 – 0.868 g/cm^3).

barriers; significant reduction of the prices of natural EO, or if possible, to produce the active components of natural EO synthetically.

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0607

Preliminary Analysis on Effects of Killing Insects and Economy in the Prevention and Treatment for Stored Grain Pests

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Abstract: During the prevention and treatment of stored grain pests, the aims should be to store grain safely and economically. Factors that need to be considered include the different methods of distributing chemicals, application of different fumigating measures, selection of different treatment times, the different pest species and selecting non-fumigation or partial fumigation depending on the assessment of the pest numbers.

Key words: prevention and treatment for insect pests; effects of killing insects; economy

Introduction

The methods and effects of killing insects in the prevention and treatment for stored grain pests have been focused on efficacy, economy and safety. In 1950's, Stern initially described the concept of economic threshold (ET), namely that prevention and treatment measures are applied when the population of pests increases to the specified density which will result in economic loss. Based on ET, Sheng Chengfa further interpreted and described the time of the prevention and treatment, investment and the survival rate of pests in 1989. For controlling pests economically, the question which needs to be answered by scientists is when the specific prevention measures should be applied to reduce the pests whose density is dangerous. In the past several years, we compared and assessed the different methods of using chemicals, the different measures of recirculation fumigation, and the treatment time for recirculation fumigation. We compared recirculation fumigation with non-recirculation fumigation when the kinds of pests and amount are at a low level. Our research shows the relationship between the effects of killing pests and ET when the prevention and treatment for stored grain pests are carried out. The optimal conditions for controlling stored grain pests were investigated.

Comparison of Traditional Fumigation and Recirculation Fumigation

Traditional fumigation using phosphine (PH_3) gas can not kill all pests because of the limited infiltration of PH_3. Better results were obtained after the combination of recirculation fumigation with traditional fumigation to treat the pests in high grain piles. After this, the use of recirculation fumigation became widespread when our old warehouses were reconstructed.

The tests were performed at Qujing Branch of China Grain Reserves Corporation, and the warehouses with facilities were chosen to test recirculation + traditional fumigation. The length of these warehouses was 51.5 m, the width was 19.8 m, and the height of the grain pile was 6.0 m. The grain tested was local round-grained rice produced in 1999. A warehouse of the same size was chosen as a control to carry out the traditional fumigation.

The dosage was 5.7 g/m^3 of aluminium phosphide (AlP) in the traditional fumigation warehouses, and the amount of total amount of AlP in each warehouse was 45 kg. Live pests were seen in the narrow space between two plastic films and the grain piles. The dosage was 3.1 g/m^3 in the recirculation + traditional fumigation warehouses and the amount of AlP in each warehouse was 25 kg, with 15 kg applied at first and another 10 kg added after 5 days. The PH_3 concentrations measured in the recirculation + traditional fumigation warehouses are shown in Table 1.

Samples were taken at intervals of 30 and 60 days after ventilation by means of a deep sampling apparatus. No live pests were found and the mortality rate was therefore 100%. The results show that the technique of recirculation + traditional fumigation can lead to thorough mixing of the PH_3 gas maintenance of high concentrations for long periods, resulting in a higher CT product. Our results suggest that the application of technique of recirculation fumigation can kill the store grain pests totally. The economical a-

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nalysis of using technique of recirculation + traditional fumigation is shown in Table 2.

Table 1. Phosphine concentrations(ppm) in recirculation + traditional fumigation warehouses.

No.	Time of day	Date											
		7.12	7.13	7.16	7.17	7.18	7.19	7.23	7.24	7.26	7.30	8.3	8.6
1	morning	382	557	605	393	403	456	556	455	376	240	181	102
	afternoon	101	402	504	358	–	345	485	–	–	–	–	–
2	morning	243	337	378	332	219	238	320	284	292	288	278	131
	afternoon	185	271	327	268	–	269	311	–	–	–	–	–
3	morninWg	360	271	411	420	332	335	330	411	358	297	192	123
	afternoon	233	472	295	382	–	340	340	–	–	–	–	–
4	morning	411	411	307	301	255	254	249	302	278	231	173	100
	afternoon	201	322	269	297	–	259	234	–	–	–	–	–
5	morning	607	669	620	424	417	395	404	501	420	285	205	110
	afternoon	476	605	561	468	–	556	446	–	–	–	–	–

Table 2. Cost analysis of different methods of fumigation

Cost factor	Recirculation + traditional fumigation	Traditional fumigation
Dosage of AlP	25 kg	45 kg
Cost of AlP	700 yuan	1260 yuan
Wages	150 yuan	270 yuan
Savings	680 yuan (savings rate = 44%)	–
Effects	100% control of pests	Some live pests, need repeating
Other cost	No difference	

Comparison of Recirculation Fumigation in Mixture of PH_3 and CO_2 With Recirculation Fumigation Through Natural Deliquesce in Distributing AlP on the Surface of Grain

The usage of recirculation fumigation with a mixture of PH_3 and carbon dioxide (CO_2) stored in steel cylinder at our testing system showed the good results. Comparison with AlP, the purchase and storage of PH_3 and CO_2 are inconvenient and their price is expensive. It costs too much to perform recirculation fumigation with a mixture of PH_3 and CO_2. CO_2 gas is purchased far from our testing warehouses still cost too much even if the generator of PH_3 gas is applied outside the warehouses. Based on the similar results obtained from the three techniques of recirculation fumigation, we suggest that the best technique for the prevention of infestations and treatment of infested grain should be recirculation fumigation with AlP placed on the surface of grain to react with the moisture in the air to promote release PH_3 gas.

Comparison of Putting AlP on the Blow-holes with Putting AlP on the Surface of Grain

This test was carried out on the blow-holes in one-stored warehouse with high space in 2005, the cloth bags containing AlP on the surface of grain were changed into putting AlP on the blow-holes by means of AlP spreading plates made by ourselves. The number of workers taking part in the toxic environment and the operation time in such environment were reduced. The dosage of AlP was decreased and the effective treatment time was extended because of the enough concentration of PH_3. Therefore, the effects of recirculation fumigation were improved obviously.

The No. 21 and No. 5 warehouses of the Qujing Branch of China Grain Reserves Corporation were chosen as testing warehouses, and the No. 22 and No. 7 warehouses were chosen as control (see Table 3 for details).

Table 3. Detailed information on test and control warehouses

	Warehouse number			
	21	5	22	7
Size(m) (L x W x H)	51 ×20 ×7.8	33 ×20 ×6.8	51 ×20 ×7.8	33 ×20 ×6.8
Grain heigh t(m)	6.1	4.7	6.1	4.7
Grain type	Indica rice	corn	Indica rice	corn
Amount(t)	3,952	2,300	3,935	2,295
Intake (month/year)	5/2003	3/2004	4/2003	4/2004

	Warehouse number			
	21	5	22	7
Moisture content(%)	12.5	13.7	12.8	13.3
Pests(per kg or per m^2)				
Sitophilus zeamais (Motchulsky)	5	4	4	3
Gelichiid moth	3	2	2	3
Troctes divinatorius Muller	uncoun – table	uncoun – table	uncoun – table	uncoun – table

The dosage of AlP in control warehouses was 3.1g/m^3 (same as the last year) and the dosage of AlP in test warehouses was 2.26 g/m^3. In the control warehouse, cloth bags containing AlP were put on the surface of grain by workers, and the amount of AlP was divided into two portions for distribution. The AlP was distributed on the blow-holes in the test warehouses; the amount of AlP was divided into two portions for distribution. Treatment times were 40 mins for the control warehouses and 7 – 10 mins for the test warehouses. The results are shown in Table 4. The test and control warehouses were checked thoroughly after fumigation and no live pests were found in either the test or control warehouses, so the level of control was 100%.

According to the test results, several suggestions can be made:

1. Regarding the distribution of PH_3 gas in the warehouses, the method of distributing the cloth bags containing AlP on the surface of grain led to fluctuation in PH_3 concentration and poor mixing because of the time required for the AlP to react with the moisture in the air, and the interval of recirculation time. The method of distributing AlP at blow-holes resulted in the continuous recirculation of PH_3 and better penetration PH_3.
2. Regarding effectiveness against pests, although the level of control was 100%, performance of differed between the two methods. The distribution of the cloth bags containing AlP on the surface of grain needed more workers. The workers had to enter the warehouses and were exposed to the toxic gas for a long time. The distributing AlP at blow-holes improved the operation conditions of the workers.
3. Regarding the benefits, the distribution of AlP at blow-holes reduced the dosage of AlP, saved the cost of protecting the workers from the toxic gas, and protected all workers who were working in the warehouses.
4. When the two methods are compared, the treatment of the wastes resulting from distributing the AlP at blow-holes were simply and quickly after the operation of the recirculation fumigation was finished.

Table 4. Phosphine concentrations(mL/m^3) in recirculation and traditional fumigation warehouses.

Time	No. 21		No. 22		No. 5		No. 7	
	maximum	minimum	maximum	minimum	maximum	minimum	maximum	minimum
6 h	11	0	3	0	9	0	0	0
12 h	130	54	33	5	97	44	17	0
1 d	218	107	213	77	278	117	250	81
2 d	205	120	382	111	195	133	360	133
4 d	173	133	358	181	170	103	323	143
6 d	132	112	280	192	151	100	301	162
8 d	275	172	320	210	303	162	330	231
10 d	231	149	288	123	251	144	278	121
12 d	176	113	181	108	162	121	172	113
15 d	121	100	131	97	117	108	142	104

The Prevention and Treatment of Psocids Using a Combination AlP and DDVP

PH_3 gas has better penetration than DDVP but DDVP is very toxic against pests. The combination of PH_3 and DDVP in a recirculation fumigation can kill stored grain pests, including psocids which are strongly resistant to PH_3. The recirculation fumigation of combining PH_3 and DDVP can not only killpsocid adults and nymphs but also eggs which are strongly resist-

ant to insecticide. Grain has been stored safely as a result of this particular technique.

The experiment was carried out in No. 21 warehouse in the Qujing Branch of the China Grain Reserves Corporation. The rice tested was 3 952 t of Indica rice taken from another province in April 2003. Grain moisture content was 13.3%, the level of impurity was 0.9%, the maximum grain temperature was 26℃, the minimum grain temperature was 15℃, and average grain temperature 18.3℃. The pests present in the rice were *Sitophilus zeamais* (5 adults/kg), gelechiid moths (2 adults/kg), *Tribolium castaneum* (2 adults/kg), and many unidentified psocids. The insecticides applied in the test were 56% AlP tablets produced in Shandong and 80% DDVP emulsifiable produced in Shandong.

Based on the total volume of each testing warehouse and the AlP dosage of 2.26 g/m^3, 18 kg of AlP tablets (56% purity) of needed to be used; based on the total volumes of the testing warehouses and the dosage of 0.3 g/m^3 DDVP, 80% DDVP emulsifiable of 2.4 kg needed to be used. Considering the property of DDVP and the distributing way of DDVP, it was difficult for DDVP to be distributed twice. DDVP Total DDVP needed to be distributed one time before AlP was distributed. The porcelain dishes containing cotton were put on the surface of grain at intervals of 50 m^2, and total 20 porcelain dishes were put on the testing warehouse. The DDVP of 2.4 kg was distributed evenly onto the 20 porcelain dishes.

Investigation of the effect of killing pests by means of the recirculation fumigation: End of the recirculation fumigation, the air blower of axial flow was employed to ventilate after the warehouse was sealed 28 days. The samples were taken for checking the pests after the toxic gas was discharged. The samples were taken again after one month and two month. No live pests were found in any samples. The rate of control was 100%, including control of psocid eggs. Based on the results, the specific technique of the recirculation fumigation can kill all pests.

Discussion and Conclusion

Usage of the Mixture of PH_3 and DDVP

Usage of the mixture of PH_3 and DDVP does not lead to antagonism and the advantages of PH_3 and DDVP are complementary. The mixture of PH_3 and DDVP can kill the stored grain pests which can be resistant to the high level of PH_3 and the therefore the grain can be stored safely. DDVP's action includes contact toxicity, fumigant toxicity and tempting pests which are living in grain piles. DDVP is good at killing psocids and mites. The operation of recirculation fumigation using a mixture of PH_3 and DDVP has been resulted in the penetration of DDVP into grain piles, and therefore prolonging the effective time of DDVP. The aim of 100% control of stored grain pests has been achieved because of the combination of the strong penetration of PH_3 and the advantages of DDVP.

Using Ventilation Instead of Fumigation

Ventilation can inhibit the growth of psocids because it decreases the temperature and humidity in the warehouse, and psocids prefer a warm and humid environment. Therefore, the measure of ventilation can also control psocids well when there are few other pests in warehouses and the density of psocids is much small. Some psocids were found in No. 24 warehouse from April to May 2004 and were also found in the three rice warehouses from October to November 2005. The measure of ventilation was employed to treat psocids and a good result was obtained. In winter, the time of ventilation was prolonged and the conditions of low temperature and low humidity lasted a long time. At the end ventilation few psocids could be found. To meet the request of storing grain greenly and to reduce the cost of storing grain, the measure of ventilation, instead of the recirculation fumigation, can be carried out to decrease the density of psocids and to control the development of their population when the density of psocids is low.

Selection of Treatment time (Low Number of Pests) and Determination of the Measures of non-recirculation Fumigation or Postponing Recirculation Fumigation

It is very important to select the correct time to perform the recirculation fumigation in the prevention and treatment of stored grain pests. When a low number of pests are found in warehouses, the major measures should focus on an intensive checking, ventilation and decreasing grain temperature. Those measures can control the occurrence and development of pests. At the two-year cycling period of corn in our warehouses, the corn moved into the warehouse is carried out the recirculation fumigation carefully. If a low number of pests are found at some parts of the warehouse in the following year, the intensive inspection and management

will be applied. Based on the situation of pests, we need to decide whether the recirculation fumigation will be performed or not at last year. If few pests are found from the grain which needs to be delivered from the warehouses that year, the grain is not fumigated. Those operations can ensure a crop of corn to be fumigated one time in. a cycling period. At a three-year cycling period, rice and wheat will be fumigated one or two time before they are delivered from the warehouses. The rate of non-recirculation fumigation's grain is increased. If pests occur much slowly, the measure of postponing recirculation fumigation may be employed to avoid fumigating the same crop of grain repeatedly in the same year.

Application of Partial Fumigation at the Large Scale Warehouses

In the practice of fumigation in warehouses, the partial infestations of stored grain pests are frequently found in large scale warehouses when the moisture content of the grain entering warehouse is not uniform. The situation of the grain graded automatically is serious and the difference of grain temperature is big. Based on the actual situation of the partial occurrence of pests, the chemicals are put on site by means of searching tube or partial fumigation on the grain surface which is covered with polystyrene plate. Partial fumigation can reduce the amount of chemicals used, avoid fumigating all of the grain stored in warehouses and delay changes in grain quality. This specific measure can lead to the obvious economical and social benefits.

Based on the comparison test of applications of different fumigating techniques, the benefit analysis of different fumigating measures, the economic analysis of the same effects of killing pests with different methods of distribution of chemicals, applied test of combination of fumigating techniques, the effect assessment of controlling pests with non-fumigation, the influence of selecting treatment time on the fumigation and the comparison of economical benefits from the application of partial fumigation. When choosing appropriate fumigation techniques people must consider the balance of effects of killing pests and economical benefits depending on different situations. The selection of specific measures needs to consider the cycling time of grain, the season of pest occurrence, the kinds and density of pests, different fumigation methods, and the technical experience of local technicians.

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0608

Efficacy of Ozone against Insect Pests in Wheat Stored in Steel Grain Bins

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Abstract: Field experiments were conducted in steel bins containing 13.6 metric tons of hard red winter wheat. One bin was treated with ozone and the second bin served as a control. Stored grain insects were placed in bins for 1, 2, 3, and 4 days exposure in sampling tubes at three ozone levels: low, medium, and high concentrations. Ozone treatments on eggs, larvae, and pupae of *Plodia interpunctella* were not effective except for pupae at 3 and 4 days exposure at the highest ozone level. *Sitophilus oryzae* adults were the most susceptible species with 100% mortality reached after 2 days exposure at the medium and high ozone levels and after 4 days exposure at the low ozone level. However, some progeny were produced at all treatment levels for all exposure periods. *Tribolium castaneum* adults had 100% mortality only after 4 days exposure at the medium and high ozone levels. No *T. castaneum* progeny were produced at the high ozone treatment after 2 – 4 days exposure. For *Rhyzopertha dominica*, *Cryptolestes ferrugineus*, and *Oryzaephilus surinamensis*, 100% mortality was never achieved and progeny were produced at all ozone levels.

Key words: ozone, stored wheat, insect control

Introduction

Alternative control measures to eliminate pests from stored products are being continually investigated because of concerns of pesticide residues in foods from grain protectants. Fumigants, which leave no pesticide residues on stored products, are effective in controlling pests of stored products but there are concerns about transporting, handling, storing, and applying these products and of insects developing resistance to them.

Electrical generation of ozone is an attractive alternative for controlling pests as it eliminates some of the concerns of using traditional post-harvest pesticides. However, there have been few published studies on its effectiveness as an insecticide. Erdman[1] observed the toxicity of ozone on two *Tribolium*spp. in laboratory trials where the insect life stages were exposed to a continuous flow of air containing 45 ppm ozone and 100% mortality was obtained in 6.5 hours or less. In a laboratory study on corn[2], 100% mortality was achieved after three days exposure of ozone at 50 ppm for adults of *Tribolium confusum* Jacquelin du Val, *T. castaneum* (Herbst), and *Sitophilus zeamais* Motschulsky, and after six days for late instar *Plodia interpunctella* (H bner). Kells et al. [3] demonstrated that 8.9 metric tons of maize treated with 50 ppm ozone for 3 days resulted in 92% – 100% mortality of adult *T. castaneum*, adult *S. zeamais*, and larval *P. interpuntella*.

In Oklahoma, wheat is the major stored grain commodity. To our knowledge, no field experiments using ozone for insect control in stored wheat have been conducted in the United States. The objective of this study was to determine the effectiveness of ozone fumigation on various stored product pests in a grain mass of wheat under field conditions during the month of October, which is the traditional time during storage when grain is fumigated in Oklahoma.

Materials and Methods

Experiments were conducted in October 2007 in central Oklahoma in two steel grain bins each containing 13.6 metric tons of hard red winter wheat (*Triticum aestivum* L.). One bin was treated with ozone and the second bin served as a control. Grain quality was poor because of the extreme wet conditions at the end of the 2007 growing season. Grain in the ozone bin was grade 4 with a test weight of 55.1 pounds per bushel, moisture content of 11.3%, and total defects (dockage, foreign material, and shrunken and broken kernels) of 3.8%. Grain in the control bin was also grade 4 with a test

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3. *Adaptive Ozone Solutions*, 1500 *West* 161*st Street*, *Suite B*, *Olathe*, *Kansas*, 66062, *U. S. A.*

weight of 55.3 pounds per bushel, moisture content of 10.3%, and total defects of 5.6%.

Ozone was produced by an OZAT Model CFS – 3A generator (Ozonia International, France) with a rated capacity of 120 g/hr on dry air but the best output we could achieve under our environmental conditions was 60 g/hr. For our study, the generator was operated at about 30 g/hr to produce the three targeted ozone concentrations of 25, 50, and 70 ppm within the bin. The ozone was introduced into the bin through a 6.4 mm inside diameter polytetrafluoroethylene tube from the generator just upstream of the fan into a 10.2 cm diameter poly-vinyl chloride pipe connected to the aeration fan transition into the plenum in the bottom of the bin. The axial fan moved 0.21 cubic meters/sec of air/ozone gas through the 2.8 m diameter by 3.4 m depth grain mass. As the ozone traveled through the grain mass, its concentration rapidly decreased due to natural decay and reaction with materials it contacted.

For testing purposes, 10.2 cm diameter poly-vinyl chloride insect sampling tubes were placed in the bins so that test insects could be easily hung in bags within the tubes and retrieved. The tubes were inserted into the grain mass to depths corresponding to the targeted ozone levels of 25, 50, and 70 ppm. Ozone rising through the grain mass entered the bottom of the insect sampling tubes and continued to flow up through the tubes thereby coming in contact in the suspended bags containing insects. A recirculation system was used to recover the residual air/ozone gas exiting the top of the grain mass and then injected it back into the bottom of the bin through a closed loop system. This minimized ozone leakage to the environment and reduced the load on the ozone generator. Ozone concentrations in the insect sampling tubes were monitored approximately every 8 hours through 6.4 mm polytetrafluoroethylene sampling lines in each of the insect sampling tubes using a Series IN – 2000 5 – channel Ozone Analyzer (IN USA, Inc., Norwood, MA, USA) that has a monitoring range of 0 – 200 ppm.

Because of limited space within the tubes, *Sitophilus oryzae* (L.), *T. castaneum*, and *Rhyzopertha dominica* (F.) were tested during week one and *Cryptolestes ferrugineus* (Stephens), *Oryzaephilus surinamensis* (L.), and *P. interpunctella* were tested during week two.

For *S. oryzae* and *R. dominica*, 25 adults of each species were placed in 7.0 × 10.2 cm cotton muslin tea bags with a drawstring (Mountain Rose Herbs, Eugene, OR, USA) containing 50 g whole wheat kernels and 20 g infested wheat kernels of the species. For *T. castaneum*, 25 adults and 20 middle-late instars were placed on 50 g whole wheat kernels, 15 g ground wheat kernels, and 2.5 g infested flour which contained eggs. For bags holding *O. surinamensis*, 25 adults were placed on 15 g ground wheat kernels and 8 g of infested rolled oats containing eggs and larvae. For *C. ferrugineus*, 25 adults were placed on 15 g ground wheat kernels, 8 g infested oats containing eggs and larvae, and 2.5 g infested flour containing eggs. For *P. interpunctella*, five pupae and five fifth instars were placed in 15 g ground wheat. Also in the bags for *P. interpunctella*, 25 eggs less than 21 hr old were adhered to double-stick tape on black filter paper strips placed in a small folded copper wire envelope to protect them from being crushed.

Stored grain insects in the bags were placed in bins for 1, 2, 3, and 4 days exposure by suspending the bags on ropes in insect sampling tubes corresponding to the targeted ozone exposure values of 25, 50, and 70 ppm. At the end of each time period, bags were removed from the sampling tubes, taken to the laboratory, and adult beetles and *P. interpunctella* larvae were removed and assessed whether alive or dead. All grain particles and dust from the bags were placed in 226.8 g glass jars fitted with lids of wire screen sandwiched between filter paper disks and held in a growth chamber at 28 C. Beetle progeny were counted at 14 and 28 days. For *P. interpunctella*, eggs were evaluated after one week to determine egg hatch and pupae were evaluated after two weeks for adult emergence.

Data were analyzed as a completely randomized design. Arcsine transformations of proportionate insect mortality values were used for percentage values before analysis to normalize data and make variances homogeneous. Statistical inferences were made after subjecting data to general linear models procedure using SAS software[4]. Treatment means were separated using least significant difference at the 0.05 level. Untransformed means are presented in figures.

Results and Discussion

The mean grain temperature in the ozone treated bin was 25.3℃ during week one and 22.3 ℃ during week two. In the control bin, the mean grain temperature during week one was

26.9 ℃ and during week two was 25.6 ℃. The temperature in the ozone treated bin may have been slightly lower than the control bin due to the movement of air/ozone through the bin during the study. Air/ozone in the external closed loop tubes would have been cooled by the ambient temperature at night resulting in cooler air/ozone being circulated through the bin.

The mean ozone levels during week one were 27.3, 3.3 ppm for the low level, 52.3, 3.5 ppm for the medium level, and 67.7, 3.5 ppm for the high level. During week two, the mean ozone levels were 27.4, 3.7, 53.0, 3.2, and 68.9, 2.2 ppm for the low, medium, and high levels, respectively.

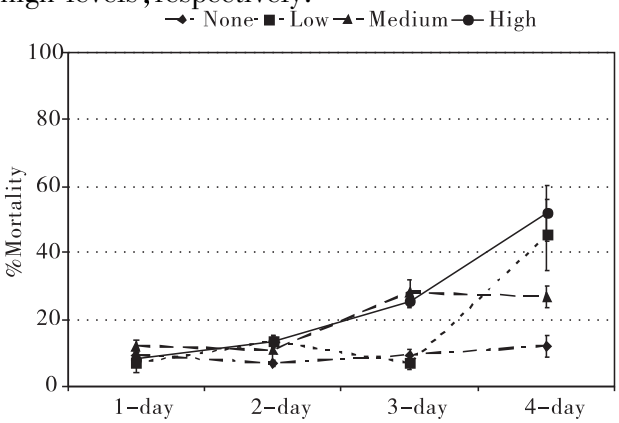

Fig. 1 Percent mortality *P. interpunctella* eggs

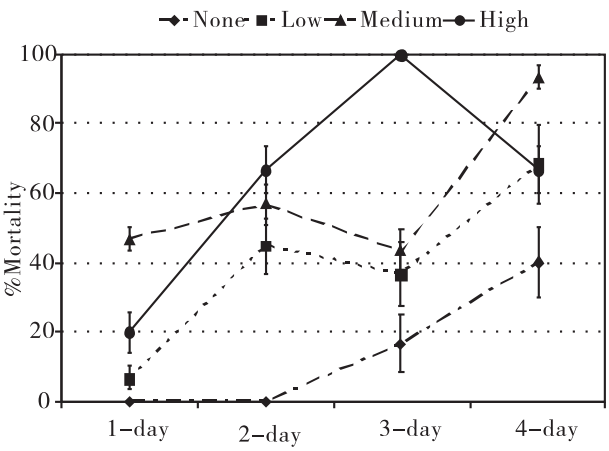

Fig. 2 Percent mortality *P. interpunctella* larvae

P. interpunctella egg mortality only reached 52% after four days under treatment at the highest ozone level (Fig. 1). Larval mortality was mixed with 100% mortality being reached after 3 days but then the mortality dropped to 66.7% after 4 days in the highest ozone treatment (Fig. 2). 100% control was never reached at the medium ozone level. Surprisingly, pupae seemed to be the most sensitive to ozone treatments. At the medium ozone level, 100% mortality was reached after 2 and 3 days but then

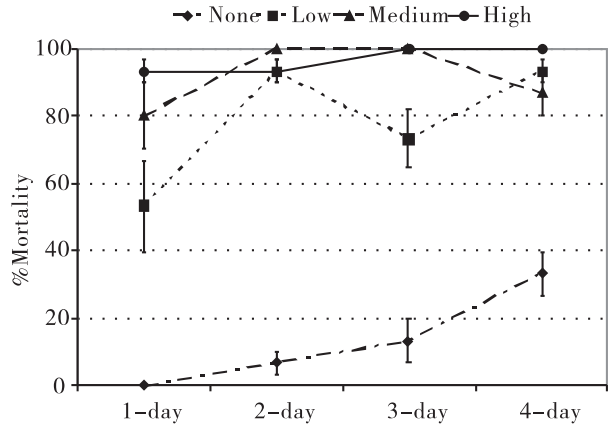

Fig. 3 Percent mortality *P. interpunctella* pupae

fell to 86.7% after 4 days (Fig. 3). At the high ozone level, 100% mortality was reached after 3 days exposure.

Mortality for *R. dominica* did not reach 100% even after 4 days exposure to the highest ozone level (Fig. 4). Progeny were produced at all ozone levels with the fewest being produced at the medium and high ozone levels (Fig. 5).

S. oryzae adults seemed to be the most sensitive to ozone treatments of the beetles tested. After 2 days exposure at the medium and high ozone levels, 100% mortality was achieved (Fig. 6). At the low level, 100% mortality was reached after 4 days exposure. However, at all ozone treatment levels, progeny were produced (Fig. 7). Evidently, some larvae and pupae inside the wheat kernels were able to survive even the high ozone treatment.

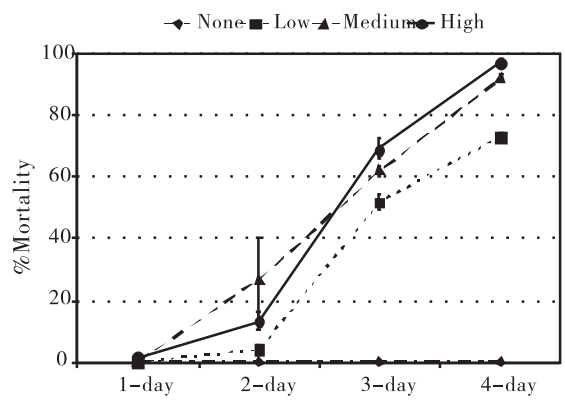

Fig. 4 Percent mortality *R. dominica* adults

No *T. castaneum* adults succumbed at the low or medium ozone levels until after 2 days exposure (Fig. 8) and the mortality was still very slight at the low ozone level. 100% mortality of adults was reached at the medium and high ozone levels after 4 days exposure. No progeny were produced at the high ozone level at 2 – 4 days exposure and only 0 – 1 progeny

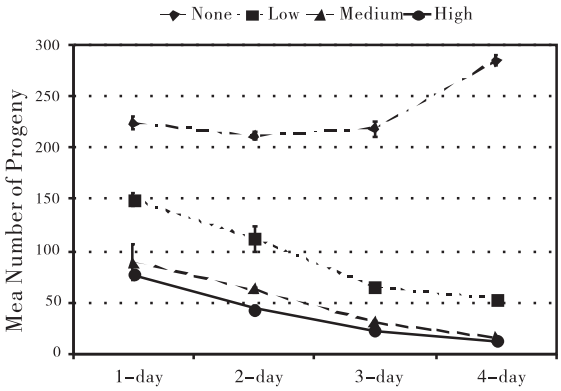

Fig. 5 Mean number *R. dominica* progeny

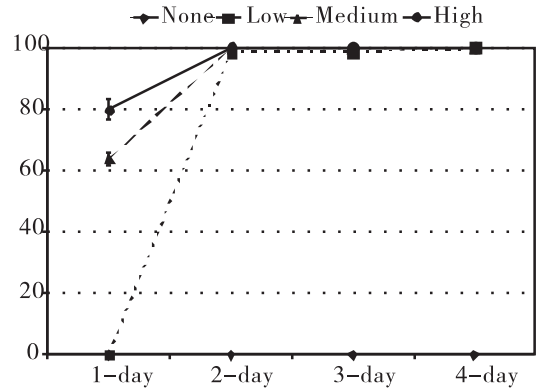

Fig. 6 Percent mortality *S. oryzae* adults

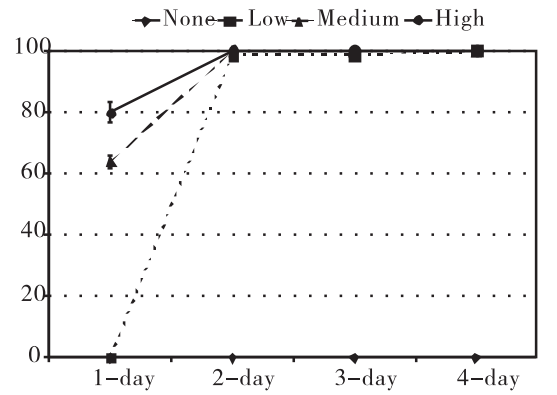

Fig. 7 Mean number *S. oryzae* progeny

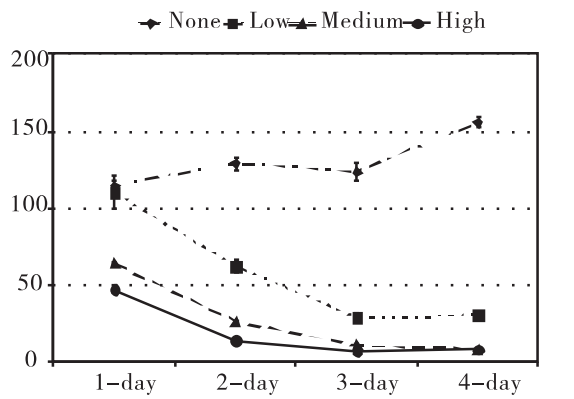

Fig. 8 Percent mortality *T. castaneum* adults

were produced at the medium ozone level during this same time period of exposure (Fig. 9).

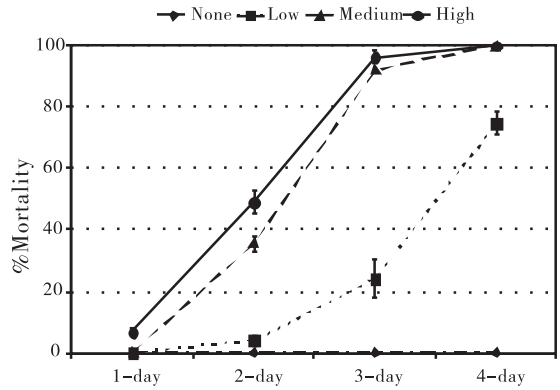

Fig. 9 Mean number *T. castaneum* progeny

C. ferrugineus adults seemed to be the toughest beetle to kill at the ozone levels tested. No adults died after 1 day exposure at any ozone levels (Fig. 10). No adults were killed at the low ozone level after 2 days exposure and there was only 1.3% mortality after 3 days at this level. Mortality only reached 88% after 4 days exposure at the high ozone level. Progeny were produced at all ozone levels with the fewest being produced at the medium and high ozone levels after four days exposure (Fig. 11).

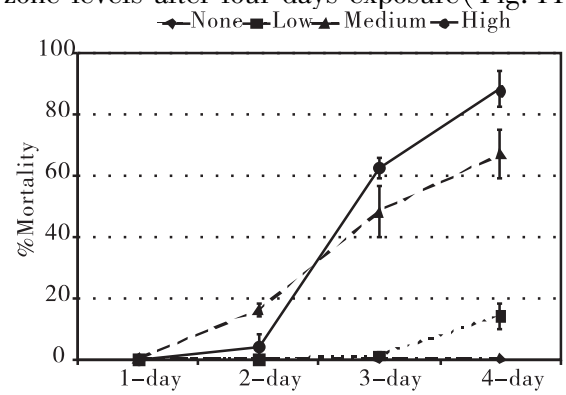

Fig. 10 Percent mortality *C. ferrugineus* adults

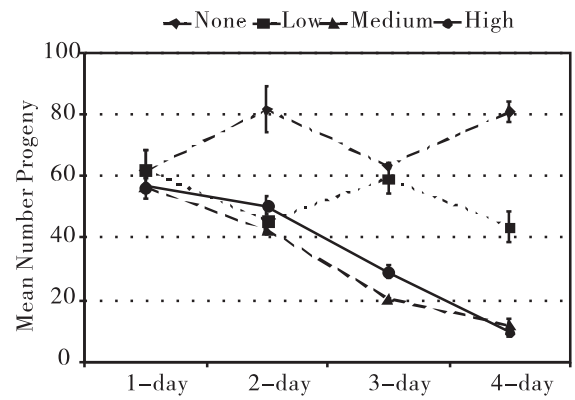

Fig. 11 Mean number *C. ferrugineus* progeny

Mortality of *O. surinamensis* adults was significantly higher at the medium and high ozone levels than at the low and no ozone levels after 2 – 4 days exposure (Fig. 12). However, 100%

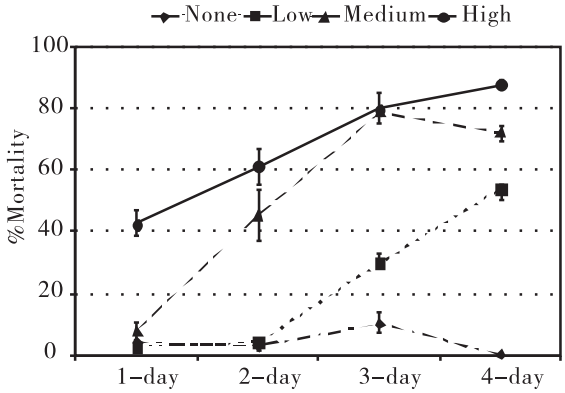

Fig. 12 **Percent mortality *O. surinamensis* adults**

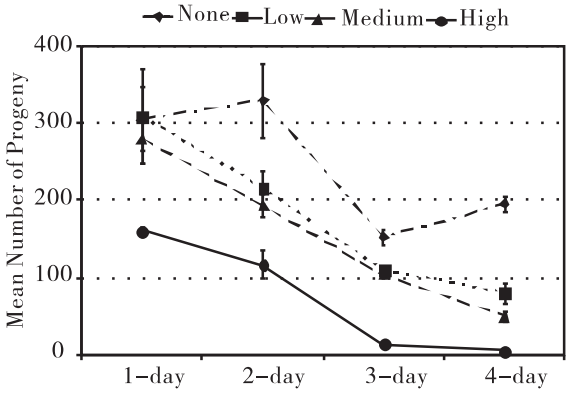

Fig. 13 **Mean no. *O. surinamensis* progeny**

mortality was never reached, even at the high ozone level. Progeny production decreased as exposure time increased but there was production at every ozone level (Fig. 13).

Overall, adult beetle mortality increased with increasing time of exposure although 100% mortality was only realized for *S. oryzae* and *T. castaneum*. Total suppression of progeny production was only seen for *T. castaneum* at the high ozone level.

To obtain total control of beetle populations, either the level of ozone concentration should be increased or the time of exposure at the tested levels should be increased. An economic analysis should be conducted to determine if ozone treatments can compare in cost to traditional methods of fumigation with products such as aluminum or magnesium phosphide.

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0609

Application of Economic Threshold Level in Stored Grain Fumigation for Controlling of Pests

Luo Fang, Li Zongliang, Yu Jieqing and Lu Xianli

Abstract: The principle of economic threshold level for storage pest control is that the cost of control is equal to, or less than, the economic loss caused by pest damage. Fumigants are economical, easy to use and efficient ways of protecting stored grain from insect pests. Of these fumigants, phosphine is most commonly used in warehouses. According to different situations of pest density and time of exposure, we use different dosages to control pests, and develop basic economic thresholds for the control of storage pests with phosphine.

Key words: economic threshold level, fumigation, Application

Introduction

Modern pest control methods are based on the economic threshold level (ETL), which combines the economic efficiency with ecological benefit during pest controlling application, and is often discussed in the economic entomology and pest control fields. Stern et al. (1959) first defined ETL as, "the density at which control measures should be applied to prevent an increasing pest population level from reaching the economic injury level". The principle of an ETL in stored pest control is that cost of control is equal to, or less than, the economic loss caused by pest damage. Insects can cause loss during grain storage, so grain pest control is a major component of the warehouse's daily work. Fumigants are cheap, easy to use and are efficient ways of protec ting stored grain from insect pests. Phosphine (PH_3) is most popular fumigant in warehouses in China. Depending on different situations of pest density and time of exposure, we use different dosages and try to reduce pest controlling costs. The aim of the study was to develop a basic rule for an ETL for storage pests in our warehouses.

Materials and Methods

The experimental barns had the same design dimensions and the grain was stored to a height of 6 m. materials and equipment used included a stored grain monitoring system, 56% purity aluminium phosphide (AlP) tablets, a phosphine concentration detector (Beijing Jiahua HL – 210) and an alarm device, and a phosphine-generator (Zhengzhou Weilai). The experimental conditions are summarized in Table 1.

Table 1. Summary of experimental conditions.

Barn number	Grain intake	Variety	Quantity(t)	Stored grain temp(℃)	Moisture content(%)	Pest species	Pest density/kg	Pressure half-life(s)
13	2005	japonica paddy	3,271	13.2	13.1	*Sitophilus zeamais*	15	50
15	2005	Indica paddy	2,963	14.8	13.2	*Sitophilus zeamais*	14 60	

Note: *S. zeamais* has only low levels of PH_3 resistance

Spot Fumigation

In a spot fumigation infested grain was covered in a bell-shape with gas-proof sheets, and fumigated by probing AlP into the grain. The dosage of AlP was 15 g/m^3. Phosphine concentrations were monitored throughout the fumigation.

Fumigation of Whole Stores

Fumigant tablets were distributed on the surface of grain and phosphine was also replenished from a phosphine-generator outside barn. The period of exposure to phosphine for No. 13 barn was 15 – 20 days, and 20 – 25 days for No. 15 barn. The concentration obtained surface using dosage is 2.5 g/m^3, and the replenished

Kunming Warehouse Directly under Central Grain Reserves, Kunming, Yunnan

concentration of phosphine is 180 mL/m^3. Five points were used for monitoring the phosphine concentration according to Technical Regulation of Grain Storage.

Results and Discussion

The total amount of AlP tablets consumption in the No. 13 barn is 16.0kg, while the amount is 24.0kg in No. 15 barn. After the released gas diffused away, the rate of mortality in samples taken from all sampling points was 100% both in No. 13 and No. 15 barns. There were no live pests present in samples taken one month later.

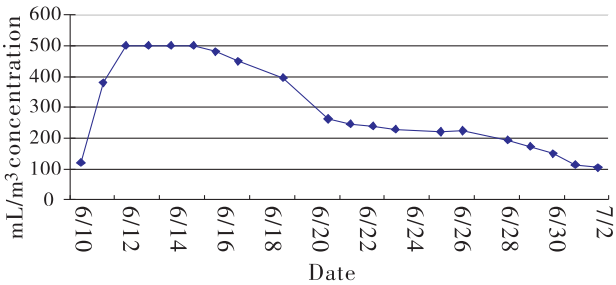

Fig. 1 PH_3 concentration during a 'spot' fumigation

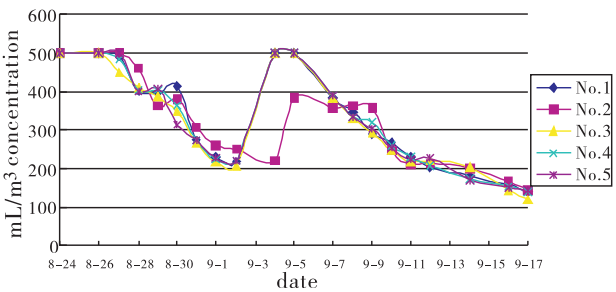

Fig. 2 PH_3 concentration in No. 15 barn

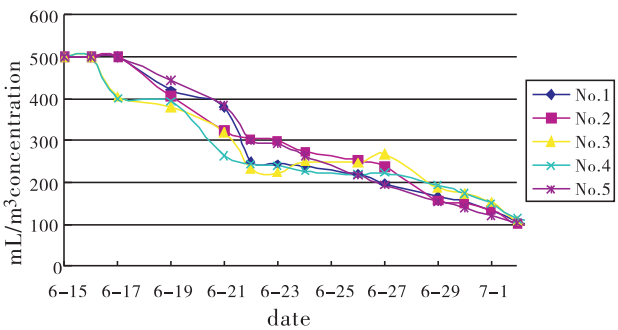

Fig. 3 PH_3 concentration in No. 13 barn

The main findings were:

- For the spot fumigation (Fig. 1), the period of exposure at a PH_3 concentration of 200 mL/m^3 must be greater than 20 days to control pests.
- For whole store fumigation of No. 15 barn (Fig. 2), the period of exposure at a PH_3 concentration of 150 mL/m^3 must be more than 23 days by replenishing phosphine concentration to maintain efficient concentrations of PH_3 on 4 September.
- For whole store fumigation of No. 13 barn (Fig. 3), the period of exposure at PH_3 concentration of 150 mL/m^3 must be greater than 16 days.

Furthermore, it is important to choose the right time and economic methods to control pest. During periods when the grain is cool, especially in winter and spring, pest densities of 5 – 9 adults/kg do not cause significant damage. What we need to do is monitor pest density rather than fumigate immediately. If the pest density is more than 10 adults/kg in summer and autumn, however, the infestation will cause an abnormal increase in grain temperature. At this point, we must disinfest the grain with the proper fumigation methods. When limited portions of the stored grain have abnormal temperature increases, only spot fumigations with phosphine are required. The fumigation dosage is commonly 6 – 15 g/m^3. When the infested portion is less than 10 m^3, the consumption of fumigant is also less. Therefore, spot fumigations with phosphine are a very economical method of pest control.

Conclusion

For the normal storage pest with low PH_3 resistance such as *S. zeamais*, when the grain temperature is lower than 15℃, the period of exposure at an effective concentration must be more than 15 days to kill all storage pests. If the pest density is more than 10 adults/kg, or the stored grain temperature rises abnormally, we treat the stored grain with spot fumigations or whole store fumigations depending on the size of the area infested and pest density. If only limited portions of the grain are infested, spot fumigations are enough to control pests, which reduces the amount of fumigant used and the cost of control.

The ETL is multi-dimension, dynamic, random, economic, ecological parameter, so it's impossible to get its exact value. What we can do is try to approach it in our research. In our daily work, the ETL is just a parameter index which tells us when we need control pests if the pest damage reaches a certain level. So application and research of the ETL in stored grain fumigation still need more experiments and works.

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0610

Sulfuryl Fluoride – Efficacy against *Tribolium castaneum* and *Ephestia kuehniella* and Residues of the Gas in Flour after Fumigations of Mills

D. Klementz[1*], W. Rassmann[2] and Ch. Reichmuth[3]

Abstract: Sulfuryl Fluoride (SF) as Profume® together with a computer program (Fumiguide®) has been registered by Dow AgroSciences in many countries for disinfestations against stored product pest insects mainly in empty structures e. g. mills. Due to strongly reduced efficacy at lower temperatures, the SF application is recommended together with thoroughly warming up the structure above 20 ℃. Based on a mill fumigation with SF (average ct – product 1,200 g · h/m, 20 ℃, 60 h), the authors discuss the efficacy of the gas towards the eggs of the Red flour beetle (*Tribolium castaneum*) and Mediterranean flour moth (*Ephestia kuehniella*). No development of eggs of *Ephestia kuehniella* occurred in 80% of the samples and in 44% of the *Tribolium* samples. The average survival rate for the moths in samples with incomplete control was 8% compared to untreated samples. This rate amounted to about 12% for the *Tribolium* samples with survivors. In large flour mills often residual flour remains inside during a fumigation due to economic reasons. This flour is not object of the treatment but picks up residues depending on the diffusion of the gas through internal walls. Data are reported on the formation of fluoride residues in flour in three such bins: 10.4 mgF^-/kg, 5.7 mgF^-/kg, and 2.9 mgF^-/kg, respectively. The European maximum residue limit for fluoride in flour will be 2 mg/kg from September 2008. One of the biggest advantages using the Fumiguide® is the precise and specific adjustment of several fumigation parameters according to the individual conditions of the fumigation. That includes the species and life stages of the pests, the gas tightness of the object, the temperature, the exposure time and the direction and strength of the wind. The impact of various parameters on effective fumigations will be discussed based on efficacy data as well as the formation of residues.

Introduction

Sulfuryl fluoride (SF) was developed in the late 1950s as a structural fumigant, mainly for termite control (Steward 1956) [1]. It is applied to buildings, which are covered with gas proof sheets or otherwise sealed. The gas provides good penetration, requires a short fumigation period of approximately 24 h against adult insects. The egg stage of many insects appears to be up to 10 times more tolerant than adult insects. SF is considered a practical alternative to MB for many uses, particularly for quarantine fumigation applications and for use in empty food processing facilities (Reichmuth et al. 1997) [2]. It is toxic to post-embryonic stages of insects (Kenaga 1957) [3] but the eggs of many species are very tolerant especially at low temperatures, requiring concentrations of over 50 g/m and exposures of up to three days for complete kill (Williams and Sprenkel 1990) [4]. Eggs of *Ephestia kuehniella* at 25℃ required a ct – product of about 1000 g · h/m to prevent hatch and 800 g · h/m to prevent emergence (Bell and Savvidou 1999) [5]. SF is currently registered for use under the trade name Vikane®. It is used in some European countries to control a wide range of pests including: wood-destroying beetles, furniture and carpet beetles and clothes moths. Research is ongoing to evaluate the potential of this chemical for timber treatment for plant quarantine purposes. Efforts are underway to develop treatment schedules to fumigate timber being imported into the USA, Europe and Japan to control wood-destroying beetles or fungal pathogens (Chambers and Millard 1995; Kappenberg 1998) [6,7].

Materials and Methods

Fumigation

The fumigation parameters are presented in Table 1. Concentrations of SF and ct – products within the fumigated object were determined by the fumigating company.

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Table 1. fumigation parameter of a mill fumigation in Germany

Fumigated object	Complete flour mill with silos, cleaning facilities and locations, stairwells and all storage halls
Location of fumigated object	Germany
Size of the fumigated object	60 000 m^3
Heating of the object	no
fumigant	Profume (99.8% sulfuryl fluoride)
Fumigated volume (e. g. silo basement, mill, cleaning facilities)	20 000 m^3
Fumigant dosage	1 200 g · h/m^3
Total SF amount, incl. replacement gas	ca. 2 000 kg
Fumigation date	2005
Fumigation time	60 h

Climate Conditions in the Mill during Fumigation

The temperature during the fumigation was recorded with nine dataloggers (Figure 1). Each of them was placed in different sample bags.

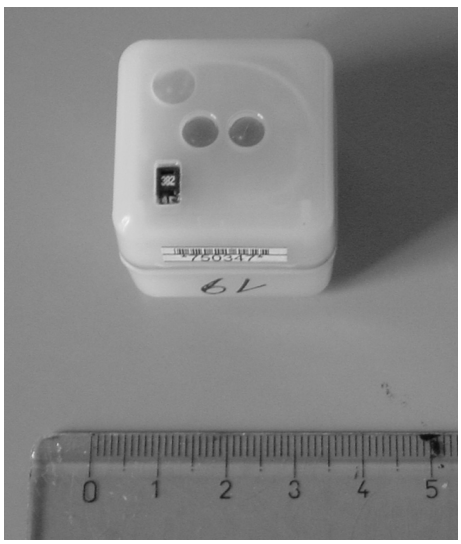

Fig. 1 Dataloggers for the detection of the temperature (mess scale in cm)

Determination of SF and Fluoride in non-fumigated Flour and Grain Silos

In large flour mills, often grain silos and bins for flour are constructed inside the whole building. For the miller, it is nearly impossible and totally unfeasible to empty these locations from the various products prior to fumigation. It would take more than a week after the fumigation to reproduce all these varieties of flour and refill the grain for the different customers. These parts of the mill are therefore not target of the treatment but pick up some gas due to diffusion of SF through walls. Since data were lacking on the possible formation of residues of SF and fluoride in flour, these in-house bins were supplied with measuring lines.

Adjacent to the fumigated area within the building, concentration of SF was automatically detected by use of a process gas chromatograph in three locations (results see Fig. 4). For this purpose, measuring lines were introduced 30 – 50 cm deep into flour. The detection of fluoride occurred electrochemically with ion-sensitive electrode. The average recovery rate for spiking samples between 1 mg/kg and 25 mg/kg, was between 91.8% and 101.2%. The quantitative detection limit was 0.5 mg fluoride/kg flour.

Three single samples of about 2 kg were taken from 30 cm underneath the flour surface with a heavy special sampling device from three bins for later determination of possible fluoride residues.

Test Insects: *Tribolium castaneum* and *Ephestia kuehniella*

Both the rust red flour beetle, *Tribolium castaneum*, and the Mediterranean flour moth, *Ephestia kuehniella*, were taken from a long lasting cultures of the Institute for Stored Product Protection of the Federal Biological Research Centre for Agriculture and Forestry (since 1. 1. 2008: Julius Kuehn-Institute, Federal Research Centre for Cultivated Plants, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection).

Tribolium castaneum was cultivated at a temperature of 25℃ and a relative humidity of 65% on a nutrient mixture with yeast and hole meal wheat flour and *E. kuehniella* at a temperature of 22℃ and the same relative humidity on a nutrient mixture of wheat and fine wheat flour. The adult beetles and moths were chosen by random selection and introduced into a vessel, which was kept in a climatized room for 1 to 4 days. Fifty samples of *T. castaneum* (50 beetles and a mixture of one to five days old eggs (more than 150 per sample)) were placed into little film boxes. The bottoms of the boxes and the caps contained pieces of very fine stainless steel gauze (100 m) (Fig. 2) to allow quick penetration of the gas and to avoid escape of freshly emerging larvae. 30 mL of fine, whole meal wheat flour served as substrate for the insects in the cages. Another set of 50 film boxes

contained 50 eggs each of *E. kuehniella*. The eggs were one to four days old and the cages supplied with 30 mL wheat as substrate.

Fig. 2 Film boxes with stainless steel gauze

One sample at a time per species and stadium was packed in a cotton bag. All the bags were placed in different locations distributed over all floors of the mill.

One of two reference samples was taken to the mill the other remained in the laboratory under culture conditions. These samples were kept at similar climatic conditions without fumigation.

Following the fumigation, all samples were collected, transported back to the lab to 25℃ and 65% r · h. and bioassayed for twelve weeks for surviving or emerging insects.

Results

Temperature in the Mill during Fumigation

The temperature in the mill during fumigation presented in figure 3 was in the range 12 – 25℃.

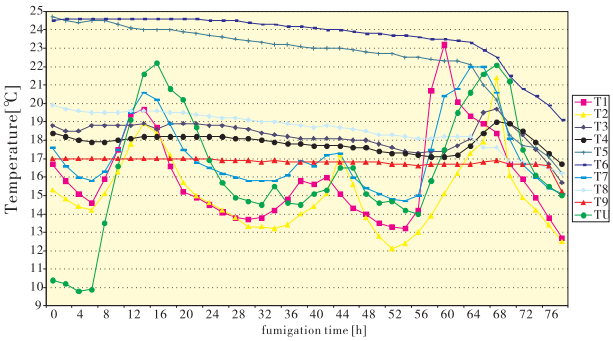

Fig. 3 Temperature on different places in the mill during fumigation with Sulfuryl fluoride

T1: 8. floor, cleaning location, on the window

T2: 3. floor, cleaning location, in one pipe

T3: 5. floor, cleaning location, on the window

T4: 3. floor, mill, in a sieve

T5: 2. floor, cleaning location, on the floor, near staircase

T6: 2. floor, mill, in one pipe

T7: 1. floor, mill, behind a metal cupboard

T8: 7. floor, storage silo, below a skylight

T9: cellar, elevator, E1

TU: behind the mill in a car

Detection of SF in Air in Flour Bins

Figure 4 describes the concentration characteristic of SF in various "untreated" silos in the fumigated mill. They picked up considerable amounts of the gas and were in so far unintentionally treated with low dosages of SF. The SF concentration 30 cm deep in the interstitial (between the particles of the flour) air in the flour within the "non-fumigated bins" reached about 60 to 80% of the values above the flour. The concentration in bin 32 and 26 were fairly similar, whereas only much less SF was detected in bin 23. The figure contains the average concentration in the flour as straight and dotted horizontal lines: bin 23: 1. 2 gSF/m, bin 26: 4. 6 gSF/m and bin 32: 4. 2 g SF/m. After about 12 h and further on, additional gas was injected to compensate for losses to ensure sufficiently high ct – products. This addition is noticeable above and inside the flour. The concentrations in Figure 4 are increasing again after previous decay. In the course of the aeration after about 60 h some additional gas diffused into the cells.

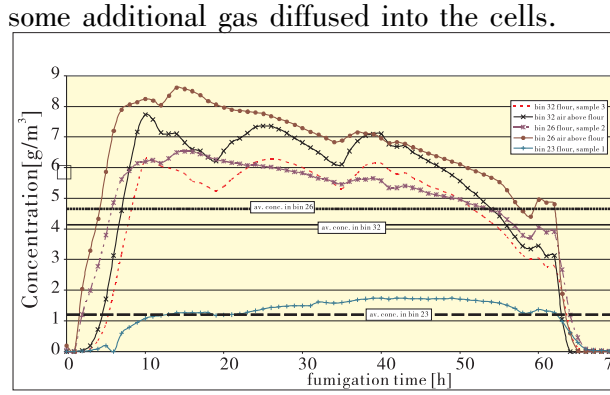

Fig. 4 Sulfuryl fluoride concentration in air during fumigation; 2 kg samples were taken for F^- residue analysis from underneath the surface (30 cm deep) in the bins 23 (sample 1), 26 (sample 2), 32 (sample3), respectively.

Detection of Fluoride in "Non – fumigated" Flour in Flour Bins

The average concentrations of SF as indicated in figure 4 and table 2 seemed to lead in tendency to higher or lower fluoride residues in the flour within the total exposure period of

60 h. The temperature was in the range of approximately 20℃. Figure 2 indicates temperatures during the treatment in various locations.

Table 2. Average SF concentration and fluoride amount in flour bins

Bin number	Sample number	SF concentration [g/m³]	Fluoride amount [mg/kg]
32	3	4.22	5.7
26	2	4.59	10.4
23	1	1.17	2.9

Biological Efficacy

Out of 50 samples with *Ephestia kuehniella*, 4 moths on average emerged in 10 samples, so 20% of the samples had survivors. For the moths, the survival percentage was 8% compared to the untreated which was 100%. With *T. castaneum*, on average 31 beetles developed in 28 samples. That means that 56% of the samples were not fully controlled. The average value of 31 survivors corresponds to 12% of survivors in the untreated references with 263 emerging beetles as 100%.

Discussion

Obviously, also untreated concrete bins within fumigated flour mill buildings pick up a certain amount of gas. If 20 g/m is calculated as the average concentration in the fumigated area (1 200 g · h/m for 60 h), about 20% of this value was determined on top of the flour bins. The F^- – residues 30 cm deep in the flour amounted on average in all three investigated bins about 6 mg/kg. In 2005, Germany had fixed a provisional maximum residue limit (MRL) of 10 mg/kg. The European legislation asks for 2 mg/kg as MRL for grain from September 2008 onwards. Therefore, this subject has to be investigated in more detail to keep this type of fumigation of large flour mills with full in – house flour bins as an option. A better sealing of these bins with residual flour from the rest of the mill might be possible to reduce the formation of fluoride residues.

The lower limit for guaranteed efficacy predicted by the Fumiguide® program is 20℃. In the presented case, the temperature in some fumigated areas was below this value. To achieve complete mortality, these areas should have been warmed up. Also the indicated obtained ct-products on average 1200 g · h/m – were seemingly not sufficient for general complete control of all test insects. The miller had indicated that he was not interested in dosages for complete control where he had not found insects prior to the fumigation. The test samples were also placed into such areas. The concept of fumigating only the critical locations with high dosage within a building with connected areas and with low dosage in some others is very questionable. Gases tend to diffuse into all corners of a fumigated object within fairly short time. In so far, such a plan is futile.

In any case, only eggs as developing stages of the test insects survived to a certain degree (8% and 12% of untreated) in a limited number of samples (20% Ek and 56% Tc of the number of samples). Bell et al. (1999 and 2003) [8,9] as shown in table 3 reported as well that the eggs of *Tribolium* spp. are especially tolerant versus treatment with SF, especially at lower temperature than 30℃.

Table 3. ct – products for 100% reduction of emergence with sulfuryl fluoride fumigation (data from * Bell et al. [8] and ** Bell et al. [9])

Ct for 100% reduction of emergence (gh/m³)			Species (most tolerant eggs)
15℃	25℃	30℃	
2016 *	764 *	–	*Ephestia kuehniella*
–	520 *	–	*Tribolium confusum*
–	1669 **	1154 *	*Tribolium castaneum*

In the presented fumigation, 1 200 g · h/m were not sufficient to obtain full control of all test insects at about 20 ℃ within 60 hours. SF has a very high potential to serve as suitable replacement gas for disinsectisation of empty premises like flour mills and other factories. If full control of all living stages of pest insects is the goal, ct – products and temperature must fit together. Also Reichmuth et al. [10] stressed this point. One of the biggest advantages using the Fumiguide® is the precise and specific adjustment of several fumigation parameters according to the individual conditions of the fumigation. That includes the species and life stages of the pests, the gas tightness of the object, the temperature, the exposure time and the direction and strength of the wind. The increase of temperature within an object can help to reduce the amount of gas.

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0611

Response of *Trogoderma granarium* (Everts) to Different Combinations of Phosphine and *Acorus calamus* Oil

Mansoor-ul-Hasan*, Muhammad Sagheer and Farooq Ahmad

Abstract: The response of *Trogoderma granarium* (Everts) to different combinations of phosphine concentrations (100ppm, 200ppm and 300ppm) and *Acorus calamus* oil doses (30, 50 and 70 μL) was studied. Percent mortality was observed for different exposure periods of 3, 5 and 7 days. Population build – up was observed after 30 and 60 days. Percent mortality increased with the increase in phosphine concentration and exposure time. In case of *Acorus calamus* oil treatments, exposure period appeared to be the most important factor affecting the percent mortality. Population build – up was reduced both with increase in dose of *Acorus calamus* oil and exposure period.

Key words: *Trogoderma granarium*, phosphine, fumigation, *Acorus calamus*, essential oil

Introduction

"Khapra" beetle, *Trogoderma granarium*, is the world's worst pest of the stored grains. (Christensen and Kaufmann, 1969). Losses caused by khapra beetle have been reported to range from 0.2% to 2.9% over a period of 1 to 10.5 months (Irshad *et. al.*, 1988). Although a native of India, this pest has gone abroad and has been reported from England, Germany, Israel, and the USA. In the Indo-Pak subcontinent, it is a very destructive pest of wheat and other stored grains, particularly in the northwestern dry regions of Pakistan and Indian states of Rajasthan, Haryana and the Punjab. Apart from wheat, the insect has also been recorded on sorghum, rice, barley, gram, maize, poppy, pulses, pistachio, walnut and other dried fruits (Azeem *et al.*, 1976; Ramzan and Chahal, 1989; Hamed *et al.*, 1989; Atwal, 1994; Khattak *et al.* 1995; Ram and Singh, 1996). Losses caused by khapra beetle have been reported to range from 0.2% to 2.9% over a period of 1 to 10.5 months (Irshad *et al.*, 1988).

Phsophine is most frequently used to protect stored agricultural products. Various stored grain insect pests including *Trogoderma granarium* (Everts.) have become tolerant to this fumigant. The substandard techniques of phosphine fumigation have led to the development of phosphine resistance in major insect pests of stored grains (Taylor, 1989; Mills *et al.*, 1990). Borah and Chahal (1979) reported that phosphine failed to control khapra beetle, *Trogoderma granarium* in warehouses in India. Tyler *et al.* (1983) documented the development of resistance in stored grain insects pests against useful insecticide, phosphine, in warehouses in Bangladesh. Appreciably high resistance was recorded in *Trogoderma granarium* strains collected from Punjab and Sindh (Alam *et al.*, 1999).

Rhizomes of sweet flag, *Acorus calamus* L. (Aracae), possesses insecticidal properties against a wide variety of insect pests. The powder and extracted oil of rhizomes act as stomach or contact poison, anti-feedant and repellent. The toxic and sterilizing effects of vapors of rhizome oil against certain insect pests have also been observed. (Sexena and Mathur, 1976; Schmidt *et al.*, 1991). Its most effective component is β – asaron (Schmidt, 1986).

In the present study response of *Trogoderma granarium* (Everts) to different combinations of phosphine and *Acorus calamus* oil has been evaluated.

Materials and Methods

T. granarium collected from various godowns of Punjab Food Department located in Faisalabad district was reared in one litre capacity glass jars containing wheat. These glass jars were placed in an incubator maintained at 30 ± 2℃ and 60% ± 5 % relative humidity.

The rhizomes of sweet flag, *Acorus calamus*, were collected from northern hilly areas of Pakistan where it grows naturally. The rhizomes were cleaned, dried and ground to a fine powder (30 mesh) and then extracted with n-hexane in the soxhlet extraction apparatus. Extracts were

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concentrated in a rotary evaporator and finally made solvent free in vacuum desiccator to obtain pure oil. The oil was stored in a refrigerator at 4℃.

Acetone was used as a solvent for *Acorus calamus* oil. Different dilutioins were prepared as under:

999 ml of acetone + 1 mL of oil = 1 liter of solution.

1 mL of solution = 1μL of oil

Phosphine gas was generated by FAO's method (Anonymous 1975). A funnel tied with thread was hanged over a cylinder with 5% H_2SO_4 solution. A pellet of aluminum phosphide wrapped in muslin cloth was dropped in solution under the funnel. Open – end burette was taken in the solution on the funnel as solution rises into the burette. As the burette filled with generated gas the level of solution go down. 5 mL gas was sucked out of it and injected into the glass jar of known volume. Again 100 mL of gas taken out from the glass jar was injected into phosphine meter to get the concentration.

Glass jars of 1 150mL capacity were used as exposure chamber. 25 grams of wheat grains were taken for each treatment. 30 grubs of *Trogoderma granarium* were placed in each glass jar. These grubs were exposed to different combinations of phosphine concentrations and *Acorus calamus* oil. Phosphine concentrations were 100, 200 and 300 ppm. The doses of *Acorus calamus* oil were 30, 50 and 70 L.

All treatments were replicated three times. There was one untreated control for each treatment.

Dosing of *Acorus calamus* oil was carried out by releasing the required volume of appropriate oil solution from an automatic pipette to a disk of 4cm diameter filter paper attached to lower surface of the lid of the glass jar.

The amount of phosphine gas required was calculated using formula as under.

Concentration × Volume of glass jar ×836. 81

Exposure periods for each treatment were 3, 5 and 7 days.

At the end of exposure period, jars were opened. The grubs of *Trogoderma granarium* were separated from the grains and mortality was assessed.

The survivors were transferred to glass jars containing untreated grains. The jars were kept at 32 ±2℃ and 65% ±5 % relative humidity. Data were collected for insect population build-up after 30 and 60 days. At the end, data were analyzed statistically by the analysis of variance, CRD test and Duncan's Multiple Range Test (Steel and Torrie, 1980).

Results and Discussion

Results of the present studies are given in table-1. Results revealed that significant difference existed between interaction of phosphine and exposure time. Percent mortality of *T. granarium* was increased with the increase in concentration of phosphine and exposure period. Maximum mortality (87. 78%) was observed at 300 ppm concentration with exposure period of seven days and minimum mortality (23. 34 %) was observed at 100ppm concentration with exposure period of three days.

According to Kashi (1982) the level of mortality increased with the increase in concentration and also by extending the exposure period. This is in accordance with our results. Present findings are also partially at par with those of Banks and Cavanaugh (1985), Winks (1986), Rajendran (1994), Proctor (1994), Irshad and Iqbal (1994) and Ahmad (1999). Our results are, however, contrary to those of Price and Mills (1988).

Results also revealed that significant difference existed between interaction of doses of *Acorus calamus* oil and exposure time. In case of *Acorus calamus* oil, mortality increased with the increase in exposure period. This indicates that the duration of exposure is more important than the dose applied.

Our findings are in accordance with the findings of Chandel *et al.* (2001); Kumari *et al.* (1999); Risha (1993) and Rasool *et al.* (2002) who observed that exposure period was much more important than dose at the levels tested.

The results of present study differ from the findings of Schmidt and Risha (1990); Risha *et al.* (1990); Schmidt *et al.* (1991) and Pierce and Schmidt (1993).

Results of present studies also revealed that when phosphine and *Acorus calamus* oil were used in combination, mortality was increased both with the increase in phosphine concentration and *Acorus calamus* oil dose as well as with the increase in exposure period.

Population build-up studies revealed that population build was reduced with the increase in dose of *Acorus calamus* oil and exposure period.

There was no effect of phosphine concentration on the population build up of the test insect.

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Table 1. Response of *Trogoderma granarium* (Everts) to different combinations of phosphine and *Acorus calamus* oil at various exposure times.

1(a): Percent Mortality of *Trogoderma granarium* (Everts)

		Phosphine concentrations						*Acorus calamus oil*					
		Phosphine used alone			Phosphine used in combination with Acorus calamus oil			Acorus calamus oil used alone			Acorus calamus oil used in combination with phosphine		
		100 ppm	200 ppm	300 ppm	100 ppm	200 ppm	300 ppm	30μL	50μL	70μL	30μL	50μL	70μL
Exposure	3 Days	23.34	41.11	50.00	33.33	55.19	62.59	10.00	11.11	12.28	12.2	12.42	14.00
	5 Days	34.45	45.56	62.22	42.21	60.00	67.04	17.78	18.89	31.11	18.00	18.59	24.00
	7 Days	40.00	68.79	87.78	51.19	72.59	92.59	38.77	43.33	40.00	39.00	44.32	41.2

1(b): Population build up of *Trogoderma granarium* (Everts)

		Phosphine concentrations								*Acorus calamus oil*							
		Phosphine used alone				Phosphine used in combination with Acorus calamus oil				Acorus calamus oil used alone				Acorus calamus oil used in combination with phosphine			
		0 ppm	100 ppm	200 ppm	300 ppm	0ppm	100 ppm	200 ppm	300 ppm	0μL	30μL	50μL	70μL	0μL	30μL	50μL	70μL
Exposure	3 Days	12.99	12.36	12.37	12.21	12.92	7.85	7.39	7.90	12.61	8.72	7.39	5.44	12.69	7.52	5.81	4.77
	5 Days	12.93	12.41	12.52	12.20	12.91	7.22	6.41	7.08	12.69	6.97	5.47	4.70	12.64	5.92	4.84	3.97
	7 Days	12.94	11.51	12.89	11.31	12.93	6.36	6.04	6.82	12.56	5.45	4.16	3.31	12.55	4.91	4.31	3.69

SESSION 7

OPERATION, REGULATION AND TECHNIQUE STANDARDS FOR CA AND FUMIGATION

Chairpersons:
Dirk Maier, USA
Zeng Ling, China

0701

Improving Structural Fumigation from Engineering Perspectives

Dirk E. Maier[1,2*], Watcharapol Chayaprasert[1] and Klein E. Ileleji[1]

Abstract: Seven sulfuryl fluoride (SF) and one MB fumigation monitoring experiments were conducted as part of regular fumigations in three flour mills. In addition to fumigant concentrations, the environmental conditions both inside and outside of the fumigated facilities were monitored during the experiments. These results showed variability in the fumigation-related parameters that had substantial impact on the success and effectiveness of each fumigation.

A CFD model was constructed based on the flour mill as an analysis tool for prediction of fumigant distribution and leakage during the fumigation process. The data from one of the fumigation experiments was used to validate the CFD model. Given the same environmental conditions and fumigation practices, the model was able to reproduce a fumigant leakage rate (i.e., Half-Loss Time or HLT) and an achieved dosage value (i.e., Ct product) similar to those observed during the actual fumigation. Thus, it was considered validated.

The validated model was used to perform eleven fumigation simulations under weather conditions of the same time period of different years (1996 – 2006). Although the simulated fumigations were performed for the same time period, year-to-year variations in weather conditions caused significantly different HLT predictions. In extreme cases, the HLT prediction can be more than 100% different (from 10.7 to 23.3 hours), yielding a difference in the achieved Ct products by more than 70% (from 476 to 840 g · h/m^3). These results implied that fumigators should quantify the effectiveness of temporary structural sealing in order to verify HLT before a fumigation, minimizing fumigant use.

An automatic fumigation monitoring and decision support system was developed. The system consists of a purge pump, port selection panel, valve control unit, gas concentration sensor, laptop computer, and decision support program. Although the regulation of dosage rate to maintain the desired gas concentration still has to be manually done by the fumigator, the monitoring and decision support system helps prevent over dosing, reduces error and risk from human mistakes, and increases the success rate of fumigation.

Key words: structural fumigation, half – Loss time, computational fluid dynamics (CFD), flour mill, monitoring system

Introduction

The phase-out of methyl bromide (MB) as the major fumigant for use in structural fumigation has warranted the industry to seek for alternative pest control measures. We define structural fumigation as fumigation performed to eradicate pest infestations in permanent enclosures (portions of and whole buildings, industrial facilities, warehouses, gas-tight chambers) that may be empty or contain goods versus fumigation of tarp-covered product stacks or of soils However, fumigation with MB alternatives is more costly and requires a higher level of stewardship to be economically competitive. Therefore, the key for successful adoption of these alternatives lies in the efficiency of its application during fumigation. Because it is not practical to perfectly seal the structure, the fumigation process can be optimized only if the dynamics of gas movement in the fumigated space and the effects of environmental conditions on the process are well understood.

In August 2004, researchers in the departments of Agricultural and Biological Engineering, Mechanical Engineering, and Entomology at Purdue University with funding from the USDA – CSREES Methyl Bromide Transition Program and in collaboration with industry partners initiated a research project with the aim to develop a comprehensive analysis tool, and an automatic monitoring and decision support system for structural fumigation. This paper summarizes the findings and explores several possibilities

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and technologies to improve the structural fumigation process.

Fumigation Experiments

Seven sulfuryl fluoride (SF) and one MB fumigation monitoring experiments were conducted as part of regular fumigations in three flour mills. The primary goals of these experiments were to gain insights into the fumigation process and acquire data for the development and validation of a Computational Fluid Dynamics (CFD) model of the fumigation process. In addition to fumigant concentrations, the environmental conditions both inside and outside of the fumigated facilities were monitored during the experiments. A weather station, which was located on the mill's roof, monitored barometric pressure, wind speed and direction, temperature, relative humidity (RH), and solar radiation. Inside each mill, a 3D anemometer monitored the inside gas velocity, a pressure sensor measured the hydrostatic pressure, six temperature/RH loggers obtained the temperature and RH profiles along the height of the mill, 24 temperature cables measured the wall surface temperatures. A fumigant concentration sensor called Fumiscope® was used to monitor gas concentrations at 1 520 locations throughout the mill. Effects of sealing on the environmental conditions in the fumigated structure were observed in all experiments. The inside temperature was always higher than the ambient temperatures and vice versa for the inside relative humidity. These results showed variability in the fumigation-related parameters that had substantial impact on the success and effectiveness of each fumigation[1]. Therefore, being able to predict some, if not all, of these parameters will lead to the improvement of fumigation efficacy.

Structural Fumigation Modeling

The primary objective of developing the CFD model was to predict fumigant distribution and leakage during the fumigation process. The data from one of the fumigation experiments was used to validate the CFD model. Chayaprasert et al.[2] discussed the modeling methodology and results in detail. A commercial CFD solver, Fluent®(Fluent Inc., Lebanon, NH), was used to construct two flow models based on a reference flour mill. It was first used to construct a model of the flow outside the reference mill for predicting stagnation pressure profiles on the structure's walls created by prevailing wind and then construct a model of the fumigation process in the mill. The domain of the external flow model was set-up as a rectangular volume such that it included the mill building and surrounding structures. Several external flow simulations were conducted to determine average stagnation pressures on the mill's walls as a result of various wind speeds and directions. The relationship between average stagnation pressures and wind velocities was then formulated. Based on the formulated relationship, given the experimental wind data, the average stagnation pressures that would have occurred on the walls during the fumigation period could be estimated. Next, the average stagnation pressures were used as boundary conditions for the internal flow model. The total dimensions of the internal flow domain were 26.5 m × 34.4 m × 27.6 m, which contained rectangular solid volumes representing milling equipment such as roller mills, purifiers, sifters, pneumatic cyclones, tanks and tempering bins.

An example of the simulation results is shown in Fig 1b which illustrates the simulated concentration curves of all monitoring points in the first five floors. The primary discrepancies observed between the experimental data (Fig 1a) and the simulation data were in the fumigant introduction phase. In the simulation, there were fewer differences in the peak concentrations among the floors. This resulted in much less time for uniform gas distribution. The differences in the simulated concentrations at all locations were within 5 g/m^3 at the fourth hour, while the same occurred approximately at the sixth hour in the field trial. However, these discrepancies were not considered critical because on average the model was able to yield a HLT value close to the HLT derived from the experimental data. The HLT of the average simulated concentration curve was approximately 17 hours, which was essentially identical to the HLT of the average experimental concentration. The underpredicted concentration resulted in underprediction of the Ct product. At the time of unsealing, the achieved Ct products of the experimental and simulated data were approximately 950 and 850 $g \cdot h/m^3$, respectively, or a difference of 10.5%.

Based on the predicted HLT and Ct results, the CFD model was considered valid. The effects of fumigation variables such as wind speed and direction, capacity and placement of circulation fans, and fumigant release time on the efficacy of the fumigation process can be evaluated using the model. The results from the

simulations will provide insight into understanding the dynamics of the structural fumigation process and help fumigators to correctly determine the amount of fumigant to be used, which in turn will yield increased efficacy and more successful fumigations.

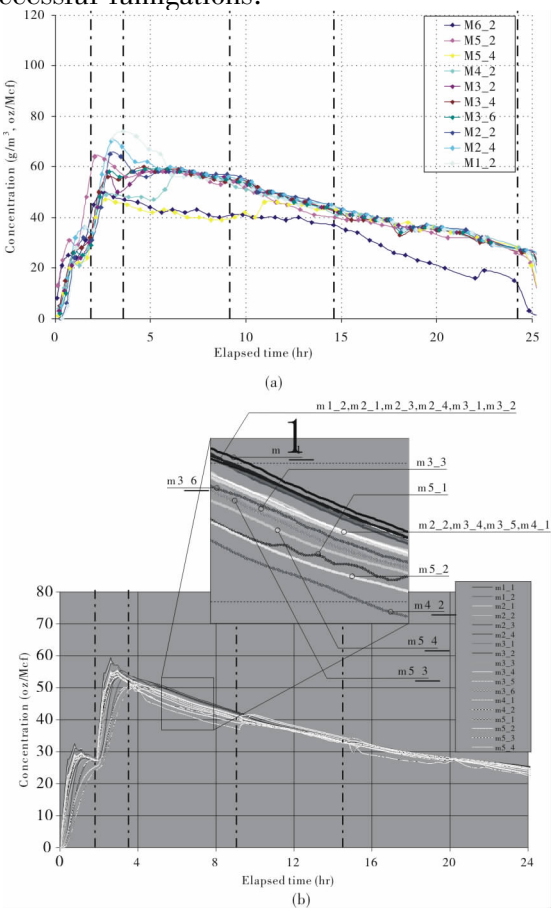

Fig. 1 (a) Sulfuryl fluoride concentration data om the fumigation experiment. (b) Concentrations obtained from the internal flow model.

Simulations of Eleven – Year Fumigations

The previously discussed CFD model was used to evaluate the effect of multi-year weather conditions on the gas leakage rate (i. e., HLT) and the Ct product during structural fumigation in the reference flour mill. Chayaprasert et al.[3] provided a complete discussion of the simulation setup and results. Eleven SF fumigation simulations were performed using historical weather data of the same time period between 1996 and 2006. It was assumed that for each year's simulation (1996 – 2006) the fumigation started at 12:00pm on the 4th of July and lasted 24 hours. Hourly average historical weather data collected at the Indianapolis International Airport were used in the simulations. For all eleven simulations, the fumigation practices (e. g., sealing quality, fumigant releases, fumigant introduction and monitoring locations) were assumed to be the same. One fumigant introduction site was located around the middle area of each floor. At each introduction site, a circulation fan (2.71 m^3/s) was placed. A total of 226.8 kg of SF was released into each floor of the mill. The first half (113.4 kg) was released at the beginning of the fumigation and the second half was released approximately two hours later. The fumigant concentrations were monitored at 18 locations distributed throughout the mill.

Although the simulated fumigations were performed with the same temporary structural sealing quality for the same time period of the 11 years, the year-to-year variations in the weather conditions caused differences in initial concentrations, HLTs and Ct products. In extreme cases, the initial concentration was almost 20% different (from 54.3 to 44.6 g/m^3) and the HLT was more than 100% different (from 10.7 to 23.3 hours), yielding a difference in the achieved Ct products by more than 70% (from 476 to 840 $g \cdot h/m^3$). This means that for a given structure even though the fumigator could maintain the same sealing quality for every fumigation, the difference between the HLT predicted based on past fumigation data and the actual HLT observed during the current fumigation could be substantial. The fumigator would either overdose in the case of underpredicted HLT or have to intermittently release additional fumigant in the case of overpredicted HLT, resulting in a non-optimized fumigation process. As a result, past fumigation data should not be the primary means for evaluating the effectiveness of sealing and the effectiveness of temporary structural sealing should be measured under controllable conditions. One standardized method used by the HVAC industry for measuring building air-tightness is the pressurization test, also known as the blower door test[4,5]. In addition, a calculation procedure, in which the result of the pressurization test is incorporated, for air leakage rates due to weather conditions has also been suggested[4]. The pressurization test and this calculation procedure could be directly applied to the prediction of HLT and Ct product, given weather forecasts for the planned fumigation period. Therefore, the fumigation performance could be substantially improved.

Automatic Fumigation Monitoring and Decision Support System

Monitoring gas concentrations for the entire fumigation duration is a labor-intensive and

tedious task. Therefore, it is typically not done on a continuous but rather on an intermittent basis (e. g., every 35 hours). However, best optimization of the fumigation process cannot be done without accurate HLT and Ct product estimations. Although prediction of half-loss time (HLT) and Ct product could be performed in advance with the application of the building pressurization test, the true HLT and Ct product can be observed only from the fumigant concentration levels inside the structure. Thus, the utilization of a fumigation monitoring system is another vital part to assure fumigation success.

Hardware Components

Fig 2 shows the hardware schematic of the automatic fumigation monitoring system. The system consists of a purge pump, port selection panel, gas concentration sensor, laptop computer and modular distributed I/O system. The purge pump (Model #2107CA20, Thomas Products Division, Sheboygan, WI) is used to draw sample gas through nylon tubing from the fumigated structure. The custom-made port selection panel is capable of handling up to 14 monitoring lines. It consists of one three-way and 14 two-way DC solenoid valves (Part #648T032 and #648T012, respectively, Neptune Research Inc., West Caldwell, NJ) each of which has an orifice size of 3.0 mm. The inlet of each valve is connected to a monitoring line and the outlet is attached to a manifold with its outlet connected to the Sulfuryl Fluoride Single Zone (SFSZ) Monitor (Spectros Instruments Inc., Hopedale, MA). The opening and closing sequence of the valves is controlled by the modular distributed I/O system called FieldPoint (National Instruments Corp., Austin, TX). The FieldPoint system consists of one interface module, one digital output module and one analog input module. The interface module, FP-1000, is the primary module which communicates with the laptop computer through a RS-232 cable. The digital output module, FP-DO-401, sends 24-volt signals to activate the solenoid valves. The analog input module, FP-AI-110, reads gas concentrations in the form of electrical signals from the SFSZ monitor. The FieldPoint system actuates the solenoid valves according to a control program written in LabVIEW (National Instruments Corp., Austin, TX), which is part of the fumigation decision support (FDS) program discussed in the next section.

Software Program

The design of the FDS program was aimed at SF and red flour beetle eggs as the primary

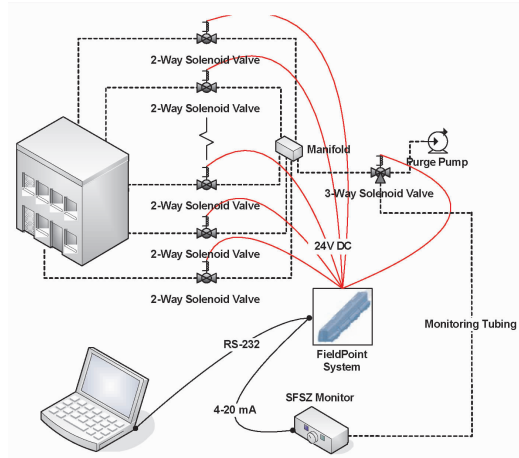

Fig. 2 Hardware diagram of the automatic fumigation monitoring system.

fumigant and target insect species/life-stage, respectively. However, the FDS program was developed on a generic platform such that it can be easily modified and customized to support other fumigants (e. g., methyl bromide or phosphine) and/or insect species/life-stages if the required dosage rate information for those particular fumigants and insect species/life-stages is available. The user specifies the number of fumigated volumes each of which is treated by the FDS program as a separate individual volume (i. e., assumes no interaction flow between volumes). Four fumigation parameters (i. e., temperature, HLT, exposure time, and size) are entered for each volume. The FDS program then provides the expected initial concentration, target Ct product, and required amount of SF. Gas concentrations in the fumigated volumes are monitored in cycles. For every new concentration reading taken, the FDS program performs a sequence of calculations to determine the Ct product that has been achieved up to the present time and to predict the Ct product that would be achieved at the end of the exposure time, i. e., the projected Ct product. As part of the fumigation control strategy, the FDS program compares between the projected and target Ct products. If the projected value is less than the target value, the FDS program displays an alarm message to the computer screen and provides a recommendation as to how much additional SF is needed and/or how long the exposure time needs to be extended in order to attain the target Ct product by the end of the fumigation.

Conclusions

The CFD fumigation model serves as a simulation analysis tool that can be used to evaluate various "what if" fumigation scenarios,

quantify the effects of weather conditions on HLT, and design possible fumigation strategies such that fumigation applications can be customized based on the prevailing site-specific conditions without the high cost of conducting full-scale fumigation experiments. Also, the established modeling methodology can serve as the basis for fumigation process modeling in any type of structure.

The experimental and simulation results showed that variations in the fumigant leakage rate from fumigation to fumigation can be substantial depending upon several factors (e. g., sealing quality, weather conditions, etc.). Therefore, in order to optimize the fumigation process, using past fumigation data as the primary means for evaluating the effectiveness of temporary structural sealing quality and predicting HLT is not adequate. Predictions of fumigation performance should incorporate quantifiable sealing effectiveness and weather information for the planned fumigation period.

An automatic fumigation monitoring and decision support system was developed based on the technologies presently available to the fumigation industry. Although the regulation of dosage rate to maintain the desired gas concentration still has to be manually done by the fumigator, the monitoring and decision support system helps prevent over dosing, reduce error and risk from human mistakes, and increase the success rate of fumigation. Currently, the system operates on the tube-and-pump principle. The time and labor needed for setting up and disassembling the fumigation monitoring system would be reduced substantially, if wireless gas concentration sensors were available.

Acknowledgements

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Early Detection of Spoiled Grain Stored in Hermetic Plastic Bags (Silo-bags) Using CO_2 Monitoring

Ricardo Bartosik*, Leandro Cardoso and Juan Rodríguez

Abstract: In Argentina about 35 million tonnes of grains were stored in hermetic systems (silo-bags) during 2007, and it is expected that in the future a bigger proportion of the grain will be stored in these silo-bags in Argentina and other countries.

The silo-bags are made of a 235 – micrometer plastic film of 2.74 m diameter and 60 m long (holding approximately 200 tonnes of wheat each). Grain quality monitoring is carried out with a standard torpedo probe. This operation has several disadvantages, including perforating the plastic film (disturbing the air tightness of the system), difficulties to target the grain spoilage area (especially when it is located in the bottom of the silo-bag), and the relatively intense labor demand. Monitoring stored grain conditions through temperature measurement is not an option, since the grain stored in the silo-bags does not increase temperature during spoilage.

A study was made with several silo-bags filled with wheat and soybean located at different farms and grain elevators in the South-West of Buenos Aires province. The silo-bags were sampled with a torpedo probe, and the corresponding value of CO_2 concentration of the silo-bag was measured. The collected grain samples were analyzed in the laboratory for moisture content (MC). Additionally, the silo-bag overall condition was evaluated (bad sealing, openings, occurrence of occasional flooding in the area of the silo-bag, etc), and evidence of spoiled grain at bag unloading was collected.

Periodic CO_2 monitoring of silo-bags allowed for the early detection of biological activity and spoiled grain. A distinctive value of CO_2 for different MC grains was established, which represents the typical atmospheric composition for a silo-bag with and without conservation problems. The silo-bags holding grains with different levels of conservation problems were identified by the unusually high CO_2 concentrations of the modified atmosphere.

Key words: modified atmosphere, biological activity, grain preservation

Introduction

In year 2007 about 35 million tonnes of grains, including soybean, corn, popcorn, wheat, sunflower, malting barley, rice, sorghum and cotton seeds were stored in hermetic systems (silo-bags) in Argentina. The end use of these crops varied from feed, wet and dry milling, seed, human consumption, brewery, oil extraction and flour milling among others.

Each silo-bag can hold approximately 200 tonnes of wheat (180 tonnes of soybean) and with the handling equipment currently available the loading and unloading operation is fast, simple and totally mechanized. These plastic bags are 60 m long, 2.74 m diameter and the plastic cover is made of three layers (white outside and black inside) with 235 micrometers of thickness.

Measuring grain temperature is the main tool used by farmers and the grain industry for monitoring proper storage conditions, since an increase in grain temperature is highly correlated with an increase in the biological activity in the grain mass. Unfortunately, this technology is not useful for monitoring storage conditions in silo-bags. It was demonstrated that temperature of the grain stored in silo-bags follows the pattern of the average ambient air through the seasons, presumably due to the high surface/volume ratio of the silo-bag, compared to a regular steel silo (this would provide the silo-bag with a high capacity to exchange heat with the air and soil)[1]. The surface/volume ratio of a 180 tonnes silo-bag is approximately 1.42, while for a standard metal bin of the same capacity (7 m diameter and 9 m height) the ratio is 44 % lower (0.79). Thus, the effect of the biological activity on grain temperature can be obscured by the ambient air temperature effect.

Monitoring the grain storage condition by probing the silo-bags with standard torpedo

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probes is a process fairly easy to implement. However, each perforation made to the plastic cover disturbs the air-tightness of the system, which limits the number of samples that can be collected from each silo-bag, and the sampling frequency. Additionally, this monitoring procedure is useful in order to obtain an idea of the overall quality of the grain stored in the silo-bag (i. e. , protein content, falling number, etc), but it is not suitable for detecting early spoilage problems (most of the spoiled grain process occurs in particular locations of the grain mass, typically in the bottom of the silo-bag where the torpedo probe cannot collect the sample). Another disadvantage of this methodology is the amount of labor and time involved.

The silo-bags are waterproof and have a certain degree of gas-tightness (oxygen (O_2) and carbon dioxide (CO_2)). As a result, respiration of the biotic components of the grain mass (fungi, insects, and grain) increases CO_2 and reduces O_2 concentrations. Thus, the degree of modification of the gas composition in the interstitial air could be related to the biological activity inside the silo-bag, and can be used as a monitoring tool to detect early spoilage problems.

Cardoso et al. [2] and Rodr guez et al. [3] studied the main factors affecting CO_2 concentration in wheat and soybean stored in silo-bags. Based on these research studies the typical CO_2 concentration of wheat and soybean stored in silo-bags without conservation problems was established. This study is about detecting conservation problems in wheat and soybean stored in silo-bags by comparing the measured CO_2 concentration with the typical CO_2 concentration values of silo-bags without conservation problems.

Materials and Methods

The tests were carried out in elevators and farms in the south east of Buenos Aires province, Argentina. The silo-bags were filled with fresh grain right after the harvest in the same plots where the crops were planted, or filled with grain coming from bins in the proximity of the elevator facility. The experiment started in January for wheat and in April-May for soybean and lasted until the silo-bags were opened for emptying (about 5 months later).

For each silo-bag two sampling locations were established. The procedure consisted of measuring first the CO_2 gas concentration with a portable gas analyzer (PBI Dan Sensor, Check-Point, Denmark), perforating the plastic cover with a needle. The gas composition was analyzed for three levels in each sampling location, close to the top of the bag, at the middle and close to the bottom.

In each sampling location grain was collected from three different levels (top = 0. 10 m depth, middle = 0. 75 m depth, and bottom = 1. 6 m depth. Total height of the bag = 1. 7 m) using a standard torpedo probe and grain MC was measured (GAC 2100, Dickey – John). After probing the silo-bags the openings were sealed with a special tape in order to restore the air – tightness.

The monitoring procedure was repeated approximately every 15 days during the entire storage period. When the grain was unloaded from the silo-bag, both grain and bags were inspected to detect spoiled grain. The silo-bag was then classified as "No evidence of storage problems" or "Evidence of storage problems" (Fig. 1).

Fig. 1 Silo-bag with spoiled grain detected during unloading.

Results and Discussion

Figure 2 shows the change of CO_2 concentration in three different silo-bags with soybean at 11.5% MC (below market MC), at 12. 9% MC (close to market MC), and 14. 9% MC (higher than market MC) (13. 5% is the base MC for marketing according to the soybean argentine commercialization standard). During the winter time (July – August) the CO_2 concentration was below 3% for all MCs. In the early spring (September) the CO_2 concentration started to increase to 9 and 10% for the silo-bags with 12. 9% MC and 11.5% MC, respectively. In October, the CO_2 concentration increased even further, up to 16% and 18% for the same silo-bags, respectively. From

then on, the CO_2 concentration decreased to about 10 to 13% and remained stable during the late spring (December). The silo-bag with wet soybean (14.9% MC) had a CO_2 concentration lower than 2% during the entire storage period. When the silo-bags with high CO_2 concentration were unloaded a significant amount of grain with severe spoilage was detected. In these two bags, a layer of 0.1 m thick at the bottom was affected by perforations in the plastic cover. This allowed the entrance of water and oxygen, creating suitable condition for mold development when the grain temperature increased in the early spring. On the other hand, the wet soybean silo-bag did not present any significant perforation in the plastic cover, so the safe storage conditions were maintained throughout the entire storage period, even during the late spring. As a result, the grain did not show any evidence of spoilage when inspected during the unloading operation.

These results showed that periodic CO_2 monitoring can be used as a tool for early detection of spoilage problems in silo-bags.

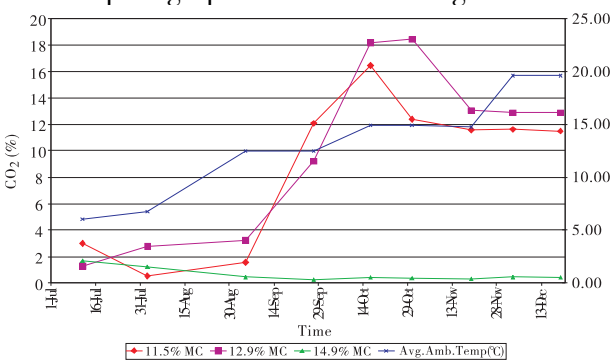

Fig. 2 CO_2 concentration during storage time of three silo-bags with soybean at 11.5% MC, at 12.9% MC and at 14.9% MC and average ambient air temperature.

Although the periodic monitoring of the CO_2 concentration allows for detecting an increase of biological activity in silo-bags during storage (Fig. 2), sometimes there is only opportunity to make a single measurement of the CO_2 concentration of the silo-bag. In this case, there is no possibility to compare results with previous values in order to determine whether the biological activity was increasing, and the grain storage condition turned unsafe. For these situations another type of approach is needed to relate CO_2 concentration with grain storage condition.

Figure 3 shows that the average CO_2 concentration for wheat silo-bags with proper storage conditions was substantially lower than the average concentration for those silo-bags with evidence of spoiled grain. At MC values below 13%, the difference between the two lines was about 10% percentage points of CO_2, while at 16% MC the difference was reduced to 7.5 percentage points. It was observed that for those silo-bags with wheat MC below 16%, the spoiled grain was localized in the bottom grain layers. In these silo-bags several perforations were observed in the plastic cover, which allowed the entrance of moisture (from rainfall) and oxygen. The perforations were caused by wild animals (armadillos), or because the silo-bag was settled on top of the residues of the previous crop (the stems perforate the silo-bag if proper care is not taken during the placement and loading operation). Another reason was improper sealing of the end of the silo-bag, which allowed the entrance of moisture and oxygen to the system. Finally, some silo-bags were placed in low lands, which were flooded after an intense rainfall. In this last situation, even an undamaged and well sealed silo-bag was affected.

On the other hand, when the wheat MC was above 18%, spoilage was observed in the silo-bag regardless of the silo-bag's structural condition (perforations or improper sealing of the ends). The excessively high grain MC resulted in high mold activity, which caused grain spoilage. Water deposition was observed at the bottom of these silo-bags, even in those bags without visible perforations. It was speculated that condensation occurred due to the day – night temperature differential. The recurrence of this process could result in significant water deposition on the inside of the plastic cover, which could produce a large amount of spoiled grain.

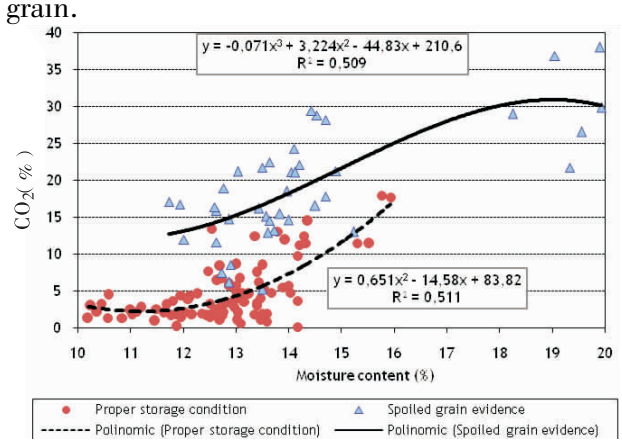

Fig. 3 CO_2 concentration at different moisture contents in wheat silo-bags with storage condition classified as "proper" and with evidence of spoiled grain.

Figure 4 shows that the average CO_2 concentration for soybean silo-bags with proper storage conditions was substantially lower than the average concentration for those silo-bags with evidence of spoiled grain. Those silo-bags with soybean under proper storage conditions always presented CO_2 values below 4%, and did not show a trend to increase CO_2 concentration with the increase of MC. On the other hand, silo-bags with evidence of spoiled soybean resulted in CO_2 concentrations as low as 6% and as high as 18%, and the average between 11.5% and 14% CO_2. Contrasting with the wheat data, there was not a clear trend to correlate the increase in CO_2 concentration with the increase of MC, neither for those bags with grain with proper storage conditions, nor for those bags with evidence of spoiled grain. The reasons that caused the grain spoilage were similar to those described for wheat silo-bags as it allowed for the entrance of moisture and oxygen: perforations in the plastic cover, improper sealing, or temporary flooding of the ground.

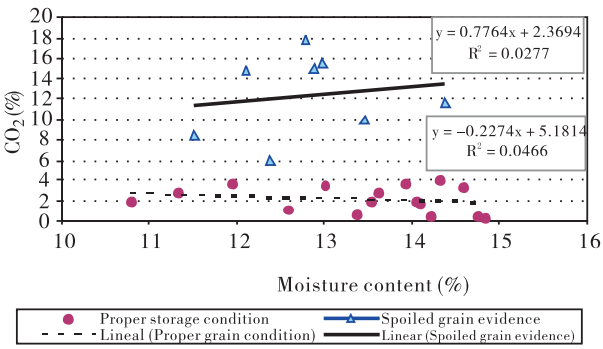

Fig. 4 CO_2 concentration at different moisture contents in soybean silo-bags with storage condition classified as "proper" and with evidence of spoiled grain.

Based on these results, the authors propose to use CO_2 monitoring for detecting spoilage problems in wheat and soybean silo-bags. In the case of wheat silo-bags, the operator measures the CO_2 concentration and collects a grain sample to determine the MC. The typical CO_2 concentration for safe wheat storage condition (at the measured MC) is obtained from Figure 3, and then compared to the measured CO_2 concentration. If the measured CO_2 concentration is below the typical CO_2 concentration, the storage condition can be classified as "safe". On the other hand, if the measured CO_2 concentration is above the typically safe CO_2 concentration, the storage condition is classified as "risky" and the operator should monitor that silo-bag closely to discard spoilage. If the measured CO_2 concentration is close or above the typical CO_2 concentration of a spoiled silo-bag, then the storage condition should be classified as "unsafe" and immediate action should be taken (i.e., unloading the silo-bag).

In the case of soybean silo-bags the grain MC is not critical to determine storage conditions (for soybean MC range between 11% and 15%), and when the measured CO_2 concentration is above 4%, the storage condition of the silo-bag should be classified as "risky". When the CO_2 concentration is close or above 11.5% – 14%, the storage condition of the silo-bag should be classified as "unsafe". Otherwise the silo-bag storage condition should be classified as "safe" (CO_2 concentration below 4%).

Conclusions

Periodic monitoring of CO_2 concentration can be used as a tool for detecting an increase in the biological activity in silo-bags and relate it to the spoiling grain process.

The expected CO_2 concentration of wheat and soybean silo-bags with safe and unsafe (evidence of spoiled grain) storage conditions was determined.

This methodology can be used for monitoring grain storability in silo-bags. The CO_2 concentration in the silo-bag is measured and compared to the expected CO_2 concentration for silo-bags with safe and unsafe storage condition, and the silo-bag storage condition is then classified as safe, risky and unsafe.

The CO_2 concentration of wheat silo-bags with safe storage conditions increases with grain MC (from lower than 5% CO_2 for 13% MC or less, to 17% CO_2 for 16% MC). Thus, the comparison of the measured CO_2 concentration with the typical CO_2 concentration of silo-bags at safe storage conditions should be related to the grain MC.

On the other hand, the MC of soybean does not substantially affect the CO_2 concentration of silo-bags with safe storage conditions (for MC soybean MC range from 11 to 15%), so any measured concentration below 4% means safe storage conditions, between 4% and 12% means "risky" storage conditions, and above 14% means "unsafe" storage condition.

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0703

The Need to Update a Practical Guide Related to the Cycle for Fumigation of Grains as a Function of Research and Technological Progress

Danilo J. Mejia Lorio

Abstract: This paper describes relevant aspects mainly related to fumigation of grains and cereals as a useful treatment to prevent post-harvest losses and improve food security. At the same time, it highlights a publication entitled: "Guide to fumigation under gas-proof sheets" prepared jointly by FAO and ACIAR in 2004. This guide is special since it is valuable as a reference text and it also contains a CD – ROM with audio visual training material on fumigation. The AGST technical unit of FAO would like to draw attention to the need to update this guide on fumigation in line with the Montreal protocol on the use of fumigants and invites storage and fumigation experts to promote activities in favour of the improvement of food security and environment protection where the INPhO web data base of FAO could play an important role in technical diffusion of this subject matter. Likewise, some FAO experiences and issues of interest related to post harvest matters and fumigation are discussed.

Introduction

The need to preserve grains and cereals after harvesting is a mandatory stage that must be faced by agricultural farmers, middlemen, traders, authorities of the national grain reserves of governments, those responsible for grain milling agro industry and many other stakeholders involved in the food chains related to grains and cereals in order to maintain good-quality grains either for consumption, processing or as a source of seeds for planting. Therefore, a basic infrastructure and application of adequate technical knowledge is essential in order to prevent postharvest losses due mainly to the attack of different pests such as insects, rodents and others.

In fact, postharvest losses could amount to as much as 80 percent, especially in developing countries and in developed countries, although postharvest losses are not as high as in developing countries, significant losses may still occur. In a study carried out by the prevention of post harvest losses programme (PFL) of FAO at the end of the 1980s 80th in Asia, it was discovered that storage is one of the most critical operations where most rice post harvest losses of occur. For a secure preservation of grains during storage, is not only important to have a hermetic structure, but also it is very important to dry the grain properly to a level of safe moisture content before it is introduced into the storage structure. The desirable moisture content for the drying of grains and cereals is shown in the following Table 1.

Table 1.

Product	Final moisture content in storage %
Maize	13.5
Sorghum	13.5
Paddy	13.5
Millet	14.5
Safflower seed	9.5
Linen	9.5
Oilseed rape	7.5
Sunflower seed	10.5
Groundnut (shelled)	8.5
Soybean	12.5

Source FAO publication, 1996: "*Secado de granos y secadoras*"

However during the storage period for dried grains and cereals, biochemical respiration, both aerobic and anaerobic, takes place at a low rate. This biochemical process is described in the following reactions shown in figure 1.

$$C_6H_{12}O_6 + H_2O = 6CO_2 + 6H_2O + \text{Heat (Aerobic respiration)}$$

or

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2 + \text{Heat (anaerobic respiration)}$$

Fig. 1 Biochemical reactions of respiration for grain and cereal during storage period

In summary, in both cases the respiration biochemical process generate as final products moisture and energy causing an increase of the internal temperature inside the store creating optimal condition for the germination of insect

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eggs and larvae growth at expense of consuming the grains substrate, which in turn provokes the quantitative and qualitative losses of the grains and cereal stored. Therefore, the fumigation process represents a very useful treatment to prevent grain losses during storage. In this context, fumigation becomes an important treatment which deserves special attention, since it allows for the protection the grain against the attacks of insects and as a result increases the shelf life of grains and cereals stored. This paper deals with the most relevant aspects to be considered for appropriate fumigation treatment at small, medium and largescale level. Likewise, this paper draws the attention to the need to have an updated guideline on fumigation which is in line with the technological progress and innovation required for grains and cereals preservation.

Background and Justification

As was mentioned above, the need to preserve the grains and cereals in storage is essential in order to avoid losses and in consequence to improve the food security of the people. This subject is of continuous importance and interest within the food chain of the agricultural sector. In this context, and due to the need to present alternative solutions, as for example, more effective and efficient technologies for protecting the grains and cereals in storage, particularly at the present time when the prices of staple food grains are increasing, this is another reason to justify why it is important to optimise, not only the production of grains and cereals, but also be more efficient in terms of managing and application of technologies at the post harvest period for grains and cereals especially during the storage operations.

Therefore, the Food and Agricultural Organization of the United Nations is concerned about the food security of their member nations and is making efforts through different activities such as projects, programs, regulation norms and publications among others to contribute significantly to improving the standard of life of people so that it is in equilibrium and harmony with the environment.

Fumigation is an important aspect of grain preservation at the small, medium and largescale level, and although it may seem a complex and hazardous technology, it is an excellent system which works effectively and efficiently when it is applied properly.

On this occasion, the Agricultural and Food Engineering Technologies Service AGST of FAO, would like to highlight an FAO publication of interest for the post harvest sector for grains and cereals during storage and which particularly refers to the fumigation treatment. It is of importance for FAO to present this useful publication on fumigation, since due to the fact that the modern trends on grain fumigation place emphasis on reducing the use of chemicals, this publication can be still considered a practical guide to be followed at present by users.

The publication in reference entitled: "Guide to Fumigation Under Gas-Proof Sheets" was produced by FAO in 2004 as a practical guide, which was compiled and prepared by the Australian Centre for International Agricultural Research (ACIAR) in Canberra, Australia. The publication comes together with a complementary multimedia CD – ROM with the same title and it is strongly recommended, in order to obtain more effective results, that the hard copy guide be used in conjunction with the electronic version contained in the CD – ROM.

Given the importance of the fumigation treatment which demands accuracy and precaution vis-vis the protection of human health and the safety of the environment, fumigation treatment must follow a certain protocol with regard to its application, and an acute sense of awareness and responsibility are very important. In this sense, the creation of a global task force of experts, that can contribute to the creation of an updated database and guidelines in line with the efficiency and the protection of health and the environment, is very important.

In fact, this publication guide along with the complementary CD, is very likely be one of the few publications, if not the only one, which have this particular type of content.

It is clear that the present guide on fumigation produced by FAO is a useful guide which contains practical issues based on the long experience and knowledge of many recognized experts. However, it is also appropriate to think that, as some fumigants are in the process of being phased out due to the fact that they cause ozone-layer depletion and due to the the protocol of Montreal, the need to revise and update the fumigation guide also becomes a matter for periodic renewal and, likewise, will need to be replaced periodically.

By means of this paper FAO would like draw the attention of the grain fumigation experts and institutions so that they may propose

new ideas that may enrich new concepts for the new version of the fumigation guide. for fumigation. FAO would be open to share future activities in this regard with other partner institutions in order to provide technical support for the prevention of post harvest grain losses and, in consequence, to ensure food security.

Fundamental Aspects to Consider for a Properly Fumigation

In this section the main terms and aspects to consider when fumigation treatment is applied are discussed. These terms and aspects are described in more details in the guideline of fumigation and also by means of the practical videos contained in the CD – Rom, which is the complementary part of the guide prepared by FAO and ACIAR.

For What Reason and for Whom this Fumigation Guide has Been Prepared

the guide has been made to tell and show users how to do undertake fumigation with phosphine and methyl bromide and using gas-proof sheets to treat bag-stacks of grain and cereals, loaded freight containers and products or cargoes like timber and machinery that can be enclosed under gas-proof fumigation sheets. The fumigation technique recommended is supported by effective scientific principles. The guide was designed for people who have some previous training in how to undertake fumigation using gas-proof fumigation sheets. The guide not intended to be a manual for people who do not have experience with fumigation nor to replace any existing local regulations on the use of phosphine, methyl bromide or other fumigants, but the guide may provide a better understanding of these regulations.

Some Terms and Basic Facts about Fumigation

a. Fumigants: these are gases of a normal temperature and due to their toxicity are used to kill pests, because they rapidly penetrate through grains and other commodities. The way fumigants diffuse during fumigation treatment depends on their physical properties. Therefore, it is important to have a good understanding of the properties of the fumigant and how they can affect the results of fumigation.

b. Fumigation: process of adding a fumigant to a fumigation enclosure with the specific purpose of killing pests.

c. Best Fumigation Practices: provide a successful treatment process and results from a practical combination of applying required procedures during fumigation to ensure that people doing the fumigation and people around the area of fumigation remain safe and are not harmed, the environment is not harmed, all life stages of all target pests are killed and the product under treatment is not damaged.

d. Fumigation enclosure: these could be permanent or temporary structures; in any case they must be well-sealed in such a way that they are sufficiently gastight to hold a toxic concentration of the gas fumigant long enough to kill target pests during a specific period of time named *the exposure period*. During the best fumigation practices, fumigation enclosures are checked to ensure that they are well-sealed to hold a fumigant over the required exposure period (s). Permanent fumigation enclosures include purpose-built fumigation chambers, some sealed grain silos, some sealed horizontal grain storages, etc. Temporary fumigation enclosures are commonly created using gas – proof plastic sheets, using a technique called sheet fumigation.

e. The exposure period: is the time required for a specific dosage of fumigant to kill target pests. The exposure period is counted from when monitoring shows that the fumigant concentration reachs an effective concentration minimum of 0. 05 g/m^3 for phosphine and 3 g/m^3 for methyl bromide. The length of the exposure period depends on the type of fumigant, physiological state of the target pest, temperature of the commodities, rate of respiration of the target pest and the dosage of fumigant applied.

f. Half-Loss Time (HLT): the time taken for one half of the original concentration of fumigant to be lost from a fumigation enclosure due to leakage or sorption. The HLT is determined only by monitoring gas concentration.

g. Fumigation sheets (Gas proof sheets, tarpaulins or tarps): are gas-retaining plastic sheets used to hold fumigants inside a fumigation enclosure during the exposure time.

h. Sealing: process which renders the fumigation enclosure gastight.

i. Leakage: is the loss of fumigant gas from a fumigation enclosure

j. Permeation: is the loss of fumigant gas from a fumigation enclosure due to "Gas-proof" Sheets, which are seldom completely impermeable.

k. Diffusion or dispersion: the process whereby a fumigant gas moves from an area of high concentration to an area of lower concen-

tration until it finds the equilibrium concentration.

l. Dosage: describes not only the amount of fumigant gas that mut be introduced, but also the length of the exposure period. It is always expressed in two parts: The amount of fumigant gas required and the period of time. For instances, X g/m^3 for 24 hours or Y g/t (grams/tonne) for 7 days.

m. Equilibrium: occurs in well-sealed enclosures after the dosage is applied, the gas concentration remains stable and equal in all parts of the fumigation enclosure and remains above an established threshold for tolerant life stages of target pests.

n. Concentration: describes the amount of fumigant in the air/atmosphere inside a fumigation enclosure and it is expressed as weight or volume of fumigant gas in a given volume of air. Common concentrations are expressed as grams per cubic metre (g/m^3); milligrams per litre (mg/L) or parts per million (ppm).

o. Monitoring: is the process of measuring the concentration of fumigant gas inside a fumigation enclosure and the area surrounding a fumigation enclosure. There are different devices and companies offering these products.

p. The threshold limit value (TLV) (occupational exposure standard) is the maximum concentration of fumigant gases established to which workers may be repeatedly exposed in the work place without harmful effects. The TLV in many countries has been set at: 0.3 ppm (0.0004 g/m^3 or 0.42 mg/m^3) for phosphine; 5.0 ppm (0.02 g/m^3 or 19.4 mg/m^3) for methyl bromide.

q. Sorption (absorption and adsorption) is the uptake of fumigant gas by the product being fumigated. When sorption is so great and concentration of fumigant inside the enclosure is reduced to less than minimum effective, it becomes impossible to kill the target pests. This situation must be corrected by the addition of more gas (dosage corrections), otherwise the treatment will fail.

r. De-sorption: is the reverse of sorption and it is the release of sorbed fumigant that was fumigated and it normally occurs at the end of fumigation or during aeration or the ventilation stage. Fumigants with a high boiling point, as for example methyl bromide, tends to be sorbed more and remain as residue for longer lengths of times than fumigants with a low boiling points such as phosphine.

s. Residues: are very small quantities of chemical left in a product after it has been fumigated. These could include, chemical from which fumigant gases are generated (e.g. aluminium phosphide formulations, fumigant gas, e.g. unchanged methyl bromide after a fumigation undertaken at a low temperatured and any compound formed when a fumigant gas reacts with the product being fumigated.

t. Aeration (airing or ventilation): is the process at the end of the exposure period, after the fumigation enclosure is unsealed, when fumigant gas desorbs and diffuses out from the product fumigated and from the fumigation enclosure.

u. Clearance: is the procedure after the aeration period when the fumigator tests the air in the workspace to make sure that the concentration of fumigant has fallen to or below the safe level and declare that the area is safe for workers.

v. Danger (exclusion, hazard or risk) area: any area near to a fumigation enclosure into which fumigant gas may escape or diffuse in dangerous concentrations is called a danger area. This area must be clearly marked in accordance with regulations.

w. The label: this is when it has been demonstrated that a chemical can be used in compliance with national regulators governing the use of insecticides, the chemical is registered for use and granted a label by a national agricultural chemical registration agency.

x. Material Safety Data Sheets (MSDS): describe the properties and hazards of a material or substance, including its identity, normal uses, ingredients, physical and chemical properties, stability, reactivity, health hazard, first aid treatment, storage, ecological information and transport and disposal considerations.

Responsibilities vs Good Fumigation

The fumigation process and its success are generally assumed to be the responsibility of only the fumigator. However this is not so, because successful fumigation relies on all people involved in any fumigation treatment and includes: the customer, the fumigator, the transport contractor and the regulatory agencies that directly affect the conduct of the fumigation.

a. Customer: they must choose qualified, approved and currently valid certification to perform fumigation among others.

b. Fumigators must have a valid license, skills and competencies, safe equipment and others.

c. Transport contractor (s): they must ob-

tain from the customer and the fumigator information about the fumigation, treatment dates, length of the exposure and aeration period, etc.

d. The regulatory agencies: national and international agencies (e. g. quarantine authorities with an interest in the way that fumigation treatment is undertaken. This may be through legislation, regulation, training and retraining, and or license.

e. Other parties: people involved in fumigation treatment may vary from place to place, for instance, in some countries the police, the fire brigade and neaby hospitals are involved.

Monitoring

This is the process of measuring the concentration of fumigant gas, both inside the fumigation enclosures and in the area surrounding a fumigation enclosure.

a. monitoring equipment: a wide variety of equipment is available for measuring phosphine and methyl bromide concentrations. The equipment must be suitable for monitoring, with regard to the concentration range, the concentration involved in the work place and the fumigant concentrations reached during fumigation treatments.

b. Fumigant concentration in the health and safety range: for measuring phosphine and methyl bromide concentration, that is around the threshold limit value which is 0. 3 ppm for phosphine and 5 ppm for methyl bromide. Different devices can be used for these purposes such as gas detectors, electronic gas – measuring equipments, dosimeters. For best fumigation practices, monitoring is essential.

Advantages and Disadvantages of Phosphine and Methyl Bromide Fumigants and Other Treatments for Grain Protection

Before a decision is taking on the selection of a fumigant, it is necessary to make an analysis of the properties and characteristics of each one and the characteristics of the product to be fumigated. Contracts or regulations may require that a specific fumigant must be used, if not so, then it is always advisable to choose the better fumigant for the job.

a. Phosphine.

Advantages:

· Not an ozonedepleting substance (environmentally safe);

· If properly used, leaves residues of no commercial significance;

· Simple application procedure using existing expertise, training and manuals;

· Disperses rapidly inside the enclosure and no fan required;

· Air – off easily after treatment and relatively easy and safe for transport in original packages;

· Is cheap;

· Not known to affect germination.

Disadvantages:

· Long exposure and airing period up to 8 days or more;

· Less effective when used at temperatures below 15℃;

· Use on a longterm basis is threatened by the development of resistance;

· Repeated application of tablets or pellets into grains may leave residues above the maximum residue limit (MRL);

· Ease of application and misunderstanding of gas loss have led to misuse and over – reliance on a single method.

b. Methyl bromide

Advantages:

· Rapid kill with 24 hours exposure broad spectrum;

· Existing expertise and training and manual.

Disadvantages:

· Cumulative poisons in humans;

· Less effective at temperatures below 10℃;

· A strong ozonedepleting substance and it will be limited to critical uses including quarantine and pre-shipment treatment. It has been phased out in developed countries and in 2015 will be phased out in developing countries;

· Leaves residues of commercial importance;

· Application fairly complex requiring electricity;

· Must be vaporised and delivered as a hot gas;

· Require fan for effective diffusion inside the enclosure;

· Airs off slowly and requires fan;

· Supplied in heavy cylinders and relatively difficult to transport;

· Becoming expensive due to the intentional phasing out process;

· Germination can be affected (varies with seeds and moisture content)

c. Other treatments

Controlled atmospheres: These are available for using as alternatives to methyl bromide. Techniques of application have been developed

that allow them to be used with grain stored in bags. Controlled Atmospheres are divided into two general classes:

1. High carbon dioxide (CO_2), in which carbon-dioxide concentrations are increased to around 70% –80%.

2. Low oxygen (O_2), where the oxygen is reduced to 1% or less.

In general, high carbon dioxide controlled atmospheres are more commonly used for des-infestation than nitrogen-based, low-oxygen controlled atmospheres because:

· They are effective over a large concentration range;

· They can be applied in a "single shot" (unlike low oxygen);

· There is a greater need for gas tightness using low oxygen atmospheres;

· The exposure period using controlled atmospheres are much longer than those required for methyl bromide or even phosphine.

At atmospheric pressure has been adequate to disinfect bagged commodities using the sealed – stack technique, this technique requires a high level of sealing to maintain carbon dioxide at the required concentration over a minimum of a 15day exposure period under tropical conditions. This technique has been successfully used in Indonesia and Vietnam for milled rice and is recommended for des-infestation of bagged organic and biodynamic grains which are becoming important in overseas markets.

Hermetic Storage

According to Varnava et al., 1995; Anon. 2002, hermetic storage, is a sstore technique used since ancient times and it is still has widespread use in subsistence agriculture, using natural materials for construction. The modern approach to the use of hermetic storage relies on plastic gastight liners to provide the hermetic storage enclosure. During hermetic storage, grains are placed in hermetic sealed enclosures that prevent air from entering or leaving. Inside the enclosure the natural respiration of the grain and any associated insects and fungi occurs. Two things happen: a) It reduces the oxygen content; b) it raises the carbon dioxide to insecticidal levels. These effects control or eliminate insect infestations. This process works better with warm conditions and well-dried grains. Modern, sealed, plastic hermetic enclosures allow grains to be stored safely, to remain fresh, free of insect infestations and to maintain the ability to germinate. Good storage results have been achieved with beans, cocoa, coffee (green), dried chillies, flour, maize, milk powder, millet, paddy, pulses, milled rice, seeds, sorghum, teff, wheat and wheat bran.

The Experience of FAO – with the Household Metallic Silos for Food Security.

The Agricultural and Food Engineering Technologies Service has introduced the household metallic silos in about 17 countries of Africa, Asia and Latin America in the last ten years. The household metallic silo is a key post harvest technology in the fight against hunger and to obtain food security. The household metallic silo may hold between 100 and 3 000 kilos of grains. A silo with a capacity of 1 000 kilos can conserve the grain needed to feed a family of five persons for one year. The silo has many advantages, including protection against rodents and other pest attacks and it is airtight and permits non-residual fumigation.

For the conservation of grains, small farmers who have benefited from silo technology are aware of that situation, and they follow a standard procedure on how to fumigate properly using the small metallic silo. This procedure includes placing the phosphine tablets on an open little paper bag on the internal surface of the grain. Normally, one tablet for each 227 kilos of silo grain capacity is recommended. For instance, a silo of 1360 kilos would need six tablet regardless as to whether it is full or not with grain.

The strategy of FAO of introducing the silo to benefited countries includes training on construction, use and handling of the household metallic silo. The fumigation recommended is the use of the phosphine treatment and hermeticity is achieved using adhesive tapes, strips of rubber or even tallow or soap. Likewise, on each silo manufactured a label must be attached to the body of the silo indicating how to use and manage the silo properly for storage of grains and cereals with emphasis on fumigation procedures. The experience with the metal silo of 120 kilos in Bolivia even demonstrated that the store and fumigation of potato seeds was adequate without affecting germination capacity.

Conclusions and Recommendations

This paper shows some relevant aspects on fumigation contained in the publication titled: "Guide to Fumigation Under Gas-Proof Sheets" produced by FAO and ACIAR in 2004.

Very likely, this is one of the few publica-

tion, if not the only in its type, related to good practices of fumigation and presented in two complementary forms: a practical hard copy guide and a useful CD – ROM with audio – visual training material.

It is a useful tool for agricultural farmers, traders, and technicians among others to prevent postharvest losses in storage and to enhance the food security through a reliable technical guide on fumigation.

Given the periodic needs to update a fumigation guide in line with the international agreements such as the Montreal protocol to protect human health and the environment, it would be highly recommendable to create a task force committed to taking care of keeping abreast of the new modern trends in this area, with emphasis on fumigation, and which could update the guide. Also, FAO would be willing to share and support these activities.

The Information Network on Post harvest Operations, INPhO web site of FAO sited at: http://www. fao. org/inpho/could be a database platform to share and update issues on fumigation treatment and progress.

Through this paper, the AGST technical unit of FAO would like to reconfirm the interest to share valuable experiences on the subject of fumigation and grain store and take this opportunity to express to the participants of this conference the idea of thinking about new ideas and sharing these with others in the future.

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0704

Introduction of the Technical Regulation for CA Storage of Grain by Purging Carbon Dioxide in China

Wang Shuanglin and Tu Jie

Abstract: The technical regulation for CA storage of grain by purging carbon dioxide in China was made, and the main technical parameters and their proofs were introduced. In the regulation, it is required to remain carbon dioxide concentration above 35 percent for 15 days for complete insect control, and the structures used for controlled atmosphere treatments must have a good gas tightness that corresponds to a decay time of 4 minutes for an applied excess pressure drop of 500 to 250 Pa in a full storage for the process to be effective and economical. The appropriate initial CO_2 concentrations under different gas tightness conditions were also stipulated.

Key words: CO_2, CA storage, technical regulation

1 Preface

Since 2001, 215 million kg capacity storehouses of CA storage of grain by purging carbon dioxide have been built in China. In order to format this technology, reduce the cost and extend it in grain storage practice, we carried out some research and proof-test, and made the technical regulations for CA storage of grain by purging carbon dioxide according to the results from the experiments conducted in China and the existing proved technique. The nomenclature and definition, principle, establishment, equipment, materials, operation requirements and evaluation of this technology were stipulated in the technical regulation.

2 Main Technical Requirements and Their Basis

2.1 Gas Concentrations and Exposure Times to Control insects

Carbon dioxide concentrations and exposure times are the key technical parameters, which have been studied widely and sound in the word. Banks (1979) suggested that an initial level exceeding 70% carbon dioxide and maintained above 35% for 10 days is appropriate for complete insect control at temperatures above 20℃[1]. At temperature decline from 18 to 10℃ or 25 to 20℃, both insects and mites in wheat and barley were killed in less than 2 week at 35% CO_2 concentration (White and Jayas 1993)[2]. Banks and Fields (1995) recommended that when the grain temperature was over 20℃, the duration for carbon dioxide concentration declining from 70% to 35% should reach 15 days for controlling most stored-product insects effectively[2].

The effect of 4 CO_2 concentrations, 25%, 35%, 45% and 80% on controlling four main insects (Sitophilus zeamais, Sitophilus oryzae, Rhyzopertha dominica, Tribolium castaneum) at 25℃ was studied at the laboratory. The result showed that the necessary exposure time for these pests effective control decreased as the CO_2 concentration increased. The result demonstrates that CO_2 concentration declining from initial high concentration to 35% naturally is reasonable and economical (table 1).

Table 1. Required exposure times for complete insect control under different CO_2 concentration.

concentration (%)	times for complete insect control (d)	
	Adult insect	All life stage of insect
35%	≥9	≥15
45%	≥8	≥14
80%	≥3.5	≥12

Experiments were carried out in Shanghai, Jiangxi and Sichuan grain depots in 2005 and 2006 to validate the new technical regulation. The result showed that all insect pests were killed when the CO_2 concentration declining from initial concentration to 35% during 15 days even under 10 – 15℃ conditions. The grain temperature in Shanghai depot was showed in table 2, and the carbon dioxide concentrations in 4 locations were showed in table 3 to table 6.

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Talble 2. Temperature of grain at No. 82 storehouse in shanghai (℃)

Date	Top layer	Second layer	Third layer	Bottom	Average
2005 – 4 – 7	12.3	10.3	9.5	10.4	10.6
2005 – 4 – 14	13.6	11.6	10.1	11.2	11.6
2005 – 4 – 21	15.2	12.4	10.4	11.5	12.4
2005 – 4 – 28	16.0	13.2	10.8	11.8	13.0

Table 3. Carbon dioxide concentration at No. 82 storehouse in shanghai

Date	Average concentration (%)	Lowest concentration (%)
2005 – 4 – 8	80.0	36.1
2005 – 4 – 10	71.6	52.8
2005 – 4 – 12	62.3	51.3
2005 – 4 – 14	57.3	48.6
2005 – 4 – 18	54.2	48.6
2005 – 4 – 20	51.4	46.3
2005 – 4 – 22	49.3	45.5
2005 – 4 – 25	48.6	44.5
2005 – 4 – 28	42.7	39.3

Table 4. Carbon dioxide concentration at No. 6 storehouse in Jiangxi

Date	Average concentration (%)	Lowest concentration (%)
2005 – 9 – 22	65.93	31.24
2005 – 9 – 23	64.92	47.10
2005 – 9 – 25	58.17	54.61
2005 – 9 – 27	55.68	49.86
2005 – 9 – 29	52.07	47.87
2005 – 10 – 1	50.91	47.04
2005 – 10 – 3	48.26	45.05
2005 – 10 – 5	45.61	43.37
2005 – 10 – 7	43.87	41.60

Table 5. Carbon dioxide concentration at No. 16 storehouse in Sichuan

Date	Average concentration (%)	Lowest concentration (%)
2006 – 6 – 18	85.5	27.1
2006 – 6 – 19	71.2	46.2
2006 – 6 – 20	63.5	52.0
2006 – 6 – 22	55.5	51.1
2006 – 6 – 26	49.9	43.6
2006 – 6 – 28	45.8	40.1
2006 – 6 – 30	42.5	37.3
2006 – 7 – 3	39.8	36.3
2006 – 7 – 4	38.0	35.3

Table 6. Carbon dioxide concentration at No. 12 storehouse in Sichuan

Date	Average concentration (%)	Lowest concentration (%)
2006 – 6 – 9	60.4	27.0
2006 – 6 – 10	57.2	49.0
2006 – 6 – 13	51.0	50.6
2006 – 6 – 16	46.1	44.5
2006 – 6 – 19	44.4	42.8
2006 – 6 – 23	43.8	42.1
06 – 6 – 27	40.6	38.5

2.2 Requirement for Gas Tightness at Grain Storehouse

Good gas tightness of grain storehouse is necessary for the process to be effective and economical. The higher gas tightness, the lower speed of the CO_2 concentration decline. But higher gas tightness also means higher cost for structures. The gas tightness should be suitable to current technique and economic actuality.

The requirement for gas tightness in the technical regulation is that the duration which pressure dropping from 500 to 250 Pa in a full storage is over 240s.

2.2.1 Feasibility in technique

Proof tests were also carried out in Shanghai City and Jiangxi Province in 2005, and in Sichuan Province in 2006. Table 7 showed the CO_2 concentration decline speed under different gas tightness of full storage. The result showed that the carbon dioxide concentration remained above 35% for 15 days economically without supplementing carbon dioxide

Table 7. Concentration decline speed under different gas tightness of full storage

Storehouse	Capacity (t)	Half life of pressure (s)	Initial CO_2 concentration (%)	Concentration decline speed
No. 82 in shanghai	3900	292	80.0	Remain above39% above 20 days

Storehouse	Capacity (t)	Half life of pressure (s)	Initial CO_2 concentration (%)	Concentration decline speed
No. 6 in Jiangxi	2800	330	65.9	Remain above 40% above 15 days
No. 12 in Sichuan	3900	245	85.5	Remain above 35% above 15 days
No. 12 in Sichuan	5200	317	60.4	Remain above 37% above 8 days

2.2.2 Feasibility in current technique and economic actuality

The gas tightness of all carbon dioxide atmosphere controlled storehouses in China was tested in 2005. The capacity of each storehouse is form 2 800 tons to 6 500 tons. The result showed that the half life of pressure of 98% of the carbon dioxide atmosphere controlled storehouses were over 240 s in China in 2005.

So, the requirement on gas tightness in the regulation is feasible in current technique and economic actuality.

Table 8. Number of the storehouse of different gas tightness in China in 2005

Location	Half life of pressure				Total
	<4 min	4 min – 4.5 min	4.5 min – 5 min	>5 min	
Sichuan Province	/	1	7	2	10
Shanghai City	1	2	4	3	10
Jiangsu Province	/	7	4	/	11
Anhui Province	/	/	2	3	5
Jiangxi Province	/	1	2	7	10
Total	1	11	19	15	46
Percentage	2%	24%	41%	33%	100%

2.3 Commendatory Initial CO_2 Concentrations under Different Gas Tightness Condition

It is effective and economical to remain carbon dioxide concentration above 35 percent after 15 days for complete insect control. Superfluous initial carbon dioxide means more cost on carbon dioxide gas, and deficient initial carbon dioxide also costs more because additional carbon dioxide must be added during the storage process. So, in order to make the process to be effective and economical, it is necessary to foreknow the suitable initial CO_2 concentrations under different gas tightness condition.

Initial CO_2 concentrations under different gas tightness condition are recommended in table 9.

Table 9. Recommended initial CO_2 concentrations under different gas tightness condition

Half life of pressure of full storage	Recommended CO_2 concentrations
240	80%
s300	70%
sAbove 360 s	60%

3 Discussion

CA storage of grain by purging carbon dioxide has been applied only 6 years in China. This technique still need to be improved and perfected in practice. It has not been applied on a large scale because of the carbon dioxide provision which causes more cost than phosphine fumigation. But CA storage of grain should have a good future among the storage technology as a green storage technology.

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0705

Factors Affecting Carbon Dioxide Concentration in Interstitial Air of Soybean Stored in Hermetic Plastic Bags (Silo-bag)

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Abstract: In 2007 about 35 million tonnes of grains were stored in hermetic systems (silo-bags) in Argentina, and about 17 million tonnes of that were soybean. The soybean stored in these silo-bags is mostly used for processing in the local industry (oil and soy – meal), but also for seeds to be used during the next planting season. The goal of this research was to conduct a series of field experiments in order to identify the main factors affecting the CO_2 concentration as an indicator of biological activity and appropriate soybean storability conditions.

The experiments consisted of monitoring the gas composition of the interstitial air, grain moisture content (MC) and temperature of several silo-bags. Additionally, the overall condition of the silo-bags was checked (broken areas, improper sealing, bottom side perforations, etc). On average, biological activity, measured as CO_2 concentration, did not increase substantially when soybean MC increased from 11% to 15.2%. The CO_2 concentration for soybean with 14% MC or lower was below 2%. The average CO_2 concentrations for silo-bags with soybean at 14% to 15.2% MC remained below 2%, however, in some silo-bags the CO_2 concentration increased to 5% at the most. The average temperature of the soybean stored in silo-bags followed the pattern of the average ambient air temperature through the seasons. There was a small increase in the average CO_2 concentration as a function of the grain temperature increase (1.5% points of CO_2 for about 10℃ of temperature increase). When individual silo-bags were analyzed, the CO_2 concentration measured during the warm storage season was up to 3 percentage points higher than when measured during the cold storage season.

Key words: grain quality, biological activity, modified atmospheres

Introduction

During the last 10 years in Argentina soybean production increased 31 million tonnes. As a result, the 2008 soybean harvest was estimated to be 47 million tonnes, representing almost half of the total grain production of the country[1]. On the other hand, the permanent storage capacity did not increase at the same pace as production, so a substantial portion of the harvest, estimated almost 35 million tonnes, was stored in a temporary hermetic storage system, called silo-bags. About half of the grain stored in silo-bags was soybean (17 million tonnes).

Each silo-bag can hold approximately 180 tonnes of soybean and with the available handling equipment is quite simple to load and unload. These plastic bags are 60 m long, 2.74 m diameter and the plastic cover is made of three layers (white outside and black inside) with 235 micrometers of thickness. The silo-bags are waterproof and have a certain degree of gas-tightness (oxygen (O_2) and carbon dioxide (CO_2)). As a result, respiration of the biotic components of the grain mass (fungi, insects and grain) increases CO_2 and reduces O_2 concentrations. When the biological activity is intense, the degree of modification of the typical atmospheric gas composition (21% O_2 and 0.003% CO_2) is greater, which would limit grain respiration and mold[2] and insect development[3,4]. It was also observed that high CO_2 concentration reduced the ability of *Aspergillus flavus* to produce aflatoxin[5].

Bartosik et al.[6] summarized previous experiences of storing grain in silo-bags, where it was demonstrated that the grain temperature in the hermetically sealed plastic bags followed the pattern of the ambient temperature throughout the year, implying that temperature of the grain mass does not reveal biological activity in the grain mass. The average moisture content (MC)

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did not significantly change during any storage experiment for both dry and wet grains. In general, no MC stratification was observed in soybean silo-bags. However, a study of MC change in individual kernels stored in silo-bags showed a slight but significant increase in soybean MC at the top layer (from 11.7 to 12.6 in 60 days)[7]. In general, when grain was stored at the market MC, no significant decrease in the quality parameters was observed during 150 days of storage, and when grain was stored above the market MC, the decrease in some of the quality parameter was observed. However, soybean stored at 12.5% MC resulted in a reduction in the germination test, although other works showed different results[8]. The increase in the CO_2 concentration was higher at the end of the storage time and also was higher in those bags with wetter grain (7.5% of CO_2 for 12.5% MC, and 16.2% of CO_2 for 15.6% MC after 160 days of storage). Based on this observations the authors hypothesized that measurement of gas composition in the interstitial air could be used as an indication of the biological activity of the grain mass in the hermetic storage system, and serve a tool for monitoring grain storability. However, a better understanding of typical CO_2 concentrations for soybean silo-bags is required to use this technology for monitoring grain storability.

Figure 1 shows a diagram of the main factors affecting respiration of the grain and microorganisms in the hermetic storage system and the relationship among them. Based on this model, the CO_2 and O_2 concentration in the silo-bag depends on the balance between respiration (consumption of O_2 and generation of CO_2), the entrance of external O_2 to the system, and the loss of CO_2 to the ambient air. The movement of gases in and out of the silo-bags depends on the gas partial pressure differential and the permeability of the system (through openings in the plastic cover, or through the natural permeability of the plastic material to the gases). Grain type and condition, MC, temperature, storage time, and O_2 and CO_2 concentrations affect the respiration rate. The temperature of the grain depends on the initial grain temperature (this effect is less important as the storage period increases), the effect of the sun radiation, the heat release from the respiration process, and the transfer of heat with the air and soil (grain temperature increases during spring and summer and decreases during fall and winter). The grain MC depends on the initial grain MC, the entrance of moisture from the outside (through openings after a rain event into broken or poorly sealed silo-bags), and the moisture released from the respiration process. Additionally, due to the day and night temperature differential, some moisture condensation can occur in the top grain layers resulting in a localized spot of wetter grain.

Thus, the goal of this research was to study the effect of grain MC and temperature on CO_2 concentration in silo-bags holding soybean.

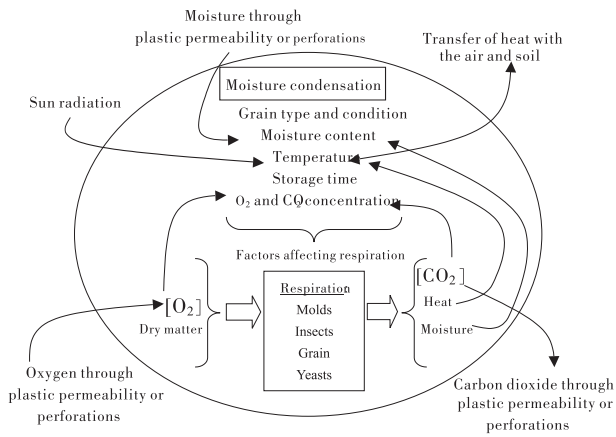

Fig. 1 Diagram of the main factors affecting respiration of grain and microorganisms in the silo-bag, the relationships among them, and the final O_2 and CO_2 concentrations.

Materials and Method

The tests were carried out at grain elevators and on farms in the South East of Buenos Aires province, Argentina during 10 months, from April 2007 to January 2008. Most of the soybean silo-bags were filled in April – May and stored until October or November. However, a small proportion of them were stored for one year period or longer. The silo-bags were sampled every 15 days during the entire storage period.

For each silo-bag two sampling locations were established. The procedure consisted of measuring first the gas concentration (O_2 and CO_2) with a portable gas analyzer (PBI Dan Sensor, CheckPoint, Denmark), perforating the plastic cover with a needle. The gas composition was analyzed for three levels in each sampling location, close to the top of the bag, at the middle and close to the bottom.

After the gas composition was analyzed, a wooden stick with three temperature sensors was inserted in the grain mass (diagonally, from the top and side to the bottom and center of the si-

lo-bag) to measure grain temperature at approximately 0.1, 0.7 and 1.4 m from the surface.

In each sampling location grain was collected from three different levels (surface = 0.10m depth, middle = 0.75 m depth, and interior = 1.6 m depth. Total height of the bag = 1.7 m) using a standard torpedo probe. Material from each one of the three sampling locations was segregated by level (top, middle, and bottom). The grain samples were stored in a hermetic plastic bag and brought to the Grain Postharvest Laboratory of the Balcarce Experimental Station of the National Institute of Agricultural Technologies (INTA). After probing the silo – bags, the openings were sealed with a special tape in order to restore the air-tightness. At the laboratory, grain samples were analyzed for MC (GAC 2100, Dickey – John).

Additional information of the silo-bag was collected, such us filling and sealing quality, history of openings, perforations due to wild animals or bad sealing after sampling, improper preparation of the soil where the silo-bag was placed (when silo-bags were assembled on top of crop residues it resulted in perforations of the bottom), silo-bags assembled in low lands with risk of flooding, and any other relevant information.

Results and Discussion

Figure 2 shows the relationship between grain MC and CO_2 concentration. The data corresponds to silo-bags without visible structural problems, although data from some silo-bags with perforations in the bottom side that were not noticed during sampling might be included.

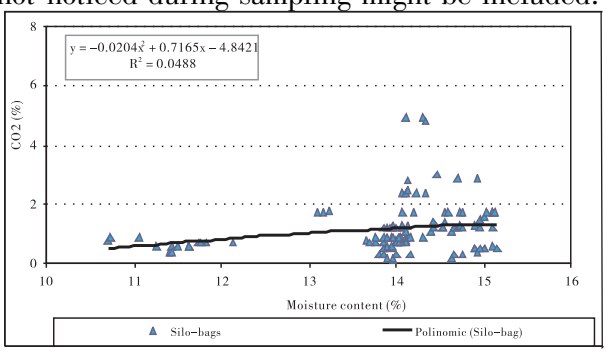

Fig. 2 CO_2 concentration in silo-bags as function of soybean moisture content.

The CO_2 concentration tended to increase very slowly with the increase in grain MC, which most likely was a consequence of the higher biological activity of wet grain (although the R^2 was very low, less than 5%). When the soybean MC was lower than 14% the average CO_2 concentration was below 2% (presumably due to grain respiration). When the soybean MC increased to the point in which molds became active (above 14%) the CO_2 concentration of some silo-bags increased up to 5%. Compared to data from wheat silo-bags[9], the relationship between grain MC and CO_2 concentration was less clear for soybean. Additionally, soybean had less biological activity at the same MC, because for 13% MC the CO_2 concentration was below 2%, while for 13% MC wheat the average CO_2 concentration was about 5%. When the soybean MC increased to 14% – 15%, the maximum CO_2 concentration was about 5% (1.5% average), while in wheat silo-bags for the same MC range the maximum CO_2 concentration was 20% and the average between 7.5% and 12%.

Figure 3 shows the relationship between the average ambient air temperature (data collected from the weather station of Balcarce Experimental Station) and the average soybean temperature in the silo-bags throughout the year.

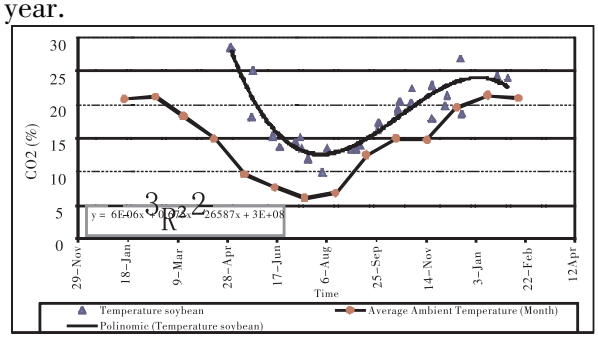

Fig. 3 Average ambient air temperature and average temperature of soybean stored in silo-bags.

This data is in agreement with the data shown by Bartosik et al.[6], where it was demonstrated that the average grain temperature follows the pattern of the average ambient air temperature. From this figure, it can be appreciated that in the early fall (April) the soybean temperature was above 25℃ (most likely soybean was harvested during a warm day). It was hypothesized that grain stored in the silo-bag can easily exchange heat with the air and soil through the large surface area. The surface/volume ratio of a 180 tonnes silo-bag is approximately 1.42, while for a standard metal silo of the same capacity (7 m diameter and 9 m height) the ratio is 44% lower (0.79). As a result, the soybean temperature decreased to about 10 – 15℃ during the winter (June – Au-

gust) as the average ambient air temperature decreased to 7℃ or lower during the same season. When the average ambient air temperature increased to 15℃ during the early spring and 20℃ later (September – November), the grain temperature increased to 15℃ and to 25℃, respectively.

Figure 4 shows the CO_2 concentration for silo-bags sampled during the winter and spring. Storage temperature affects biological activity, reducing the respiration rate of grain and microorganisms. During the winter time, when the grain temperature was below 15℃, the CO_2 concentration was below 2% for soybean with 13% MC or less, below 3% for soybean between 13 and 14% MC, and below 5% with soybean between 14 and 15.3% MC. When the grain temperature increased during the spring (up to 25℃, Figure 3), the CO_2 concentration increased about 2.5% – 3% points for all MC values (up to 4.5% CO_2 for soybean below 13.5% MC, and up to 8% CO_2 for soybean between 13.5 and 15.2% MC). However, the average CO_2 concentration did not increase substantially with the increase in temperature, as shown in the trend lines.

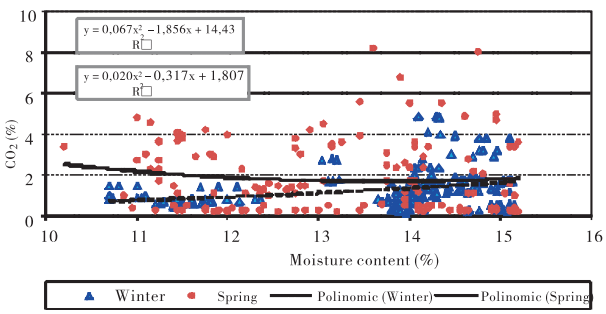

Fig. 4 CO_2 concentration at different grain moisture content for silo-bags sampled during the winter and spring.

Conclusions

On average, the increase of biological activity, measured as CO_2 concentration, did not increase substantially when soybean MC increased from 11 to 15.2%. The CO_2 concentration for soybean with 14% MC or lower was below 2%. The average CO_2 concentrations for silo-bags with soybean at 14 to 15.2% MC remained below 2%; however, in some silo-bags the CO_2 concentration increased to 5%.

The average temperature of the soybean stored in silo-bags followed the pattern of the average ambient air temperature through the seasons. There was a small increase in the average CO_2 concentration as a function of the grain temperature increase (1.5% points of CO_2 for about 10℃ of temperature increase). When individual silo-bags were analyzed, the CO_2 concentration measured during the warm storage season was up to 3 percentage points higher than when measured during the cold storage season.

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0706

Some Keys and Discussion about Recommended Regulation of Phosphine Fumigation for Chinese Grain Storage

Wang Dianxuan* and Bian Ke

Abstract: A recommended state standard for phosphine recirculation fumagation in grain storage industry in china was made. The basic condition is required on gastightness, equipment, instrument and relative materials. The scheme should be made according to the condition of grain quality, insects and their resistance, stacking method, climate and temperature of grain, safety issues and so on. The monitoring on phosphine, concentration maintaining by fumigant reinforcing, and insect detection by test insect cages as keys are recommended during the fumigation. The dosage of tablets of aluminium phosphide is determined by the planned phosphine concentration according to the insect species, resistance, density of insect per kilogram of grain, temperature of grain, schemed fumigating time, gastightness et al. The process about tablet applying methods or phosphine generator operating, phosphine recirculation time, concentration monitoring time and some issues on fumigant distribution is suggested. On the other hand, the recommonded data are effective for insect pest control in many cases. There are some technical issues which should be discussed due to a huge of changes happened in practice in last several years.

Key words: phosphine, fumigation, grain storage, regulation, China

Introduction

Since 1998, A new type of horizontal warehouses had been widely used in state grain storage in china since 1998, which are 48 to 60 meters in length by 21, 24, 27 and 30 meters width and capable of holding a grain mass in 6 meters or more in height. And some huge squat bins and concrete silos had been constructed. These facilities were equipped with closed-loop fumigation (CLF) equipment for the effective phosphine distribution. The CLF system consists of some fixed or mobile pipes which were connected with the wall of storage by a sub-floor ventilation system. Phosphine-air mixtures can be recirculated through the pipes, ventilation ducts, grain mass and headspace in the storage[1]. In some cases, the top grain was sealed by plastics. Some piloting pipes were posted under of the plasic sheeting for fumigant so that there was no phosphine distributing in the headspace.. The phosphine was usually applied in one of the following ways: one, aluminium phosphide tablets were placed on the surface of the grain mass; two, the tablets was dropped into water, and then phosphine with a few carbon dioxide was put into entrance of vetilation duct. The phosphine in the storage can be sampled by some sampling pipes located in grain bulk and monitored by electronic monitor or detecting tubes. For the effective application of the CLF system and pest control, a recommended regulation of phosphine recirculation fumigation for Chnese grain storage industry had been made. There were still some problems in practical fumigation according to the results from a communication survey about phosphine fumigation which was carried out from 246 state grain depots, in 2005 – 2007 in China. In order to make the CLF system application and insect pest control be more effective, a recommended regulation of phosphine recirculation fumigation for grain storage industry in China was made, whichmainly focusing on phosphine concentration, exposure time, phosphine applicating methods, tolerance or resistance of insect to phosphine and so on.

Phosphine Concentration for Fumigation

The phosphine concentration was the most importantfactor to kill insects, which not only deponded on the dosage of applied phosphine or aliuminium phosphide, but also on the gastightness of warehouse and other factors such as enviroment temperature, grain and so on[2]. In the standard the concentrations are suggested according to tolerance or resistance of insect to

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phosphine, temperature of grain and exposure time(Table 1).

Table 1. Recommended phosphine concentration under different temperature and exposure time

Species	Temperature※ (℃)	Concentration in different exposure time(mL/m³)		
		≥14 d	≥21 d	≥28 d
Susceptible pests-Sitophilus zeamais Latheticus oryzaeTribolium confusemOther susceptible species	>25	200	150	100
	20 – 25	250	200	150
	15 – 20	–	250	200
Tolerance pests-Rhyzopertha dominicaSitophilus oryzaeTribolium castaneumMoths and other resistant species	>25	300	250	200
	20 – 25	350	300	250
	15 – 20	–	350	300

※: temperature: point of pest exists in grain mass

The fumigation in most grain depots at the recommened concentrations were effective in practice. But there were cases that the concentration is not big enough to kill insects completely. According to the survey from 246 grain depots, the survey results shew that the lowest phosphine concentratiuon should be bigger than that in the table 2 [3]. As the data in table 2, the phosphine concntration killling all pests completly was changeable due to the insect torlerance, exposture time and practical storage. Generally, the concentration to kill pests completly in south china was bigger than the central or north one. That might be affected by the higher tolerance or resistance of insect to phosphine, the environment teperature, humidity, generations of insect reproduction, which were helpful for survival of insect after each fumigation in south China[4]. It indicated that the range of recommended concentration had significance effect for fumigation from table 2.

Table 2. Lowest limitation of effective phosphine concetration in grain depots in China

Depot name, Province	Lowest PH_3 (mL/m³)	Location in China	Depot name, Province	Lowest PH_3 (mL/m³)	Location in China
Huadu, Guangdong	350	South	Nanjing, Jiansu	400	East
Sanya, Hainan	200	South	Anqing, Anhui	300	East
Shenzhen, Guandong	300	South	Shanghai	300	East
Wuzhou, Guangxi	300	South	Wenzhou, Zhejiang	200	East
Beihai, Guangxi	200	South	Laiwu, Shandong	200	East
Nanning, Guangxi	300	South	Qingzhou, Shandong	250	East
Ningdu, Jiangxi	300	South	Rizhao, Shandong	150	East
Pingxiang, Jiangxi	200	South	Shenqiu, Henan	350	Central
Chenzhou, Hunan	200	South	Xuchang, Henan	200	Central
Hengyang, Hunan	300	South	Wuhan, Hubei	350	Central
Fuzhou, Fujian	200	Southeast	Macheng, Hubei	210	Central
Zhangzhou, Fujiang	250	Southeast	Nanyang, Henan	300	Central
Xiamen, Fujian	300	Southeast	Zhumadian, Henan	250	Central
Jintang, Sichuan	180	Southwest	Changchun, Jlin	100	North – east
Luzhou, Sichuan	260	Southwest	Nongan, , Jilin	100	North – east
Zunyi, Guizhou	250	Southwest	Haerbin, Helongjiang	150	North – east
Kunming, Yunnan	180	Southwest	Mudanjiang, Helongjiang	160	North – east
Lanzhou, Gansu	150	West	Haicheng, Liaoning	200	North – east
Xi′an, Shanxi	100	West	Jianping, Liaoning	100	North – east
Dezhou, Shandong	150	East	Taiyuan, Shanxi	150	North – west
Jiana, Shandong	150	East	Xiangyuan, Shanxi	200	North – west

Exposure Time

The grain can be stored for a longer time 3 – 5 years for wheat, 3 years for paddy. The recommended expsoure time of phosphine fumigation was more than 14 days, 21 days and 28

days respectively in the different condition including phosphine concentration, resistance level of insect, insect species and temperature. The time would be shorter or longer due to the different concentration of phosphine Usually, the biger was the concentration, the shorter was the exposure time. The higher level of phosphine is suggested for the serious insect exsiting, for that there were many lesser grain borers in grain mass at a high temperature. However, It was economic to maintain the phosphine at a suitable low concentration especially for less insects in the grain. In fact, it was not easy to know that insects were killed completely or survival in a sealed warehouse or grain bulk. To set insect cages was a useful method to detect the effect of fumigation according to the mortilaty of insects. Some authors had reported the practical exposture time in different cases[4]. It was seemly needed much longer time to kill some special insects completely such as rusty grain beetle and psocides in certain level of phosphine. It was necessary that exposture time was more than 30 days under the concentration of 300 mL/m^3 or bigger[4]. Generally, the exposture time of phosphine for complete control in China was bigger than that of some published reoprts such as 6 – 9 days[5,6], Price and Mills, 1988 and > 7 days[9] Sayaboc et al., 1998.

Phosphine Concentration Maintaining and Gastightness

In order to kill all pests during fumigating, maintaining effective concentration of phosphine was principal key in the whole process. There had been a requirement for gastightness, and the time to pressure decay from 500 Pa to 250 Pa was more than 40 seconds for horizontal storage, 60 seconds for squat bin, empty for vertical silos. It was recommended that the time to niuus pressure getting back from 500 Pa to 250 Pa was over 90 seconds for the storage sealed with plastic sheeting in flat warehouse. How to maintain the phosphine concentration was a difficulty in many fumigations, and it was mainly due to the gastightness insufficiency of warehouse. Phosphine supplement was a useful way of maitaining the concentration in production. Aluminium phosphide tablets were put into ventilation duct meanwhile CLF system was runing. On – site phosphine generator was also used for fumigation.

Resistance or Torlerance of Insect

There were a few research result on insect resistance to phosphine around China[6,7]. And some other authors had given some reports on it. Most of resistance insects are from several provinces of south china. Some results had been given an output from programs coperation between China and Australia on the project PHT9415, Phosphine resistance in insect pests of stored grain, and project PHT98 – 137, Integrating effective phosphine fumigation practices into grain storage systems in China, Vietnam and Australia. The relaitive results had been reported[7,8,10,11,12,13]. Yan et al (2005) had reported some on the lastest development of it. According to the measurement, the resistance factor of rusty grain beetle was not too big in the species. But this beetle had a higher resistance than that of *Sitophilus zeamais* which was susceptible to phosphine [10]. So for the total population extinction in the case of insect pest existing in fumigation, relative tolerance has more significance than resistance perhaps. All above, it was difficult to know the real resistance or torlerance of insect to phosphine.

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0707

Regulation and Management of Fumigations through the Australian Fumigation Accreditation Scheme (AFAS)

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The Australian Quarantine and Inspection Service (AQIS) relies on effective methyl bromide fumigation treatments to address quarantine risks associated with imported cargo (including wooden packing and materials).

The Australian Fumigation Accreditation Scheme (AFAS) was established to address ineffective fumigation performance.

At present, AFAS has been fully implemented in Indonesia, Malaysia, Thailand and India. Full implementation in the Philippines and Papua New Guinea is expected in late 2008 and in the People's Republic of China in mid -2009. Singapore, Sri Lanka and New Zealand are at various stages of participation.

AFAS objectives are to:

Provide capacity building assistance to quarantine regulatory authorities to effectively monitor quarantine treatments;

Enhance compliance with Australian quarantine requirements through the registration of overseas fumigation companies that are eligible to participate in AFAS;

Facilitate trade between AFAS participating countries and Australia by ensuring that fumigation treatments are performed effectively;

Provide improved communication and feedback with participating countries to maintain compliance with AFAS requirements.

The above objectives are achieved through:

Training of overseas quarantine officials and fumigators in effective methyl bromide fumigation techniques;

Accreditation of individual fumigators who have been assessed as competent against AFAS requirements by the quarantine regulatory authority;

Registration of AFAS approved fumigation companies by the quarantine regulatory authority, that are then eligible to perform methyl bromide fumigations on consignments bound for Australia;

Training of government officers as methyl bromide fumigation trainers to ensure sustainability of AFAS;

Training of government officers in effective auditing techniques;

Auditing of registered fumigation companies by the quarantine regulatory authority to ensure continued compliance with AFAS requirements;

Annual Joint System Reviews of AFAS operations conducted by AQIS and the relevant quarantine regulatory authority.

Benefits gained from AFAS are not only limited to trade facilitation and increased confidence in fumigation performance. A positive outcome has been the substantial reduction in the use of methyl bromide in accordance with the Montreal Protocol. AQIS has calculated that effective, first time fumigations performed under AFAS has resulted in a reduction of approximately 95 tonnes of methyl bromide alone in Indonesia and Malaysia since AFAS implementation in 2004 and 2005 respectively.

AFAS is fundamentally a management system that can and is being expanded to incorporate additional quarantine treatments such as ethylene oxide (ETO), heat treatment, and other emerging fumigation treatments such as sulfuryl fluoride.

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0708

Phase-out of Methyl Bromide in Grain Storage in Indonesia

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Abstract: Indonesia has ratified the Montreal Protocol and its amendments. This ratification shows the Indonesian commitment to actively contribute to preserving the global environment particularly in the context of ozone layer protection. The wide use of methyl bromide as an Ozone-Depleting Substance (ODS) in grain storages in Indonesia must be phased-out. The Government of Indonesia through the Ministry of Environment has decided to phase-out the use of methyl bromide in grain storages/non Quarantine and Pre-Shipment (non – QPS) by the end of 2007. The program on the phase-out of the use of methyl bromide in grain storages was financially supported by the Multilateral Funds through the United Nations Industrial Development Organization (UNIDO) and was carried out from 2006 to 2007. The Indonesian Ministry of Environment coordinated the implementation of this program. The abovementioned Ministry has appointed SEAMEO BIOTROP the Southeast Asian Regional Center for Tropical Biology as the National Implementation Institute (NII) to carry out the program for the phase-out. The subject of the program is "Preparation and Organization of the Training of Trainers and Workshop-Phase-Out of the Use of Methyl Bromide in Grain Storages in Indonesia". The activities covered seminars and training courses participated by staff involved in fumigation or pest management in grain storages in several provinces in Indonesia. The aim of conducting these activities were to improve their knowledge and skills in the field of phosphine fumigation and integrated storage pest management (ISPM) as an alternative technology to replace methyl bromide in grain storages. The program on phase – out of the use of methyl bromide was terminated in December 2007. Starting January 1, 2008 the use of methyl bromide in grain storages in Indonesia is prohibited. The Ministry of Environment of the Republic of Indonesia has been monitoring the ex-users of methyl bromide in the non-QPS sector who have already replaced methyl bromide with phosphine and ISPM so that they will not use methyl bromide again.

Key words: phase-out, non-QPS, methyl bromide, phosphine fumigation, integrated storage pest management (ISPM)

Introduction

Methyl bromide is an active ingredient pesticide and it is usually used for soil treatment before planting, fumigation in storages, and also for treatment of Quarantine and Pre-Shipment (QPS). It is considered an Ozone Depleting Substance (ODS).

In an effort to overcome pest problems in grain storages, one of the common control techniques implemented is chemical control, mainly fumigation. Methyl bromide has been widely used, because of its superiority compared with other fumigants, i. e. quite short application time, easy to be conducted, and relatively low price. Therefore, there has been a tendency of increased use of methyl bromide from year to year.

Based on the Montreal Protocol and its amendment, the use of methyl bromide as an ODS must be phased-out in line with the agreed schedule. The Government of Indonesia through the Ministry of Environment has decided to phase-out the use of methyl bromide in grain storages/non-Quarantine and Pre-Shipment (non-QPS) by the end of 2007.

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The program for the phase-out of the use of methyl bromide in grain storages in Indonesia was conducted from 2006 to 2007. This activity received financial support from the Multilateral Fund through the United Nations Industrial Development Organization (UNIDO). The Ministry of Environment of the Republic of Indonesia coordinated the implementation of this program. The Southeast Asian Minister of Education Organization (SEAMEO BIOTROP) Regional Centre for Tropical Biology was appointed by the Ministry of Environment of the Republic of Indonesia as the National Implementation Institute (NII) to conduct this program. The subject of the program was "Preparation and Organization of the Training of Trainers and Workshop-Phase-Out of the Use of Methyl Bromide in Grain Storages in Indonesia.

Starting 1 January 2008, the Indonesian Government has banned the importation of methyl bromide for fumigation in storages and soil fumigation. This was related to the regulation issued by the Minister of Trade Republic of Indonesia No: 51/M - DAG/PER/12/2007 on importation of methyl bromide for Quarantine and Pre-Shipment (QPS) purpose.

Although the use of methyl bromide in grain storages has been phased-out, the Ministry of Environment Republic of Indonesia has been conducting the monitoring of the use of methyl bromide in grain storages. Monitoring has been conducted so that ex-users of methyl bromide in the non-QPS sector who have already replaced methyl bromide with phosphine and ISPM will not use methyl bromide again.

Preparation and Organization of the Training of Trainers (TOT) and Workshop

The activities covered seminars and training courses participated by the staff involved in fumigation or pest management in grain storages in several provinces in Indonesia to improve their knowledge and skill in the field of phosphine fumigation and integrated storage pest management (ISPM) as a replacement for methyl bromide in grain storages. The details of the program activities were to 1) conduct a public awareness seminar at the beginning and at the end of the project, 2) prepare the compilation of a module and a manual on the Training Course on Phosphine Fumigation Good Practices and Integrated Storage Pest Management, 3) conduct some training courses on Phosphine Fumigation Good Practices and Integrated Storage Pest Management, 4) monitor the implementation of the training.

The program on phase-out of the use of methyl bromide was terminated in December 2007.

Seminar on the Program of Phase-out of the Use of Methyl Bromide in Grain Storage in Indonesia

The seminar on the Program of Phase-out of the Use of Methyl Bromide in Grain Storage in Indonesia was held at Harris Hotel, Jakarta on 14 February 2006. The aim of the seminar was 1) to socialize the program at the beginning of the project, and 2) to give additional input/perceptions to experts in the fields of fumigation, agriculture, grain storage and related institutions to be more aware of the ozone layer damage due to the use of methyl bromide.

There were 56 participants coming from various institutions such as government agencies, Indonesian Pest Control Association (IPCA), and agencies involved in the food and feed sectors. The first session started with the presentation of the Ministry of Environment on "Strategy and Program on the Protection of Ozone Layer in Indonesia", followed by the "Explanation on the Program of Phase-Out of Methyl Bromide in Grain Storage in Indonesia". The second session was a panel discussion on "Challenge of Phase-Out of the Use of Methyl Bromide in Indonesia". In this panel discussion session five papers were presented by the Department of Trade, Department of Agriculture (represented by Plant Quarantine Service of Agriculture, Quarantine Agency, and Center for Permit and Investment), Department of Health, and the Indonesian Pest Control Association (IPCA). The seminar concluded that the participants agreed to replace methyl bromide with phosphine and implement ISPM in grain storages in Indonesia.

Compilation of Module and Manual for Training Course on Phosphine Fumigation Good Practices and for Training of Trainers on Integrated Storage Pest Management (ISPM)

The objective of the compilation of the module and manual for the Training Course on Phosphine Fumigation Good Practices was to help the participants with the understanding of the given subjects/topics during the training and also as a guide book to carry out fumigation in the field. The module contains the subjects/topics related to, for example, Problems on Global Environment, Towards the Phase-out of the Use of Methyl Bromide in Storages in Indonesia, General Description of Phosphine Fumi-

gation, and Phosphine Fumigation on Some Commodities. The manual is a practical guide for field workers of storage pest control or operators in charge of fumigation. The manual contains general knowledge on phosphine and techniques of phosphine fumigation good practices, i. e. , Introduction of Fumigation, Technical Requirement, Phosphine Fumigant, The Process of Fumigation, Process of Residue Handling, Failure in Fumigation, Storage and Transportation of Phosphine, and List of Important Storage Insect Pests which could be Controlled by Phosphine Fumigation.

The objective of the compilation of the module and the manual for Training of Trainers on Integrated Storage Pest Management (ISPM) was to increase the participants' understanding of the given subjects/topics during the training and provide a guide book to implement ISPM in the field. The module of the training contains the subjects/topics related with the training, i. e. , Problems on Global Environment, Towards the Phase-out of the Use of Methyl Bromide in Storages in Indonesia, Integrated Storage Pest Management, Introduction of Storage Insect Pests, Ecology of Storage Insect Pests, Storage Fungi in Grains, Population Monitoring of Storage Insect Pests, Sampling Technique for Commodities, and Pesticide Application for Controlling Insect Pests. The manual serves as a practical guide for field workers to carry out integrated storage pest management activities. The topics in the manual cover Introduction, Introduction of Storage Insect Pests and Storage Fungal Species, Design and Structure of Warehouse, Storage Sanitation and its Surrounding Environment, Implementation of Initial Standard Quality Regulation of Grains, Inspection and Monitoring, Management of Stock, and Pesticides and Fumigants Application.

1st Training Course on Phosphine Fumigation Good Practices, and 2nd Training Course on Phosphine Fumigation Good Practices and the Use of Phosphine Fumigation Equipment

The 1st Training Course on Phosphine Fumigation Good Practices, the 2nd Training Course on Phosphine Fumigation Good Practices, and the Use of Phosphine Fumigation Equipment were held at SEAMEO BIOTROP, Bogor, Indonesia, on 27 – 29 March 2006 and 13 – 14 August 2007, respectively. This first training was attended by 32 participants and 2 observers, while the second training was attended by 41 participants and 2 observers.

The objectives of these trainings were to provide the participants with the understanding and skill to properly conduct phosphine fumigation. The difference in the two training courses was at the second training course participants received grant equipment for phosphine fumigation and ISPM from UNIDO through the Ministry of Environment and SEAMEO BIOTROP.

The activities of the trainings included lectures and hands-on exercises on good fumigation practices and the use of some fumigation and ISPM equipment that were granted to the participants. UNIDO granted the same kind of equipment in two phases. The first phase was delivered to the beneficiaries with the technical assistance through the activities of the 2nd Training Course on Phosphine Fumigation Good Practices and the Use of Phosphine Fumigation Equipment. The equipment consisted of PH_3 meters, sand snakes, plastic fumigation pipes, full face masks, filter canisters, plastic sheet, and ISPM equipment, i. e. , grain moisture testers, thermo-hygrometers (in/out), light traps, bait traps, card traps, yellow sticky traps, and knapsack sprayers. The equipment for calibrating PH_3 meters was donated to SEAMEO BIOTROP.

The second phase was carried out through the activities of the Workshop on Demonstration of Leak Detector that was held at SEAMEO BIOTROP, 20 February 2008. The equipment consisted of PH_3 meters, sand snakes, leak detectors, and ISPM equipment, i. e. , grain moisture testers, thermo – hygrometers (in/out), and light traps.

The training courses were successfully conducted. The success of the training was concluded based on the results of early and final evaluations of the participants. The evaluation form contained questions related to ozone layer, storage pest, fumigant and fumigation. The final evaluation showed a significant increase in the results (scores) which means that the participants' knowledge on the subjects had improved.

Training of Trainers on Integrated Storage Pest Management (TOT on ISPM)

The Training for Trainers on Integrated Storage Pest Management was held at SEAMEO BIOTROP (24 – 28 April 2006). The objectives of this training were to provide the participants with the understanding and skill on the techniques of integrated storage pest management so that the participants could share their knowledge gained in this training with other

staff members in their respective institutions as well as with other parties involved in storage pest management.

The training course was held successfully. There were 10 participants coming from food industries and related institutions. The success of the training was based on the final evaluations that showed a significant improvement in the knowledge of the subjects as compared to the evaluation at the beginning of the training. The evaluation form contained questions about the ozone layer, storage pests, and integrated storage pest management.

TOT on ISPM: On-Site Development (Monitoring)

TOT on ISPM: On-site Development (Monitoring) was the continuation of TOT on ISPM. After the completion of the training course on ISPM, the participants should implement their knowledge gained in this training in their home region. The results of the implementation were monitored by the training committee of SEAMEO BIOTROP together with the staff of the Ministry of Environment and National Project Coordinator (NPC).

The objective of this activity was to evaluate each participant's implementation of their knowledge that was gained during Training for Trainers on ISPM.

Based on the results of the completed checklist filled out during the monitoring, ISPM was mostly implemented in all institutions of the participants, although it has not been fully implemented in all. In general, the policy of ISPM implementation at all institution of the participants was supported by the management side.

Regional Training Course on Integrated Storage Pest Management

The Regional Training Course on Integrated Storage Pest Management was conducted as a follow up of the TOT on ISPM at SEAMEO BIOTROP on 24 to 28 April 2006. The participants of the TOT on ISPM were obligated to transfer their knowledge gained from the training to other staff members in their work places as well as to other personnel who are responsible for storage pest management in their region.

The objectives of this training were to distribute the ISPM knowledge and technology as a replacement for the use of methyl bromide in grain storages in the respective areas of the participants,.

The materials for this course were similar with the materials given to the participants of the TOT on ISPM. Therefore, the participants were given the modules and manuals of Integrated Storage Pest Management as well as Phosphine Fumigation Good Practices.

In these training courses, SEAMEO BIOTROP collaborated with the supervisor management of the participants who paid serious attention to the success of the ISPM program, especially Perum BULOG.

Representative in 7 provinces were trained. The time schedule of these training courses is shown in Table 1.

Table 1. The time schedule of the Regional Training Courses on ISPM

No	Province	Venue and Time	Number of Participant
1	DKI Jakarta and West Java	SEAMEO BIOTROP, Bogor, 21 – 23 November 2006	24
2	Central Java	PT Garudafood, Pati, 4 – 5 January 2007	13
3	East Java and East Kalimantan	Sidoarjo, 28 – 30 March 2007	23
4	Banten	Serang, 23 – 25 May 2007	11
5	South Sulawesi	Makassar, 18 – 20 June 2007	22

The practical work activities that received serious attention were preparing, installing and collecting of data from various insect traps, i. e., carton trap, bait trap, sticky trap, and also light trap. This was due to the fact that the use of various insect traps to determine the population of storage insect pests was a new technique for staff members of Perum BULOG. Participants were very enthusiastic because of the superiority in the use of these insect traps to determine the storage insect pest population which does not require sampling of rice. Especially for light traps, it has more advantage in trapping moths which could not be trapped by other traps (carton and bait traps). The use of light traps supported the policy of Perum BULOG, as their warehouses should be free from *Ephestia*. Storage insect population is usually monitored by sampling of milled rice using a probe (a spear sampler). The participants expected that the monitoring technique using insect traps could be adopted and implemented at Perum BULOG circle. Nevertheless, this technique has its weaknesses, i. e., there is no correlation between data determined and the real population. Further research is still needed.

Seminar to Socialize the Program on Phase-Out of the Use of Methyl Bromide in Grain Storage in Indonesia

This seminar was the last activity of the project on Preparation and Organization of the Training of Trainers (TOT) and Workshop, Phase-Out of the Use of Methyl Bromide in Grain Storage in Indonesia.

The goals of the seminar were to disseminate the results of the program on phase-out of the use of methyl bromide in grain storage in Indonesia, and to plan a policy that will be implemented to control the use of methyl bromide after its phase-out in Indonesia.

The seminar was held at SEAMEO BIOTROP, Bogor, on 20 November 2007. This seminar was attended by 78 participants coming from various institutions such as UNIDO, Ministry of Environment, SEAMEO BIOTROP, Bogor Agricultural University, Perum BULOG, Department of Health, Department of Agriculture, Customs Office, Department of Trade, fumigation service companies involved in the Indonesian Pest Control Association (IPCA), agrochemical companies, estate enterprises, wood packaging enterprises, food and feed industries, methyl bromide importers, etc.

The seminar was divided into two sessions, i. e., panel discussion and discussions concerning the expectation and the follow-up activity for the program of phase-out of the use of methyl bromide in grain storages in Indonesia.

Conclusions

The activities on the Preparation and Organization of Training of Trainers and Workshop of Phase Out of the Use of Methyl Bromide in Grain Storage in Indonesia were conducted based on the cooperation between the Ministry of Environment with United Nations Industrial Development Organization (UNIDO) and SEAMEO BIOTROP. The Letter of Contract No. SPK – 01/Dep. III/LH/01/2006 was well implemented.

In general, the participants considered that the training was very well organized. They expected that the duration of the training should be longer and sustainable. The enterprise management of the training participants supported very much the implementation of ISPM to replace the use of methyl bromide in controlling storage pest in their enterprises. To support the implementation of ISPM at Perum BULOG, its enterprise management gave a very good response by increasing the number of training participants, either through the joint trainings planned by Perum BULOG and organized by SEAMEO BIOTROP, or a training organized by PERUM BULOG with National Project Coordinator and SEAMEO BIOTROP scientists as the resource persons. This means that getting the ball rolling has a significant effect on transferring the knowledge and technology on ISPM and phosphine fumigation The Indonesian Pest Control Association (IPCA) expects a sustainable cooperation with SEAMEO BIOTROP in implementing training on fumigation, either for fumigators or superintendents.

Although the project activities on the Phase-Out of the Use of Methyl Bromide in Grain Storage in Indonesia has ended, the Ministry of Environment is expected to pay continuous attention, especially on controlling the use of methyl bromide after its phase-out starting January 2008 for its use in grain storages for Non-QPS. Coordination among related institutions and the institution responsible to control the use of methyl bromide is needed in order to guarantee that the users of methyl bromide, who have already replaced the use of methyl bromide with phosphine, will not use methyl bromide again. Consequently, decisions made by several related departments concerning the prohibition of using methyl bromide in grain storages in Indonesia and its sanction are needed. Therefore, regulation and sanction for offenders either importers, distributors or users of methyl bromide, especially for grain storages in Indonesia should be immediately prepared.

0709

Efficacy of Sulfuryl Fluoride on Stored Grain Pests in a Warehouse Trial in China

Zeng Ling*, Zhang Xinfu, Xian Qing and Chen Jiadong

Abstract: Sulfuryl Fluoride is widely applied and is considered as a feasible substitute for methyl bromide in many countries. Efficacy of sulfuryl fluoride on stored grain pests, including *R. dominica*, *S. oryzae*, *and T. castaneum*, in a warehouse trial were undertaken in Guangzhou City of Guangdong Province, China in May 2006. According to the trial results, the doses of 40 – 5 g/m^3 with 48h – 7d exposures gave complete mortality of adults, larvae and pupae of insect species, but couldn't complete control eggs. After exposed to sulfuryl fluoride 30 – 40 g/m^3 for 48 h, the sulfuryl fluoride residues in paddy, flour, wheat, milk, rice were below the maximum levels in food premises. It is concluded that sulfuryl fluoride is an effective fumigant for control stored grain pests in China. These results will support sulfuryl fluoride product for registration.

Key words: sulfuryl fluoride, fumigation, stored grain pests

Introduction

Lesser grain borer *Rhzopertha dominic* (Fabricius), rice weevil *Sitophilus oryzae* (Linnaeus) and red flour beetle *Tribolium castaneum* (Herbst) are three important stored grain pest species. They can cause reduction in weight and quality of stored grain [1]. At present, the control of stored grain pests are largely relied on the use of chemical insecticides, especially fumigants. Methyl bromide (MB) as a fumigant permitted for use in grain and dry food products, has been widely used for half a century [2]. However, the use of methyl bromide is being phased-out due to its ozone-depleting properties [3,4]. Under the Montreal Protocol, methyl bromide is being phased out in developed countries and developing countries by 2005 and 2015[5]. Therefore, there is an urgent need for suitable alternatives to replace methyl bromide.

A lot of studies showed that sulfuryl fluoride (SO_2F_2) has been potential to control a wide variety of postharvest pests[6-9], including quarantine pests[10,11]. It could be a good candidate, as it can be utilized under almost the same conditions as MB, particularly with regard to its exposure time[12,13]. This fumigant has been registered for structural fumigations against termites, wood boring beetles and pantry pests for nearly 40 years[14]. Sulfuryl fluoride, presently marketed under the trade name ProFume®, has now been registered for insect pest control in food commodities and food processing facilities in the USA and in some countries in Europe[15-20].

In China, sulfuryl fluoride has been used to fumigate a variety of buildings and non-edible commodities[21-23], but has not been used in food premises until the end of 2007. Efficacy of sulfuryl fluoride on stored grain pests, including *R. dominica*, *S. oryzae*, and *T. castaneum*, in a warehouse trial were undertaken in Guangzhou City of Guangdong Province, China in May 2006. The results presented in this paper from upper trial carried out according to the method to meet the registration requirements of Institute for the Control of Agrochemicals, Ministry of Agriculture (ICAMA), China. ICAMA is the national authority responsible for pesticide registration and supervision. Using these results, the efficacy data from several other research laboratories, Shandong Longkou Chemical Factory will submit its sulfuryl fluoride product for registration in the ICAMA utilising dosages based on the target species, lift stage.

Materials and Method

Insect Pests

Species tested had included *R. dominica*, *S. oryzae*, and *T. castaneum*. *R. dominica* and *S. oryzae were reared on whole wheat*, *T. castaneum* was reared on a 3∶3∶1 mixture of broken wheat, rolled oats and yeast. The cultures were maintained at 25 ± 1℃ and 70% – 80% r. h. From these cultures, adults of three species (1

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-2 weeks old) were taken for preparation of mixed-age cultures. About 300 adults of *R. dominica*, *S. oryzae*, and *T. castaneum* were released separately into 200g of their respective rearing media in glass jars, and cultured them for 60 days to make that there were adults, eggs, larvae and pupae in each jar. Rearing media containing mixed-age cultures of the insects were weighed in 50g into cloth bags (20cm, 15cm size) and the bags were placed individually in the warehouse. In each fumigated warehouse, three cloth insect bags was hanged at upper point, middle point and lower point separately for each specie as three replicates for each dose of sulfuryl fluoride + CO_2 mixtures, with an equal number (three) of untreated control replicates

Fumigation

Fumigations were carried out in six small warehouses which were the same size. The size of each warehouse was: length × width × height $= 6m \times 3.5m \times 3m = 63m^3$.

Sealing was undertaken to improve the warehouses fumigant gas retention properties. All of doors and windows were mainly sealed with polyethylene sheeting and double sealed them with sealing adhesive, newspaper and paste. Performed sealing of door after introduction tube well placed.

The fumigant sulfuryl fluoride (99.8%, produced and provided by Shandong Longkou Chemical Factory) was contained as a pressurised liquid in steel cylingders. The cylingders, which remained outside the fumigated warehouses when in use, were connected using a short length of copper tubing fitted with rubber tubing that could be closed by pinch clamp. Sulfuryl fluoride and CO_2 were taken separately from two compressed gas cylingder into the same application mixing bottle, then injected sulfuryl fluoride + CO_2 mixtures in the bottle into the test warehouse with a 1cm outside diameter and 0.7cm inside diameter polyethylene tube (introduction tube). Fixed one end of the introduction tube in the middle of the warehouse, the other end was introduced to the outside of the warehouse through sealed door and connected with application mixing bottle to perform introduction. The application mixing bottle was a 5 000mL aspirator bottle for distilled water. The quantity of sulfuryl fluoride to be introduced was calculated according to the intended dose. The dosage of CO_2 was calculated on the basis of $100g/m^3$ of the warehouse volume.

Mixed-age cultures of different insect species were exposed to sulfuryl fluoride + CO_2 mixtures for 48hours ($40g/m^3 + 100g/m^3$ and $30g/m^3 + 100g/m^3$, active ingredient, the same below) and 7 days ($10g/m^3 + 100g/m^3$ and $5g/m^3 + 100g/m^3$). A reference fumigation was carried out. This was with 56% aluminium phosphide tablets, produced by Henan Zhengzhou Pesticide Factory, at $3.36\ g/m^3$ (active ingredient; the dosage of tablet was $6g/m^3$). Controls were placed in another warehouse at the same temperature.

Observations on Mortality

Following fumigation respectively, the test insect bags were taken out. The contents of the bags were transferred to individual glass jars (250mL size) and maintained at 27℃ and 70% r. h. in the laboratory at the Guangdong Institute for Cereal Science Research, for mortality assessments, which were completed after 7 days and 60 days. Rearing media inspection was carried out by cutting open the whole wheat. Counts of live and dead insects were made according to species. The efficacy was evaluated according to the formula below:

$$\text{Mortality}(\%) = \frac{\text{The number of dead insects}}{\text{Test insects}} \times 100$$

$$\text{Corrected mortality}(\%) = \frac{\text{Mortality of the treated} - \text{Mortality of the control}}{100 - \text{Mortality of the control}} \times 100$$

Determination of Residues

At the same place as the cloth insect bags, three cloth bags (25cm, 20cm size) with one of kinds of foods, including paddy, flour, wheat, milk powder and rice bought in market or supermarket, were hanged separately at the upper point, middle point and lower point in each fumigated warehouse as three replicates, each bag of food was about 500g.

At the end of fumigations, the fumigated warehouses were taken outdoors and opened to enable the fumigant to escape. 24 hours later, the residues of sulfuryl fluoride in different fumigated foods were determinated by GC external reference method in accordance with Chemical Reagent General Rules for the Gas Chromatography (GB/T9722-1988)[24].

Results

Temperature

Data on temperatures for the outside of the warehouse during fumigated treatment are presented in Table 1. The target temperature of 25℃ was attained.

Table 1. Average, minimum, and maximum temperatures for all fumigations treatments during the exposure time

℃	1st day	2nd day	3th day	4rd day	5th day	6th day	7th day
Average	26	25	25	25	24.5	24.5	25
Minimum	23	22.5	23	24	22	23	24
Maximum	27	27	25.5	25	24.5	25	25.5

Efficacy

The efficacy of sulfuryl fluoride on adults of tested insect species was shown in Table 2. The result showed that doses of 40 – 5 g/m^3 with 48hours – 7days exposures gave complete mortality of all adults. Rearing media inspection was carried out by cutting open the whole wheat, no live larvae and pupae were found in all fumigation treatments. Following fumigation, for efficacy assessments on eggs, which were completed after 60 days by counting of live adults of insect species. Average number of adults of insect species after 60 days was given in Table 3. The result revealed that doses of 30 – 40 g/m^3 with 48hours exposures were completely control eggs of *R. dominica* and *S. oryzae*, but tested doses couldn't complete control eggs of *T. castaneum*.

Table 2. Efficacy of sulfuryl fluoride on the adults of insect species

Dosage (g/m^3)		Exposure time	Repeated times	Corrected mortality %		
				R. dominica	*S. oryzae*	*T. castaneum*
SO_2F_2	40	48h	3	100	100	100
	30	48h	3	100	100	100
	10	7d	3	100	100	100
	5	7d	3	100	100	100
PH_3	3.36	7d	3	100	100	100
Control			3	0.2**	1.1**	0.33**

Note: Average mortality of Control.

Table 3. Average number of adults of tested insect species after 60 days

Dosage (g/m^3)		Exposure time	Repeated times	Average number of adults		
				R. dominica	*S. oryzae*	*T. castaneum*
SO_2F_2	40	48h	3	0	0	38.3
	30	48h	3	0	0	58
	10	7d	3	0	1	37
	5	7d	3	0.3	1.3	39.7
PH_3	3.36	7d	3	0	0	0
Control			3	21	39.3	>300

The residues of sulfuryl fluoride in different foods

Foods were exposed to sulfuryl fluoride 30 – 40 g/m^3 for 48 h. After 24h of the end of fumigation, the sulfuryl fluoride residues in paddy, flour, wheat, milk, rice were showed in Table 4. According to Maximum Levels of Contaminants in Foods (GB2762 – 2005)[25], the maximum levels of sulfuryl fluoride were 1.0 mg/kg for rice and flour, 1.5 mg/kg for others. Table 4 showed the residues of tested foods were below 1.0 mg/kg which were lower than the maximum levels in food premises.

Table 4. Residues of Sulfuryl Fluoride after Fumigation in Foods

Samples	Sulfuryl fluoride fumigant dose (mg/kg)	
	40 g/m^3	30 g/m^3
paddy	<1.0	<1.0
flour	<1.0	<1.0
wheat	<1.0	<1.0
milk	<1.0	<1.0
rice	<1.0	<1.0

Conclusion and Discussion

According to the trial results, the doses of 40 – 5 g/m^3 with 48h – 7d exposures gave complete mortality of adults, larvae and pupae of insect species. The doses of 30 – 40 g/m^3 with 48hours exposures were completely control eggs of *R. dominica* and *S. oryzae*, but the doses of 40 – 5 g/m^3 with 48h – 7d exposures couldn't complete control eggs of *T. castaneum*. After exposed to sulfuryl fluoride 30 – 40 g/m^3 for 48 h. the sulfuryl fluoride residues in paddy, flour, wheat, milk, rice were below the maximum levels in food premises. It is concluded that sulfuryl fluoride is an effective fumigant for control stored grain insects in China.

The fumigation dosage of sulfuryl fluoride that can control the eggs effectively in the field warehouse is still to be further study.

As a high-performance pesticide, when using, we should perform sealing of fumigation grain mass and grain warehouse carefully to improve airtightness and to enhance the pest control effect. Moreover, sulfuryl fluoride belongs to the toxic gas, during operation, we should pay e-

nough attention to the safety problems, and operators should be trained before operation.

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0710

A Review of the Global Applications of ECO_2 FUME and $VAPORPH_3$ OS Cylinderized Phosphine Fumigants for Stored Products Disinfestation

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Abstract: For decades phosphine has been the world's most widely used and cost effective fumigant for stored product protection. In response for the need to eliminate the associated risks of fire or explosion, need for deactivation, disposal and direct contact with the fumigant, ECO_2 FUME, a non-flammable cylinderized formulation of 2% phosphine and 98% carbon dioxide (CO_2) by weight, was developed and made available in the market in late the 1980's. By the year 2000, $VAPORPH_3OS$, a cylinderized formulation of 99.3% phosphine, entered into the market for large scale application using a safe and effective CYTEC approved on – site mixing equipment with CO_2 or air.

This paper describes the up to date history and the different global applications of ECO_2 FUME and $VAPORPH_3OS$ as fumigant for food commodities such stored grains, oilseeds, nuts and beans, fruits and vegetables, animal feed and feed ingredients and non-food commodities such as tobacco, cut flowers and foliages, tires and structural fumigation. ECO_2 FUME was first introduced in Australia during the late 1980's for stored grains, oilseeds and nuts both for un – sealed and sealed vertical silos and horizontal sheds. ECO_2 FUME is also used in Australia and New Zealand for export cut flowers and foliages. $VAPORPH_3OS$ was first introduced in China in 2000 at Dalian Grain Export Terminal using the on – site mixing with CO_2 technology. In North America, ECO_2 FUME was commercially used starting in 2000 in sealed storages for grains, nuts, dried fruits, tobacco, flour, processed foods and feeds as well as structural fumigation. With the development of the Horn Diluphos System (HDS) for safe on – site mixing of $VAPORPH_3OS$ with air in 2004, $VAPORPH_3OS$ has become an increasingly popular phosphine fumigant as a practical and convenient approach for large scale fumigation of grains, oil seeds, nuts and fruits and vegetables in Australia, USA and South America.

Introduction

Phosphine has been for decades the world's most cost effective and widely used fumigant for stored product protection against insect pests. With the introduction of ECO_2 FUME and VAPOR PH_3OS cylinderized phosphine fumigants, the usual disadvantages associated with the solid phosphine formulation of being self-igniting when exposed to air and the need for deactivation and disposal of unspent residue have been overcome.

ECO_2 FUME is a liquefied gas mixture of 2% phosphine and 98% carbon dioxide (CO_2) by weight making it a non-flammable and ready to use. It comes in high pressure aluminum or steel cylinders with a net fumigant weight of 31 kg. It requires simple dispensing equipment designed to deliver the fumigant as quickly or slower as required by each individual application. $VAPORPH_3OS$ is 99.3% phosphine by weight and is designed for use with approved blending equipment for on – site dilution with CO_2 or air in non-flammable proportions. It comes in steel cylinders with a net fumigant weight of 18 kg and just recently 22 kg. $VAPORPH_3OS$ is most suitable for larger storage volume applications where it is not practical to store, handle or transport large numbers of cylinders, price sensitive applications such as grains and for locations that conduct frequent fumigations.

ECO_2 FUME and $VAPORPH_3OS$ have the advantages of being safer, greener and faster. Safer because it is applied externally to the fumigation structure which eliminate confined space entry, reduce worker exposure and eliminate retrieval of partially spent fumigant. It is greener because there is no waste product or residue that requires waste deactivation or disposal. It is environmentally friendly due to its non-ozone depleting property. It has non-phytotoxic property to sensitive commodities such as cut flowers, fruits and vegetables. The required fumigation time is relatively faster than the solid phosphine formulation since it is easily applied as gas mixture to quickly distribute and achieve uniformly the target concentration. There is

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more effective control of target insects due to better gas distribution and maintenance of target concentration which leads to decreased amount of phosphine applied.

Applications in Australia

ECO_2 FUME (formerly known as Phosfume) was first commercially applied in Australia in 1988 by BOC Gases Australia who produced and patented the phosphine/CO_2 blend and developed special dispensing equipment for fumigating grains and oilseeds in unsealed and good sealed silos and horizontal sheds. This was in conjunction with the CSIRO patented fumigant application technologies called SIROFLO, SIROCIRC and SIROFUME. To date, there are over 150 million tons of grains and oil seeds that have been fumigated with ECO_2 FUME. In 1999, CYTEC Industries Inc. acquired the ECO_2 FUME global fumigant business from BOC Gases including all patents, trademarks, registrations and pending registrations. The SIROFLO dispensing equipment was further improved during the early 2000 for safety and less maintenance. The improved versions were developed by GasApps Australia, ABB Grain Ltd and CYTEC.

SIROFLO is a continuous slow addition of ECO_2 FUME in an air stream such that phosphine concentration is diluted from 20,000 ppm to about 30 ppm before introducing it into the bottom of the silo or shed and exit at the top of the grain for an exposure period of up to 28 days (see Figure 1). The bottom and the walls of the storage should be reasonably gas – tight to ensure that with the low positive pressure of the gas mixture the fumigant leaks outwards through the storage structure, and there is only minimum ingress of air that could locally dilute the fumigant concentration. The long exposure period to low phosphine concentration will allow the killing of all stage of insects including the less susceptible egg and pupae stages. With the development of increased resistance of some insects species (lesser grain borer and rice weevil) a new set of fumigation protocol was established in 2004 covering minimum phosphine concentration of 70 – 700 ppm for 3 – 21 days at 15 – 30℃.

SIROCIRC is similar to SIROFLO except insofar as it includes a recirculation duct connected between the storage roof and the fan inlet. This allows the recovery of phosphine from the headspace above the grain and its recirculation through the grain mass. At least 90% of phosphine can be recycled in a reasonably well sealed storage. While SIROFLO is a set-and-leave operation, SIROCIRC requires a reduction in the fumigant flow-rate once phosphine begins to recycle back from the top of the storage. This can be done manually, but control is facilitated by the use of an automatic electronic controller that intermittently adjusts the fumigant flow to generate a near-constant phosphine concentration in the delivery duct.

Large storages such as big silos in grain terminals and horizontal sheds have employed the use of on-site mixing of VAPORPH$_3$OS with CO_2. GrainCorp large storage facilities in Queensland, NSW and Victoria have used on – site mixing equipment developed by CYTEC and GasApps Australia.

SIROFUME differs from the other two in being a "one-shot" technique wherein gaseous phosphine is dumped into the head space of a sealed storage. Nowadays, this fumigation approach is mostly used with VAPORPH$_3$OS using an on – site phosphine/air mixing equipment.

ECO_2 FUME is also used in Australia and New Zealand for pre-shipment treatment of exported cut flowers and foliage. At normal atmospheric pressure, the protocol used is 700 ppm of phosphine for 15 hours at minimum temperature of 15℃. In New Zealand, a shorter exposure of 3 – 4 hours is adopted at 700 ppm and minimum 15℃ with the use of a vacuum chamber at 70 mm Hg absolute pressure. ECO_2 FUME is used to a relatively limited extent for quarantine treatment of imported grains, flours, oil seeds and nuts that come in shipping containers.

With the development and commercialization of the Horn Diluphos System (HDS), (a CYTEC approved phosphine/air on-site mixing equipment) in 2004, VAPORPH$_3$OS became an increasingly popular fumigant for cost effective, flexible and convenient way of fumigating grains and oilseeds in sealed storages (vertical silos, horizontal sheds and bunkers). The HDS fumigation equipment is manufactured and supplied by Fosfoquim SA in Chile. The HDS comes in three size models (HDS 801.2 kg phosphine/hr, HDS 2003 kg phosphine/hr and HDS 80012 kg phosphine/hr) which cater to a wide range of storage capacities ranging from 1 000 tons to 300 000 tons. The VAPORPH$_3$OS phosphine fumigant in combination with the HDS fumigation equipment is now widely used by the three Australian bulk handling companies (GrainCorp,

ABB Grain and CBH Group) which handle over 90% of harvested grains and oilseeds in the country. Figures 24 show examples of current applications of VAPORPH$_3$OS with the HDS.

Applications in China

China is the first country to commercially apply VAPORPH$_3$ OS at Dalian Xizui Grain Terminal by on-site mixing with bulk CO_2 in 2000. The on-site mixing of VAPORPH$_3$OS with bulk CO_2 produces the ECO_2 FUME blend which is introduced for fumigation with the SIROCIRC fumigation system developed by GasApps Australia and constructed by Grain Tech System Pty Ltd. This grain terminal has a 1 million ton grain capacity divided into a block of 144 × 3000 ton sealed silos and another block of 20 × 30000 ton sealed silos both equipped with SIROCIRC fumigation system. The fumigation system is composed of 1) a 5-ton bulk liquid CO_2 tank and VAPORPH$_3$OS cylinders storage, 2) on-site mixing system (ECO_2 FUME mixer), 3) ECO_2 FUME delivery pipe work and 4) SIROCIRC system. Some components of the fumigation system are shown in Figures 5. During fumigation, a phosphine concentration of 100 ppm is maintained through out the grain mass for a period of 18 days enough to kill all stages of insects.

Applications in North America

Commercial applications of ECO2FUME in the USA started by fourth quarter of 2000 after full registration both for non-food and food use was granted by August 2000. ECO_2FUME is applied into the sealed fumigation structure by direct injection using simple and quick dispensing equipment with variable fumigant flow rates.

Tobacco fumigation is among the first commercial application of ECO_2FUME in the USA. Tobacco bales stored inside large warehouses are fumigated by first sealing the warehouse and injecting ECO_2FUME from a bank of cylinders in manifold located outside the warehouse. A phosphine concentration of 250 ppm is maintained for a period of 96 hours to achieve successful fumigation. The use ECO_2FUME has the advantages over solid phosphine formulation of 1) quick dispensing and attainment of target concentration throughout the structure, 2) no dependence on humidity and temperature for reaction, 3) eliminate dust or solid waste generated from using solid formulation, 4) no waste deactivation, 5) eliminate waste disposal and 6) no ammonia residue released that require additional scrubbing.

Among the different commercial applications of ECO_2FUME and VAPORPH$_3$OS in the USA is either methyl bromide or solid phosphine formulation replacement as below (Figure 6).

1. In-transit fumigation of flour and rice in rail cars using ECO_2FUME

2. Rice, wheat, corn and other grain fumigation in sealed vertical bins using ECO_2FUME or VAPORPH$_3$OS

3. Fumigation of almonds, walnuts, and pistachio nuts with VAPORPH$_3$ OS previously were using methyl bromide in fumigation chambers/containers and metal phosphide in metal storage bins

4. Bagged and bulk seed in cold storage warhouses with ECO_2FUME

5. Fumigation of stacked raisins under tarp and other dried fruits using ECO_2FUME or VAPORPH$_3$OS

6. Structural fumigation (e. g. flour mill, empty warehouse) using ECO_2FUME in combination with heat and CO_2

7. Bunker storage using VAPORPH$_3$OS

The dosage recommendation for ECO_2 FUME and VAPORPH$_3$OS in the USA varies in phosphine concentration of 2001000 ppm for 36 hours to 6 days at above 26℃ depending on the commodity, target insects and sealing degree of the fumigation structure. There is also a protocol with shorter exposure period of 24 hours at above 26℃ using a phosphine concentration of 5001000 ppm. Rodents and other vertebrate pests in storages may be controlled with short-term fumigations within 1 to 4 hours after achieving distribution of phosphine throughout the structure.

Only ECO_2 FUME is currently used in Canada for similar range of applications as in the USA but using the same dosage rate of 2001000 ppm phosphine concentration for an exposure period 214 days and temperature range of 0 – 16 or above.

Applications in Latin and South America

Chile is the first country in the world which has commercially applied VAPORPH$_3$OS (TK Gas brand name in Chile) for fumigation of export fruits and vegetables. Fosfoquim SA developed the Horn Diluphos System (HDS)

fumigation machine to safely mix VAPORPH$_3$ OS and air and deliver the phosphine air mixture at small to high flow rates into different sized sealed fumigation structures. Fosfoquim SA has also formed a fumigation company which provides fumigation services to fruit exporters. A fleet of fumigation vans and trained fumigators provide mobile fumigation service to all customers in Chile. Fumigation services provided by Fosfoquim include fruit and vegetable fumigation which accounts for a major portion of the fumigation services. Figure 7 is a sample setup of fruit fumigation using the HDS 800 in a fumigation van.

There are many advantages of using VAPOR PH$_3$ OS in combination with the HDS for fruits and vegetables fumigation as follows:

· Phosphine eliminates the target pests in fruits, like mealy bugs, Pseudococcus spp; apple moth, Cydia pomonella; eulia, Proeulia spp; fruit tree weevil, Naupactus xanthographus; mediterranean fruit fly, Ceratitis capitata; fruitfly, Bactrocera spp, Anastrepha spp; and Thrips spp.

· No changes in taste, smell, texture, color or shelf life of the fruit, if fumigation has been conducted at low temperature. This is mainly because it is possible to fumigate at low temperature with the cooling system running (Figure 1).

· It is not necessary to heat fruits up before fumigation. Therefore, the shelf life of the fruits is extended.

· There are no residues after fumigation on the fruits and no product residues that have to be deactivated and disposed after fumigation.

· Cylindered phosphine does not produce ammonia and it is, therefore, not phytotoxic.

· The fumigation can be done in the same cooling chambers where the fruit is stored prior to shipment.

· There is no need to fumigate at the port of arrival, since the fumigation can be done at the processing plant before shipping.

· The fruit can be delivered immediately upon arrival at the port since the fruit is already fumigated and inspected, increasing capacity of the ports to receive fruits, or giving other ports, that do not have fumigation facilities, an option to deliver fruits to other parts of the receiving countries.

· This fumigation technique has no environmental problems, since only hydrogen phosphide is applied, which is readily deactivated by sunlight upon release into the atmosphere. Therefore there is no damage to the ozone layer, as it occurs with other fumigants used for fruit fumigation.

· The fumigation is operator friendlier than methyl bromide. The gas concentration can be monitored exactly with different electronic devices in order to ensure minimum exposure of fumigators and plant workers to the gas.

· Gas injection even in the largest fruit fumigation cool houses takes less than one hour and aeration systems are installed in order to allow aerating chambers in less than 90 minutes.

· As the method permits applying the gas from outside the facility, the gas concentration can be changed at any time during the fumigation.

· The gas can be applied to a totally sealed structure without increasing the pressure, if the gas from the cylinders is mixed with air taken from the inside of the structure to be fumigated.

· The HORN DILUPHOS SYSTEM allows stopping gas dispensing at any time during gas injection (Figures 2, 3, 4, 5 and 6). Unlike from what had always been thought, there are no corrosion problems on the cooling.

Tables 1 and 2 show the extent of fresh fruits and vegetables fumigation conducted by Fosfoquim in terms of type and destination country from 20052008. The top six export fruit type fumigated are apples, prunes, grapes. peaches, oranges, and pears. The top six destination countries are Mexico, Iran, Korea, Japan, Bolivia and China.

In Trinidad and Tobago, ECO$_2$ FUME is used for cargo container fumigation, shiphold fumigation, warehouse fumigation, storage bin treatment and fumigation of grains on pallets and in silos. ECO$_2$ FUME is dispensed using simple and quick dispensing equipment into a sealed structure.

Summary

ECO$_2$FUME and VAPORPH$_3$OS are cylinderized phosphine fumigants that offer safer, greener and faster advantages for disinfestation of food commodities such stored grains, oilseeds, nuts and beans, fruits and vegetables, animal feed and feed ingredients and non-food commodities such as tobacco, cut flowers and foliages, tires and structural fumigation.

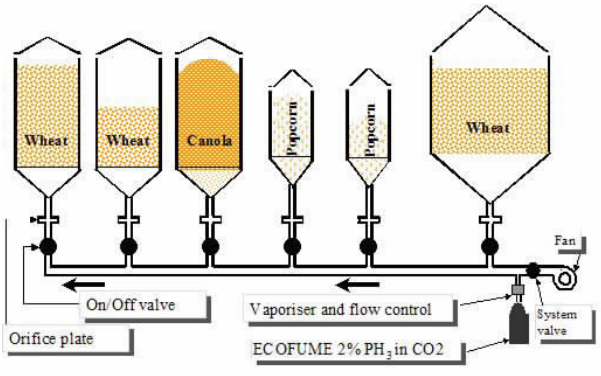

Fig. 1 Schematic of SIROFLO fumigation system for unsealed silos.

Fig. 2 The 300,000 ton capacity sealed horizontal shed at CBH Kwinana Grain Terminal Western Australia with insert HDS 800/VAPORPH$_3$OS fumigation setup.

Fig. 3 An 8000 – ton sealed silo with the HDS 800/VAPORPH$_3$OS fumigation setup at ABB Wallaroo Grain Terminal in South Australia.

Table 1. Volume of fruit fumigation covered by Fosfoquim SA based on fruit type from 2005 to June 2008.

Fresh Fruit	Volume of Froit Fumigation(m^3)				Sub Total
	2005	2006	2007	2008	
Garlic	810	86	0	0	896

Fig. 4 A 20000 – ton bunker with the HDS 800/VAPORPH$_3$OS fumigation setup at GrainCorp Nhill grain storage center in Victoria Australia.

Fig. 5 Dalian Phase – 2 silo block of 20 bins by 30 000 ton capacity each. The CO_2 storage – tank and ECO$_2$FUME on – site mixing enclosure are dwarfed by the silos. Around the lower part of each silo is the fumigant delivery ducting.

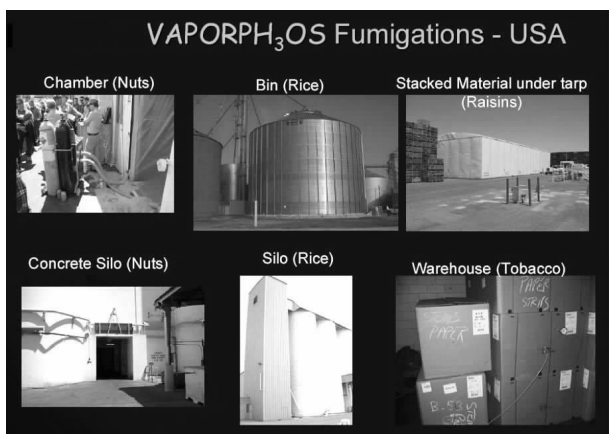

Fig. 6 Methyl bromide replacement with VAPORPH$_3$OS for fumigations in the USA.

Fresh Fruit	Volume of Froit Fumigation(m^3)				Sub Total
	2005	2006	2007	2008	
Parsimon	83	2.474	2.187	734	5.478
Onions	0	0	856	0	856

Fig. 7 The HDS 800 in a fumigation van with a two-hose connection to a fruit fumigation chamber.

Fresh Fruit	Volume of Froit Fumigation(m^3)				Sub Total
	2005	2006	2007	2008	
Chenies	0	5.744	426	0	6.170
Prunes	34 056	82 260	11 363	11 978	139 657
Clementines	4 786	11 214	6 232	0	22 232
Apricots	2 706	249	354	83	3 392
Pomegranate	0	0	0	1 246	1 246
Kiwifruits	462	7 716	9 206	856	18 240
Lemons	2 614	166	4 428	8 565	15 773
Apples	58 843	286 807	279 202	107 873	732 725
Quinces	83	1 065	660	1 704	3 512
Oranges	10 098	34 541	35 648	513	80 800
Nectarines	5 321	3 415	581	2 545	11 862
Avocado	2 656	1 638	1 280	0	5 574
Pers	6 867	18 264	13 875	16 507	55 513
Grapefruit	0	4 783	2 376	249	7 408
Grapes	688	14 178	33 134	52 284	100 284
Total	189 561	523 086	404 423	211 495	1 328 565

$1 m^3$ = 22 standard fruit trays 11.5million travs in 2006

Table 2. Volume of fruit fumigation covered by Fosfoquim SA based on destination countries from 2005 to June 2008.

Destination Country	Fruit Volume Fumigated(m^3)				Sub Total
	2005	2006	2007	2008	
Argentina	0	2 659	3 372	332	6 363
Bolivia	0	11 926	24 000	19 031	54 957
Brazil	0	0	415	0	415
China	0	0	19 370	3 226	22 596
Colombia	0	0	4 072	1 354	5 426
Korea	0	2 250	32 516	29 919	64 685
Costa Rica	0	83	640	83	806
Ecuador	0	0	2 266	1 852	4 118
Spain	0	0	582	0	582
Europe	0	1 092	4 770	5 032	10 894
Hong Kong	0	0	166	0	166
Netheriands	0	0	320	0	320
India	0	0	6 000	332	6 332
Iran	0	44 149	40 860	49 739	134 748
Japan	0	7 508	37 260	17 050	61 818
Far East	0	0	5 829	0	5 829
Mexico	49 694	368 079	196 214	72 978	686 965
New Zealand	0	0	684	817	1 501
Panama	0	0	6 195	3 004	9 199
Peru	0	166	1 181	700	2 047
Russia	0	0	2 064	166	2 230
Taiwan	0	0	1 092	1 162	2 254
USA	0	415	12 749	4 635	17 799
Venezuela	0	0	83	0	83
Other markets	139 867	84 759	1 723	83	226 432
TOTAL	189 561	523 086	404 423	211 495	1 328 565

0711

Factors Affecting Carbon Dioxide Concentration in Interstitial Air of Wheat Stored in Hermetic Plastic Bags (Silo-bag)

Juan Rodríguez[1], Ricardo Bartosik[*1], Leandro Cardoso[1] and Diego Crocce[2]

Abstract: In 2007 about 35 million tonnes of grains were stored in hermetic systems (silo-bags) in Argentina, and about 5 million tonnes of that was wheat. The wheat stored in these silo-bags was mostly used for milling (internal and external market), but also for seeds for the next planting season.

The goal of this research was to conduct a series of field experiments in order to identify the main factors affecting the carbon dioxide (CO_2) and oxygen (O_2) concentrations, as an indicator of biological activity and appropriate wheat storability conditions.

The experiments consisted of monitoring the gas composition of the interstitial air, grain commercial quality, grain moisture content (MC), and grain temperature of several silo-bags.

The main results indicated that the CO_2 concentration of wheat stored in hermetic plastic bags increased with grain MC. At MC below 13% the CO_2 concentration was below 5%, and as mold become active at higher MC the CO_2 concentration increased to 30% for MC of 19%.

Silo-bags with good quality wheat resulted in lower CO_2 concentration than silo-bags with poor quality wheat at the same MC, implying that poor quality wheat had higher biological activity (CO_2 concentration up to 7 percentage points higher).

The effect of average grain temperature on CO_2 concentration became substantial when grain MC was above 14%. For silo-bags with wheat MC higher than 14% the CO_2 concentration was higher during the warm season than during the cold season, and this difference was up to 7 percentage points when wheat MC was between 16% and 17%.

Key words: grain quality, biological activity, modified atmospheres

Introduction

In 2007 about 35 million tonnes of grains were stored in hermetic systems (silo-bags) in Argentina, and about 5 million tonnes of that was wheat. The wheat stored in these silo-bags is mostly used for milling (internal and external market), but also for seeds for the next planting season.

Each silo-bag can hold approximately 200 tonnes of wheat and with the available handling equipment is quite simple to load and unload. These plastic bags are 60 m long, 2.74 m diameter and the plastic cover is made of three layers (white outside and black inside) with 235 micrometers of thickness (Figure 1).

The silo-bags are waterproof and have a certain degree of gas-tightness (oxygen (O_2) and carbon dioxide (CO_2)). As a result, respiration of the biotic components of the grain mass (fungi, insects and grain) increases CO_2 and reduces O_2 concentrations. When the biological activity is intense, the degree of modification of the typical atmospheric gas composition (21% O_2 and 0.033% CO_2) is greater, which would limit grain respiration and mold[1]

Fig. 1 Picture of a 200 – tonne capacity (60 m long and 2.74 m diameter) hermetic storage plastic bag (silo-bag).

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and insect development[2,3]. It has also been also observed that high CO_2 concentration reduced the ability of *Aspergillus flavus* to produce aflatoxin.

Bartosik et al.[4] summarized previous experiences of storing grain in silo-bags, where it was demonstrated that the grain temperature in the hermetically sealed plastic bags followed the pattern of the ambient temperature throughout the year, implying that temperature of the grain mass does not reveal biological activity in the grain mass. The average moisture content (MC) did not significantly change during any storage experiment for both dry and wet silo-bags. In general, no MC stratification was observed in wheat silo-bags. When the grain was stored at the market MC, no significant decrease in the quality parameters could be observed during 150 days of storage. However, when grain was stored above the market MC, the decrease in some of the quality parameter could be observed. The increase in the CO_2 concentration was higher at the end of the storage time and also was higher in those bags with wetter grain (13.0% of CO_2 for 12.5% MC, and 22.8% of CO_2 for 16.4% MC after 100 days of storage). Based on these observations the authors hypothesized that measurement of gas composition in the interstitial air could be used as an indication of the biological activity of the grain mass in the hermetic storage systems, and as a tool for monitoring grain storability. However, a better understanding of typical CO_2 concentrations for wheat silo-bags is required to use this technology for monitoring grain storability.

The CO_2 and O_2 concentration in the silo-bag depends on the balance between respiration (consumption of O_2 and generation of CO_2), the entrance of external O_2 to the system, and the loss of CO_2 to the ambient air (Fig. 2).

The movement of gases in and out of the silo-bags depends on the gas partial pressure differential and the permeability of the system (through openings in the plastic cover, or through the natural permeability of the plastic material to gases). Grain type and condition, MC, temperature, storage time and O_2 and CO_2 concentrations affect the respiration rate. The temperature of the grain depends on the initial grain temperature (this effect is less important as the storage period increases), the sun radiation, the heat release from the respiration process, and the transfer of heat with the air and

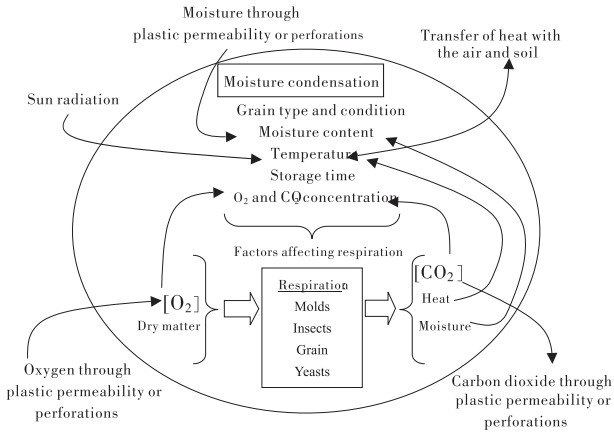

Fig. 2 Diagram of the main factors affecting respiration of grain and microorganisms in the silo-bag, the relationships among them, and the final O_2 and CO_2 concentrations.

soil (grain temperature increases during spring and summer and decreases during fall and winter). The grain MC depends on the initial grain MC, the entrance of moisture from the outside (through openings after a rain event into broken or poorly sealed silo-bags), and the moisture released from the respiration process. Additionally, due to the day and night temperature differential, some moisture condensation can occur in the top grain layers resulting in a localized spot of wetter grain.

Thus, the goal of this research was to study the effect of grain MC, grain temperature and grain quality on CO_2 concentration in silo-bags holding wheat.

Materials and Method

The tests were carried out at grain elevators and on farms in the south east of Buenos Aires province, Argentina, through three storage seasons (from January 2006 to May 2008). Most of the wheat silo-bags were filled in December – January and stored until June or July. However, a small proportion of them were stored for more than one year.

For each silo-bag two sampling locations were established. The procedure consisted of measuring first the gas concentration (O_2 and CO_2) with a portable gas analyzer (PBI Dan Sensor, CheckPoint, Denmark), perforating the plastic cover with a needle. The gas composition was analyzed for three levels in each sampling location, close to the top of the bag, at the middle and close to the bottom.

After the gas composition was analyzed, a wooden stick with three temperature sensors was inserted in the grain mass (diagonally, from the

top and side to the bottom and center of the silo-bag) to measure grain temperature at approximately 0.1, 0.7 and 1.4 m from the surface.

In each sampling location grain was collected from three different levels (top = 0.10 m depth, middle = 0.75 m depth, and bottom = 1.6 m depth. Total height of the bag = 1.7 m) using a standard torpedo probe. Material from each one of the three sampling locations was segregated by level (surface, middle, and interior). The grain samples were stored in a hermetic plastic bag and brought to the Grain Postharvest Laboratory of the Balcarce Experimental Station of the National Institute of Agricultural Technologies (INTA). After probing the silo-bags the openings were sealed with a special tape in order to restore the air – tightness.

Additional information of the silo-bag was collected, such us filling and sealing quality, history of openings, perforations due to wild animals or bad sealing after sampling, improper preparation of the soil where the silo-bag was placed (when silo-bags were assembled on top of crop residues it results in perforations of the bottom), silo-bags assembled in low lands with risk of flooding, and any other relevant information.

At the laboratory, grain samples were analyzed for MC (GAC 2100, Dickey – John) and commercial quality according to the Argentina wheat quality standard[5]. Later, each sample was qualified as good quality wheat or poor quality wheat according to Table 1. The monitoring procedure was repeated approximately every 15 days during the entire storage period.

Table 1. Limits of the quality factors of the argentine wheat commercialization standard used to categorize the grain samples as good quality or poor quality.

Grain quality	Quality	Test weight	Foreign matter	Damaged kernel	Broken and vane kernels
Grade		(kg/hL)	(%)	(%)	(%)
Good quality	1	79.0	0.2	1.0	0.50
	2	77.5	0.6	1.5	0.85
Poor quality	2	76.0	0.8	2.0	1.20
	3	73.0	1.5	3.0	2.00
	out of standard				

Results and Discussion

Figure 3 shows the relationship between grain MC and CO_2 concentration. The CO_2 concentration increased with the increase in grain MC, which is a consequence of the biological activity. When the wheat MC was lower than 13% the average CO_2 concentration was below 5% (presumably due to grain respiration). When the wheat MC increased to the point at which molds became active (between 13.5 and 14.5%) the CO_2 concentration increased to 15% with wheat at 16% MC, and to 30% with wheat MC above 19%. The data shown in Figure 2 corresponds to silo-bags without visible structural problems, although some silo-bags with perforations in the bottom side that were not noticed during sampling might be included.

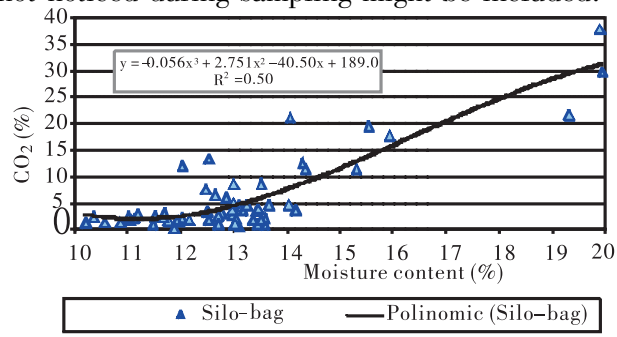

Fig. 3 CO_2 concentration in silo-bags as function of wheat moisture content.

Figure 4 shows the difference in CO_2 concentration of silo-bags with good and poor quality wheat for different grain MCs. Poor quality grain has more percentage of foreign matter, broken kernels and damaged kernels. As a result, for the same MC level, silo-bags holding poor quality wheat resulted in higher CO_2 concentration than silo-bags filled with good quality wheat, due to the higher biological activity of the poor quality wheat. The difference between both grain types increased from about 5 percentage points of CO_2 for 13% MC to 7.5 percentage points for 15.5% wheat MC.

Figure 5 shows the CO_2 concentration for silo-bags sampled during the warm and cold storage season. Storage temperature affects biological activity, reducing the respiration rate of grain and microorganisms. At low grain MC there was almost no difference in CO_2 concentration for the warm and cold season, while for MC above 14.0% (presumably MC at which mold become active) the difference in CO_2 concentration during the warm season (January – March) was up to 7 percentage points higher than during the cold season (May – July).

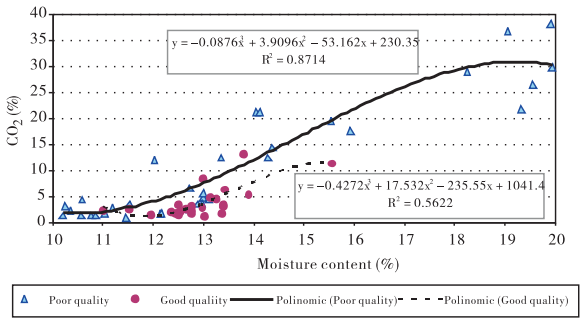

Fig. 4 CO_2 concentration at different grain moisture contents for silo-bags with good and poor wheat quality.

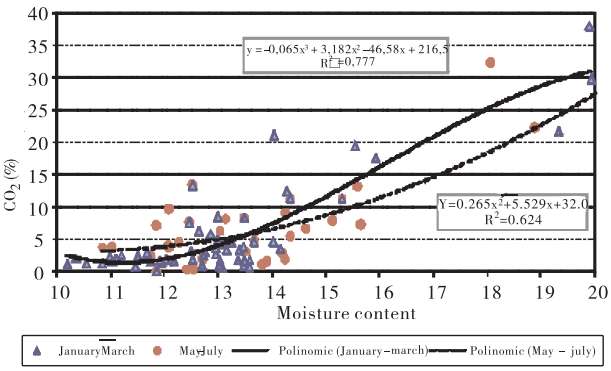

Fig. 5 CO_2 concentration at different grain moisture content for silo-bags sampled during the warm storage season (January – March) and the cold storage season (May – July).

This study showed that the main factor affecting CO_2 concentration in wheat silo-bags was MC, which increased the CO_2 concentration from 5% to 30% when the wheat MC increased from 14% to 19%. Grain quality was less important, although poor quality wheat had from 5 to 7.5 percentage points higher CO_2 concentration than good quality wheat. It was also showed that the effect of quality increased with MC. Finally, the effect of temperature was important for wet wheat (up to 7 percentage points higher), but for wheat below 14% MC the effect of temperature was not noticeable.

Conclusions

The CO_2 concentration of wheat stored in hermetic plastic bags increased with grain MC. At MC below 13% the CO_2 concentration was below 5%, and as mold became active at higher MC the CO_2 concentration increased to 30% for MC of 19%.

Silo-bags with good quality wheat resulted in lower CO_2 concentration than silo-bags with poor quality wheat at the same MC, implying that poor quality wheat had higher biological activity, (CO_2 concentration up to 7 percentage points higher).

The effect of average grain temperature on CO_2 concentration became substantial when grain MC was above 14%. For silo-bags with wheat MC higher than 14% the CO_2 concentration was higher during the warm season than during the cold season, and this difference was up to 7 percentage points when wheat MC was between 16% and 17%.

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SESSION 8

CA AND FUMIGATION——INSECT RESISTANCE AND MANAGEMENT STRATEGIES

Chairpersons:
Pat Collins, Australia
Cao Yang, China

0801

DNA Testing for Phosphine Resistance ——The Future of Resistance Monitoring and Management

David I. Schlipalius[1,2], Rajeswaran Jagadeesan[3], Yosep Mau[3], Patrick J. Collins[1,2] and Paul R. Ebert[3*]

Abstract: In recent years, monitoring programs have detected increasing levels of resistance to phosphine among pest insect species. Effective management of resistance requires knowledge of the frequency and distribution of resistance genes within populations. Such knowledge-based management strategies will require rapid, high-throughput resistance assays. Our research is focussed on genomic approaches to identifying phosphine resistance genes in two of the major worldwide pests of stored products, *Tribolium castaneum* and *Rhyzopertha dominica*. These genes will then be used in rapid diagnostics to provide data on resistance gene distribution.

Our results to date indicate that in both *T. castaneum* and *R. dominica*, high-level resistance is mediated by two major genes, each of which confers weak resistance, butwhich interact synergistically to provide high-level resistance. For *R. dominica*, the genes appear to be the same in all outbreaks yet studied, which suggests they are conserved in most (if not all) cases of high level resistance. This observation enhances the validity of a DNA based test for phosphine resistance, at least in a great majority of cases.

Data produced by DNA testing can be used to model the spread of resistance, to indicate pest management strategies that may be deemed at "high-risk" of promoting resistance as well as to assess the probability of success or failure of future resistance management strategies. As DNA testing and the more traditional resistance bioassays have complementary advantages and limitations, it will be most effective to apply both techniques in a program for resistance monitoring

Key words: phosphine resistance, monitoring, genomics

Introduction

In 2002, worldwide annual post-harvest crop losses directly due to insects and mites in food storage were conservatively estimated at approximately 5% translating to approximately 120 – 160 million tons of cereal product each year. This is despite the widespread use of chemical treatments to control these pests[1]. In 2008 dollars and using the current wheat price of approximately US $320/ton, this translates to a global loss of more than US $38 billion per year due to insects in cereals alone. Given the recent rate of increase in the cost of food, especially grain crops, this estimated annual cost is set to increase.

One major chemical treatment, phosphine fumigant, has been in use for more than 50 years. This is remarkable longevity for an insecticidal compound. However, the increase in the use of phosphine in recent decades is associated with a parallel increase in both the frequency of occurrence and the absolute level of resistance. Even so, when used appropriately, phosphine is the most economically and environmentally sound routine commercial treatment for stored grain. Since no alternative fumigants match the value of phosphine, the arguments for maintaining the effectiveness of this important chemical is clear.

Since the 1980's, Australia has had the unique distinction of maintaining a long-term program for monitoring resistance to phosphine and grain protectants. The result of this long-term program has enabled the detection, culturing and characterisation of a number of resistances in various pest species from across Australia[11-14].

Currently, the technique most often used in resistance monitoring involves a bioassay of resistance in which insects are exposed to an insecticide for a set period of time at a dose that discriminates between resistant and sensitive strains of insect. Usually this discriminating

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dose is marginally higher than the $LD_{99.9}$ of a susceptible strain of pest insect. Sometimes, such as with phosphine in the Australian resistance monitoring program, a second discriminating dose may be used to distinguish between-weak' andstrong' resistances. In this way the occurrence of resistance can be detected in the field and the number of strains exhibiting a given level of resistance can be documented.

In Australia, resistance 20 – 25 times higher than the basal level of tolerance of fully susceptible beetles, was first reported in the lesser grain in the mid – 1970s[2] and the frequency of weak resistance has gradually increased from that time[7], whereas strong resistance was not detected until 1997[8]. Resistance has also been reported to be increasing in many countries around the world[9,10] but especially in Asia and the Indian subcontinent[11-14].

One limitation of the bioassay is that it is a slow, labour-intensive process that is unable to detect heterozygous carriers of resistance genes. However, it is currently the only method that is able to detect unknown resistances in field – collected populations. In contrast, DNA testing of resistance genes is rapid, high-throughput, and is able to detect resistance genes in heterozygous carriers who do not express the resistance phenotype. The DNA assay can even be used to detect the presence of the resistance gene in dead insects. The two techniques are complementary, however, as the DNA test cannot detect novel resistance mutations, whereas the slower bioassay can be used to assess the resistance levels of any insects.

Recent Work

The majority of our recent work has focussed on the genetic basis of phosphine resistance. We have shown that in the Australian strains of *R. dominica* the majority of the strong resistance trait was controlled by two independently assorting genes, named *rph*1 and *rph*2, that individually conferred a weak resistance(– 25X and – 12. 5X respectively), but together act synergistically to confer a strong resistance(> 250X)[15,16]. This unique situation in which no individual gene can confer high-level resistance provides an opportunity for effective management of resistance in the field. One potential barrier to resistance management is that laboratory experiments reveal no detectable fitness costs associated with the resistance genes[15], which has consequences for modelling of resistance outbreaks.

We have also found through complementation analysis with several independent phosphine resistance outbreaks across Australia, the genes were the same in each case[17]. In fact, the phenomenon of limited numbers of resistance genes appears to be the case for insecticide resistances generally[18]. This indicates that the number of phosphine resistance genes is likely to be limited and that genetic tests should be robust.

We have also extended our search for resistance genes to *Tribolium castaneum*, a pest insect for which the genome sequence has just recently been published[19], an advance that will greatly facilitate isolation of resistance genes. Our recent results have shown that, as with *R. dominica*, two genes act synergistically to confer high-level resistance(R. Jagadeesan, *unpublished*), although we have yet to determine whether the same genes confer resistance in each of the two species. Identification and comparison of the resistance genes in the two species will allow us to make more broadly relevant predictions about phosphine resistance.

Implications

Onstad[20] highlights three issues that are critical for the evaluation of all resistance monitoring plans: goals, precision and cost. We believe that application of DNA testing to monitoring will greatly increase the scope of the first and make significant improvements to the logistics of the latter two of these critical issues. To highlight these improvements, we provide a brief overview of current and proposed methods and discuss their relative merits.

Monitoring

Current bioassay techniques for monitoring resistance have a major strength in that they can detect resistance genes in field – collected populations of insects regardless of whether those particular resistances have been documented or characterised previously. It also requires a minimal amount of specialist training to set up and uses basic, non-specialist laboratory equipment. However, bioassays are a slow, labour intensive process and require a minimum number of insects collected from the field, which are usually bred through to F_1 or F_2 generations to generate enough material for robust confirmation of resistance. This process takes months to do. Also, since bioassays rely on a 'phenotype'(i. e. the actual expression of resistance in the insect) and insecticide resistances are generally reces-

sive, this type of assay does not detect insects that are heterozygotes for resistance gene alleles (i. e. 'carriers' of the trait). Heterozygotes only have one copy of the resistance gene/allele, whereas expression of resistance requires two copies, one from the female parent and one from the male parent. This means that if resistance is rare or uncommon in a population, then the bioassay technique will likely be unable to detect it.

Looking ahead, diagnostic DNA tests will able to distinguish phosphine resistance genotypes from field-collected insects without a requirement of breeding or exposure to the chemical. In fact, the insects to be assayed need not be of any particular age and dead insects can be assayed just as readily as living insects. DNA tests are also rapid (requiring only hours or days) and many samples can be processed in parallel. This can save months of work and provide high-quality, uniquely informative data at the same time.

The data from DNA tests are unambiguous, which facilitates information sharing between laboratories as the assays are not influenced by the age of the insects or the culturing conditions. Also, DNA itself can be transferred over large distances, eliminating the need to transport living cultures, which avoids quarantine restrictions between countries. DNA tests are also scalable, in that they can be performed in small labs, or in large centralised labs depending on the research model adopted. In fact, they provide much more flexibility for small labs since no culturing of insects is needed, thus reducing labour costs and space requirements (eg. constant temperature cabinets).

While DNA tests are very good at rapidly and effectively identifying insects that carry known resistance genes, the tests cannot readily detect novel resistances. Bioassays, however, are good for detecting novel resistances, despite being slow and expensive for routine resistance monitoring. Therefore, we envisage a synthesis of the two methods, whereby the molecular tests are augmented with periodic bioassays to ensure that new resistance genes do not escape detection.

Future Directions

The utility of our DNA tests will extend beyond the very practical identification of resistant insects in commercial settings. We will also use the tests to gather data on the distribution and frequency of individual resistance genes in the field. This information will be integrated into resistance monitoring programs and pest ecology projects that seek to identify the primary factors that select for resistance as a means of assessing the effectiveness of various resistance management strategies. Our goals in using DNA tests can also be stated as a series of questions that they will be used to answer, namely: Where, when and how many resistance genes or resistant individuals exist in a particular setting This information allows one to answer the question: Are resistance management strategies working Ultimately, we wish to answer: What are the factors that pose the greatest risk of generating or increasing phosphine resistance in insect populations on farms and in bulk storages Identifying the genes directly responsible for resistance is the first step in developing the tools to answer these questions.

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Molecular Cloning and Sequence Analysis of Four New cDNA Fragments of Cytochrome P450 from *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelididae)

Jiang Hongbo, Wang Jinjun*, Xu Yongqiang and An Fengming

Abstract: P450 enzymes are an important metabolic system involved in the metabolism of a phenomenal number of endogenous and exogenous compounds. In China, Liposcelis bostrychophila is a dominant species in stored products and the resistance to PH_3 has been a severe problem. In order to reveal whether P450 enzymes were involved in the resistance to PH_3 or not and the molecular mechanism of resistance, four different P450 cDNA fragments (243 bp) were cloned from the susceptible strain of L. bostrychophila, according to the RT – PCR strategy with a pair of degenerate primers designed on the basis of the heme-binding region of CYP6 family. The putative conserved domains of P450 were found in fragments 1, 2 and 4, by the Blast search in GenBank database. It was the first time that P450 genes had been cloned from L. bostrychophila, which verified the existence of P450 enzymes in L. bostrychophila. The homologous alignment by ClustalX (Ver. 1.81) indicated that the fragments 1, 2 and 4 shared higher identities of deduced amino acid sequences with certain known members of CYP6 family (42% – 67%, 42% – 56% and 41% – 66%, respectively), while fragment 3 showed lower identities (38% – 56%). And the result was further tested by the phylogeny analysis by MEGA (Ver. 3.1) utilizing the UPGMA (unweighted pair group method with arithmetic mean).

Key words: *L. bostrychophila*, P450, RT – PCR, homologous analysis

1 Introduction

P450 enzymes (mixed function oxidases, cytochrome P450 monooxygenases), one of the most important enzyme systems involved in insecticide detoxification or activation, are a complex family of heme containing enzymes found in most organisms. Various kinds of P450 enzymes have been found in animals, microorganisms and plants, and classified into more than 36 families [1,2]. P450 enzymes bind molecular oxygen and receive electrons from NADPH to introduce an oxygen atom to the substrate. In insects, the diverse functions of P450 enzymes range from the synthesis and degradation of ecdysteroids and juvenile hormones to the metabolism of xenobiotics [3,4]. P450 enzymes play important roles in adaptation of insects to toxic compounds in their host plants and are involved in metabolism of all commonly used insecticides. P450 enzymes metabolize organophosphorus insecticide compounds to more active toxicants by activation of a P = S bond to a P = O bond [4,5,6]. However, in general, P450 enzymes mediate metabolic detoxification of other insecticides.

So far, the nomenclature of P450 gene has been established to designate all gene members of the P450 super-family with a CYPprefix, followed by a numeral for the family, a letter for the subfamily, and a number for the individual gene [4,7]. This system defines that members of a family share > 40% identity in amino acid sequence, and members of a subfamily share > 55% identity [4].

Because of the property of instability, most researchers prefer molecular methods to biochemical methods in the insecticide resistance mechanism of P450 enzymes study. The first insect P450 gene (CYP6A1) was isolated from an insecticide-resistant strain of housefly, *Musca domestica* [8]. After that, more and more P450 genes were cloned. To date, more than 1958 P450 genes have been registered in the GenBank database. In China, the biochemical characteristics of P450 enzymes in insects were given more attention and the molecular cloning just started recently. Three new full-length cDNA were cloned from *Aedes albopictus* [9]. A xanthotoxin-inducible cytochrome P450 cDNA (CYP6B8) was isolated from *Helicoverpa zea* [10], and the full length of $CYP6BF_1$ was obtained from

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Plutella xylostella through the SMART (Switching Mechanism At 5' end of the RNA Transcript) technique [11]. Apart from these, a few cDNA fragments were also reported. Nine CYP4 fragments from *Culex pipiens* Pallens [12], two CYP6 [13] and ten CYP4 [14] fragments from a susceptible strain of *Helicoverpa armigera* were cloned successively. Besides, two new P450 cDNA fragments were gained from deltamethrin resistant strain of *Musca domestica* using differential display PCR technique [15].

Liposcelis bostrychophila, an important stored-product insect pest, is worldwide and commonly found in various processed and unprocessed dry foods in households, granaries, and warehouses [16]. Outbreaks of *L. bostrychophila* have been reported in humid tropical countries such as Indonesia, Malaysia, Singapore, the Philippines, Thailand, the People's Republic of China and India [17,18]. Routine fumigations of warehouses and storage facilities with methyl bromide have failed to control the pest [19]. In addition, the rapid development of resistance to physical and chemical treatments by the psocids has also been reported [20]. In Australia, detection of high level resistance to phosphine in psocids infesting stored commodities [21] has elevated their pest status enormously and put them alongside the major beetle pests [22]. The metabolic resistance mediated by P450 enzymes may be the most important resistance mechanism[3,6,23,24] while the study about the enzymes was lack in psocids. The objectives of this study were to clone the P450 genes and analyze their functions to supply some basic information for further study on the molecular resistance mechanism of psocids.

2 Materials and Methods

2.1 Insects

The susceptible strain of *L. bostrychophila* was cultured in the Key Laboratory of Entomology and Pest Management, Southwest University, Chongqing. The insects were reared on a diet consisting of whole wheat flour, skimmed milk and yeast powder (10∶1∶1) in an air-conditioned room at 27 ± 1℃, RH 75% – 80% and a scotoperiod of 24 h [25]. This strain was not exposed to blended gas or insecticides for 16 years.

2.2 Isolation of Total RNA

Total RNA was isolated from *L. bostrychophila* adults using TRNzol Reagent (Tiangen). One thousand healthy adults of *L. bostrychophila* were homogenized with at least 1 mL TRNzol Reagent in the glass homogenizer. The particular process of total RNA extraction and purification was carried out following the manufacturer's instructions of the reagent kit. Finally the total RNA (A_{260}/A_{280} = 1.8) dissolved in 40 μL DEPC treated H_2O for future use.

2.3 Synthesis of the First Strand cDNA

The first strand cDNA was synthesized by M – MLV RTase cDNA Synthesis Kit (Takara). The reverse transcriptional system included 2 μL total RNA, 1 μL Oligo (dT_{15}) and 8 μL DEPC treated H_2O at 70℃ for 5 min in water and for 10 min on ice. It was then mixed with 5 μL M – MLV 5 × Reaction Buffer, 1.25 μL dNTPs, 1 μL rRNasin and 4.75 μL DEPC treated H_2O at 42℃ for 5 min. Then 1 μL M – MLV reverse transcriptase was added to the mixture above. At last, the mixture was kept at 42℃ for 1 h, 95℃ for 5 min and hold at 4℃.

2.4 PCR Amplification

PCR cloning was carried out using the Takara PCR Amplification Kit (Takara). The 25 μL reproducing system contained 2 μL cDNA template, 2.5 μL 10 × Reaction Buffer, 4 μL 25 mM $MgCl_2$, 4 μL 2.5 mM dNTPs, 3 μL 10 mM forward or reverse primer, 0.5 μL 5 U/μL Taq DNAse and 6 μL DEPC treated H_2O. The system was kept at 94℃ for 2 min, then 30 cycles of polymerase chain reaction (94℃ for 1 min, 50℃ for 1 min, 72℃ for 1 min), 72℃ for 5 min, and was finally hold at 4℃. The degenerate primers used in PCR cloning were designed based on the homologous region of CYP6 family and their nucleotide sequences were as follows [26].

Forward: 5'–CGGARACNHYNMGNAARTAYCC–3'

Reverse: 5'–CGGGNCCNKCNCCRAANGG–3'

Where, H, K, M, N, R and Y are the IUB (International Union of Biochemistry) standard code for degenerate bases. H = A/C/T, K = G/T, M = A/C, N = A/C/G/T, R = A/G and Y = C/T.

The PCR products were purified and recovered by Gel Extraction Mini Kit (Watson Biotechnologies, inc) and ligated to pMD – 18T vector (Takara) at 16℃ overnight. The ligation solution was transformed to *Escherichia coli* competent cell HB101 (Takara). After that, the transformed competent cells were transferred onto LB medium containing Ampicilin, X-gal

and IPTG at 37℃ for 12 h. In succession, the bacteria colonies screened and the white colonies were selected to culture in the LB medium containing no Ampicilin at 37℃ for 12 h. Finally, PCR amplification was applied for plasmid DNA isolated from the culture to confirm the positive cloning that would be sequenced.

2.5 Sequence Analysis

The obtained sequences were translated into proteins. Searches for similar sequences were carried out by Blast Search of GenBank. The multiple alignments of deduced amino acid sequences with other members of CYP6 and CYP3 family were processed using ClustalX software (Ver. 1.81), meanwhile, the identity comparison was executed by Blastp in GenBank. The phylogenetic relationship was analyzed by MEGA(Ver. 3.1).

3 Results

3.1 The Agarose Gel Electrophoresis of RNA

The integrity of mRNA isolated from *L. bostrychophila* was showed indirectly by the agarose gel electrophoresis of rRNA(Fig. 1).

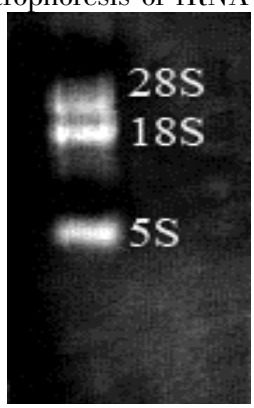

Fig.1 The agarose gel electrophoresis of total RNA

The three electrophoresis strips marked with 28S, 18S and 5S represented 28S rRNA, 18SrRNA, and 5S rRNA, respectively. The proportion of 28S and 18S seemed to be 1:1 in virtue of strips brightness and width.

3.2 The Agarose Gel Electrophoresis of PCR Products and Their Sequences

The PCR products amplified with a pair of degenerate primers were displayed with the agarose gel electrophoresis(Fig. 2). Four different cDNA fragments, all 243 bp in length, were obtained through the RT – PCR. The corresponding nucleotides sequences and the deduced amino acid sequences (underlined sequences were used for designing degenerate primers) were showed in Fig. 3. According to the Blast search in GenBank database, the putative conserved domains of P450 were found in fragments 1, 2 and 4 while no in fragment 3. Meanwhile, fragments 1, 2, and 4 shared higher identities(42% – 67%, 42% – 56% and 41% – 66%, respectively) than fragment 3 to other members of CYP6 family(38% – 56%), based on the identities comparison of the cloned sequences with corresponding part of other members of P450 gene(Table 1).

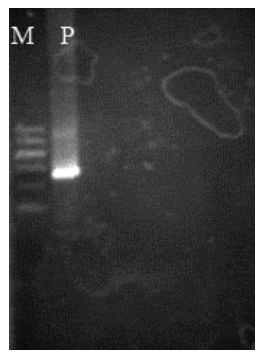

Fig.2 The agarose gel electrophoresis of the cDNA fragments from PCR cloning. Lane M represents molecular marker, lane P represents PCR product

Table 1. Identities comparison of the cloned sequences with corresponding part of other members of P450 gene

P450 members	Source	Identities of the deduced amino acid sequences(%)			
		Fragment 1	Fragment 2	Fragment 3	Fragment 4
CYP3A1	*Rattus norvegicus*	45	42	46	45
CYP6A2	*Tribolium castaneum*	67	56	46	66
CYP6B1	*Papilio polyxenes*	48	47	41	50
CYP6B2	*Helicoverpa armigera*	46	45	38	45
CYP6D1	*Musca domestica*	46	45	41	47
CYP6D2	*Drosophila melanogaster*	42	45	41	41
CYP6E1	*Culex pipiens quinquefasciatus*	48	48	50	50
CYP6G1	*Apis mellifera*	62	56	43	61

P450 members	Source	Identities of the deduced amino acid sequences(%)			
		Fragment 1	Fragment 2	Fragment 3	Fragment 4
CYP6J1	*Blattella germanica*	53	48	42	50
CYP6K1	*Apis mellifera*	62	56	43	61
CYP6P2	*Anopheles gambiae*	55	56	42	51
CYP6N3v3	*Aedes albopictus*	52	51	45	55
CYP6AY1	*Nilaparvata lugens*	53	50	56	56
CYP6Y1	*Anopheles gambiae*	46	43	43	43

3.3 Homology Analysis of the Sequences

The deduced amino acid sequences, 80 amino acids in length, were aligned by ClustalX (Ver. 1.81) with CYP6 family P450 genes from other insects and CYP3 family P450 genes from rats. By virtue of the alignment, it was concluded that the regions where the degenerate primers were designed were highly conserved, and those were also the conserved regions of CYP6 family (Fig. 4). The dendrogram of the cDNA frgments and certain members in P450 gene family (Fig. 5) was drawn by MEGA 3.1 utilizing the UPGMA. Fragments 1, 2, and 4 were found more closely related to CYP6A2 from *Tribolium castaneum*, while fragment 3 shared higher homology to CYP6AY1 from *Nilaparvata lugens*.

```
Fragment 1
        1 gaaactcttaggaagtacccagtggcaggagtaataattaggcaatgtaataaagactat
          E  T  L  R  K  Y  P  V  A  G  V  I  I  R  Q  C  N  K  D  Y
       61 aaaattccggatagcgatatggtgattcccaaaggaacttctacgcatataccgatttat
       21  K  I  P  D  S  D  M  V  I  P  K  G  T  S  T  H  I  P  I  Y
      121 tcccctcattacgattcaaagtattttccgaatcctgagaaatttgatccggatcgtttc
       41  S  P  H  Y  D  S  K  Y  F  P  N  P  E  K  F  D  P  D  R  F
      181 acagaagaagtcaaatctcaacgacctcgttatgcatatttacctttcggcgaaggaccc
       61  T  E  E  V  K  S  Q  R  P  R  Y  A  Y  L  P  F  G  E  G  P
      241 g

Fragment2
        1 gaaaccccaagcaaatatccagtagcgggattattgcttcgagaatgcaacaaagattat
          E  T  P  S  K  Y  P  V  A  G  L  L  L  R  E  C  N  K  D  Y
       61 aaagtcccaaatagcgacatggtaataccgaaagggacttcgatacaagtacccatttat
       21  K  V  P  N  S  D  M  V  I  P  K  G  T  S  I  Q  V  P  I  Y
      121 tccctccattatgatccaaaatatttccctgatccacaacgttttgatccggatcgtttc
       41  S  L  H  Y  D  P  K  Y  F  P  D  P  Q  R  F  D  P  D  R  F
      181 aaagaagaagtcaaatcccaacgacatcgttacgcttatttaccgttcggtgaaggcccc
       61  K  E  E  V  K  S  Q  R  H  R  Y  A  Y  L  P  F  G  E  G  P
      241 g

Fragment3
        1 gagacgcctagaaagtaccctcccctaccttttgttgttaagaaggtgtacaaaagcgtac
          E  T  P  R  K  Y  P  P  L  P  L  L  L  R  R  C  T  K  A  Y
       61 aagattccggattccgatgtaattttagaaccaggaaatctggttttttcccgacatat
       21  K  I  P  D  S  D  V  I  L  E  P  G  N  L  V  F  F  P  T  Y
      121 tcttatcaacgcgatccagaatattatccggatccggaggaattccgaccagaaagattt
       41  S  Y  Q  R  D  P  E  Y  Y  P  D  P  E  E  F  R  P  E  R  F
      181 tcaccggaaaatcgggaaaaactggtagctcatacgattttgccctcggcgacggaccc
       61  S  P  E  N  R  E  K  L  V  A  H  T  I  L  P  F  G  D  G  P
      241 g

Fragment4
        1 gaaacaactagaaaatatccacctgctggtgtagtaagcagagcatgctcaagagactat
          E  T  T  R  K  Y  P  P  A  G  V  V  S  R  A  C  S  R  D  Y
       61 caaatccctgatttctgatgtgaccattgaaaaaggtatacaagttataatacccgtgttt
       21  Q  I  P  D  S  D  V  T  I  E  K  G  I  Q  V  I  I  P  V  F
      121 ggacttcatcatgatgaaaaatattttccaaatccggaaaagttcgatcctgatcgattt
       41  G  L  H  H  D  E  K  Y  F  P  N  P  E  K  F  D  P  D  R  F
      181 acggaagagggaaaagcctcccggccaaattatacgtacttgccgttcggagctggaccc
       61  T  E  E  G  K  A  S  R  P  N  Y  T  Y  L  P  F  G  A  G  P
      241 g
```

Fig. 3 The nucleotides and deduced amino acid sequences of four cDNA fragments (the letters underlined are the nucleotides sequence of the primers)

4 Discussion

Gene cloning by PCR using degenerate primers derived from conserved amino acid sequences from other species has been proved to be a powerful method to obtain related DNA sequences from the target species. Although the P450 super-family has a very divergent sequence and the overall homology may be less than 40% even within the same family, particularly in insects (Wang and Hobbs, 1995), there are still some conserved regions preserved during evolution. And the degenerate primers used in the experiment were designed based on the conserved region of CYP6 family (heme – binding region).

Though the primers applied were degenerate, the agarose gel electrophoresis of the PCR products showed good specificity. Four different cDNA fragments, similar to previous reports in length (Ai et al., 2004a; Li et al., 2005), were obtained after eight.

```
Fragment1       ETLRKYPVAGVIIRQCNKDYKIPDSDMVIPKGTSTHIPIYSPHYDSKYFPNPEKFDPDRF
Fragment2       ETPSKYPVAGLLLRECNKDYKVPNSDMVIPKGTSIQVPIYSLHYDPKYFPDPQRFDPDRF
Fragment4       ETTRKYPPAGVVSRACSRDYQIPDSDVTIEKGIQVIIPVFGLHHDEKYFPNPEKFDPDRF
CYP6A2          ETLRKYPAASIITRTCVKDYKIPDQDIVIEKGTSVIIPVLGIHHDEKFYPNPEKFDPERF
CYP6G1          ETLRKYPPLGFLDRVALHDYKIPNSDVTIDKDTPVIIPMIAFHYDPKYFPNPEKYDPLRF
CYP6K1          ETLRKYPPLGFLDRVALHDYKIPNSDVTIDKDTPVIIPMIAFHYDPKYFPNPEKYDPLRF
CYP6B1          ETLRKYPVADFTQRNAKTDYVFPGTDITIKKGQTIIVSTWGIQNDPKYYPNPEKFDPERF
CYP6B2          ETLRMYSIVEPLQRKAIRDYKLPGTDVVIEKDTVVLISPRGIHYDPKYYDNPKQFNPERF
CYP6E1          ETLRKYPPVANLFREITKNYKVPETDITLEKGYRVVIPVYGIHHDPDIYPNPEVFNPERF
CYP6N3v3        ESLRKYPPASNLTRIVSKDYKLPNCDVVLQQGSTIIVPVYALHHDAEYYPNPEKYDPDRF
Fragment3       ETPRKYPPLPLLLRRCTKAYKIPDSDVILEPGNLVFFPTYSYQRDPEYYPDPEEFRPERF
CYP6AY1         ETMRKYPPVSVLARVCTKPYTIPGTKISIDPGTSVAIPVYSFHHDHKYFPDPETFDPERF
CYP3A1          ETLRLYPIGNRLERVCKKDVEI--NGVFMPKGSVVMIPSYALHRDPQHWPEPEEFRPERF
CYP6Y1          ETLRKHPPVAILERNADKDYRLPDSGLLLRRGQKIMIPIYAMHHDPAHFPEPEQYRPERF
CYP6P2          ETLRKYPPLETVTRAPEHDYTVPGTAHVIPKGTMIQIPIYALHHDAQYYPDPERFDPERF
CYP6D1          ETTRKYPGLPFLNRKCTQDFQVPDTKLTIPKETGIIISLLGIHRDPQYFPQPEDYRPERF
CYP6D2          ETIRKYPGLPFLNRECTEDYPVPGTNHIIAKGTPILISLFGMQRDPVYFPNPNGYDPHRF
CYP6J1          ETLRKYPAIPFLDRRCQEDYPL-TQDLMLPAGTGVYIPVYALHHDSKYFPSPAKFDPERF
                *:  :.     *      .     :      ..  . : *  : .*  : * **

Fragment1       TEE-VKSQRPRYAYLPFGEGP
Fragment2       KEE-VKSQRHRYAYLPFGEGP
Fragment4       TEE-GKASRPNYTYLPFGAGP
CYP6A2          TEE-NKAARHHYAHLPFGEGP
CYP6G1          SEE-VKKTRPSYVYMPFGEGP
CYP6K1          SEE-VKKTRPSYVYMPFGEGP
CYP6B1          NPE-NVKDRHPCAYLPFSAGP
CYP6B2          FAE-EVGKRHPCAYLPFGLGP
CYP6E1          IPE-LSTNRHPMAYLPFGEGP
CYP6N3v3        TSE-EVAKRNPYCFLPFGEGP
Fragment3       SPE-NREKLVAHTILPFGDGP
CYP6AY1         SPE-NQEKSINYTYLPFGAGP
CYP3A1          SKE-NKGSIDPYVYLPFGNGP
CYP6Y1          SPD-EVARRDPYCYLPFGEGP
CYP6P2          RPE-VANARPAYVYMPFGEGP
CYP6D1          ADE-SKDYDPA-AYMPFGEGP
```

Fig. 4 Homologous analysis of cloned P450 amino acid sequences from *L. bostrychophila* susceptible strain and corresponding sequences of other sources of P450 members

" – " indicates the gaps, " * " indicates the completely conserved residue, " : " indicates one of the " strong " groups (STA, NEQK, NHQK, NDEQ, QHRK, MILV, MILF, HY, FYW) is fully conserved, ". " indicates one of the "weaker" groups (CSA, ATV, SAG, STNK, STPA, SGND, SNDEQK, NDEQHK, NEQHRK, FVLIM, HFY) in amino acids is fully conserved[27].

White plaques (positive cloning) were sequenced, suggesting that more than one gene were cloned. It verified the diversity of P450 genes [6].

The degenerate primers were derived from the conserved region (heme-binding region) of CYP6 family [26]. The putative conserved domains were found in fragments 1, 2, and 4 through the Blast search while no in fragment 3, and it might suggest that fragment 3 was not conserved in the putative conserved domains of CYP6 family or fragment 3 may be a pseudogene evolved from a gene of CYP6 family [29,30].

It is known that the identity comparison is different from homologous analysis. Thus, two methods were used in the process of homologous analysis. That is to say, both sequences identity and phylogeny were analyzed here. And the analysis revealed that the results of the two methods were approximately identical. According to the homologous analysis, the four fragments were probably assumed to be new members of CYP6 family, suggesting P450 genes were cloned from *L. bostrychophila* for the first time. Fragments 1, 2, and 4 shared higher identity to CYP6A2 from *T. castaneum*. The identities amounted to 67%, 52% and 66%, respectively, while an identity of 56% was found between fragment 3 and CYP6AY1 from *N. lugens*. These probably suggested that fragments 1, 2, and 4 belonged to CYP6A subfamily and fragment 3 might be classified to CYP6AY subfamily.

Most of the deduced amino acid sequences were about five hundred residues long, and the four fragments obtained from *L. bostrychophila* just accounted for one eighth of the P450 gene full length. Consequently, the nomenclature and classification of these P450 genes could not be carried out until the gene full lengths were obtained and identified.

Whatever, it was the first time that the P450 genes were cloned from *L. bostrychophila*. Undoubtedly, the fragment cloning was the fist step for the acquisition of gene full length and the results will contribute to the thorough understanding of P450 genes soon and molecular resistance mechanism mediated by P450 enzymes in further study.

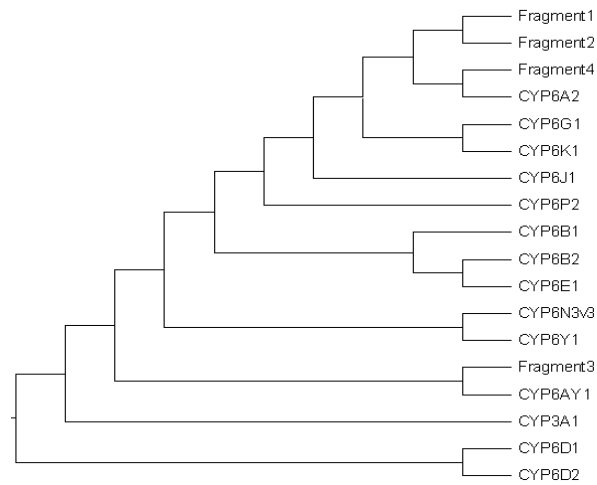

Fig. 5 Dendrogram of certain members in P450 gene family

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0803

Resistance and Genetic Differentiation of *Rhyzopertha dominica* to Phosphine among Different Geographical Populations in China: a Preliminary Study

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Abstract: In this article, the mortality effect of the most world's used fumigant, phosphine, was studied on one of the most important species of stored-product insect, the lesser grain borer *Rhyzopertha dominica* (Fabricius). In parallel, genetic differentiation among various resistant populations of *R. dominica* was investigated using amplified fragment length polymorphism (AFLP). The LC_{50} values from 6 different geographical populations, showed values between 0.05 mg/L and 3.25 mg/L. The populations Banan, Chengdu, Shayang, Zhucheng, Yangchun, Xuchang were, respectively, the most sensitive to the most resistant. In addition, AFLP results using *Eco*R I/*Hpa* II and *Eco*R I/*Msp* I primers, separated populations into a phylogenetic tree. The dendogram obtained with the primers *Eco*R I/*Msp* I could be easily explained by a geographical hypothesis but the pattern obtained with *Eco*R I/*Hpa* II primers could not. Two populations (Yangchun and Zhucheng) with distant geographical origins but with a high resistance to phosphine were clustered together. The influence of the phosphine treatment, and therefore, resistance of *R. dominica* to this insecticide, on the genes is one hypothesis resulting from this preliminary work.

Key words: *Rhyzopertha dominica*, geographical populations, phosphine, resistance, AFLP, genetic diversity

Introduction

The lesser grain borer, *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae). *R. dominica.* is a harmful grain pest originating from tropical areas but actually distributed throughout the world. Both larvae and adults cause serious damage to grain such as rice, maize, wheat, paddy. It is described as the most important pest of stored wheat in Brazil [11] and it attacks the storage of paddy rice too [13]. Several resistant strains of *R. dominica* have been described [10].

The main method used for controlling stored-product insects world-wide, including China, is the fumigation with phosphine gas [1]. It is commonly used against all species of stored-product insects including *R. dominica.* Resistance of stored-grain insects to phosphine was detected in 33 of 82 countries surveyed[3] and stored product insects are described as resistant to the compound in more than 45 countries[1,4]. Resistance to phosphine has been reported occurringin several economically important species, including*Sitophilus oryzae* (F.), *Sitophilus zeamais* (Motschulsky), *Tribolium castaneum* (Herbst) and *R. dominica*[3,9,11].

Several studies have dealt with the problem of the resistance to insecticides, particularly to the phosphine. But few of them studied the genetic resistance[5,16]. In these articles, authors observed the involvement of resistance genes, and also their inheritance. Contrary to other disciplines where genetic resistance and population genetics are strongly described, no data exist on a possible relationship between resistance evolution and genetic polymorphism in stored-product species. Amplified fragment length polymorphism (AFLP) is a technique which could help to understand this resistance [17]. AFLP is used to analyze genetic diversity, to identify biotypes, to construct linkage genetic maps, to study population genetics, gene location and genetic polymorphism[12,8,2,7,14].

The objective of this research was to test the hypothesis of genetic variation among six populationsof *R. dominica* populations in China. Using both classical measures of mortality

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and AFLP, we aimed to described a possible genetic differentiation among populations with different levels of resistance.

Material and Methods

Insects and Rearing Conditions

Populations of *R. dominica* were collected between 14 May and 4 September, 2007 in six cities of China: Banan (Chongqing province), Chengdu (Sichuan), Shayang (Hubei), Yangchun (Guangdong), Zhucheng (Shandong) and Xuchang (Henan).

Xuchang, Shayang, Zhucheng and Yangchun populations were collected from central storages. Banan population came from a rice mill And the Chengdu population was from Chengdu Grain Storage Research Institute. Populations were then reared in our laboratory with cracked wheat at a moisture content of 13 1%. Rearing temperature was maintained at 30 ± 1℃ and relative humidity was 75 5%. Adult *R. dominica* 14 days old were used in phosphine resistance assays.

Phosphine Bioassays

Phosphine was obtained by a reaction of zinc phosphide (Jining City Yimin Chemical Plant. Concentration) and sulfuric acid. The Lethal Concentration 50 (LC_{50}) values of *R. dominica* to the fumigant phosphine were detected using an FAO method. After 20h treatment, each sample of tested insects was transferred into glass tubes with creaked wheat. Mortality was recorded 2 weeks later after the transfer of adult insects. Each treatment was undertaken three times.

DNA extraction

We adopted phenol-chloroform extraction method and tested OD 260/280 from 1.6 – 1.9[18]. Seven adults of *R. dominica* were suitable for DNA extraction.

Amplified Fragment Length Polymorphism (AFLP)

DNA was digested with *Hpa* II, *Msp* I and *Eco*R I restriction endonucleases (MBI Fermentas, EU). Then, for the ligation, used adapters were prepared by the mixing of two adapters (MBI Fermentas, EU): *Hpa* II/*Msp* I adapter was prepared by mixing 5' – GACGATGAGTC TAGAA – 3' and 5' – CGTTCTAGACTCATC – 3' adapters and *Eco*R I was prepared by mixing 5' – CTCGTAGACTGCGTACC – 3' and 5' – AATTGGTACGCAGTC – 3' adapters. DNA fragments were ligated to *Hpa* II/*Msp* I and *Eco*R I.

Pre-amplification was performed with preselective primers *Hpa* II/*Msp* I (5' – GATGAGTCTAGAACGGT – 3') and *EcoR* I(5' – GTAGACTGCGTACCAATTCA – 3') (Shanghai Generay Biotech Co, China). Pre-amplification was performed with 0.1 μL of each *Eco*R I and *Hpa* II/*Msp* I preselective primers (100μM), 0.5 μL dNTPs (10 mM), 2 μL Mg^{2+} (20 mM), 2.5 μL 10X PCR buffer (500 mM KCl; 100 mM, Tris – HCl; pH 8.3; 15 mM $MgCl_2$) and 0.2 μL Taq DNA polymerase (5U/μL) and 5μL of ligation solution in a total volume of 25 μL. The PCR reaction was performed at 94℃ for 3 min, following by 20 cycles of 94℃ for 30 s, 56℃ for 1 min, 72℃ for 1 min, and a final elongation of 72℃ for 5min.

Selective amplification used two selective primers *Hpa* II/*Msp* I(5' – GATGAGTCTAGA ACGGT – 3') and *Eco*RI + ATA (5' – GTAGACTGCGTACCAATTCATA – 3'), each containing three selective nucleotides. Selective amplification was performed with 0.05 μL of each selective primers (100μM), 0.3 μL dNTPs (10mM), 1.2 μL Mg^{2+} (20mM), 1.5μL 10X PCR buffer (500 mM KCl; 100 mM, Tris – HCl; pH 8.3; 15 mM $MgCl_2$), 0.2μL Taq DNA polymerase and 3 μL of preamplification solution in a volume of 15μL. The PCR reaction was performed with a first denaturation cyle at 94℃ for 3 min, following by 13 cycles (94℃ for 30 s, 56℃ for 1 min, and 72℃ for 1 min) during which the annealing temperature was decremented 0.7℃ each cycle. The PCR continued with 23 cycles (94℃ for 30 s, 56℃ for 1min, and 72℃ for 1 min) following by a final elongation of 72℃ for 5 min.

Amplifications were carried out in PTC – 200 DNA machine (MJ, USA). The reaction products were separated on denaturing 6% polyacylamide gels adopting at 70W power with a DYCZ – 20C electrophoresis machine (Beijing Liuyi Instrument Factory, Beijing, China).

Data Analysis

Linear regressions of mortality and LC_{50} values were obtained with SPSS 10.0 (Statistical Package for Social Science, Chicago, USA). AFLP results were analyzed by a cluster analysis. Each strain was scored 1 for the presence or 0 for the absence of each band. A dendrogram was obtained using the Unweighted Pair – Group Method using the Arithmetic Averages (UPGMA) with NTSYSpc version 2.10p (Applied Biostatistics, Inc., New – York, USA).

Results

Mortality of *R. dominica*

The six populations of *Rhyzopertha dominica* showed a range of sensitivities to phosphine. Mortality values, expressed as LC_{50}, fluctuated between 0.05 mg/L and 3.30 mg/L (Table 1). The highest resistance factor was almost 66. Populations could be ordered by sensitivity to the phosphine in 3 groups. The most sensitivewere the populations from Banan and Chengdu presenting almost the same low LC_{50}, almost 0.05 mg/L. Then one population, from Shayang, had an intermediate mortality value with a 0.59 mg/L LC_{50}. Then, the three populations from Yangchun, Xuchang and Zhucheng had the highest LC_{50} values (3.19 – 3.29 mg/L).

Table 1. Analysis of mortality of *Rhyzopertha dominica* populations to phosphine. Linear regression was performed for each population. The bioassays were performed in triplicate.

Origins	Regression of the linear regression of mortality	LC_{50}/mg/L	95% Confident limit
Banan	Y = 2.27 X + 4.61	0.0495	0.207 – 16.7
Chengdu	Y = 2.24 X + 2.91	0.0498	0.00271 – 0.184
Shayang	Y = 2.73 X + 0.629	0.585	0.0167 – 2.03
Yangchun	Y = 2.52 X + 0.379	3.19	1.447 – 107.0
Xuchang	Y = 5.84 X – 2.99	3.25	0.633 – 4.90
Zhucheng	Y = 4.85 X – 2.51	3.29	0.0735 – 4.08

AFLP Analysis

The tree obtained with both *Msp* I and *Hpa* II bands clustered populations into two groups (Fig. 1). The first group included the Banan and Chengdu populations. The second group was itself separated in two groups of two populations: on the one hand Shayang and Xuchang populations, on the other hand, Yangchun and Zhucheng populations.

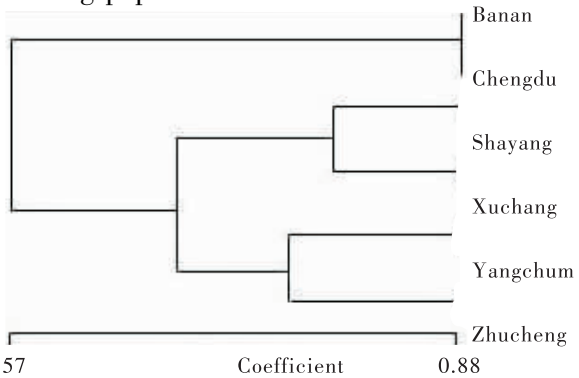

Fig. 1 Dendrogram of *Rhyzopertha dominica* populations based on amplified fragment length polymorphism (AFLP) analysis using the number of bands, of *Msp* I and *Hpa* II, coding by absence/presence. Analysis of bands, performed in duplicate, was based on the analysis of 50 bands for *Eco*R I/*Hpa* II and 52 bands *Eco*R I/*Msp* I.

The dendogram obtained with *Msp* I bands alone showed similar relationships, although the one obtained with *Hpa* II bands showed a different pattern (Fig. 2). The tree obtained with Hpa II, like the other two, divided *R. dominica* populations into two major clusters. The major difference was in the subdivision of the second cluster, the first cluster included Banan and Chengdu populations. For the four other populations, Zhucheng population was nearest with Shayang and Xuchang populations. Yangshun population was by itself.

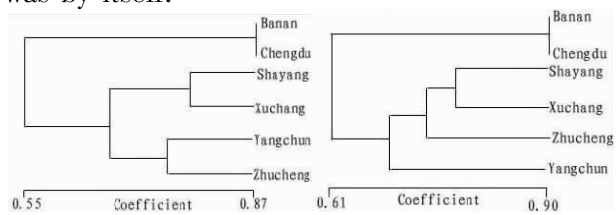

Fig. 2 Dendrogram of *Rhyzopertha dominica* populations based on amplified fragment length polymorphism (AFLP) analysis with bands *Hpa* II (on the left) and bands of *Msp* I (on the right). Each band analysis, performed in duplicate, was coding by absence/presence. Analysis is based on the analysis of 50 bands for *Eco*R I/*Hpa* II and 52 bands *Eco*R I/*Msp* I.

Discussion

The dendograms obtained from analysis of *Hpa* II/*Eco*R I and *Msp* I/*Eco*R I bands, showed three final clusters: Benan – Chengdu, Shayang – Xuchang and Yangchun – Zhucheng. We propose a geographical explanation for this pattern. Indeed, Chengdu and Banan (the first cluster) are cities close together. Similarly, the cluster Shayang – Xuchang could be explained by the proximity of these two cities. However, a geographical hypothesis cannot be used to explain the cluster Yangchun – Zhucheng, as Yangchun is in the south of China and Zhucheng on the East coast and closer to

Xuchang or Shayang. In contrast, the pattern obtained with *Msp* II primers can be explained with a geographical hypothesis alone. However, the results obtained with both primers or only with *Hpa* I cannot be explained by geography alone. Looking at both dendograms and the mortality data, two points need to be highlighted: the differences in mortality values between the two close (genetically and geographically) populations, Shayang and Xuchang, and the relatedness of the Yangchun and Zhucheng populations in spite of their geographical remoteness. The influence of phosphine treatment and/or the resistance of *R. dominica* to this insecticide, on the *Eco*R I/*Hpa* I restriction sites is one hypothesis that could explain the relatedness of Yangchun/Zhucheng .

These two particular last points are encouraging although we didn't reach our major objective. Indeed, none of the studied bands showed specifically a region involved in phosphine resistance. Several explanations could explain this failure. Compared with classical samples in AFLP studies (e. g. 38 *Aedes aegypti* samples from Mexico[14] or 133 pollen beetles samples from Sweden[6]), there was a lack of the diversity in the sample. Concerning the method, while in this study only two pairs of primers were used without combination, four primer combinations were used on pollen beetles [6] and three on *Aedes aegypti* individuals[14].

However, we managed to obtain some interesting results with this preliminary experiment using AFLP method on a species of stored-product insects,. Knowing that both molecular and classical studies demonstrated that resistance can occur by a genetic change at a single locus[15], we are currently developing the method to study more populations within China. The final objective is to study genes involved in resistance in *R. dominica* populations internationally. In the same way, the influence of generation time, the role of spatial factors and insecticide treatment on population differentiation[6] can be studied. Moreover, we are currently completing this phylogeographic study with experiments based on mtDNA sequence analysis from cytochrome oxidase I and II.

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0804

How to Effectively Control Phosphine-resistance Development in Stored Grain Insects by Integrated Pest Management

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Abstract: The paper elucidated how to effectively control phosphine-resistance development in stored grain insects by integrated pest management. This is achieved by detecting phosphine-resistance in major stored grain insects in Fujian province, comparing with investigation results of phosphine-resistance in stored grain insects in the same ecological area in recent years, and taking into account insect pest control experience of grass-root grain storage units in vertical administration system of State Grain Reserves. This offered some valuable information for the development of integrated pest management in grass-root grain storage units.

Key words: stored grain insects, phosphine, resistance, integrated pest management

1 Introduction

The insect resistance to insecticide inevitably results from the continuous use of pesticides whose selective action might induce gene mutation, bringing about insect resistance and pesticides gradually become ineffective. As a major fumigant, PH_3 has been extensively used worldwide for over 60 years, and its excellent fumigation effect has been absolutely affirmed. However, many stored grain insects have produced serious resistance due to the objective factors, and its unscientific and irrational use during its long application period. According to the investigation of grain storage workers in China, the major stored grain insects, such as *Sitophilus zeamais* Motschulsky, *Rhyzopertha dominica* (Fabricius), *Tribolium castaneum* (Herbst) and *Cryptolestes ferrugineus* (Stephens), etc., have produced strong resistance to phosphine, furthermore, increasing with time, in southwest China, central China and south China where stored grain insects severely occur. In the 1980s, PH_3 resistance coefficient of an *R. dominica* strain in Guangdong province unexpectedly reached 1160 times. The enhanced PH_3 resistance led to incomplete fumigation effect, and even no effect. About 50% stored grain requires two PH_3 fumigations, which is very common in high temperature and humidity areas in China. A global investigation from 1972 to 1973 showed that about 10% tested insects collected from 40% countries produced PH_3 resistance, which has been found in Australia, Bangladesh, U. S. A., India, east Asia, Africa and America. Therefore, the increasing insect resistance has become a global issue, augmenting the control cost and threatening the continuous PH_3 use.

In order to understand the major grain insects phosphine resistance in Fujian, supervise and offer valuable information for the future stored grain insects control, the stored grain insects resistance degree to phosphine in Fujian province was detected.

2 Materials and Methods

2.1 Insects and Their Incidental Grain

Test insects were collected from deputy depots and depots in Fujian branch company, directly under China Grain Reserves Corporation (in the remainder of this paper referred to as Fujian branch company). Several stored grain insects, which are difficult to control in common management, were selected based on practical experience of grass – root grain storage units. Three species: *Cryptolestes ferrugineus* (Stephens) *Sitophilus zeamais* Motschulsky, and *Rhyzopertha dominica* (Fabricius) were selected, including 6 samples (Table 1). The grain collected with the insects has been stored for 2 – 4 years in Fujian. The grains were produced in both local field and other provinces of China and from foreign field. The tested insect have propagated for several generations, and basically adapted to the stored grain environment in Fujian province.

1. *Quanzhou Depot State Grain Reserves*, *Quanzhou* 362113)
2. *Fujian Branch of China Grain Reserves Corporation*, *Fuzhou* 350001)
3. *Putian Depot State Grain Reserves*, *Putian* 351158)
4. *Fuzhou Depot State Grain Reserves*, *Fuzhou* 350101)

Table 1. The basic information of the tested insects

No.	Insect	Collecting place	Incidental grain kind	Grain producing time	Grain producing place
1	*C. ferrugineus*	Xiamen grain purchasing and storage company	Wheat	2004	Henan
2	*C. ferrugineus*	Pucheng county grain trade company	late indica rice	2006	Fujian
3	*R. dominica*	Quanzhou Depot Directly Under Central Grain Reserves	late indica rice	2004	Anhui
4	*R. dominica*	Pucheng county grain trade company	late indica rice	2004	Fujian
5	*S. zeamais*	Putian Depot Directly Under Central Grain Reserves	Wheat	2004	Import
6	*S. zeamais*	Putian Depot Directly Under Central Grain Reserves	Corn	2005	Liaoning

2.2 Measurement of the Resistance to Phosphine

The standard FAO recommended method[7] was used to measure resistance to phosphine. Phosphine was prepared from reaction of zinc phosphide and 10% H_2SO_4 solution, and the insects were exposed for 20 h and 72 h.

The method to measure knockdown resistance[4]: 10 one-week adults were put into a fumigation flask with a known volume. Phosphine was injected into the flask corresponding to dosage of 2.0 mg/L, then the response of tested insects was observed. The adult was confirmed as "knocked down" by phosphine when it exhibited convulsion symptoms. The adult knockdown time was recorded. Each strain was tested 3 times.

The results of exposing for 20h and 72h were analysed by Probit analysis, and the results were analysed by DPS software.

3 Results

3.1 Measurement of the Phosphine Resistance

The results of the phosphine resistance measurement are provided in Table 2, which showed that the phosphine resistance of *C. ferrugineus* in Fujian province significantly increased as compared with historical resistance data in the same ecological area. In particular, the tested insects collected from Xiamen grain purchasing and storage company reached 449 times, which increased 2.8 times compared to 160 times of *C. ferrugineus* in Jianyang, Fujian province detected by Yan Xiao-ping in 2004[3]. The results accorded with the great difficulty of *C. ferrugineus* control in grass – root grain storage units at the present time. The phosphine resistance level of *R. dominica* and *S. zeamais* was slight low, exhibiting susceptible or moderate, which basically equaled with the historical record

Table 2. The results of the phosphine resistance of stored grain insects in Fujian branch company

No.	Insect	Regressione quation	LD 50.00	Range value	Resistance coefficient	Grain producing place
1	*C. ferrugineus*	Y = 2.04X + 3.58	4.94	4.16 – 6.12	449.14	Henan
2	*C. ferrugineus*	Y = 2.31X + 3.64	3.86	2.90 – 17.48	350.51	Fujian
3	*R. dominica*	Y = 0.70 X + 5.95	0.043	0.0007 – 0.12	5.39	Anhui
4	*R. dominica*	Y = 0.52X + 5.30	0.26	0.016 – 0.50	32.64	Fujian
5	*S. zeamais*	Y = 0.65X + 6.55	0.004	0.000 – 0.014	0.6	Import
6	*S. zeamais*	Y = 0.95X + 5.84	0.12983	0.00004 – 0.39	18.55	Liaoning

The phosphine resistance of the tested insect species were different. *C. ferrugineus* was the most resistant among stored grain insects in Fujian, and its resistance coefficient reached 449 times, the phosphine resistance of *R. dominica* and *S. zeamais* was comparatively low. As far as the origin of the gain was concerned, the phosphine resistance level of the stored grain

insects in other provincial and imported grain was lower than that from local grain. For the incidental grain kind, the phosphine resistance level of the stored grain insects in paddy rice was stronger than that in corn, wheat and other grains. For the management condition, the phosphine resistance level of the stored grain insects in depots directly under Central Grain Reserves was lower than that in local deputy depots.

3.2 The Reasons about Origin of the Phosphine Resistance

3.2.1 Geographical environment factor Since the climatic condition of high temperature and humidity offered favorable environment for occurrence and reproduction of the stored grain insects, in addition to long-term pesticide selection, the phosphine resistance level of a portion of insects in Fujian was higher that in other provinces. Therefore, the phosphine resistance level of the stored grain insects in grain from other provinces was lower than that in local grain. Furthermore, because the *R. dominica*, *S. zeamais* and other insect species diffused into Fujian in recent years, their adaptability to climatic environment were slight weak, and their resistance level was instead lower than that of the same ecological area, and easy to control.

3.2.2 Storage equipment factor. The storage equipment of local deputy storage corporations was very old, and its gas-tightness condition was poor. The lack of gas-tight structures resulted in a great deal of fumigant leaking out before evenly distributing deep within the grain mass after fumigation application, unevenly distributing among grain mass, or not keeping effective fumigant gas concentration for enough time, and so on, then incomplete fumigation effect against insects among every portion of the grain mass.

Sealing of the doors and windows of the warehouse before the fumigation did not satisfy the requirements for an effective fumigation.

3.2.3 Human factor During the past, insect pest control practices, the human factors, such as insect pest control conception and incorrect fumigation manipulation, etc., occasionally resulted in the fumigation failure, accidentally enhancing the phosphine resistance.

(1) Unreasonable fumigationSome grassroot grain storage units immediately implemented fumigation once insects appeared, even if grain temperature was less than 15℃. Under these conditions, the insects reproduce very slowly, fumigant could not adequately diffuse among the grain mass, and the fumigation effect is very poor at too low temperature.

(2) Unreasonably reduce dosage Some people think that low phosphine concentration is in favor of enhancing fumigation effect, meanwhile, keeping low phosphine concentration in grain mass for a long period could kill the existing insects and prevent insects occurring. However, they ignored that only appropriate high phosphine concentration could kill some resistant insects. Because of low dosage and poor gas-tightness, it is difficult to get the effective lethal concentration. Even if the effective lethal concentration is obtained, the CT value is too low, resulting in not killing the insects, instead inducing insect resistance.

(3) Unreasonably increase dosage Some people mistakenly thought that application dosage was too low when they could not obtain the expected fumigation effect using preventative fumigation method and dosage at some places. In order to kill the insects, they unreasonably increased dosage next time, resulting in hidden insects receiving over-high phosphine dosage or concentration. The insect could revive after implementing fumigation for a period of time, and not be 100% killed.

(4) Fumigation seal time was not enough In early phosphine application instruction, some made suggestions to seal for 3 to 5 days, later, seal for 7 to 10 days after fumigant application. The seal time in these suggestions is far from enough at present concept. Many factors, such as grain mass temperature, application dosage, insect pesticide tolerance, etc., can impact phosphine fumigation seal time required. Consequently, the fumigation seal time cannot regulate in one criterion.

(5) Lack of concentration detecting measures. A lot of phosphine fumigation were implemented according to fumigant dosage. The grain quantity, dosage and gross application amount were taken into account during the fumigation. Owing to lack of instruments to measure gas concentration, a series of issues, such as if the effective fumigation concentration quickly reached in the warehouse or grain mass after application, if there were existing fumigation dead zone or local low concentration, and if the effective fumigation concentration could be kept for enough time, etc., might inevitably bring unreasonable fumigation.

(6) It is very common that many depots directly implemented fumigation without investigating stored grain insects stage and resistance before fumigation. This only killed adults and

susceptible strains, then the other stage insects and resistant individuals survived, and the more resistant insects soon again occurred after fumigation.

(7) The single use of aluminium phosphide as a fumigant for a long time has induced insect resistance. In addition, most depots did not consider the effect of micro-airflow in grain mass and applied the fumigant at same place. This also is one reason responsible for insect resistance.

After the establishment of vertical administration system of State Grain Reserves in 2000, the insect control concept has changed. The human factors noted above have basically disappeared during the course of insect control in depots directly under Central Grain Reserves now. This is one reason for the phosphine resistance level of the stored grain insects in depots directly under Central Grain Reserves lower that in local deputy depots.

4 Discussion

4.1 Coincident Degree Between Resistanceand Practical Management Difficulty

C. ferrugineus was the most resistant in view of the results, and the phosphine resistance of *R. dominica* and *S. zeamais* was not severe. The phosphine resistance of *R. dominica* and *S. zeamais* from other depots was comparatively weak except Shaowu depot with resistance coefficient 32.64 times of *R. dominica*. There is a huge difference from other provincial detecting results, but it is consistent with daily storage management practice in Fujian.

In the early of 1980s, *R. dominica* was seldom found in Fujian. At that time Fujian Province was self-sufficient for grain. After then, with the adjustment of agriculture plant structure, more and more grain was transferred into Fujian, along with *R. dominica*. Although the warehouse conditions were very poor, mortality of *R. dominica* was very high after fumigation, the common application dosage basically killed all of the insects. However, since the late 1980s, many depots have reported that *C. ferrugineus* could survive the fumigation. Because of poor warehouse condition and technique at that time, the issues could be resolved only by increasing the dosage, but it was not completely resolved. Because repeat fumigation screened *C. ferrugineus* resistance, the resistant strain continuously reproduced, and the resistance increased stage by stage.

The major stored grain insect resistance in Fujian is basically equal with national average level in China. But it also appeared some local characteristics, for instance, *R. dominica* showed low resistance, or even no resistance in Fujian, however prominent resistance in other provinces, particularly, Guangdong, Anhui, etc., locating southern high temperature and humidity areas. The phenomenon is very hard to understand. In our opinion, most paddy rice in Fujian was bought from Anhui and Jiangxi, and after the *R. dominica* came into Fujian along with the grain, most stored grain temperature due to low and quasi-low temperature grain storage implemented by Fujian branch company, Central Grain Reserves, was too low to be suitable to *R. dominica* development.

4.2 About Fumigation Management of Stored Grain Insects

Insecticide resistance results from continuous selection with insecticides. Insecticide kinds used in stored grain insects control are very limited, and fumigant kinds are even rarer. Therefore, insect resistance is unavoidable. However, as long as deeply understanding about insect resistance, taking scientific and rational control measures, the development of insect resistance can be delayed. The following major measures are summarized based on recent several years practical experience.

4.2.1 Execute integrated control Carefully execute the control principle " prevent first, integrated control" , seriously improve storage condition, enhance the quality of stored grain, reinforce daily management, and prevent insect infestation. Timely, harmonious using various methods, to the best avoiding apply insecticides or not using insecticide, is the precondition of prevent insect resistance development.

Firstly, strictly check on the grain quality before storage, intentionally reduce the kinds and quantity of stored grain insects during the grain entering warehouses, prevent the outer insect entering warehouses, concentrate several extensively distributed important insects into some portion avoiding to diffuse into other warehouses with no or a few insects. Meanwhile, various manual or mechanical equipments can be used to clean out grain impurity and outer insects.

Secondly, change the insect reproduction condition. Try to deteriorate the living environment condition of stored grain insects, so as to prevent and restrain stored grain insects happening. During the grain entering warehouses, try to decrease grain impurity and moisture,

some depots with good storage equipments can implement low and quasi-low temperature grain storage.

Cleanliness and sanitation control. Thoroughly and frequently maintain cleanliness and sanitation inside and outside the warehouses, carefully disinfect empty warehouses before putting the grain into the warehouses, and then apply defending line avoiding insects diffusing.

Strengthen behavioural control. Behavioural control can be taken when the insect density is lower than common insect grain standard. For example, moths can be controlled by sealing the grain surface with plastic film or other materials from late winter to early spring. All kinds of insects can be trapped and killed based on their up-climbing, chemical tropism, photokinesis, etc., generally, including probe trap and kill, lamplight trap and kill, noxious bait trap and kill, etc., subsequently, decreasing insect density and insecticide application times.

4.2.2 Enhance control quality Improving insecticide application environment, selecting rational insecticide application method, dosage and formulation, trying one fumigation to completely kill all kinds of insects, development stages, leaving no living insects, are the foundation of preventing insect resistance. The action characteristics of phosphine should be fully understood before fumigation, taking scientific, rational and effective application techniques, avoiding non-effective fumigation due to unreasonably increasing dosage, executing fumigation under poor sealing condition, and over-high concentration at the beginning of the fumigation and too low concentration towards the end of the fumigation. For fumigant selection strategy, phosphine should be protected as a priority and used, not being substituted by other fumigants. For phosphine application, we should develop scientific application techniques; implement fumigation according to actual phosphine concentration and fumigation effect, not simply based on dosage and experience.

Keeping rational phosphine concentration is extremely important. The effective phosphine concentration differs depending on warehouse, insect species, insect stage, resistance degree in practical fumigation. Generally speaking, in order to get ideal fumigation effect, phosphine concentration must be carefully detected, and ensure keeping effective phosphine concentration in each portion of the grain mass, which is very important for avoiding fumigation failure and inducing insect resistance. In other words, phosphine fumigation should be implemented based on concentration and fumigation effect. The reference parameters, 100 – 350mL/m^3 fumigation concentration and sealing over 14 – 28 days, were recommended in LS/T1201 – 2002 *Fumigation Regulation of Phosphine Recirculation* issued in 2002 according to different insects condition and grain temperature, etc.. In recent years, application practices of grass-root grain storage units, the reference parameters can basically successfully guide practical fumigation practices. But for some especially high resistant or endurance insects, such as *C. ferrugineus*, the practical fumigation concentration should be higher, and the sealing time should extend for 30 – 45 days.

Keeping phosphine concentration effective and even distribution is closely relating to application techniques, methods, instruments. The application techniques have been helpfully explored in China, and the more practical techniques include slow-releasing application, phosphine generator application outside warehouse, cylinder fumigant containing PH_3 : CO_2 mixture application, and so on. Recirculation technique can effectively accelerate phosphine even and quick distribution in huge and deep grain mass.

4.2.3 Shift or change of insecticides According to current insect resistance development condition, selecting different kinds insecticides, and designedly periodically shifting insecticides is very important to control resistant insects, especially selecting new insecticides and intentionally shifting insecticides to avoid cross-resistance happening. It was reported that the adults of phosphine resistant *R. dominica* and *Sitophilus oryzae* strains did not produce cross-resistance to malathion, deltamethrin, etc.. thus, grain protectants can be used to control phosphine resistant insects. In addition, synergist also can be used to enhance toxicity. The common synergist is PBO (piperonyl butoxide). Pyrethroid mixing with synergist can increase control effect。

Furthermore, mixing different insecticides can enhance controlling effect. Different mechanism insecticides being used together can not only successfully overcome insect resistance, but also enhance controlling effect, reduce dosage and control cost. Zhengzhou Grain College validated that CO_2 could obviously enhance PH_3 toxicity to *Tyrophagus putrescentiae* by mixing different proportion PH_3 and CO_2 to control *T. putrescentiae*.

5 Prospect

Chemical control is inevitable to generate resistance, as well as grain and environment contamination. With the increasing concern about green food, non-chemical control are more and more highlighted. Therefore, most experts and enterprises are positively researching non – chemical control, such as controlled temperature storage, controlled atmosphere storage, biological control, etc., in China, and this is a necessary development orientation for the future. The researchers have got some achievement, for example, China Grain Reserves Corporation implemented controlled atmosphere storage experiments in tens of depots in 2007. Ideal fumigation effect was realized by injecting CO_2 or nitrogen, integrating sealing and low oxygen storage, storage time was prolonged, and stored grain quality was maintained. Moreover, a portion of depots of China Grain Reserves system plan to use cold source from ambient liquefied natural gas storage stations to realize quasi – low temperature grain storage, so as to greatly reduce insect control cost. Most experts and scholars are researching plant materials and natural enemies to control insects in China. For example, garlic volatiles possesses strong inhibition or contact action against *R. dominica*, *Tribolium castaneum*, cereal psocid. Aloe extract had strong inhibition against *R. dominica* population, etc.. It can be reliably predicted that these non – chemical control methods must be popularized in practical production, bring about a revolution of insect control methods, arouse far – reaching effect on insect control.

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Studies on Prevention of Resistance in *Cryptolestes Ferrugineus*

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Abstract: In this study we have compared the efficacy of different fumigation methods for control of *Cryptolestes ferrugineus* in maize, wheat and paddy, including: the recirculation fumigation with DDVP (by surface application) and AlP (application to surface and at intake), recirculation fumigation under film with DDVP (by intake) and AlP (application at intake and under the surface with small cloth-bag). The experimental results indicate that the last method can control *cryptolestes ferrugineus* effectively in imported wheat and prevent other insect pests at the same time.

Key words: *Cryptolestes ferrugineus*, resistance, prevention

Introduction

Cryptolestes ferrugineus (Stephens) (Coleoptera, Cucujidae Latreille) reproduces 3 – 6 generations per year and is a secondary pest. It over-winters as an adult, surviving on drier grain granule chippings, flour and dust, and its ability to tolerate low temperature is weak. Many individuals have obvious resistance to phosphine and the adult is good at flying. When it present in high numbers, it will cause grain the stack to generate heat and mould growth in part or the entire warehouse. The main damage occurs in cereals, oilseeds, flour and so on, especially in oilseeds and flour.

Resistance to phosphine is becoming broader and graver in stored grain worldwide (Rajendran, S. 1989; Price, L. A. and Mills, K. A. J. 1988; Dyte, C. E., Mills, K. A., and Price, N. R. 1983). Since the 1990s, there have been many reports in succession about serious resistance to *Cryptolestes ferrugineus* in Guang Dong province and Hunan province, China (Yu Wenjiang et al., 2004; Jiang Zhongzhu, Liu Xiaofu et al. 1990; Zhang Xinfu, Chen Jiadong, et al. 1985). In Zhejiang province, we find it is difficult to control *Cryptolestes ferrugineus* with ordinary phosphine dosages and it quickly re-established after fumigation. The National Grain Reserve Jinhua Depot is located in the Jinqu Basin, which experiences high temperatures and high humidity, the average annual temperature is above 22℃ and the mean temperature of grain stack is above 18℃ over half the year. Annual average relative humidity is 70%, and above 70% over 6 months. So relative humidity in the storage is above 65% over half a year. These conditions favour *Cryptolestes ferrugineus* reproduction. *Cryptolestes ferrugineus* is the most difficult pest to control with chemical treatments. It has obvious resistance to phosphine. The resistance factors (Rf) of the *Cryptolestes ferrugineus* is 121.6. There is currently no preventative or chemical control method effective against this species.

In recent years, staff at Jinhua Grain Depot, State Grain Reserves, have explored various methods to control *Cryptolestes ferrugineus*. We investigated the insects, their living habits and the effect of fumigation each year, and have accumulated a large amount of data. We found that fumigation with AlP and DDVP had greatest effect. Based on previous experimental data, we compared different fumigation methods that have been used in maize, wheat and paddy to control insect pests. We adopted two methods: recirculation fumigation with DDVP (by surface application) and AlP (by surface and by intake), the recirculation fumigation under plastic sheet film with DDVP (by intake) and AlP (application at intake and under the surface with small cloth-bag). We compared and analysed the effect of these control methods with the aim of developing an optimal technique to prevent *Cryptolestes ferrugineus* infestation.

1 Materials

1.1 Details of Field Trials are Listed in Table 1

1. *Jinhua Grain Depot, State Grain Reserves. Jinhua City, ZheJiang Province, China* 321018
2. *Zhejiang Branch Co., Central Reserves of Grain Management Co. Hangzhou City, ZheJiang Province, China* 310013

1.2 Materials

1.2.1 Gas Tightness of Storehouse. Half-life when warehouse pressure falls from 500 Pa to 250 Pa: No. 23, 42 sec; No. 35, 40 sec; No. 22, 44 sec; No. 2, 45 sec; No. 9, 43 sec; No. 7, 41 sec; No. 10, 40 sec.

1.2.2 PH_3 monitoring instruments: HL-210 type detecting instrument (Beijing Xinghualaoke Trade Ltd).

1.2.3 Grain temperature monitoring system: GSM grain electron temperature measureing system (Shaanxi Academy of Food Science and Edible Oil).

1.2.4 Recirculation Fumigation System: Stationary recirculation fumigation system (Beijing China Grain Science & Technology).

1.2.5 Sampling in Bulk: Grain multifunctional sampling system 1 500 kW, (Chengdu Grain Storage Research Institute).

1.2.6 Gas-proof material: adopt 0.16 mm PVC film

1.2.7 Fumigation chemicals 56% ALP; 和 85% ALP (Jiangsushuangling Chemical Industry Ltd.); DDVP (Nan Tong City, Jiangsu Province product)

2 Methods

2.1 Sealing Methods

2.1.1 Storehouse Sealing

In all experimental storehouses, the method of "two layers of non-woven fabric and three thickness of glue" was adopted to seal between the wall and precast floor slab and between the precast floor slabs. All doors, windows, gable axials and aeration ducting were sealed gas-tight using 0.16 mm PVC film.

2.1.2 Surface Sealing

The surface of grain mass was covered with 20 cm thick wraps that were filled with bran, and then used to seal 0.16 mm PVC film.

2.2 Fumigation Methods

2.2.1 Determining the Dosages

Factors taken into account to calculate dosage included: storage location, design and volume, insect density, type and resistance levels, climatic conditions and grain variety and sorption potential. The AlP dosages applied were: in the storehouse No. 23, No. 22, No. 9: 150, 150 and 154 gm/tonne, respectively. DDVP dosage was 4 kg for each. In the warehouse No. 35, No. 2, No. 7, No. 10, ALP dosage were 152, 153, 153, 155 g/tonne respectively, and DDVP dosages were 6, 4, 4 and 4 kg, respectively.

2.2.2 Application Method

The insecticides were applied manually. AlP: in storehouses No. 23, No. 22 and No. 9, was applied to the surface on a plastic tray, and to the air-vents in two sacks. In the warehouse No. 35, No. 2, No. 7 and No. 10, a small cloth-bag was put on the surface and two sacks containing AlP were placed in the air-vents. When the phosphine concentrationwas less than 200 ppm, more AlP was applied. In warehouses No. 23, No. 22 and No. 9, DDVP was sprayed on old sesame sacks which were placed on the grain surface. In warehouses No. 35, No. 2, No. 7 and No. 10, DDVP was sprayed on old sesame sacks which were placed on intake in the augur. Fumigation methods, application methods, and dosages are detailed in Table 2 for all warehouses.

2.2.3 Recirculation and measuring phosphine Concentration

Recirculation and concentration monitoring methods were carried out according to the recommendations of "Aluminum phosphide circular current fumigation operation standards". We used these guidelines as general principle but also considered the reality of the fumigation at the same time. After applying the chemicals, recirculation was continued for 48 h. The concentration was determined at 8 o'clock in the morning each day, and afterwards phosphine was recirculated for 2 – 3 hours in each 24 hours. After the recirculation was ended, the fumigant concentration was checked again. Concentration was monitored until the gas was cleared and AlP residue removed. There were 5 check points (a centre and 4 others around it), and each point distributed in three tiers. Gas dispersal was expressed by standard square difference (S); S value is getting smaller as approaching 0, explaining the data fluctuation less, concentration distribution homogeneity increases more.

2.2.4 Evaluation of the Fumigation Effect

Efficacy of the fumigation was measured as "time interval for no insect detection". Methods used to determine efficacy included: (1) As soon as ventilation period ended, examine grain for insect mortality. (2) Take random 1kg samples from the grain and incubate these in isolation in the laboratory at 25℃ for 30 days—this will indicate survival of immature stages. (3) Monitor grain stack for insects.

3 Results and Discussion

3.1 Integrated Fumigation Effect on *Cryptolestes Ferrugineus* and Other Insect Pest

After ventilation, we inspected grain in the seven warehouses. The mortality of *Cryptolestes ferrugineus* was 100% without exception. Random samples of grain were removed for isolated incubation but no insects were detected after 30 days. In follow-up inspections, *Cryptolestes ferrugineus* were only found in warehouses No. 22 and No. 23, where the time interval without insects was 88 days and 80 days, respectively. Insects were not detected in any other warehouse. A few mites were detected in some samples from warehouse No. 35, No. 10 , No. 7, No. 9, at 4 months after ventilation (1/kg、1/kg 、2/kg 、3/kgrespectively). Up to the present, *Cryptolestes ferrugineus* and acaridae have not beenfound in warehouse No. 2. To sum up, efficacy of the treatment in each of the warehouses was as follows: No. 2 > No. 35, No. 10 > No. 7 > No. 9 > No. 22, No. 23. Detailed results are shown in Table 3.

3.2 PH_3 Concentrations over Time

PH_3 concentrations at each check point are shown in Table 4. Phosphine distributed comparatively equality in No. 10 warehouse because of three "非" shape PVC circular pipes (with 6% orifice coefficient) placed under the grain mass surfaces at a depth of 60 cm, which facilitated homogeneous dispersal of the gas. But from the mortalities, the infection of distribution homogeneity was less. Phosphine concentration was equal to or greater than 300 ppm for 20, 16, and 22 days in No. 35, No. 2, No. 22 storages, respectively; PH_3 concentration was equal to or greater than 200 ppm for 28, 25 and 23 days in No. 2, No. 35, No. 10 storage, respectively; and, PH_3 concentration was equal to or greater than 100 ppm for 38 and 37 days in No. 22 and No. 9 storages, respectively. Prevention of infestation was achieved in storages No. 2, No. 35, No. 10, where phosphine concentration was maintained at equal to or greater than 200 ppm for a long time, whereas the effect was relatively poor in No. 22 and No. 23 because of loss of fumigant from the warehouse. These results demonstrate that complete control of resistant *Cryptolestes ferrugineus* can be achieved if the phosphine concentration is maintained at equal to or greater than 200 ppm for a long time.

3.3 The application Technique and Recirculation

Our trials indicate that effective control of grain insect pests can be achieved by recirculating fumigant using the following methods: by the aluminum phosphide film with small cloth-bag and by the combination of application of phosphine and DDVP at intake. Investigating the possible cause, we conclude that air tightness was crucial, which was directly correlated with PH_3 concentration and lowering the CT value. Gas tightness was obviously improved by covering the grain with PVC film and when the time to half pressure decay from −500Pa to −250Pa was more than 90 sec. The CT value was higher, so the effect of fumigation was better.

3.4 Effect of the Different Grain Types on Insect Control

Most even dispersal of PH_3 occurred in the storehouse containing wheat, dispersal was lowest in the paddy stored storehouse and it was between in the corn storehouse. When adopting recirculation fumigation under film, the effectiveness of fumigation was best in wheat, the corn and paddy took second place. The possible cause was the different sorption properties of grain types, which caused the PH_3 concentration discrepancy. Previous research (Jiang Liguo et al. 1999) indicated that sorption of PH_3 capability in turn was paddy > corn > wheat, it is compatible with our result.

3.5 Effect of Applying AlP and DDVP Together

The mortality of insect pests was 100% in 7 warehouses, the control effect was obvious especially to *Cryptolestes ferrugineus*. The possible cause may be that phosphine acts on insect energy metabolism system, insecticidal effectiveness is strong, penetration and diffusion of gases is good, sorbs less, desorbs rapidly, yet some insect has produced strong resistance to phosphine. DDVP acts on insect nervous system, it can induce insect movement from the grain mass. The effect is rapid, the "knock down" activity is strong, especially to insects of Coleoptera and Lepidoptera. The shortcoming is that volatilization and diffusion is slow, permeation is weak (Shen Zonghai, 1997). The current results indicate that fumigation with phosphine and DDVP together can complement each other, had a synergistic effect, and enhanced the insecticidal effect.

4 Conclusion

4.1 Fumigation with PH_3 only for long term, additionally grain is mainly exchanged from other province in ZheJiang Province, so as to the insect pest is introduced along with the grain. So the *Cryptolestes ferrugineus* appear strong resistance and its source is broad. These bring difficulties to insect control in stored grain.

4.2 This investigation indicates the optimal control method: recirculation fumigation under film, mixing AlP (the combination of application at intake and under the surface with small cloth-bags) and DDVP (intake application). The DDVP dosage is 1.3 g/m^3 and the PH_3 effective concentration is equal to or greater than 200 ppm for more than 28 days.

4.3 Because sorption capability of grain types varies, the effect of fumigation is different. From the experiment, when adopting recirculation fumigation under film with intake and under surface application, the effectiveness of fumigation is best in the wheat stored storehouse, the corn and paddy take second place.

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Table 1. Details of trials

NO.	Type	Volume (ton)	Type	Quantity (ton)	Storage date	Moisture content (%)	Test weight (g/L)	Foreign matter (%)	Brown rice recovery (%)	Average temperature (℃)	Species and density			
											Angoumoisgrain moth (per kg of grain)	Sitophilus zeamais (per kg of grain)	Cryptolestes Ferrugineu (per kg of grain)	Acaroid mites (per kg of grain)
23	Large warehouse	2720	corn	2627	2007.04	13.0	719	1.0	/	17.4	/	8	8	mass
35	Large warehouse	4070	corn	3800	2007.06	13.2	720	0.6	/	19.8	2	3	8	mass
22	Large warehouse	2720	import wheat	2750	2004.11	10.4	803	0.7	/	14.1	/	/	12	mass
2	Large warehouse	2720	import wheat	2700	2004.10	11.3	816	0.8	/	16.5	/	/	12	mass
9	Large warehouse	2720	early paddy	2047	2005.12	12.8	/	1.0	75.0	16.5	/	/	14	mass
7	Large warehouse	2720	early paddy	1946	2005.10	12.4	/	1.0	76.0	19.9	/	/	14	mass
10	Large warehouse	2720	early paddy	2012	2007.04	13.5	/	1.0	76.4	25.0	/	7	7	mass

Table 2. Fumigation method, application way and dosage

NO.	Fumigation method	ALP application way and dosage		DDVP application way and dosage	
		application way (first application + second application) (kg)	Dosage of each ton grain (tons/kg)	application way (kg)	Dosage (g/m^3)
23	Interval application, recirculation	surface(9 +4.5) + intake(4 +1)	150	surface(4)	0.8
35	Interval application, recirculation under film	under film with small cloth – bag (12 +7) + intake(4.5 +1.5)	152	intake(6)	1.1
22	Interval application, recirculation	surface(9.3 +4.7) + intake(3 + 1.3)	150	surface(4)	0.8
2	Interval application, recirculation under film	under film with small cloth – bag (8.6 +4.7) + intake(3 +1.3)	153	intake(4)	1.3
9	Interval application, recirculation	surface(6 +3.3) + intake(2.7 + 1.3)	154	surface(4)	1.1
7	Interval application, recirculation under film	under film with small cloth – bag (6.1 +3.3) + intake(2 +1.3)	153	intake(4)	0.8
10	Interval application, recirculation under film	under film with small cloth – bag (5.6 +2.7) + intake(3.4 +1.3), three "非 – shape" PVC circular pipes with 6% orifice coefficient were placed under grain mass surfaces 60 cm	155	intake(4)	1.1

Annotate: ALP dosage convert into powder

Table 3. Fumigation effectiveness

NO	ifferent PH_3 concentration maintaining time			otal exposure time (day)	PH_3 concentration while deflating (ppm)	The temperature where insects happened during fumigation		Mortality (%)	Insects species and density (per kg of grain)		None insects intervals (day)	Integrated effect evaluation of fumigation
	100	300	200			Max (℃)	Min (℃)		Cultivated for 30d	After the deflation		
23	9	19	35	40	47	31.0	25.5	100	None	1 cryptolestes ferrugineus, after 81d	80	*
35	20	25	36	40	56	33.4	29.2	100	None	1 acaroid mites, after 100d	No main insects	****
22	13	20	38	42	48	27.0	25.0	100	None	1 cryptolestes ferrugineus, after 89 d	88	*
2	16	28	32	35	53	27.7	25.0	100	None	None	up to the present	*****
9	11	20	37	41	42	29.8	27.2	100	None	3 acaroid mites, after 93 d	No main insects	**

NO	ifferent PH_3 concentration maintaining time			otal exposure time (day)	PH_3 concentration while deflating (ppm)	The temperature where insects happened during fumigation		Mortality (%)	Insects species and density (per kg of grain)		None insects intervals (day)	Integrated effect evaluation of fumigation
	100	300	200			Max (℃)	Min (℃)		Cultivated for 30d	After the deflation		
7	10	22	35	38	52	30.6	26.3	100	None	2 acaroid mites, after 79 d	No main insects	***
10	11	23	36	42	50	35.0	29.7	100	None	1 acaroid mites, after 104 d	No main insects	****

Table 4. The change of PH_3 concentration in experimental warehouse every 3d

No.		23	35	22	2	9	7	10
First application time		2007.6.19	2007.7.19	2007.8.6	2007.8.21	2007.7.24	2007.8.10	2007.6.22
1st	southeast	380	600	360	550	496	570	560
	northeast	406	580	410	318	486	278	530
	northwest	520	600	>1000	458	291	420	535
	southwest	492	570	945	324	450	488	540
	center	452	610	300	460	272	765	425
	S	58	16	340	99	109	181	53
4th	southeast	456	450	645	>1000	414	446	332
	northeast	326	410	540	890	390	368	328
	northwest	418	460	>1000	890	368	318	318
	southwest	398	408	>1000	895	412	402	312
	center	438	470	384	>1000	350	590	300
	S	50	29	277	59	28	104	13
7th	southeast	368	493	660	680	330	440	312
	northeast	238	472	484	600	334	260	308
	northwest	300	492	925	710	338	380	305
	southwest	354	470	334	585	320	380	300
	center	356	418	384	615	314	286	290
	S	54	30	240	54	10	74	8
10th	southeast	346	382	570	535	286	325	218
	northeast	216	377	416	386	296	162	214
	northwest	278	380	452	476	294	283	204
	southwest	318	378	370	324	280	280	199
	center	316	340	410	376	242	186	210
	S	50	18	76	85	22	70	8
13th	southeast	258	296	328	428	144	210	158
	northeast	127	303	292	326	240	152	148
	northwest	154	294	316	382	179	182	138
	southwest	276	304	336	254	154	179	139
	center	266	268	360	306	160	166	150
	S	70	15	25	68	38	22	8

0806

Review: Resistance to Insecticides in Stored-product Insects and Its Mechanisms

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Abstract: In this review, we briefly describe the major species of stored-product insects. We concentrate particularly on the economic importance of the control of these insect pests, highlighting a final cost of more than one billion of dollars world-wide. Then we describ in detail and focus our review on the different mechanisms of resistance described in these species. This work is centred on the main ways the resistances described most recently in these insects. First, we detail common description of species resistance throughout the world. We observed 27 recurrent species involved in resistance cases occurring on 5 continents. Resistance is commonly expressed as a factor. Field-collected strains expressed 1.3 to 1 194 fold resistance compared with sensitive strains according to the considered species and insecticide. Described resistance mechanisms include: behavioral resistance, particularly described with *Oryzaephilus surinamensis*, the involvement of detoxifying enzymes (particularly esterases, monooxygenases and transferases) and studies on the genetic resistance, involving the Kdr mutation mechanisms, the transmission of the genes of resistance. We detail all these mechanisms and give some advice on the need for further research some possible future studies, emphasising the need for more collaboration between laboratories from different countries. We dedicate a section to cross-resistance and the multiple resistance due to the many mistakes and different meaning we can find in several articles. Finally, we conclude with pathways that could be followed to increase our knowledge of the development of resistance. We think that studies on genetic diffusion in species and populations of stored-product pests are necessary. These studies are needed to understand the development and the diffusion of the resistance to insecticides.

Key words: stored-product insects, resistance, insecticides

Introduction

Somta *et al* described the problem of the Mungbean, *Vigna radiata* (L.) Wilczek, attacked by one species of stored-product insect[104]. Mainly produced in Asia, the Mungbean infestation can result to a total destruction within 3 – 4 months[6]. And knowing that this crop is a major source of dietary protein for poor people and an important nitrogen-fixing legume in tropical cropping systems, we can measure the several consequences related to this insect diseases. Unfortunately, this example is only one among several others.

Classical argumentation to highlight stored-product articles was an economic argue. But with the actual world-wide repartition of the majority of the major pests and with the resistance development to all tested insecticides, the stakes are at least double today. First, we have a real competition between several species for a raw material which the food, included the species *Homo sapiens sapiens*. The second argue, more and more taking in account by necessity, is the environmental problem. Develop here in some words: find environmental importance[19]. The stake of these researches is simple to understand: decrease diseases of stored-product insects to increase the quantity of food. With the actual scenario of lack of food in several country on several continent and the future scenario about the feeding of the world wide population, the argument is not necessary to be presented again for the necessity of all these works. Existing projections indicate that future population and economic growth will require a doubling of current food production, including an increase from 2 billion to 4 billion tons of grains annually[110]. The economic cost is always remains to us at the beginning of each articlebut it remains difficult to have an idea about the real cost of these pests and or the real disease.

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We can find, here or there, some estimations. Insects and mites are responsible for deterioration of stored food and they cause yearly losses estimated at about 30% of 1800 million tons of stored grain [52]. Another one talk aboutbut we didn't find any study treating about the total cost(treatment, lost, lack of food for human). In spite of all the pesticide used/misused, insects still destroy over 30% of the world's food crops each year. Over 2 billion tons of grains are produced yearly for food and feed, providing roughly two-thirds of total direct and indirect protein intake[110]. When we observed the stock market values exchanges for maize or wheat in the American continent or in Europe, the price tonnage lies beyond the 200 $/ton (i. e., tender wheat reached 385 $/ton in January 2008). Although we are unable to give a precise number, we can estimate a global worldwide cost of at least one hundred billion dollars.

About 300 different species of stored product pests may be encountered with only about 18 species of primary economic importance. Stored product insects are adapted to infesting raw grains and cereal products, and present a constant threat to these commodities worldwide. These insect pests survive on dry, stored cereals and legumes in raw or processed form, and they are maintained year after year in storage systems by residual grain remaining in bins, poor sanitation in mills, food-processing facilities and warehouses, and storage, and immigration form natural(rodent caches, bird nests, wooded areas) and other infested sites. Two major groups of insects harbour the mostly economically important post-harvest insect pests: Coleoptera (beetles) and Lepidoptera(moths and butterflies). To notice that several Coleopteran and Lepidopteran species attack crops both in the field and in store. Post-harvest insect pests may be primary, i. e. able to attack intact grains such as the genus *Sitophilus*, while others are secondary pests, attacking already damaged grains or grain products such as the genus *Tribolium*. The Table 1 is a list of the most studied pests until 1995.

Table 1. List of stored-product speciesand their location in the recent studies coming from 2 major journal treating the resistance in stored-product insects: Journal of Stored Products Research(1995 – 2007) and Journal of Economic Entomology(1997 – 2007)

Order	Family	Species	Location of studied populations
Coleoptera	Anobiidae	*Lasioderma serricorne*	UK
	Anthribidae	*Araecerus fasciculatus*	India
	Bostrichindae	*Prostephanus truncatus*	Benin, Mexico
		Rhyzopertha dominica	Australia, Brazil, India, Morocco, China, USA
	Bruchinae	*Callosobruchus chinensis*	Uganda, Nigeria, Thailand
		Callosobruchus maculatus	India, Nigeria, Ghana, Uganda, Brazil, Burkina Faso, Cameroon
		Acanthoscelides obtectus	Mexico, Greece, Colombia
		Zabrotes subfasciatus	Mexico
	Cucujidae	*Oryzaephilus surinamensis*	Australia
	Curculionidae	*Cylas formicarius elegantus*	India
		Sitophilus granarius	former Yugoslavia
		Sitophilus oryzae	Australia, Brazil, Ethiopa, Morocco, India, USA, Greece
		Sitophilus zeamais	Australia, Brazil, Benin, Mexico, Togo, USA
	Dermestidae	*Trogoderma granarium*	Asia/Africa
	Laemophloeidae	*Cryptolestes ferrugineus*	China, Australia, USA
		Cryptoletes pusillus	USA
	Silvanidae	*Oryzaephilus surinamensis*	Australia, Wales, England, USA, Turkey, Israel
	Tenebrionidae	*Tribolium castaneum*	Australia, England, Ivory Coast, Brazil, Israel, Nigeria, Morocco, India, Belgium, France, USA, Turkey
		Tribolium confusum	Israel, Greece
Hymenoptera	Ichneumonidae	*Diadegma insulare*	Mexico and USA

Order	Family	Species	Location of studied populations
Lepidoptera	Pyralidae	*Amyeolis transitella*	USA
		Cadra cautella	Korea
		Ephestia cautella	Australia, Turkey, Israel
		Ephestia kuehniella	Greece
		Plodia interpunctella	USA
	Tortricidae	*Cydia pomonella*	USA
Psocoptera	Liposcelididae	*Liposcelis bostrychophila*	China, USA, UK

To illustrate local diseases, we chose to cite just few examples coming from 3 different continents, Africa, South America and Asia. In East Africa, infestation of pigeonpea by *Callosobruchus chinensis* starts in the field and, once infested seeds are stored, there is rapid pest multiplication and destruction of seeds which may reach 100% within a very short time[101]. In Nigeria, about 30 000 tons is lost annually due to only one pest, *Callosobruchus maculatus* (F.) (Coleoptera; Bruchidae). To notice that under traditional storage conditions, 100% infestation of cowpea occurring within 3 to 5 months of storage is common[67]. In Asia, rice is an important staple food where paddy fields are harvested 1 – 3 times in a year and then stored before consumption. India produces hundred millions tons of paddy rice annually which is stored for 3 – 12 months as paddy by farmers, traders and by the public sector agencies. Only a small fraction of the paddy rice is stored in silos, which do not have the facilities for aeration and drying[14]. Outdoor storage of paddy rice is common in China[89] and other Asian countries[99,88]. Again, outdoor storage does not give the same advantage as the silo, and losses are difficult to estimate. InLatin America, losses in maize caused by *Prostephenus truncatus* is well studied and varied from 9 to 45% depending upon the period of storage[39,59,81,76,66]. Latin America produced nearly half the world's supply of dry beans[18]. But almost 80% of beans are produced on small-scale farms without silos. Post-harvest crop losses are predominantly caused by coleopteran species from the family Bruchidae.

Actually the easiest method of controlling insects is with insecticides. In fact, insecticides are generally the most effective management tool and in many instances provide the only feasible method of reducing insect pest populations or reducing them to acceptable levels[50,84]. We can observed than less and less experiments were done with Organochlorine and organophosphorous insecticides, 1st generation of chemical insecticides. Historically, they were replaced by pyrethroids, especially deltamethrin, very efficient against arthropods. Two fumigants are currently used for the protection of stored foods: phosphine and methyl bromide. However, the use of methyl bromide was restricted due to its ozone depleting properties[121] and it must be used after careful consideration because of its very high toxicity to warm-blooded animals[28]. Phosphine remains one the most used insecticide. In parallel, carbon dioxide is an important factor affecting the efficacy of controlled atmosphere treatments for pest mortality[115]. More recently, scientists have tested natural oils, which demonstrated a great and new interest of scientists and partners for environmental compounds in the recent future. Essential oils are an alternative to the currently used of fumigants. Papachristos and Stamopoulos[82] insisted on the different methods we can use them, i. e. as fumigants[108], contact insecticides[94], repellents[91], antifeedants[51] and also affect some biological parameters such as growth rate, life span and reproduction[47]. The other used insecticides finding in the literature are diverse: microbial pesticides, insect growth regulator, synergist[27]. Integrated pest management system to control insect pests should combine the parasitoid and host plant resistance[98].

Most recent research advances in insect topics, especially in resistance domain and in molecular and genetic advances, are well done on Diptera, particularly on Drosophila, the model, and on mosquitoes, the most injury. And reviews on resistance to insecticides in insects existed, especially developed on Diptera. For example, the two articles from Hemingway and Ranson[55] on one hand and Li *et al*[74] on the other hand described precisely biochemical and molecular mechanisms developed by insects. Hemingway and Ranson[55] described mecha-

nisms resistance based on mosquitoes studies involving esterase, gluthation-S-transferase and monooxygenases activities, target site modification, acetylcholinesterase activities, GABA receptor modification and sodium channels, also enhancing the mutation in structural genes, gene amplification and transcriptional regulation. Li *et al*[74] described these mechanisms more in depth with the involvement of transposable elements, allochemical tolerance and several molecular mechanisms of metabolic resistance (upregulation, coding sequences changes, catalytic site). In parallel, stored-product insects do not lack research too: several articles were published and a journal, Journal of Stored Products Research, was created in 1965. Unfortunately, we can't find synthesis of resistance mechanisms to insecticide on insect stored products.

Described Resistances

In recent years, more than 504 species of insects and mites with insecticide resistance have been recorded and there is still a steady increase in resistance to specificchemicals, with many species now resistant to several groups of insecticides[38,68]. Insects have successfully adapted to most insecticides by becoming physiologically or behaviorally resistant to them[93]. In post-harvest ecosystems, the development of insecticide resistance is of great concern to many people. Cases of resistance of insect-stored products to grain protectants [3,29], and fumigants[21,72] have been classically documented.

Resistance to insecticides such as malathion, pirimiphos-methyl, fenitrothion has been reported, for example, in *Rhyzopertha dominica* (F.), *Sitophilus oryzae* (F.), *Sitophilus zeamais* (Motschulsky), *Tribolium castaneum* (Herbst)[41,42]. *S. zeamais* is resistant to DDT and deltamethrin too[75]. Resistance to DDT and pyrethroids was reported in the early 1990s and in recent years a few instances of organophosphate as well as pyrethroid resistance have been reported[36,90]. Insecticide resistance has a patchy distribution in Brazilian populations of maize weevil without significant spread, suggesting that the grain trade within the country and local selection are probably major forces driving the evolution and spread of insecticide resistance in this case [42,36,90,35]. Perez-Medoza[84] described also resistance in strains of *S. zeamais* in Mexico. But in this country, maize weevil resistance to deltamethrin and permethrin is in its initial stages because these insecticides were registered as grain protectants in Mexico only after 1992[84]. Another example could be *Oryzaephilus surinamensis* (L.) in Australia. This insect was decribed resistant to commonly used pesticides such as fenitrothion, pirimiphos – methyl and chlorpyrifosmethyl[9]. The field-collected strains showed resistance to DDT(1.3 – to 14.1 – fold), lindane (4.7 – to 20.9 – fold), malathion(1.6 – to 31.4 – fold), pirimiphos – methyl (3.0 – to 3.7 – fold), deltamethrin(1.2 – to 1.8 – fold), and permethrin (2.3 – to 3.5 – fold). In Morocco, 50 of 51 studied insect populations have been detected resistant[13]. Several other workers also detected resistance to this fumigant in different parts of the world[107,77,112,126,5]. Cases of resistance to used fumigants(phosphine but also methyl bromide and ethylene dibromide) has been well documented[21,72,58]. Similarly, extensive use of controlled atmosphere in insect control could lead to selection of insect populations resistant to hypercarbia and hypoxia[30,31,115]. At least 11 species of stored-product insects are now known to have developed resistance to phosphine[23], which has been linked to selection pressures exerted by repeated ineffective fumigations in situations where phosphine gas was rapidly lost due to leakage[49,112,13].

But generally, insecticide resistance studies are frequently involve only detection bioassays, with folder of resistance. Unfortunately, in the majority of these descriptions of insecticide resistance, we are unable to find experiments on the involved resistance mechanisms.

Behavioral Resistance

This last years, few studies dealt with this method of resistance. Experiments were principally done with *Oryzaephilus surinamensis* and several insecticides. Barson *et al*[8] demonstrated that avoidance behaviour in *O. surinamensis* was not only dependent on the relative sensitivity of each strain to the toxicant, but also to the quality of the diet in terms of nutritional value and egg-laying sites. Sparks *et al*[106] identified the role of insect mobility in avoidance behaviour and Wildey[120] demonstrated the importance of insecticide formulation on contact repellency. At least, Watson and Barson insisted on the effects of the high insecticide concentrations on a avoidance behaviour of *O. surinamensis*[116]. When applied at the highest tested doses, permethrin, pirimiphos-methyl and etrimfos

caused disorientation. Tested insects demonstrated evidence of avoidance behaviour to high insecticide concentrations[116]. But, studies of behavioral resistance on stored-product insects, principally due to the non application of their results on the field, were not further developed.

Detoxifying Enzymes

Resistance suppression by a particular synergist suggests detoxification enzymes are involved in the resistance mechanism. The use of insecticide synergists for providing preliminary evidence on the resistance mechanism has seldom been fully explored in stored-grain insect pests (e. g., (Guedes et al.[41]; Guedes and Zhu[45]) and in resistance studies with *S. zeamais*. Classical involved enzyme studies in other field research, the 3 most studied families: esterases, monooxygenases and glutathione transferases.

Esterases

Considerable focus on the role of esterases have been described in pyrethroid tolerance. A clear link was shown between the levels of esterases and populations of *Nilaparvata lugens* from pyrethroid treated and non-treated areas[53]. Kranthi et al.[65] and Gunning et al[48]. described the same correlations for *Helicoverpa*, respectively in India and Australia. Esterases have been involved in German cockroach pyrethroid resistance[86,95,83]. Lee and Clark[69,71] suggested that the pyrethroid was being sequestered in the haemolymph through a high affinity binding site on carboxylesterases. In other cases no relationship has been found between esterase levels/patterns and pyrethroid resistance[79,7,46,1]. Despite the high level of variability in the esterases among the populations of *Liposcelis bostrychophila*, Ali and Turner[1] were unable to link this variability with the permethrin tolerance. The involvement of esterases in resistance to organophosphorus insecticides in *Tribolium castaneum* was shown early by Dyte and Rowlands[33]. Triphenyl phosphate, a carboxylesterase inhibitor, was used as an indicator for carboxylesterase involvement in malathion resistance in laboratory tests[33]. Malathion resistance in most *T. castaneum* strains is due to this mechanism[80,118,109]. To terminate, grain storage and warehouse operators should be aware that controlled atmosphere (CA) treatments can induce the esterase enzymes which in turn can promote selection of pest strains resistant to CA treatments[16] and the rates of development of CA resistance are similar to those recorded for laboratory induced resistance to fumigants[30]. Many similarities exist between CA resistance and resistance to methyl bromide[78,113,115].

Glutathione-S-transferases

In the same way, glutathione-S-transferases are often described in insect resistance to insecticides. The involvement of GSTs in the defense against not only organophosphates, but also organochlorines and cyclodienes, is widely reported and continues to attract attention[123,124,34]. Some involvement of glutathione-S-transferase has been suggested in German cockroach[122] and the mite *Varroa jacobsoni* Oud.[56] resistance. Kranthi *et al*[65] suggested that the synergist insensitive tolerance of *Helicoverpa armigera* (Hubner) was due to some sort of nerve insensitivity and a similar mechanism was suggested by Yu and Nguyen[125] for *Plutella xylostella* (L.)[1]. GST activity levels towards the substrate chlorodinitro benzene (CDNB) were always higher, but not always significantly in the resistant populations when compared with the susceptible population. The usually higher GST activity of the resistant population is a likely consequence of their distinct selection history[42,90,34], which also seems to lead to differences in fitness cost associated with insecticide resistance in these populations[36,44,34]. The higher catalytic activity of GSTs provides support for the hypothesis of their involvement in the resistance to this insecticide group in some maize weevil populations. GSTs may act as binding proteins increasing the activity of other pyrethroid detoxification enzymes such as esterases[40,64]. An alternative explanation for the GST role as a binding protein is that the higher GST activity levels in pyrethroid resistant populations of maize weevil, as reported here, may be favoring their direct catalytic activity over pyrethroids as earlier recognized[125], or their activity as antioxidant agents decreasing the oxidative stress initiated by pyrethroids as more recently suggested[114]. Either way, there seems to be an involvement of enhanced GST activity in pyrethroid resistance in Brazilian populations of maize weevil, but this resistance mechanism is apparently secondary in importance to altered target site[42,36,90], and is not as stable based on demographic and physiological studies with these same populations of *Sitophilus zeamais*[35,44,34].

Monooxygenases

The monooxygenases are a complex and a large family of enzymes, known to be involved in adaptation of insects and active in the metabolism of all known – insecticides. A number of mechanisms have been suggested recently to explain tolerance or resistance to pyrethroid insecticides. Microsomal cytochrome P450 dependent mono-oxygenases have been shown to be important in some Lepidoptera[2,65], house flies[63], headlice[54] and *Blatella germanica* (L.)[95,96][112]. Cyt P450 is a well-known and well-described enzyme against used-insecticides. For example, the indirect synergist-based evidence of Turner *et al*[111] suggested that the detoxification mechanisms in *L. bostrychophila* are of the microsomal mono-oxygenase type[1]. But more interesting in our area of investigation, recent studies turned towards the possible involment of Cyt P450 on natural insecticides. A large number of essential oils extracted from various spice and herb plants have already been screened for toxicity as potential fumigants. Monoterpenes rich in essential oils also showed their strong fumigant toxicities against several different stored-grain insect pests[100]. Monoterpenes can be degraded by the cytochrome P450 – dependent monooxygenase system. In insects, 1, 8 – cineole was metabolised to 2b – hydroxycineole when the pyrgo beetle, *Paropsisterna tigrina* Chapuis, was fed leaves of the Australian tea tree, *Melaleuca alternifolia* (Maiden and Betche) Cheel[105]. Essential oils or monoterpenes can induce the concentration and aldrin epoxidase activity of cytochrome P450 – dependent monooxygenase in rats and insects[17,57,70]. Collins *et al*[26] also reported the 21. 9 – fold higher aldrin epoxidase activity and 12. 5 – fold higher concentration of cytochrome P450 in a CM – resistant strain, VOSCM in comparison to VOS48. Therefore, cytochrome P450 monooxygenase activity is presumably related to the detoxification of essential oil or monoterpenes in *O. surinamensis*[70,68]. Against botanical insecticides, previous cited examples suggested that the cyt P450 monooxygenases are involved with the appearance of resistance to essential oil vapour. . Pretreatment of the insects with diethylmaleate, an inhibitor of the glutathione S-transferases[87,117], caused a partial suppression of resistance to lavender essential oil vapour. Conversely, triphenyl phosphate, an esterase inhibitor[41,45], did not show any degree of synergism indicating that these enzymes are not involved in the detoxification of lavender essential oil vapour by *Acanthoscelides obtectus*[82]. These studies studies suggested that Cyt P450 and GST play a role in the resistance to lavender essential oil vapour, but not the esterases.

Acetylcholinesterase

The mode of action of fumigant toxicity of essential oil or monoterpene against insects may also be the inhibition of acetylcholinesterase (AChE)[91]. They determined that five monoterpenes inhibited AChE activity in the electric eel and killed adults of the red flour beetle, *Tribolium castaneum* (Herbst). The enhanced carboxyl esterase and anti-oxidation enzymes (superoxide dismutase and catalase) activities could reduce the effects of these toxic products on insects resulting in the insects' resistance to CO_2 increasing. Although the resistance mechanisms of dichlorvos have not been elucidated, it is well known that organophosphate pesticides exert their neurotoxic effects by inhibiting the enzyme acetylcholinesterase (AChE), thereby prolonging the residence time of acetycholine at cholinergic synapses and producing hyperexcitation of cholinergic pathways. Recent study by Leong and Ho[73] showed that the AChE activity was inhibited by DDVP in both *L. bostrychophila* and *Liposcelis entomophila*[29].

Genetio Resistance

Genetic results on insecticide resistance of stored-product insects are not so abundant as in mosquitoes and drosophila studies. *Tribolium castaneum* and *Rhyzopertha dominica* are the two species given us the more recent results, in party due to the work ofBeeman and Collins.

Malathion-specific resistance has been particularly described in *T. castaneum*. Even ifmost studies concluded that this resistance is controlled by a single factor[10,118], evidences were described involving a second allele giving a weaker resistance to malathion, also segregated at this locus[11].

On the same way of scientific contradictions, Lindane resistance has been reported as being multifactoral and, later, this resistance was described as controlled by a single semi-dominant gene located on chromosome III.[11,24]. This two examples reflect the lack in the genetic knowledge on the resistance of stored-product insects.

Resistance to pyrethroids in *T. castaneum* strains was finally described to be controlled by at least two incompletely dominant, autosomally

inherited factors[24]. In this same article except, the inheritance of this resistance was lightly studied too. Collins suggested that resistance was autosomally inherited and maternal effects were absent, estimating that independent genes controlling response from the response of F1 backcrosses were two or three[24]. Assie *et al.*[4] showed that the increase in the high malathion-specific resistant strains is due to a genetic background and could depend also to changes occurred in environmental parameters. They also suggested that two generations of selection may be sufficient to detect the potential for the increase of resistance[4].

As suggested by Collins, extra resistance genes, with different characteristics, may have evolved in different regions. Resistance to phosphine in *R. dominica* species is complex and Collins is not sure about he number of involved genes, writing "least two, and possibly five, different genes"[25,97]. These limited data are a real lack in our research field. Contrary to other disciplines where genetic resistance is strongly studied as the same as the population genetic of insects inside a country, a continent, even the world, we can't find actually works and cooperation about this subject. I insist too about the phylogeography problem. No data exist about a possible relationship between resistance evolution and genetic polymorphism on stored-product species. These data could be helpful to understand where these species are originating from, to define some natural enemies in the original area, to understand the world-wide spread of a species, to study the differences of the resistance evolution between the different geographical areas.

In this way, the very detailed article of Black and Vontas[15] could be very useful. They described in details all the actual sequencing methods used nowadays (more than 20). They explained single nucleotide polymorphism mechanisms involved in each methods, their individual advantages and lacks, with a cost study of each of this techniques. As write before, to study the involved genes among some populations, or some species , more cooperation between laboratories from developed countries, able to have the most recent techniques and able to currently use them, and laboratories from developing countries, more involved in sampling and resistance studying, need to set up. For example, our laboratory just defined some RFLP primers and finished our first study on *R. dominica* and we ought to used them on several populations coming from the 5 continents.

Cross Resistance or Multiple Resistance

Example: Kdr Resistance

Actually, an observed resistance to one insecticide is often related to a (some) previous used one(s). And we can read everywhere that it is a cross resistance. For example, one study on the more known resistance mechanisms in stored-product species on *Sitophilus zeamais* coming from Brazil[42]. "The knockdown resistance is due to the alteration in the site of action of insecticides in the resistant insects. Cypermethrin and permethrin were never used against stored grain pests in Brazil. The detection of resistance bioassays using pyrethroids synergized with piperonyl butoxide and pirimiphos-methyl provided further evidence that KDR is the resistance mechanism involved. Therefore, population of S. zeamais seems to have a pyrethroid target site alteration that provides cross-resistance (i. e., resistance to two or more insecticides due to the same mechanism) to the three pyrethroids. These results support the contention that cross-resistance to pyrethroids in Brazilian populations of maize weevil usually occurs in seed storage facilities which were subjected to heavy DDT use in the past"[42].

Other Citations

In the literature, concerning the stored-product insects, we found different used of the expressions cross-resistance. Guedes et al[42] wrote "This cross-resistance between DDT and pyrethroids became known as knockdown resistance" instead of talking about the involved mechanism. We can also find some supposition about a cross-resistance mechanism without any explanation: "The increased tolerance for the essential oil may be the result of cross-resistance"[70]. Again we can wonder if talking about cross-resistance is relevant between an essential oil and one of its compounds: "a chlorpyrifos-methyl resistant strain was cross-resistant to essential oil obtained from Eucalyptus globulus (Labill) and its primary monoterpene, 1,8 – cineole"[68]. Often, assumptions about involvement of some cross-resistance were expressed to explain some observed resistance, without any evidence and mechanism explanations: "our results suggest that the resistance to these compounds is likely caused by cross-resistance from another compound used as a grain protectant in

Brazil"[90]. We also can find cross-resistance between insecticide families, and no insecticides themselves: "were either highly resistant to malathion or were cross-resistant to organophosphates and pyrethr
oids"[62].

Some times, we can find some precaution in the used of cross-resistance and multiple resistance expressions. Fragoso *et al*[34] called to mind "*the possibility of cross and multiple resistance*" and introduced us a important notion for field observation with an observed resistance probably due to "a result of cross-selection by another insecticide".

Definitions

With these several examples, we can observe that authors used "cross-resistant" to described as well the resistance itself, the resistance to insecticide or its mechanisms. No more rigor is observed concerning the use of cross-resistant, cross-resistance, multiple resistance and cross-selection. Definitions about cross-resistance and multiple resistance have evolved since 1979, date of the first finding of these words. Chapman and Penman described cross-resistance as a resistance to one compound conferring resistance to other compounds of the same group , and multiple resistance was described when resistance has been developed to compounds from a number of structurally different groups[22]. In herbicide resistance, we have two other definitions for cross-resistance and multiple resistance. A cross-resistant weed biotype possesses resistance to several herbicides through a single resistance mechanism. An altered site of action does not necessarily provide cross-resistance to all herbicides with the same site of action. A weed biotype with multiple resistance possesses two or more distinct resistance mechanisms. A water hemp biotype has been identified that has altered binding sites for both the triazine and amino acid synthesis herbicides[85].

In stored-product articles, cross-resistance is described occurs when a population (or strain) of insects that has developed resistance to one insecticide exhibits resistance to one or more insecticide(s) it has never encountered. Cross-resistance is different from multiple resistance, which occurs when insects develop resistance to several compounds by expressing multiple resistance mechanisms. Here we are far away form the first definitions. Although I am almost agree with this multiple resistance definition, even if it is incomplete, I prefer to define the cross-resistance as defined by Ishaaya[60] describing the cross-resistance as following. Cross-resistance appeared when a selected strain(or population, or species) with insecticide A become resistant to an insecticide B; in parallel, this strain selected by the insecticide B must become resistant to the insecticide A. Then, we have a complete description of a cross-resistance, and we are able to work on the involved mechanisms into cross-resistance. About the field strain, we'd better use the term of multiple resistance, including the developed resistance to one insecticide leading to the resistance to one or more insecticide(s) with a single or multiple resistance mechanisms. Finally, agree or not with this definition is not the final point we wanted to highlight here. We though it is dangerous to use, in science, some bad words for good ideas. We need to be vigilant about our word choices.

Conclusions

Lateness in Fundamental Knowledges

This review gave a state on our current knowledge on the resistance of stored-product insects to insecticides. Involved laboratories, published articles and dedicated newspapers, or in part, to this discipline are numerous. Work that has been performing in each laboratory, on each species with each of insecticides is consequent. But we can deplore some lack in the fundamental accumulated knowledge. As an excuse, often, laboratories have contact with administrators or farmer, expecting applied and rapid results with precise data useful in the field. We can understand the importance of immediate results of resistance study for associates, but as scientists, we needn't forget the importance of knowledge about species history, wide spread, development of genetic tools, evolution of pest management.

The solving of the problem of resistance (after first acknowledging its existence, which is unfortunately the major published article on the resistance in stored product insects) includes accurate forecasting based on the previous history of pesticide use and making available research data on resistance parameters[37,103,61].

As the number of insecticides and fumigants for insect control have decreased, low cost, convenient to use and environmentally friendly alternatives need to be developed[82]. But knowledge on resistance need to evolve fas-

ter than this new discoveries. Actually, this is an hypothesis from a utopia.

Considering the mechanisms described, the involved enzymes in the biochemical resistance, the discovered genes in the genetic resistance, we can obviously conclude that, indeed necessary, the made works are not innovative. Nothing new has been discovered compared to drosophila, mosquito and even crop insects. Considering the consequent use of phosphine in close space in stored-products, we can fairly wonder why were we unable to be so innovative. With Phosphine : innovative method of resistance All species resist by the same way Resistance due to fumigant or to phosphine : what about the next generation of botanical fumigants with the integrated pest management.

Collaborations, a Way for the Genetic Diffusion of Resistance

I want to insist of the lack of cooperation between laboratories from different countries working on the same species and insist on the need to create some collaborations between us. The other major relevant deficiency is the absence of phylogeny and phylogeography studies on the stored-product insects, to determinate the origin point of these species and to determinate whether a natural enemy exists or if biological compounds from this geographical origin, due to the co-evolution plant-insect, could be used. Furthermore, the study of insecticide resistance should employ evolutionary and ecological approaches to explore its genetic basis, together with the way selection acts to bring genetic changes, and the use of this knowledge to delay the onset of resistance[4].

The other lack is the absence of the use of new techniques in the stored-product studies. As explained before, fundamental and applied studies of stored-product diseases can't go one without the other. The price of these methods in the developing countries need to be compensate by the laboratory from developed countries with more collaborations. The complete review of Black and Vontas[15] could help laboratory to choose the involved method and also the laboratory. Indeed, with method keywords and clearest laboratory website, to find some scientists to collaborate has become easier. On the way of the new tools, modelling of the evolution of the resistance need to be considered. With insecticide treatment archives, cases of insecticide resistances, number of studied species and insecticides, involved mechanisms and future genetic results, we should be helpful to predict the rate of development of resistance to insecticide. Moreover, the effect of population density, plant/insect interaction on the resistance level could be integrated into theoretical models[4].

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A Field Trial of Phosphine Fumigation on a High Resistant Strain of Rusty Grain Beetle in Paddy Rice Stored in Horizontal Storage

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Abstract: A phosphine fumigation trial was conducted on bulk paddy rice stored in a horizontal warehouse (10. 19. 13. 8 m). The rice was covered with clear PVC sheeting (0. 14 mm thick) that was sealed to the walls by pushing it into a slot with a rubber pipe. Phosphine was applied as aluminium phosphide three times during the fumigation at application rates of 6, 3 and 1 g/m^3 of tablets. The aim was to determine the suitability of the storage for fumigation and the application rate needed to control very highly resistant *Cryptolestes ferrugineus*. Cages of highly resistant (x328) and susceptible *Rhyzopertha dominica* and a strain of *C. ferrugineus* collected from the paddy were placed under the sheeting at the beginning of the fumigation. Phosphine concentration was monitored during the fumigation. Despite gas leakage due to the poor condition of the warehouse, phosphine concentration was maintained at > 200 mL m^{-3} for more than 35 days. Insect cultures could be clearly observed through the covered plastic sheeting during the fumigation. There were no active adults of susceptible or resistant *R. dominica* after 3 and 15 days, respectively. However, *C. ferrugineus* adults could be observed moving in the cages until day 30. No insects were present in the grain six months later when the sheeting was removed. The results indicate that very highly resistant strains can be controlled if a sufficient concentration of phosphine is maintained for along enough exposure period. The appropriate exposure period can be accurately determined by observing the response of the insects infesting the grain mass.

Key words: phosphine fumigation, rusty grain beetle, exposure time

Introduction

In local grain storages in southern China, paddy is usually stored in bulk or in bag stacks in warehouses that are not suitable for phosphine fumigation due to poor gas - tightness. However, insect pest infestation is always a serious problem in this region due to the warm, moist climate and the grain is often fumigated with phosphine (tablets of aluminium phosphide) as there is no other practical means of control. Under these conditions, there are usually some survivals of insect pests and resistance to phosphine has developed so that control failures have become more and more common, especially in some species or populations of insect pests. The Rusty grain beetle, *Cryptolestes ferrugineus*, has become a serious pest because it is now the most difficult species to control with phosphine.

Herein, we describe a field trail that was carried out on bulk paddy rice stored in a horizontal warehouse where the rice was covered with clear PVC sheeting that was sealed to the walls by pushing it into a slot with a rubber pipe. Phosphine was applied as aluminium phosphide three times during the fumigation. The aim was to determine the suitability of the storage for fumigation and the application rate needed to control very highly resistant *Cryptolestes ferrugineus*.

1 Materials and Methods

The trial was conducted on bulk paddy rice of 193 tonne stored in a horizontal warehouse (10. 19. 13. 8 m) in Minzhong Grain Depot, Zhongshan, Guangdong Province, China. The rice was covered on top with clear PVC sheeting (0. 14 mm thick) that was sealed to the walls by pushing it into a slot with a rubber pipe. The moisture content of rice was 12. 7%. Insect infestation was 12 adult insects per kilogram, including rice weevil (*Sitophilus oryzae*), lesser grain borer (*R. dominica*) and rusty grain beetle (*C. ferrugineus*) in the paddy. The temperature of paddy rice was 20 - 26℃ and ambient conditions in the warehouse were 26 - 32℃ and 70% - 90% relative humidity.

New PVC sheeting was used and checked against daylight for holes before use. Any holes were sealed with glue. Sheets were joined to each other by overlaping them about 40 mm and

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heat sealing.

The bag-stack was tested for gas-tightness by applying a negative pressure. A vacuum hose was inserted through the plastic sheeting and air withdrawn. Only 80 Pa of highest negative pressure was read from a water-filled manometer. The gas-tightness was not high enough for a single application of fumigant, so second and third supplements were carried out to ensure an effective concentration during the fumigation period.

Four groups of insect cages of highly resistant (x328) and susceptible *Rhyzopertha dominica* and a strain of *C. ferrugineus* collected from the paddy were placed under the sheeting at the beginning of the fumigation. The cages consisted of 50 adults insects, in culture medium, contained in glass tubes (70 mm long × 10 mm diameter) covered at each end with gauze to allow free flow of gases. Several tubes were glued into the sheets to facilitate insect checking and tablet application.

Phosphine was applied as aluminium phosphide three times during the fumigation at application rates of 6, 3 and 1 g/m^3. The tablets were put in cotton bag (10 cm × 8 cm, 150 g aluminium phosphide per bag) and inserted into the grain mass 20 – 30 cm in depth through the PVC sheeting, which was sealed after the application. The second and third applications were decided according to the phosphine concentration motored in progress. Nylon tubing for gas sampling, 3 mm inner diameter was inserted through small holes into the sheeting at four points (Figure 1). Phosphine concentration was measured at intervals of 24 hours (at 11.00 am each day), using an electronic monitor (model HL – 210, Xinjialiang Co., Beijing, P. R. China) with a range of 0 – 1000 mL/m^3, and phosphine detection tubes (ALARM brand, Hebi Gas Detecting Tube Manufacture, Hebi, Henan Province). Phosphine concentration was measured in units of mL/m^3 (ppm).

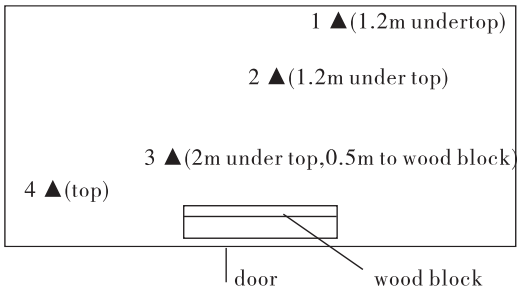

Fig. 1 Sketch of gas sampling for phosphine monitoring

2 Results

Phosphine concentration of four monitoring points is shown as Table1 and Figure2.

Table1. Phosphine concentration of four monitoring points (mL/m^3)

date	time (d)	Point1	Point2	Point3	Point4
April. 15	1	51	108	414	22
April. 16	2	176	384	540	59
April. 17	3	386	510	725	173
April. 20	6	570	950	985	320
April. 22	8	875	902	1200	306
April. 24	10	915	820	945	234
April. 27	13	776	608	656	194
April. 29	15	636	528	512	185
April. 30	16	520	468	382	152
May. 2	18	657	398	724	228
May. 3	19	766	380	846	344
May. 6	22	768	344	662	334
May. 7	23	685	454	572	170
May. 8	24	617	482	532	346
May. 9	25	612	466	556	388
May. 12	28	610	378	658	405
May. 13	29	610	354	622	395
May. 14	30	582	326	505	444
May. 15	31	548	308	451	370
May. 19	35	286	206	328	58

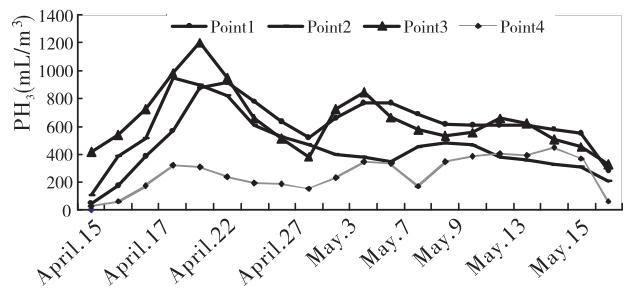

Fig. 2 Phosphine concentration of four monitoring points

Our results (Table 1 and Fig. 2) indicate that phosphine concentration reached a higher level on the second day after tablet application. The peak concentration occurred in about eight days. When the concentration at point 4 was less than 200 mL/m^3, the second 3 g/m^3 of tablets was added. Again, the third application (another 1 g/m^3 of tablets) was carried out when phosphine concentration began to decrease. The lower the dosage of tablets used, the lower the

peak concentration monitored. Among four monitored points for phosphine, point 4 always gave lowest concentration due to a fine slot existed on the wall near the point. Despite gas leakage due to the poor condition of the warehouse, phosphine concentration was maintained at >200 mL/m^3 for more than 35 days.

There were no active adults of susceptible or resistant *R. dominica* after 3 and 15 days, respectively. However, *C. ferrugineus* adults could be observed moving in the cages until day 30. There were no survivals in the culture sampled from grain mass and cages after 14 days. No insects were present in the grain ten months later when the sheeting was removed. The results indicate that very highly resistant strains can be controlled if a sufficient concentration of phosphine is maintained for a long enough exposure period. The appropriate exposure period can be accurately determined by observing the response of the insects infesting the grain mass.

3 Disscussion

The purpose of this work was to determine if a leaky warehouse could maintain phosphine concentrations long enough and at high enough concentrations to control resistant insects by supplementing phosphine dosing. Complete control of both the test insects and the natural infestations suggest that the fumigations may be successful against resistant strains prevalent in China. A weakness with this trial was that the test insect samples contained adult insects and probably some eggs and we have no evidence of other stages being present. However, further culture of insects in cages and samples from grain mass indicate that the trial methodology was successful in achieving complete control of the test resistant insects.

Several authors have characterised high resistance in *R. dominica* and Dr. Collins has summarised their results at doses measured in these trials. At 0.2, 0.3, 0.5 and 0.7 g/m^3, 10, (Collins et al. 2005), 8 (Collins et al. 2005, Rajendran and Gunasekaran 2002), 6 – >9, (Collins et al. 2005, Liang et al., Price and Mills 1988) and >7 (Sayaboc et al. 1998, Rajendran and Gunasekaran 2002) days are required for complete control, respectively. Fewer data are available for resistant strains of other species. Resistant *S. oryzae* populations can be very difficult to control: all life stages of a strain from Bangladesh were controlled in 10 days at 0.47 g/m^3 (Price and Mills 1988), and a resistant strain from Australia was controlled in 10, 7 and 5 days at 0.3, 0.5 and 0.7 g/m^3 (Daglish et al. 2002), respectively, while Nayak et al. (2003) report that it took 11 days to control a resistant strain from China at 0.3 g/m^3. Price and Mills (1988) achieved 98.8% control of resistant *C. ferrugineus* at 0.47 g/m^3 in 10 days. *L. entomophila* requires 7 days at 0.3 g/m^3 (Nayak et al. 2003) or 9 days at 0.27 g/m^3 Pike (1994) for complete control. Therefore, based on theworst – case' of published reports, phosphine doses required to control all documented resistant strains are: 0.2 g/m^3 for >10 days, 0.3 g/m^3 for 10 days, 0.5 g/m^3 for 10 and 0.7 g/m^3 for >7 days. This trial provided that a high enough concentration for long enough is indeed necessary to successfully control higher level of resistant strains, especially for rusty grain beetle.

In conclusion, insects with different levels of resistance to phosphine require different concentration and exposure times for complete control. An extraordinarily long time with higher concentration is necessary for successful fumigation of some insects resistant to phosphine such as rusty grain beetle. During fumigation, supplementary application of phosphine is a useful approach to maintain effectiveconcentration in a leaky warehouse.

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Studies on Development of Resistance in Different Strains of *Trogoderma granarium* (Everts) to Phosphine Fumigation in Southern Punjab, Pakistan

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Abstract: The study was conducted to determine the response of different strains of *Trogoderma granarium* (Everts) to phosphine concentrations. Thepest was exposed to 200, 400, 600 and 800 ppm concentrations of phosphine gas in glass jars under control conditions following CRD, replicated four times along with a control treatment. The observations regarding percent mortality of grubs of *T. granarium* were recorded after 1, 3, 5, 7 and 15 days of application of phosphine gas. The results revealed that maximum mortality of the pest was recorded to be 81.67 % in jars where 800 ppm concentration of the gas was applied 47.55, 61.42 and 70.33% mortality was observed by 200, 400 and 600ppm concentration. Shah Sadardin strain showed maximum mortaility (68.35%) followed by Tounsa Sharif strain (45.95) and D. G. Khan strain (43.60%).

Key words: phosphine, *Trogoderma granarium*, concentrations, exposure intervals.

Introduction

Grain storage is one of the most important tasks confronting the grain handling agencies and the stored grain entomologists of the world today, because of an admitted fact that post harvest losses of grains in Pakistan are 10% – 15% (Jilani, 1981). Main reasons of these losses are: lack of sanitary conditions in and around the storage system, leaky godowns, improper application of fumigants and non-availability of trained manpower in food handling agencies. There is no denying the fact that the biological agents are responsible for major part of the post harvest losses. Insect pests especially coleopterans are very important biological loss causing agents. The stored grain losses by these insect pests have been calculated to be 3.6 to 25.5 % (Irshad and Baloch, 1985). Ahmad (1984) reported that 6.75 million tones of wheat that remained in stores for a period of 6 to 12 months suffered for loss of 0.169 tones due to these insect pests. Among the coleopterous insect pests in storage systems, *Trogoderma granarium* (Everts) is considered to be the world's worst pest of stored grain (Christensen and Kaufmann, 1969). In Pakistan, it is a very destructive pest of wheat and other stored grains, particularly of the North-Western Dry Regions.

Phosphine fumigation is the principal and efficient method to control stored grain insect pests but lack of education and training in grain storage management, resulting in improper exposure periods, gas leakage and sub-lethal concentration make this tool in-effective. The substandard techniques of phosphine fumigation have led to the development of phosphine resistance in major insect pests of stored grains (Mills, 1983; Taylor, 1989; Mills *et at*, 1990). Borah and Chahal (1979) reported that phosphine failed to control khapra beetle, *Trogoderma granarium* in warehouses in India. Tyler *et al.* (1983) documentedthe development of resistance in stored grain insects pests againstuseful insecticide, phosphine, in warehouses in Bangladesh. Appreciably high resistance was recorded in *Trogoderma granarium* strains collected from Punjab and Sindh (Alam *et al.*, 1999).

The present project has been carried out to determine mortality in different strains of *T. granarium* collected from D. G. khan District at different concentrations (200 ppm, 400 ppm, 600 ppm, 800 ppm and 0 ppm as control) of phosphine at different exposure periods. The results of present study provide useful information for the effective management of T. *granarium*.

Materials and Methods

The research project was conducted in the Grain Research, Training and Storage

Management Cell, Department of Agri. Entomology, University of Agriculture, Faisalabad, Pakistan. Large number of live adults and grubs of *T. granarium* were collected from various in-

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fested godowns in Dera Ghazi Khan, district of punjab. Three strains viz., D. G. Khan city, Shahsadardin and Tehsil Tounsa sharif were used for experimentation. These strains were kept in wide mouth glass jars covered with muslin cloth. Each strain was labelled and reared separately in the laboratory for two months. The adults of *T. granarium* of each strain were sieved and placed in breeding containers (wide mouth glass jars) in the medium ofuninfested wheat grains. The jars were placed in Gallenhamp incubator at 35 ± 1℃ and 65% ± 5% relative humidity and were kept there for three days. The insects were removed from the above jars and transferred to new containers. The media in which the adults of the test insect were retained initially for three days contained sufficient number of eggs. The larvae first appeared after four days with the majority appearing after six days. Progenies removed from these stocks were supposed to be of same age. It was further kept for another period of five days. The larvae were allowed to flourish under the optimal development conditions, i. e. 35 ± 1℃ and 65% ± 5% relative humidity.

For phosphine gas generation, a funnel tied with thread was hanged over a cylinder filled with 5% sulphuric acid solution. A tablet of aluminium phosphide (Agtoxin) wrapped in muslin cloth was dropped in the solution in such a away that it went directly under the funnel in the solution. This funnel was tied with the thread below the burrete. The air in the burrete was sucked out with the help of a syringe through rubber septum, until the solution rose in to the burrete up to the mark. Phosphine gas liberated and accumulated in the burrete over acidified water-When it was filled with gas, 5 mL of gas was sucked out with the help of an air tight syringe and was injected into the sealed glass jar of known volume (1125 cc); 50 mL of gas sample from the jar-was taken and injected in to the Harris conductivity meter (Harris, 1986) for measuring gas concentration.

100 grams of wheat grains were taken in each glass jar and amount of phosphine for each glass jar was calculated by using following formula:

Phosphine required = Concentration × Volume of jar × 836.81

A 500μL (micro-litre) syringe was used to inject phosphine concentration into the jars. For each exposure period (1, 3, 7 and 15 days) four concentrations of phosphine were injected into the jars. A complete test (i. e. for single concentration) had four repeats of 25 grubs for each exposure period with one control. Data were collected after each exposure period and corrected mortality calculated by using Abbot's formula as recommended Busvine (1980). Finally the data were analyzed statistically.

Results and Discussion

The results revealed that maximum mortality of the pest was recorded to be 81.67 % in jars where 800 ppm concentration of the gas was applied 47.55%, 61.42% and 70.33% mortality was observed by 200, 400 and 600ppm concentration. Shah Sadardin strain showed maximum mortaility (68.35%) followed by Tounsa Sharif strain (45.95) and D. G. Khan strain (43.60%). It was observed that mortality increased as the concentration of the gas was increased with the increase at all exposure periods as depicted by Table1

Table 1. Comparison of mean values regarding percent mortality of different strains of *Trogoderma granarium* (Everts) against different concentrations of phosphine gas at various exposure periods.

CONCENTRA TIONS (ppm)	POST TREATMENT MORTALITY (%)				AVERAGE
	1 DAY	3 DAYS	7 DAYS	15 DAYS	
0	0.00 m	0.00 m	3.33 1m	5.331	2.17e
200	18.67 k	39.33 h	60.67 f	71.67 e	47.58d
400	22.67 j	60.33 f	75.67 d	87.00 c	61.42 c
600	29.00 i	73.33 de	84.00 c	95.00 b	70.33 b
800	42.67 g	86.67 c	97.33 ab	100.0 a	81.67 a
Average	22.60 d	51.93 c	64.20 b	71.80 a	

Means sharing similar letters are not significantly different by DMR Test at $P = 0.05$

Table 4. b. Comparison of mean values regarding percent mortality of different strains of *Trogoderma granarium* (Everts) in phosphine gas treatments at various exposure periods.

Strains	Post treatment mortality (%)				AVERAGE
	1 DAY	3 DAYS	7 DAYS	15 DAYS	
D. G. Khan	5.801	42.40 h	59.00 e	67.20 d	43.60 c
Tounsa Sharif	7.401	46.40 g	60.20 e	69.80 c	45.95 b
Shah Sadardin	54.60 f	67.00 d	73.40 b	78.40 a	68.35 a
Average	22.60 d	51.93 c	64.20 b	71.80 a	

Means sharing similar letters are not significantly different by DMR Test at $P = 0.05$.

Table 3. LC_{50} values(ppm) of phosphine for different strains of *T. granarium* (Everts) for various exposure periods.

Strains	1 day	3 days	7 days	15 days
D. G. Khan	13058.06	422.567	238.025	185.759
Taunsa Sharif	1491.33	361.828	159.324	91.551
Shah Sadardin	195.721	70.283	65.601	52.747

The results revealed that maximum mortality(100 percent) of the pest was observed at 15 days exposure period under 800 ppm concentration. The results indicated that maximum mortality of the pest was observed at the maximum concentration of the gas with maximum exposure period. All the concentrations and exposure periods differed significantly in response to pest mortality.

Compared with those of Winks et al. (1980) who reported that period of exposure over 10 days proved lethal for stored grain pests. Similarly Borah and Chahal (1979) reported that *T. granarium* had become resistant to phosphine in certain areas. The present findings are also in conformity with those of El – lakwah et al(1989) who reported that the effectiveness of phosphine was reduced for short exposures and increased for longer exposure. A general statement was given by Shroff and Dhuri(1991) that phosphine was an ideal fumigant for the control of storage insect pests. The present findings are in partial agreement with those of Aheer et al. (1993) who tested various fumigants under control conditions @ 30 tablets per 28.3 m^3 and reported 100 percent mortality. The present findings cannot be compared with those of Winks et al. (1980), Ranjendaran(1982), Bell et al. (1984), Desmarchelier and Wohlgemuth (1984), Reichmuth(1985), Bell(1985), Price and Mills (1988), Ahmad (1989), Ali et al. (1989), Udeaan (1990), Ahmad et al. (1993), Molinari et al. (1993), Muller (1994), Sharma and Karla (1995), Bell and Wilson 1995), Gibe et al. (1997), Sharma and Karla(1998), Sarfraz et al. (2000), Rajendran et al. (2001), Mordkovich (2002), Gooch (2002), Roels et al. (2002), Dalish et al. (2003) and Qin et al. (2003) because of differences in their materials and methods.

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SESSION 9

ACHIEVEMENTS OF CA AND FUMIGATION AND DEVELOPMENT TRENDS

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0901

Recent Developments in Hermetic Storage Technology Using Sealed Flexible Storage Structures

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Abstract: Restrictions due to the adverse effects of pesticide residues in food and the environment have resulted in the imposition of strict limitations on pesticide registration by regulatory agencies. Consumer demand for chemical-free and insect contamination-free products has increased the attention for the application of non-residue organic technologies for the protection of stored grain. Among the new gaseous application technologies that have successfully replaced fumigants are the production of modified atmospheres (MAs) through the use of biogenerated MAs, for insect control and for quality preservation of seeds, stored paddy, polished rice, wheat, pulses, coffee beans, cocoa beans, and high moisture corn. Biogeneration takes advantage of the atmospheric gas composition produced by the respiratory metabolism of the biological agents of the grain bulk which prevents insect development and suppresses microflora activity. Sufficiently sealed structures enable insects and other aerobic organisms in the commodity, and/or the commodity itself, to generate an MA by reducing the O_2 and increasing the CO_2 concentrations. Freshly harvested high moisture corn has been successfully stored under hermetic conditions, maintaining its quality prior to subsequent drying or processing into feeds or ethanol. A recent development is the use of MAs in a low-pressure environment or use of CO_2 to accelerate the process. These niche applications of MAs have resulted in very promising treatments with market acceptability. As discussed and illustrated, hermetic storage for dry commodities is now used in 31 countries for storage of a number of important commodities. The growing number of types of hermetic containers for various applications is documented. This ranges from small portable containers of 60 kg to 1 tonne, called SuperGrainbags™, to a series of large flexible storage structures, called Cocoons™, TranSafeliners™ and Bunkers™, ranging from 5 tonne to 30 000 tonne capacity. Economic analysis as reported by studies and field trials is provided for representative applications, including rice and cocoa.

Key words: hermetic, controlled atmosphere, modified atmosphere, pesticide-free, Cocoons, SuperGrainbags, TranSafeliner, molds, insects, grain storage, seed storage, long-term storage, fumigation, disinfestations, organic, V - HF, G - HF.

Introduction

Hermetic storage technology has emerged as a significant alternative to other methods of storage that provide means of commodity protection from insects and molds, especially in hot and humid climates. This technology, also termed sealed storage, airtight storage, or assisted hermetic storage is a form of biogenerated modified atmosphere. Hermetic storage is based on the principle of generation of an oxygen-depleted, carbon dioxide-enriched interstitial atmosphere caused by the respiration of the living organisms in the ecological system of a sealed storage[1,2,3]. Hermetic storage generates a modified atmosphere in an environmentally safe and sustainable manner that eliminates the need for chemical treatments or fumigants.

Modern hermetic storage results from important scientific work carried out at the Agricultural Research Organization in Israel[4]. Only in the last several years has hermetic storage emerged as an important, widely used alternative method of post harvest storage. This is due, in part, to increasing concerns about the use of residual pesticides which endanger the fumigator, the environment, and the consumer, as well as to growing field experience.

1 Applications of Hermetic Storage

The applications for which hermetic technology has been most widely accepted are:

· Long-term storage of cereal grains, primarily rice, corn, barley, pulses, and wheat.

· Long-term storage of a variety of seeds to preserve germination potential and vigor.

· Quality preservation of high - value commodities, such as cocoa and coffee.

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3. GrainPro Philippines Inc. Subic Bay Freeport Zone 2222, Philippines

1.1 Low Oxygen Modified Atmosphere to Eliminate Infestations

A sufficiently low oxygen and elevated CO_2 atmosphere is created through a natural metabolic process based on insect respiration and/or the respiration of other microorganisms within a sealed storage system. When a level of less than 2% oxygen is reached mortality of all insect stages is achieved rapidly[1]. When needed, this process can be accelerated by the use of "Vacuum – Hermetic Fumigation" (V – HF) or "Gas – Hermetic Fumigation" (G – HF), using injected CO_2.

1.2 Prevention of Ingress of Water vapor

By preventing the entry of additional water vapor into a hermetically sealed container, dried commodities are protected from humidity. This prevents a rise in their moisture content beyond their critical moisture level, thus overcoming the limitations that make conventional silos unsatisfactory in tropical climates[5].

1.3 Protection from Rodents

Properly designed hermetic storage, because of its slippery surface, when kept taut, is highly rodent resistant. Rodent resistance is provided by using tough, slippery materials such as flexible PVC (typically 0.83 mm thick), and tensioning straps, which prevent rodents from getting a tooth hold[6,7,8].

1.4 Preservation of Germination and vigor in Stored Seeds

The International Rice Research Institute (IRRI) in the Philippines has extensively tested and recommends the use of hermetic storage for rice seeds and paddy[9]. A PhilRice study compared: hermetic storage (HS), air-conditioned storage (ACRS), and cold room storage (CRS). All performed substantially the same. The study concluded that for storage for nine months or more, hermetic storage provided better performance as compared to previously used techniques but at a lower overall cost, and that after 3 months conventional storage (CTRL) was inadequate to meet the certified seed 85% germination requirement[10] (Tables 1 and 2).

1.5 Quality Preservation Aroma and flavor

Sealed hermetic containers preserve the quality of aromatic dried plant material such as spices. Hermetic storage traps the aromatic volatiles that are emitted by such commodities and maintains the aroma and flavor of such commodities as coriander, turmeric tuber, chili pepper, coffee (Fig. 1), cocoa and basmati rice[1,11].

Table 1. Mean percent germination rate of Mestizo 1 (PSB Rc72H) hybrid paddy seeds stored under different storage technologies and durations

	Storage duration (months)			
Method	0	3	6	9
HS	1.13	3.82	3.22	8.42
CRS	0.96	0.38	0.64	0.56
ACRS	0.84	0.76	8.58	38.27
CTRL	0.35	16.95	79.40	147.84

Table 2. Mean percent adult insect density per kg sample of Mestizo 1 hybrid paddy seeds stored under different storage technologies and durations.

	Storage duration (months)			
Method	0	3	6	9
HS	1.13	3.82	3.22	8.42
CRS	0.96	0.38	0.64	0.56
ACRS	0.84	0.76	8.58	38.27
CTRL	0.35	16.95	79.40	147.84

2 Examples of Current Large Scale Applications

2.1 Overview

Hermetic storage of maize, as well as sorghum, beans, and rice is currently used on a large scale in a number of countries such as Ghana, Philippines, Rwanda, South Sudan and Sri Lanka. Cocoons™ are used for long-term storage in Rwanda (Figures 2 and 3). A Rwandan

Fig. 1 Storing high quality coffee using GrainShade to prevent moisture migration and preserving its quality.

Ministry of Agriculture report on long-term corn storage concludes "after more than 30 months of

storage insects present in the grains were all dead and no re-infestation was recorded; grains remained identical in appearance and preserved their germination[12]."

Fig. 2 Corn storage in a Cocoon™ of 150 tonnes in the process of sealing, Rwanda, in 2007. (Courtesy of GrainPro Inc.)

2.2 Hermetic Storage of Rice

As a result of extensive studies at IRRI[9] and later by PhilRice[10], over the last 10 years, the benefits of storing both rice and rice seeds in hermetic storage are now well understood and in widespread use, particularly in Asia. The Cocoons shown in Figure 4 are used in a warehouse of the National Food Authority of the Philippines, to store rice paddy safely for up to one year. Hermetic storage applications for rice and/or rice seed are currently found in such countries as: Cambodia, East Timor, Indonesia, India, Pakistan, Sri Lanka, and Vietnam[13].

Multi-tonne storage containers called Cocoons are currently in use in sizes from 5 tonnes to 1000 tonnes capacity. IRRI itself has also adopted the use of the portable hermetic storage liners called SuperGrainbags™, now available with capacities of 10 to 1000 kg. SuperGrain Bags serve as liners for either polypropylene or jute outer bags (Figure 5). Recently, a hermetic liner for 20' and 40' shipping containers called TranSafeliner™ became available (Figure 6).

Fig. 3 Hermetic warehouse storage of corn, Rwanda, in 2007 (Courtesy of GrainPro Inc.)

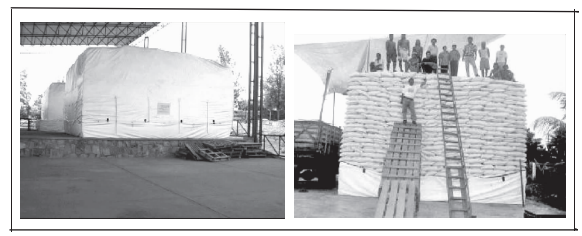

Fig. 4 Cocoons™ in the Philippines warehouse of National Food Authority

Fig. 5 Supergrain Bags™, filled with paddy seed. IRRI, Los Banios, Philippines

Fig. 6 TranSafeliner™ installed in 20 – ft. shipping container, 2008. (Courtesy GrainPro Philippines, Inc.)

2.3 Hermetic Storage of Corn

Cocoons are widely used in Rwanda, Ghana and the Philippines for storing both shelled and unshelled corn, typically in capacities ranging from 50 to 150 tonnes. Similar results were obtained for corn when stored in 60 kg capacity SuperGrain Bags. The large flexible hermetic storage units are generally used at the village level, but also as strategic reserves to prevent famine at the district level[1,13,14]. In 2007,

100,000 Super Grain Bags were delivered to Ghana for a variety of uses, including household use.

2.4 Medium and large Scale Storage of Wheat and Barley

Hermetic storage of wheat in "Bunkers" with capacities ranging from 10 000 to 30 000 tonnes was first introduced in the early 1990's, as shown in Fig. 7. Hermetic storage of wheat, stored at or below its critical moisture content of 12.5 %, provides storage without significant degradation of quality, including maintenance of baking qualities, for up to 4 years[15,16,17]. In Cyprus such Bunkers allowed quality preservation of barley for 3 years, with total losses of 0.66% to 0.98%, and with germination remaining above 88%[17].

Fig. 7 GrainPro Bunker™ storing wheat in Cyprus.

2.5 Hermetically Stored Pulses (beans)

Beans in storage are subject to invasive pests such as *Callosobruchus maculatus* and *C. chinensis*, which are controlled through hermetic storage In Rwanda and Ghana, storage of beans in Cocoons of 20 to 150 tonne capacity has permitted groups of farmers to hold their crops off the market while waiting for more favorable market prices[12].

2.6 Cocoa Storage under Tropical Conditions

Cocoa's critical moisture level of 6% at 30 ℃ is typically exceeded in storage, often at 7% – 8%. This leads to the growth of molds. When cocoa beans are stored hermetically, oxygen levels are typically depleted to less than 2% within as little as a week, thereby preventing the growth of molds (as well as protecting against insects and oxidation effects)[18]. In a 6-week trial by the Ghanaian Cocoa Board, three Cocoons were loaded with cocoa. By the end of the trial, oxygen levels in all 3 Cocoons had reached 0% and complete insect mortality was observed.

2.7 Coffee Quality Preservation

Field data from Costa Rica shows that preventing the penetration of external humidity alone has proved sufficient to protect coffee bean quality for up to 9-months[11]. Coffee is now stored commercially in portable SuperGrainbags, or in larger Cocoons for storage to preserve quality, and also, for long transit – time shipments in shipping containers without refrigeration[19], using SuperGrainbags, or TranSafeliners™. Hermetic coffee storage of green coffee beans is now practiced in Costa Rica, East Timor, Ethiopia, Jamaica, Hawaii, Peru, and the continental United States. A recent U.S. scientific study of coffee and its processing effects concludes, "overall it appears that under standard warehouse conditions, long – term storage in GrainPro [hermetic storage], compared to jute, may preserve coffee much better, leading to moisture content in the desired range and ultimately to better cup scores"[20]

3 Economics of Hermetic Storage

Studies performed by PhilRice[10] compared four forms of seed storage: unprotected control stored in a warehouse in bags (CTRL), hermetic storage (HS), cold room storage (CRS), and air-conditioned storage (ACRS). The report calculated the total storage cost of the three technologies meeting the certified seed 85% germination threshold, and of the unprotected control (CTRL): CTRL $ 2.50/20 kg bag; HS $ 2.52/20 kg bag; ACRS $ 2.63/20 kg bag; CRS $ 4.20/20 kg bag. By nine months, all three remaining methods provided similar and adequate germination rates, but CRS and HS provided the lowest insect count, and by month 6, hermetic storage provided the lowest total cost (Table 3).

Field experience in Africa has shown that Cocoons are being used successfully where previous storage attempts using metal or concrete silos failed[5]. In the case of a high value crop such as cocoa, weight loss of 1% to 2% per month during six-month storage periods has been due to the damage caused by insects, while in hermetic storage no weight loss is observed[18]. The cost of hermetic storage using Cocoons, ranges from $ 20 per tonne to $ 80 per tonne, with a useful life of 10 – 15 years, resulting in a per year depreciation of $ 1.33 to $ 8/tonne/year. The value of storing crops safely for months after harvest to take advantage

of much higher prices is illustrated in a study performed in Rwanda on beans, sorghum and corn, which resulted in an average payback of 97 days[21].

Table 3. Cost comparison, in Philippines of using four storage methods for preserving Mestizo 1 (PSB Rc72H) hybrid paddy seeds (Using $1 = 50 pesos)

Particulars	Storage Period (Months)							
	Three Months				Six Months			
	CTRL	HS	CRS	ACRS	CTRL	HS	CRS	ACRS
Investment Cost ($ US)	82,250	1,744	12,820	16,230	82,250	1,744	12,820	16,230
Operating Expenses								
Fixed Cost	18,095	488	2,820	3,570	18,095	488	2,820	3570
Variable Cost	6,896	16	728	250	12992	16	1.376	379
Total Operating Expenses	24991	504	3.548	3,820	31,086	504	4,196	3,950
Capacity, # of Bags	10,000	200	1,000	1,500	10,000	200	1,000	1,500
Cost per Bag, ($ US)	2.50	2.52	3.55	2.55	3.11	2.52	4.20	2.63

CTRL = unprotected control stored conventionally in a warehouse in bags; HS = hermetic storage; CRS = cold room storage; and ACRS = air – conditioned storage[10].

4 Conclusions

Hermetic storage is a sustainable, cost effective, user-friendly and environmentally benign technology that renders post-harvest use of pesticide, fumigants, and climate control unnecessary. The technology has already been adapted for the protection of many different commodities in quantities ranging from that of conventional grain bag size to many thousands of tonnes. Applications of hermetic storage are very likely to expand even more rapidly in the future, as the available forms of hermetic storage continue to increase and more users experience and understand the advantages of this "green" technology.

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0902

The Fumigation Bioactivities of Three Kinds of Plant Extracts on Four Species of Important Stored-grain Insects

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Abstract: Plant extracts were extracted by Soxhlet extractions with anhydrous diethyl ether from *Citrus suavissima* Tanaka, *Ailanthus altissima* L. and *Capsicum frutescensp* L., and their fumigation bioactivities on *Sitophilus oryzae*, *Oryzaephilus surinamensis*, *Tribolium castaneum* adults and *Liposcelis paeta* nymphae at a specific concentration of 1∶15 of plant extract : acetone by volume were detailedly investigated in the paper. The results showed that all of the plant extracts have significant fumigant activities on *O. surinamensis* adults and weak fumigant activities on *Sitophilus oryzae*, *Tribolium castaneum* adults and *Liposcelis paeta* nymphae. The highest corrected mortality of *O. surinamensis* adults reached more than 99%.

Key words: plant extracts, stored – grain insects, fumigation bioactivities

Introduction

The widespread and intensive use of synthetic insecticides for the control of stored-grain insects has led to serious problems including insecticide resistance, poisoning of workers, rising cost of production, and reduction of natural enemies in stored grain ecosystems. Development and implementation of alternative control strategies and integrated pest management systems have recently been considered to be the only solution to combat this increasing insecticide-resistant insect pests[1-3]. In this regard, plant-based insecticides may provide potential alternatives to currently used insect-control agents because they constitute a rich natural source of bioactive chemicals with complicated action mechanism, to which the insect pests are difficult to produce resistance, are readily biodegradable, often of low mammalian toxicity, and pose no or low danger to the environment if used in small amounts[4-6]. Many Chinese plants are potential sources of pesticides and have been shown to contain potent fumigation to many major stored-grain insects[6,7]. Base on the above background, the extracts from *Citrus suavissima* Tanaka, *Ailanthus altissima* L. and *Capsicum frutescensp* L. were tested for their potential fumigant activities against four major stored grain insects, *Sitophilus oryzae*, *Oryzaephilus surinamensis*, *Tribolium castaneum* adults and *Liposcelis paeta* nymphae.

Materials and Methods

1.1 Insects

All of the test insects were obtained from laboratory cultures maintained in the dark in incubators without exposure to any insecticide at 27 ±2℃ and 75% ±5% r. h. at the institute of stored product insects of Henan University of Technology. The food media used were washed, sterilized wheat with about 13.5% equilibrium moisture contentfor *S. oryzae*, wheat flour, rolled oats and yeast (6∶3∶1, w/w/w) for *O. surinamensis*, wheat flour and rolled oats (6∶1, w/w) for *T. confusum*, and wheat flour, degrease milk powder and yeast (1∶1∶1, w/w/w) for *L. paeta*. Healthy and consistant developmental insects were randomly chosen for tests.

1.2 Preparation of Plant Extracts

The *A. altissima* bark was collected in Henan, central China, October 2006, dried at room temperature, ground to powder, and screened by 60-eye sieve. 50 g of powder was extracted by Soxhlet extractions with 250 mL anhydrous diethyl ether until the distilled liquid was colorless. The solvent was evaporated under vacuum in a rotary evaporator. The essential oil was stored in airtight fuscous glassware in a refrigerator at 4 ℃, and evenly diluted with actone (analytical purity) at the proportion of 1∶15 (v/v) for the following tests.

The *Citrus suavissima* Tanaka bark and *Capsicum frutescensp* L. fruit were bought in market, and their extracts preparation were the

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same as the *A. altissima* bark extract.

1.3 Fumigant Activity

Fumigant activities to *S. oryzae*, *O. surinamensis*, *T. castaneum*, *L. paeta* were respectively carried out with 30 insects exposed in a 250mL flask sealed with a rubber stopper, holding 10g wheat. 1mL diluted plant extract was evenly applied to a Whatman No. 1 filter paper strip (7 cm × 9 cm), which was dried in air for 10 min and then fixed on the stopper by a staple at one end, and the equal amount of actone was applied alone as control. The stopper was tightly stuffed to make the filter paper suspending in the top, avoiding the filter paper contacting the flask wall. The flask was placed in the incubators at 27 ± 2 ℃ and 75% ± 5% r. h.. Five replicates were conducted. The number of dead insects was recorded after 4 d.

2 Results

The plant extracts from *C. suavissima*, *A. altissima* L. and *C. frutescensp* showed strong fumigant activities against *S. oryzae* and *O. surinamensis* adults, particularly the *A. altissima* L. bark extracts showed the most potent fumigant activities against *S. oryzae* and *O. surinamensis* adults, and the corrected percentage insect mortality reached 81.9% and 99.3% respectively. But all of the three plant extracts from *C. suavissima*, *A. altissima* L. and *C. frutescensp* showed slighty weak fumigant activities against *T. castaneum* adults and *L. paeta* nymphae (Table 1).

Table 1. The fumigant activities of the plant extracts against four species of important stored - grain insects (the corrected percentage insect mortality %)

plant extracts	*S. oryzae*	*O. surinamensis*	*T. castaneum*	*L. paeta*
A. altissima bark	81.9	99.3	5.3	44.2
C. frutescensp fruit	60.6	98.0	1.4	57.4
C. suavissima bark	56.4	96.0	2.6	19.7

3 Discussion

Many plant extracts and their constituents have been studied to possess potential as alternative compounds to currently used insect-control agents[6-8]. The plant extracts from *C. suavissima*, *A. altissima* L. and *C. frutescensp* also showed strong fumigant activity against *O. surinamensis* and *S. oryzae* adults. Further research on how to use the plant extracts as fumigants effectively for the control of insects in stored products is necessary. The activities of plant extracts and their pure constituent level along with structure-activity relationships against different developing stages, especially the eggs and pupae, of the major stored grain insects, are urgently required.

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0903

Achievements of Modified Atmospheres and Fumigation in Israel

S. Navarro

Abstract: Biogenerated modified atmospheres (MAs) successfully replaced fumigants for insect control and for quality preservation of stored products in Israel. CO_2 based MA was used in specially constructed rigid structures for the preservation of organic wheat. The rigid metal structure was equipped with pressure relief valve and gas purge system. Plastic structures suitable for long-term storage systems, as well as intermediate storage of grain in bags or in bulk have been developed and applied in Israel. These storage systems based on the hermetic principle are: 1) Bunker storage for conservation of large bulks of 10 000 to 15 000 tonnes; 2) Flexible silos supported by a weld-mesh frame of 50 – 1 000 tonnes capacity for storage of grain in bulk or in bags; 3) Liners for enclosing stacks of 10 – 50 tonnes capacity termed storage cubes, and designed for storage at the farmer-cooperative and small trader level.

Insect control and quality preservation of stored cocoa beans was achieved as a methyl bromide alternative, by employing the use of biogenerated MAs. A method of treatment based on hermetic storage against the large narcissus fly (*Merodon eques*) was developed. The MA is created due to the respiration of the bulbs that deplete the O_2, thus controlling the maggots of *M. eques*. This method fully replaced the methyl bromide for treatment of narcissus bulbs for quarantine applications. Nitidulid beetles were shown to be sensitive to low O_2 or high CO_2 concentrations for disinfestation of dry fruits. Special portable chambers made of flexible tarp-like sheeting provide the benefit of treatment in any location with low pressure treatments. Under vacuum, the chamber wall shrinks over the commodity indicating retention of vacuum. Uses of these flexible chambers for narcissus bulbs, dry fruits or museum artifacts are in current use. For the disinfestation of dates, ethyl formate is being successfully tested to replace methyl bromide.

Key words: postharvest systems, grain storage, dates, narcissus bulbs, methyl bromide alternatives, hermetic storage, modified atmospheres, nitidulid beetles, ethyl formate.

Introduction

The objective of modified atmosphere (MA) treatment is to attain a composition of atmospheric gases rich in CO_2 and low in O_2, or a combination of these two gases at normal or altered atmospheric pressure within the treatment enclosure, for the exposure time necessary to control the storage pests or to protect the quality of the stored product. MA offers an alternative that is safe and environmentally benign, to the use of conventional residueproducing chemical fumigants for controlling insect pests attacking stored grain, oilseeds, processed commodities and packaged foods. Pioneering modern MA storage[1,2,3,4,5] have demonstrated the broad use of safe, pesticide-free MA and hermetic storage suitable for many commodities and seeds, particularly in hot, humid climates. In this paper, are reported the development work and achievements that have resulted in such technologies, which are currently applied in Israel.

Types of Structures Used For MAs

Rigid Structures

Existing silos were modified to provide a high degree of hermetic seal for the application of MA using CO_2[6]. Others were purposely constructed for the application of MA using CO_2 from pressurized cylinders. The gastight metal silos were used for organic grain for which conventional fumigation was not acceptable. These silos were equipped with specially designed pressure relief valves and gas expansion chambers to permit high velocity gas purge[6] (Fig. 1).

Flexible Structures

In Israel, the manufacture of PVC liners that conform to required specifications of resistance to adverse climatic conditions, gas-permeability, and physical properties, enabled the development of three storage systems based on the hermetic principle. These are:

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– Bunker storage for conservation of large bulks of 10 000 to 15 000 tonnes capacity[7,8].

– Flexible silos supported by a weld mesh frame of 50 – 1 000 tonnes capacity for storage of grain in bulk or in bags[9,10].

– Liners for enclosing stacks of 10 – 50 tonnes capacity Volcani cubes termed also GrainPro Cocoons™, and designed for storage at the farmer-cooperative and small trader level[11]. These structures are in current use for capacities of up to 300 tonnes for bagged storage of cereals.

The problem of applying present-day technology to provide hermetic storage for subsistence farmers lies in the need to provide an easily sealable low-cost container of 50 – 100 kg capacity. The most recent attempt to address this problem has been through the construction of a small granary for use by small scale farmers, suitable to store up to 1 000 kg, termed GrainSafe™[12]. This granary was equipped with an upper collapsible sleeve for loading and a lower collapsible sleeve for unloading. The hermetic flexible bag was inserted into a rigid sheath surrounding the vertical sides of the hermetic bag (Fig. 2). An additional development has been the use of hermetic storage bags called SuperGrainBags™, designed to hold 50 kg of paddy or corn. These gastight liners are now available with capacities of 1 000 kg. SuperGrainbags™ serve as gastight liners for outer bags made of either polypropylene or jute.

Experience Gained Using Flexible Liners

Our accumulated experience of hermetic storage using several types of flexible liners for above – ground storage, in – the – open, under tropical and subtropical conditions[7,8,9,10,11,13,14], is summarized in the following observations:

Structural Durability

The use of PVC-based sheeting without mesh reinforcement produces a material of suitable strength and elasticity for storing grain. This material was formulated to have a high resistance to solar UV irradiation. Rodents find it difficult to gain a tooth-hold on the smooth surface. This has been corroborated by laboratory studies using wild-caught roof rats and house mice. Liners have been used continuously for over 10 years, and though they have lost some plasticity, permeability to gases decreases as the plasticizers evaporate. This characteristic renders the liners more effective with time in retaining gas concentrations.

Moisture Migration

Diurnal temperature fluctuations, accentuated by solar radiation on liners, followed by rapid cooling at night, cause successive moistening and drying cycles at the upper grain surface. This may result in gradual moisture accumulation, particularly during the transient seasons between summer and winter when temperature fluctuations are greatest. The result is that initially dry grain may rise to above critical moisture levels enabling limited microfloral spoilage to occur. For bunkers of 12 000 to 15 000 tonnes capacity, the condensation phenomenon has been eliminated by leveling the peaked apex (with a ridge of less than 2 m) to a slightly convex, wide apex of bunker cross-section (with a ridge of more than 6 m) which is sufficient to permit rain-water run-off[5,15]. For dry grain kept in "cocoons" in subtropical climates, moisture migration is not a pronounced phenomenon. However, for storage in the tropics, moisture migration is more pronounced because the initial grain moisture is closer to its critical level. Moisture migration has been solved by placing a reflective cover over the cocoons (Fig. 3).

Generation and Application of MA

Supply of Gases from Tankers

When the target MA gas composition is < 1% O_2 or high CO_2 concentration, a commonly used method is to supply N_2 or CO_2 from pressurized cylinders or tankers. For small-scale applications of up to 50 tonnes capacity containers, pressurized cylinders are sufficient (Fig. 4). For largescale application of N_2 or CO_2, vaporizers are essential.

Exothermic Gas Generators

For on-site generation of MAs by combustion of hydrocarbon fuel to produce a low O_2 atmosphere containing CO_2, commercial installations termed exothermic gas generators or gas burners are available. Therefore, their use in the grain industry requires several adaptations, like adjusting the equipment to obtain an O_2 level of < 1%; utilization to full advantage of 13% – 15% CO_2; and removal of excessive humidity from the generated atmosphere. Full-scale field trials using catalytic burners[16] to provide a low O_2 gas mixture have proved successful.

Biogeneration of MAs

A form of biogeneration of MA is hermetic storage. A high level of gastightness is required for a structure to be suitable for hermetic stor-

age of dry grain. Experience has shown that hermetic storage in flexible plastic storage systems, under subtropical climatic conditions, continues to offer an excellent solution, provided there is a certain degree of tolerance to the presence of insects at critical areas in the storage structure (at the grain surface, where moisture condensation is likely to occur). At the end of long-term hermetic storage, when unloaded grain is destined for immediate consumption, the risk of spreading insect infestation was found to be negligible. Insect control success due to the hermetic storage treatments is comparable to conventional fumigants (over 99.9% kill), and losses due to insect activity are minimal (0.15% loss in weight for a storage period of 15 months)[7].

Low Pressures (Vacuum treatment)

The introduction of flexible transportable sealed chambers made of welded PVC liners has opened new opportunities to implement low pressures (vacuum) as a competitive and affordable treatment to control storage insect pests[17]. Under vacuum, these chambers shrink tightly over the periphery of the commodity. The system is sealed by an airtight zipper and is able to retain vacuum. At the base of the chamber there exists an inlet hose which enables connection to a vacuum pump to create the prerequisite low pressure. Our studies showed that it is not a practical approach to attempt to hold a pressure below 45 mm Hg because of the energy required for prolonged operation of the pump. Conversely, pressures above 55 mm Hg prolong the time to achieve kill. In contrast to fumigations where schedules are provided by defining dosages to be applied for a predetermined time, at a set temperature range, low pressure treatment schedules must be presented as exposure times at both, a temperature range and a relative humidity that is in equilibrium with the commodity moisture content.

Achievements on Specific Target Pests and Method of Control and Preservation

Insect control and preservation of organic cereals, pulses, nuts and flours using vacuum

Ten durable commodities; corn, corn chips, garden peas, chick peas, wheat, wheat flour, rice, sun flowers seeds and semolina, were exposed to five days vacuum treatment[17]. Corn, garden peas, chick peas and sun flower seeds were stored in 1 – tonne capacity bigbags. Wheat, rice and semolina were stored in 50 kg bags and corn chips and wheat flour were stored in 25 kg bags loaded on wooden pallets. In all tested commodities the treated product was well preserved and in cases where initial infestation was detected, complete mortality of insects was observed. The advantage of this treatment is that no toxic chemicals are employed. In comparison with phosphine, exposure times to provide kill are comparable and the exposure time of five days falls within a range suitable for quarantine treatments where no rapid treatment is essential. Where the commodity can be placed in flexible liners, and packed in a manner that can withstand the low pressure, vacuum treatment can provide an appropriate solution. The transportable system was made of flexible PVC, which has been in use commercially for hermetic storage of grain and other commodities to control insect disinfestation by naturally obtained modified atmospheres[18]. For the disinfestation of durable commodities, these flexible storage containers can be considered for the application of vacuum as an alternative to treatments with methyl bromide and other toxic fumigants.

Disinfestation of Dates Using MA

As a potential alternative to methyl bromide fumigation, the influence of different MAs in causing emigration of *Carpophilus* spp. larvae from dates was compared with that of methyl bromide[11,12]. A concentration of 35% CO_2 was found to cause an emigration similar to methyl bromide. This method has been used for several years in the largest packing house in Israel. Laboratory experiments were carried out to investigate the influence of different modified atmospheres (20% CO_2 in air or 2.8% O_2 in N_2), low pressures alone or methyl bromide alone in causing nitidulid beetles to emigrate from infested dates[12]. At 4 hours exposure and at 26℃, the treatments that had a marked influence in causing insects to abandon the infested dates were: a low pressure of 100 mm Hg, and 2.8% O_2 in N_2, both of which caused over 80% of the initial insect populations to emigrate from the fruit. In addition to causing emigration of nitidulid beetles from dates, CO_2 atmospheres were found effective for long term preservation of the dates[19,20].

Control the Large Narcissus Fly Using Biogenerated Atmospheres

The large narcissus fly *Merodon eques* F. attacks narcissus bulbs and also bulbs of other geophytes. This species has not been recorded

in the USA; and is therefore included within quarantine requirements that demand total mortality prior to export to the USA[21]. Fumigation with methyl bromide (MB) has been used to eliminate narcissus fly infestation in flower bulbs due to its rapid killing time (4 hours). However, MB is also known to cause damage to the bulbs. Therefore, our initial trials were aimed at finding alternative treatments to MB so as to prevent phytotoxicity. These trials were carried out in flexible plastic chambers that replaced the previously used rigid fumigation chambers (rooms).

In experimental procedures, due to the respiration of the newly harvested narcissus bulbs, there was an extremely rapid depletion of O_2 within the sealed gastight enclosure where the bulbs were stored[22]. This procedure also revealed that significant anoxia was achieved within less than 20 hours (less than 0.1% O_2 and about 15% CO_2) during treatment at 28℃ to 30℃ and the possibility arose of using this method alone as a control measure[23]. This use of bio – generated modified atmosphere utilizing the bulb respiration alone was adopted by farmers as an alternative to methyl bromide; offering a practical solution in specially designed flexible treatment chambers (Fig. 5)[24].

Quality Preservation of Stored Cocoa Beans Using Biogenerated Atmospheres

Intermediate moisture contents (at equilibrium air relative humidities of 65% to 75%) of stored commodities are inevitable in tropical climates due to the difficulties in maintaining safe moisture contents for long-term storage. Under hermetic conditions, stored commodities with intermediate moisture contents generate modified atmospheres due to the respiration of the microflora and the commodity itself. Data was shown for insect control and for quality preservation of stored cocoa beans by employing a novel approach through the use of biogenerated modified atmospheres as a methyl bromide alternative. The respiration rates of fermented cocoa beans at equilibrium relative humidities of 73% at 26℃ in hermetically sealed containers depleted the oxygen concentration to < 1% and increased the carbon dioxide concentration to 23% within six days. Laboratory studies in Israel were implemented under field conditions in a cocoa bean storage facility. A hermetically sealed flexible structure containing 6.7 tonnes of cocoa beans at an initial moisture content of 7.3% (70% equilibrium R. H.) was monitored for oxygen concentration and quality parameters of the beans[25] (Fig. 6). The measurements showed a decrease in oxygen concentration to 0.3% after 5.5 days. No insects survived the oxygen depleted biogenerated atmosphere. These encouraging results reveal the possibility of utilizing biogenerated atmospheres in integrated pest management (IPM) for quality preservation (by preventing the development of FFA, molds, and mycotoxins), and insect control of cocoa pests.

Preservation of High Moisture Corn Using Biogenerated Atmospheres

Under humid and warm conditions harvested grains are susceptible to molding and rapid deterioration. Therefore, they should be dried to safe moisture levels that inhibit the activity of microorganisms. Drying to these moisture levels is not economical for farmers in developing countries. Laboratory studies were carried out on the effect of various moisture contents on the quality of corn grains in self-regulated modified atmospheres during hermetic storage[26]. Laboratory results experiments indicated that corn at the tested moisture levels can be stored satisfactorily under sealed conditions in which self-regulated atmospheres provide protection against microflora damage. Further large scale trials were carried out to evaluate the economic feasibility of storing high moisture corn. Shelled corn of 26% moisture content was stored in a Cocoon under hermetic conditions for 96 days to demonstrate the effectiveness of maintaining its quality prior to subsequent drying or processing into feeds or ethanol. The initial oxygen concentration dropped within one day and remained at an average of 0.54% throughout the storage period. No significant change in starch content was observed throughout the storage period. Corn in the control bags deteriorated after three days and temperature increased to 55℃. The high moisture corn in the Cocoon™ initially had 59 ppb of aflatoxin which increased to 90 ppb after one week of storage and remained at that level for 96 days. Feeding trials indicated that the corn from hermetic storage was palatable to cows and swine. Results of the study indicate that wet corn can be safely stored for extended periods of time without significant increase in aflatoxin, and without significant changes in starch.

Disinfestation of Dates Using Ethyl Formate

Laboratory fumigation tests were carried out using the gas mixture of CO_2/Ethyl formate

(83∶17 w/w) to control nitidulid beetles larvae. Mixed populations of *C. hemipterus* and *C. mutilatus* larvae obtained from the field were tested in an incubator at 30 ± 1℃. The effectiveness of CO_2/Ethyl formate in causing emigration of *Carpophilus spp*. larvae from artificial feeding sites was tested. Mortality at exposure to 420 mg/L resulted in complete kill and average disinfestation value of 69. 6% was recorded within 12 h exposure. Commercial scale pilot fumigation trials were carried out that yielded promising information on the disinfestation capacity of the gas mixture during short exposure times.

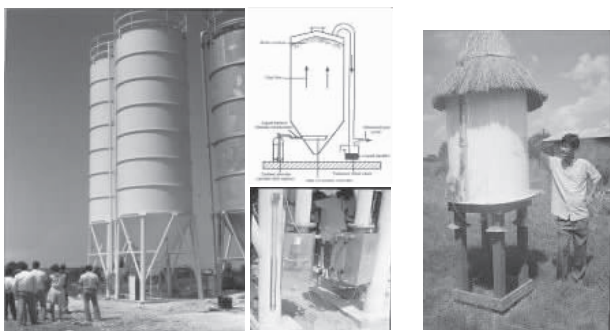

Fig. 1 Application of carbon dioxide based MA on a silo bin and the schematic presentation of the application process.

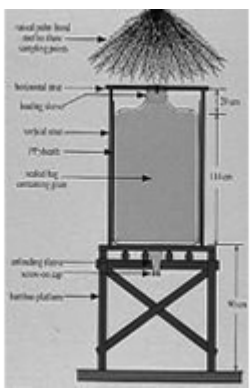

Fig. 2 Hermetic granary for use by small scale farmers, suitable to store up to 1,000 kg, termed GrainSafe.

Fig. 3 Moisture migration has been solved by placing a reflective cover over the cocoons.

Fig. 4 For small-scale applications of up to 50 tonnes capacity containers, pressurized cylinders are sufficient.

Fig. 5 Bio-generated modified atmosphere for the control of narcissus fly utilizing the bulb respiration alone in specially designed flexible treatment chambers.

Fig. 6 Bio-generated atmospheres for the control of cocoa beans insects utilizing respiration of cocoa beans in a hermetically sealed cocoon.

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0904

Research Progress on Modified Atmosphere Technologies in China

Fu Pengcheng and Tao Cheng

Abstract: We summarize here the progress on Modified Atmosphere (MA) technologies that have been made since the 1960's in China, including natural low-oxygen and artificial low-oxygen, application with deoxidizers, "double-low" and "three-low" storage, MA with carbon dioxide and nitrogen-enrichening, and sealing materials and technologies for grain storage. Research Progress on MA Technologies in China has vaulted into the ranks of the advanced countries in the world.

Key words: China, grain, modified atmosphere

Compared with conventional techniques of grain storage to prevent and control insect pests, inhibit growth of grain fungi, and postpone changes in grain quality, MA, which works through altering gas elements in well sealed granaries, is a more important and complicated technology for grain storage. In the last several years, many continuing studies have been conducted by the regional grain administration throughout China, and many application technologies have been developed and significant progress has been made. Research Institutes and Academies have solved the key technologies for MA application by brainstorming projects.

1 Research on Natural Low-Oxygen and Artificial Low-Oxygen

Oxygen concentration can be lowered and carbon dioxide concentration can be increased through natural respiration of grain and microbes. (1) natural low-oxygen. In 1974, experiments on natural low-oxygen for pests control in grain storage were made at a grain depot under the grain administration of Jia county, Zhejiang province. The study showed that rice weevil, flour beetles, and sawtoothed grain beetle were controlled in 48 hours because the concentration of oxygen was lower than 0.5%. When the moisture and temperature of the grain was higher, the rate at which oxygen concentration was lowered increased. Different grains and grain products had different rates at which oxygen was lowered. For example, wheat > japonica rice > Keng paddy > flour[1]。 (2) artificial low-oxygen. ① In 1972, an experiment on low-oxygen for grain storage was conducted by the Second grain depot under Nanchang Grain Corp., Jiangxi province. It showed that the concentration of oxygen had been lowered to 3% – 6% by the end of the experiment, that circulation of low-oxygen with carbon burning could be done easily and at low cost, and this resulted in good pest control[2]. ② In 1974, an experiment on deoxidation by microorganisms was conducted at the grain depot under the grain administration of Jia county, Zhejiang province. It showed that by cultivating *Saccharomyoes carlsberqcnsis* on bran and chaff, one kilogram of substrate could consume 30 – 50 liter of oxygen every 24 hours[3]。 ③ In 1975, an experiment on speeding deoxidation by microorganisms was conducted by the grain administration of Xiangtan region, Hunan province. It showed that the content of carbon dioxide is 5 – 6 times higher than control group when cultivating *Mucor* mucedo on rice bran to lower oxygen concentration, and the effect would be better by cultivating Saccharifying Strains and Yeast on rice bran substrate to lower oxygen concentration[4].

2 Application Technologies Research on Free-Oxygen Absorber for Grain Storage

Application technologies research on Free-Oxygen Absorber for grain storage began in the 1980s in China. ① In 1982, relevant research was conducted by the Second purchasing and supplying grain depot and Fudan University biology department, Shanghai City. It showed that packaging rice with a Free-Oxygen Absorber packet would better inhibit fungi and improve storage quality than vacuum storage, but the Free-Oxygen Absorber is expensive[5]. ② In 1983, Lu Xiyu et al., showed that an iron deoxidizer had more potential than sodium hyposulfite[6]. ③ In 1985, Huang Zhiliang et al.

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showed that polyethylene polyester film had better sealing performance than polyethylene film for rice. The moisture and temperature of grain had little effect on deoxidant effects, and heat and moisture produced during the deoxidization process had little impact on grain storage stability[7]. ④ In 1986, Ni Zhaozhen et al. conducted studies on the composition of different iron deoxidizers, the absorbing abilities of deoxidizers with different carriers, actual oxygen consumption, and the amount of time it took to absorb the oxygen. ⑤ In 1987, He Qihua et al. conducted experiments on salted peanut and in-shell peanut storage with deoxidizers, and they showed that germination ability and other physiochemical indices were always better than with conventional techniques of grain storage [9]. ⑥ In 1988, they showed that F_X – B deoxidizer had excellent application prospects.

3 Research on "Double-low" and "Three-low" Storage

As with the development of fumigation with hydrogen phosphide and grain storage technologies with low temperature and MA in the 1980s, "double-low" and "three-low" storage has been developed in China and been improved and perfected constantly. Here "double-low" storage means grain storage technologies combining "low-oxygen and low-fumigants", and "three-low" storage means combination grain storage technologies, including "low-temperature", "low-oxygen" and "low-fumigants". In recent years, development of these technologies has advanced further, and as an example of application on grain storage, has been written into the China Grain Professional Criterion "Technical Criterion of Grain and Oils Storage".

① In 1976, Wang Zhengqun et al. conducted experiments showed that the combination of natural low-oxygen with low-fumigants provided effective grain storage [11]. ② In 1980, experiments with the combination of natural low – oxygen and low concentration PH_3 fumigation determined the quantities of PH_3 required[12]. ③ In 1980, Liu Weichun wrote a paper "application technologies on 'three-low' grain storage", which introduced requirements and application methods for "three-low" technology[13]. ④ In 1980, through the paper "MA synergism on pH_3 fumigation and its technologies application", Liang Quan et al. showed that lowering the oxygen concentration to 12% and increasing the carbon dioxide concentration to 4% –8% improved PH_3 efficacy significantly for control of the five main grain storage beetles. The synergism index would account for pests group resistance against PH_3, but the relativity between synergism index with pests group had not been found. When postponing the sealed time the effect of pests control will be increase. Combination MA with pH_3 fumigation can degrade fumigants quantity and improve the effect of pests control, Thus they can be taken as a kind of fumigation strategies against resistance pests [14]. ⑤ In 1980, in the paper "research about carbon dioxide influence on pests control with low concentration PH_3", Qiu Shijie et al. indicated that when the concentration of carbon dioxide and oxygen were respectively higher or lower than their homologous concentration, the lethal ratio of red flour beetle and maize beetle with PH_3 was improved. At a temperature of 30℃, carbon dioxide content greater than 11.8%, and oxygen concentration below 10.6%, fumigation with PH_3 at 0.009mg/L for 3 days caused adult pest death. However, in a normal atmosphere, the same effect would be achieved at 0.015mg/L, so AlP quantity can be saved 40%[15]。 ⑥ In 1980, according to the research on "MA and grain storage pests control", Liang Quan et al. showed that at a grain temperature of 20℃ and moisture content below 15%, common adult beetles could be completely controlled. At an oxygen concentration of 5%, the time would be more than 27 days. At 3%, the time would be more than 21 days. At 2%, the time would be more than 16 days. The time to death would be halved if grain temperature was raised 5℃. ⑦ In 1980, according to "discussion on different oxygen concentration and effect of insecticide under MA" conducted by Zhe Jiang grain research institute, they found that when concentration of oxygen was below 2%, the exposure time of maize weevil, red flour beetle, sawtoothed grain beetle, and lesser grain borer would be about 96 hours. In the range of the limited lethal oxygen concentration of 2% –5%, the lethal ratio was in direct proportion with the exposure time, and time was inversely related with moisture. If carbon dioxide was filled up with 2% –5% content of oxygen, the lethal time of tested grain pests would be shortened, and have obvious synergism. But when the concentration of oxygen below 2%, then filled carbon dioxide up would not have obvious synergism. Relative pest sensitivity against low-oxygen was sawtoothed grain beetle > flour beetle > maize weevil > lesser grain borer and adults > larvae > egg > pupae[17]。 ⑧ In 1980, ac-

cording to the paper of "discussion on low-oxygen low-fumigants and grain sealing storage" made by grain administration of Fengxian county, Shanghai city, they showed that when oxygen concentration decreased to 3% and carbon dioxide raised to 7% – 8%, each 25 – 35 ten thousands kilograms grain need one kilogram AlP [18]. ⑨ In 1985, according to "summary of comprehensive application technologies on 'three-low' grain storage" made by Jiangxi grain and oils grain storage Corp., four – way operations were tested, such as deoxidization by sealing→ pest control by fumigation → temperature decrease by aeration → keeping low temperature; temperature decrease by aeration → pest control by fumigation → temperature decrease by aeration → keeping low temperature; pests control by fumigation (or chemicals mixture) → temperature decrease by aeration → keeping low temperature; putting low temperature grain into granary → pest control by fumigation (or chemicals mixture) → temperature decrease by aeration → keeping low temperature[19]. ⑩ In 1985, Jin Yinggui reported that the criterion requirement on "double-low" grain storage technologies would be established in Chengdu city.

4 Research on MA Technologies with Carbon Dioxide

Since the 1960s, many systematic studies on MA technologies with carbon dioxide have been conducted in China, including effect of pest control with CO_2, CO_2 synergism effect on fumigation with PH_3, CO_2 inhibition effect on grain storage mould, effect on grain qualities alteration with CO_2, and qualities alteration after MA with CO_2. In the last 10 years, much further research has been conducted.

① In 1997, Li Qiantai et al. conducted research on effects of different CO_2 concentrations (15%, 20%, 25%, 30%, 35%, 40%, 45%) on grain storage pest control at different temperatures (32℃, 28℃) and relative humidity (70%, 50%), and showed that there was an obvious effect at a CO_2 concentration of 15%[21-23]. ② In 2002, Deng Yongxue et al. exposed lesser grain borer adults and eggs, larva, pupae, and adults of confused flour beetle to 35% CO_2 and 11% O_2, 35% CO_2 and 21% O_2, 75% CO_2 and 11% O_2, 75% CO_2 and 21% O_2 for 12, 24, 36, 48, 60, 72, 84, 96, 108, and 120 hours, respectively, and showed that adult lesser grain borers were sensitive to these MA conditions and LT_{50} values were 34.6, 42.0, 18.6, and 17.8 hours respectively. Adult confused flour beetles were tolerant to 35% CO_2, 11% O_2 and 35% CO_2, 21% O_2 MA conditions, after treatment for 120 hours, and the lethal ratio was below 12%. All of the eggs and larva were sensitive to these four kinds of MA conditions. Pupae had stronger tolerance to 35% CO_2、11% O_2 than 35% CO_2、21% O_2, and their LT_{50} was 2248.3 hours and 124.5 hours respectively. The order of confused flour beetle sensitivity to MA conditions was as follows: egg > larve > pupae > adult[24]. ③ Based on a number of previous studies, four CO_2 MA grain storage demonstration granaries were built in Mianyang, Sichuan Province, and designed by Chengdu grain storage research institute. Excellent pests control with CO_2, inhibition of mould and postponing grain qualities alteration have been achieved in actual granaries. ④ Chengdu grain storage research institute also has undertaken design and construction of another four large CO_2 MA depots in Shanghai, Jiangxi, Jiangsu, and Anhui province, of which the largest single capacity reached up to seven thousands tons and the total capacity under CO_2 MA reached up to 21 500 tons.

5 Research on MA with Nitrogen – enrichening

Following application of MA with CO_2, many studies on MA with nitrogen-enrichening have been carried out. ① During 1968 ~ 1970, the practice of sealing grain with a polyethylene sheet for storage had been adopted by the grain storage Corp. in Shanghai. About ninety millions tons of grain has been preserved by enrichening with nitrogen after vacuum, there were no heating, no pests, and no mould in the grain bulks during storage [26]. ② In 1972, in an experiment on rice storage with nitrogen – enrichening conducted by the grain and oils storage Corp. in Shanghai, it was shown that the quality of rice increased as the concentration of nitrogen increased, and *Penicillium* and *Aspergillus candidus* growth increased in final period of storage when concentration of nitrogen below 95%[27]. ③ In 1975, through an experiment on low-oxygen storage conducted by the Chengdu grain storage research institute, it showed that there was not only low cost by nitrogen-enrichening, but also such qualities as reducing sugar, non-reducing sugar, viscosity, pH of rice water, and content of dry matter in rice water were better than in the control group[28]. ④ In 1976, an ex-

periment on deoxidization and nitrogen-enrichening with molecular sieves for grain storage was reported by the grain and oils Corp. in Xiangtan region, Hunan province. ⑤ In 1982, in the paper "the experiment on deoxidization and nitrogen-richening with 5Åmolecular sieve for grain storage" by Shanghai grain storage Corp., they showed that the sieve generator, which produced quantities at 20m^3/h and concentration beyond 98%, could be used for grain storage to control pests[30]. ⑥ In 1983, through the paper "research on deoxidization and nitrogen-richening with molecular sieve for grain storage", Jiang Zhongzhu showed that grain qualities could be maintained better and the content of oxygen could be degraded faster this way[31]. ⑦ In 1983, through the paper "research on carbon molecular sieve nitrogen generator", Cheng Zhiyuan showed that a carbon molecular sieve generator worked better than, and it had such advantages as low energy consumption, simple operation technology, and low cost. When concentration of nitrogen was 98%, the producing nitrogen ratio and recovery rate were better[32]. ⑧ An experiment on inhibiting mould growth was conducted by the grain administration of Fushun county, Sichuan province, and showed that pests could be controlled effectively when decreasing the concentration of oxygen to the limitation of 2% by the RSL - 180 nitrogen generator[33]. ⑨ In 2005, at the Nanjing depot directly under the centre grain storage Corp., studies showed that grain storage pests could be inhibited effectively and grain temperature in the upper bulks could be decreased by producing nitrogen with a nitrogen generator so that concentration of nitrogen circulation fumigation reached 95% under the sheet for 16 - 20 days,[34]. ⑩ Since 2008, the China grain reserve Corp. extended the capabilities of MA with nitrogen to 0.5 million tons at ten depots directly under the centre grain storage Corp. in Lower-and-Middle Section of Yangtze River and south China, with plans for an additional 1.5 million tons by 2010.

6 Research on Sealing Materials and Technologies for Grain Storage

It was the key for the MA operation to be successful and low cost to determine whether maintaining the airtight quality of the grain warehouse was beneficial or not. Through numerous experiments, relevant points with airtight quality have been solved in China by researches on MA technologies with airtight materials and economic analysis.

①In 1979, in the paper "grain storage with carbon dioxide MA", Tang Zhengjia et al. measured carbon dioxide leakage through a plastic sheet [35]. ② In 1980, through research on sealing materials and technologies conducted by Shanghai grain research institute, the performances of plastic sheet and complex sheet, made in China, were tested, and the results from full - scale experiments were reported. ③ In 1983, conglutinate technologies of sheets in sealed storage were discussed by JiuLongPo district grain Corp., Chongqing city[37]. ④ In 1983, preliminary research on airtight material in granary was conducted by Zhang Ziquan[38]. ⑤ In 1983, Xu Yuanzhang et al. showed that two layers of No 10 asphalt and paper chart ash had better airtight performance by determining the air tightness of four coating materials[39]. ⑥ In 1984, Teng Jianping et al. determined gas permeation ratio through different films, oxygen consumption by paddy with different moistures, the requirement time of paddy anoxybioticly under well sealed conditions, paddy with different moisture contents sealed by polyvinyl chloride film of different widths, and oxygen content in grain bulks in storage [40]. ⑦ In 1984, in the paper "ways adhering plastic film with granary walls" by the storage department in grain administration of Mianyang region, Sichuan province, experiments on adhesives and paints were undertaken. Experiments were conducted on plastic plate gluing ways, gluing ways with adhesives, gluing ways with paints and sealing ways by olefin with comprehensive considerations of air tightness, strength of gluing, and cost. Original investment of plastic plate gluing ways was high, however, if used for enough times, costs could be spread over time[41]. ⑧ In 2002, in the construction of Mianyang MA grain storage granary, Sichuan province, the difficulties of selection of sealing materials and technologies operation were overcome by Tu Jie et al. They found that the half - loss time at 500Pa in airtight performance of an MA empty granary reached beyond 12 minutes, and in a kernel granary it reached 5 minutes and 16 seconds.

7 Summary

As China's national economy develops rapidly, green foods have become popular by more and more people. MA has been regarded as a non-chemical storage technology which has been accepted generally in the world and will

have more and more application in China.

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0905

An Urban Eradication of Khapra Beetle in Western Australia

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Abstract: The khapra beetle (*Trogoderma granarium*) is one of the most serious pests of stored grain and is a regulated quarantine pest in most countries. In April 2007 khapra beetle larvae and adults were found in a suburban residence and personal effects of a family that had migrated to Perth, Western Australia two weeks earlier. Immediate and uncompromising action was taken through industry and government collaboration to quarantine the home and fumigate with methyl bromide at the internationally agreed khapra beetle rate of 80g/m^3. Technical issues are described whereby the two-storey home was shrink-wrapped to ensure that gas concentrations were maintained and monitored for 48 hours. A number of social challenges were encountered dealing with nearby families during the treatment and removing the malodour from the property afterwards. A two-year trapping program was undertaken to validate the complete eradication of the pest.

Key words: khapra beetle, *Trogoderma granarium*, fumigation, eradication, biosecurity

Introduction

The khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae) is a stored product pest of great significance and is nominated as one of the 100 worst invasive species worldwide[1]. It infests grain and cereal products, particularly wheat, barley, oats, rye, maize, rice, flour, malt, and noodles, although it will feed on almost any dried plant or animal matter[2].

Khapra beetle's importance however, lies not only in its capacity to cause serious damage to stored commodities, but also the impact it has on trade for countries that have established infestations. Quarantine regulation can also be imposed if the pest is detected in export produce, containers or packaging from any country. In 2007 – 2008, news media reported on numerous khapra beetle related quarantine incidents involving rice, soybeans and cotton moving between Russia, Uzbekistan, Pakistan, Mexico, U. S. A. and China[3].

Identification of khapra beetle is difficult[4] and Australia was inadvertently recorded as a "khapra beetle country" in the 1950's due to a misidentification of a non-pest native beetle[5]. It took many years of international lobbying to be removed from this list and even today there are occasional databases that report the error[6]. To protect its reputation as an exporter of clean grain, Australia has maintained a rigorous protocol of pre-loading inspection of export ships to ensure there is no residual infestation especially given the khapra beetle can exist without food in a fumigation-tolerant state of facultative diapause for many years[7].

Most years khapra beetle are intercepted at ports by Australian Quarantine Inspection Service (AQIS) pre-shipment inspectors in vessel holds and especially ship's stores requiring treatment before loading. The khapra beetle has never been found in Australia so it was with the greatest concern that a post-border detection of khapra beetle was recorded in April 2007 infesting personal effects in a suburban household in Perth, Western Australia.

This paper reports as a case study, the immediate and uncompromising action taken by Australian government and grain industry to fumigate the incursion and the ongoing trapping to reinforce complete eradication.

Methods

Diagnosis and Response

The khapra beetle incursion was initially reported by a couple who had emigrated to Australia from the U. K. two weeks earlier. They were disturbed by the presence of beetles, lar-

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vae and cast skins throughout their belongings which had taken 6 weeks to arrive by ship. They sought help from a commercial pest controller who recognized the suspicious beetles from various literature and reported it to the Department of Agriculture and Food, Western Australia (DAFWA) who sent an inspector to collect specimens. Adult and immature specimens from a breeding population were identified by the DAFWA taxonomist based on examination of larval and adult stages of the insect. The insect identification was later confirmed by CSIRO Entomology, Canberra.

An assessment by the Australian Bureau of Agricultural and Resource Economics (ABARE) of the potential economic impact of khapra beetle in Western Australia[8], based on costs associated with export market losses ranged from $ 46 million to $ 117 million/year, while the present value of costs over a 30 year period ranged from $ 200 million to $ 1.6 billion. The incident was considered by the Australian Consultative Committee on Emergency Plant Pests to be a detection of an Emergency Plant Pest and consequently the processes as defined under the Emergency Plant Pest Response Deed and PLANTPLAN would be followed requiring immediate eradication.

The spread potential from the suburban site was considered low given that the infested house is 22 km from the nearest grain handling facility and about 40 km from the grain export terminal. The khapra beetle does not fly and its wider dispersal is usually dependant on human activity so the decision was made to fumigate the entire house and contents with methyl bromide under plastic sheeting at the internationally recognised "khapra beetle rate".

This is also the current recommended rate to be used by AQIS for khapra beetle fumigation (T9056) of 80g/m^3 methyl bromide for 48 hours at 21 ℃ at normal atmospheric pressure with an end point concentration at 48 hours of 20g/m^3. The fumigation must undergo monitoring at 24 hours to ensure a minimum concentration of 24g/m^3 and an additional 8g/m^3 for each 5 ℃ the temperature is expected to fall below 21 ℃ to a minimum of 10 ℃. It is the minimum temperature during the course of the fumigation that can be used for the calculation of the dose. AQIS does not accept dosage compensation for temperatures above 21 ℃ or below 10 ℃.

Western Australian bulk grain handler Co-operative Bulk Handling (CBH Group, http://www.cbh.com.au) were the obvious choice to perform the fumigation given their expertise in large sheeted fumigations. CBH Group have an interest in exporting clean grain and immediately agreed to do the work on a cost-recovery basis.

The residents were immediately moved to hotel accommodation with only the clothes they were wearing and their laptop PCs. To minimize the chance of the khapra beetle spreading while preparing for the fumigation, the house and car were treated internally by a commercial pest controller with Permethrin and the exterior and gardens with Bifenthrin. Cardboard removalist cartons, a known harbourage of khapra beetle, were loaded into a fumigation trailer and treated with methyl bromide on-site then buried. Additional high-risk personal items and packaging remaining in the adjoining garage were placed into sealed quarantine plastic bags, sprayed with Pestigas. The sealed quarantine bags were opened prior to the house fumigation to ensure gas penetration.

Treatment

The two-year-old two-storey townhouse was covered with plastic sheeting on May 3, 2007 by contractors Under-Raps (http://www.underraps.com.au) who specialise in encapsulation and shrink wrapping, using industrial grade 200

Fig. 1 The two-story townhouse before and after shrink-wrapping

low density polyethylene plastics. Shrink wrap plastics have several advantages over older

techniques using tarpaulins or canvasses. They fit more tightly around the structure, reducing wear and tear in windy conditions and the plastics can be welded together onsite using hand held heat guns, and shrink tapes.

Three gas introduction points and four electric fans were placed throughout the house however, given the superior state of sealing afforded by the shrink-wrap process, the methyl bromide was able to be introduced through only one point in the upstairs roof space. The other introduction points were not required because the gas dispersed throughout the house and adjoining garage rapidly and evenly.

Six gas monitoring points and one temperature probe were installed in areas that were considered to be the most demanding. Two hours after introduction of 100 kg of methyl bromide five of the monitoring points showed the maximum concentration readable by Drager tubes of 80g/m^3 and one point at 68g/m^3. The average recorded could have been well over 80g/m^3 if the gas monitoring equipment was able to read higher. After 24 hours the average concentration was 39.8g/m^3 and at 48 hours 30.8g/m^3. The average temperature over 48 hours on the concrete lower floor was 20.7 ℃. Ambient wind speeds were quite low averaging 16.7 km/h during the fumigation

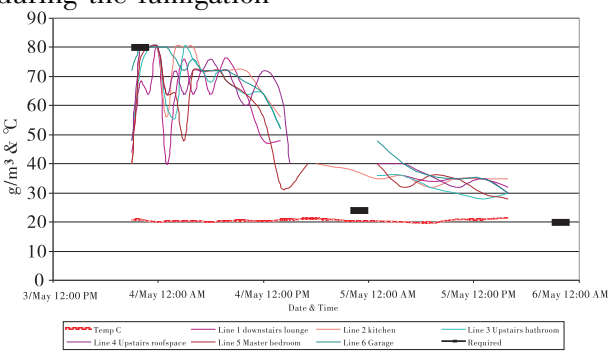

Fig. 2 Methyl bromide gas concentrations and temperature throughout the treatment period

Monitoring data shows a very effective fumigation with the khapra beetle rate exceeded. Occasional dips over one reading are suspected to have been caused by inadequate flushing of the long monitoring lines before taking the reading.

Aeration

Aeration began on the late evening of May 5 by gradually cutting holes in the wrapping. Ambient wind speeds continued to be low averaging 15.4 km/h during the aeration. Within 12 hours the methyl bromide concentration in the house was down to the TLV (threshold limit value) of 5 ppm however there were pockets of higher concentrations in household items like cushions, bins, hot water bottles and unopened confectionery. As a result it was not until May 11 before the house could be cleared for occupation. This delay required the hiring of security guards to protect the open house.

Post-treatment Cleaning

Professional cleaners were engaged as soon as the house was habitable and they cleaned and deodorised all floors cupboards, blinds, windows (inside and out) and steam-cleaned the carpet. Unfortunately there was a distinct malodour throughout the house that was particularly bad in a carpeted room downstairs. This odour apparently had sulphide and butylketone components because it smelled very bad and the decaying body smell attracted many blowflies and flesh flies to the house adding to the resident's displeasure. The smell appeared to be coming from the low quality rubber carpet underlay.

The house was not considered habitable due to the malodour which Chemistry Centre (WA) were engaged to analyse and identify. CCWA tested for sulphur and phosphorus compounds with an AP2Ce flame spectrophotometer-based hand held chemical warfare agent detector. Ammonia, carbon monoxide and volatile organic compounds at a detection limit of 1 ppm were analysed with a Hapsite portable gas chromatograph operating in high sensitivity mode. Two SUMMA canisters were also used to collect air samples from inside the house for laboratory analysis at CCWA. No volatile organic compounds were detected above ambient (1 ppm (V)) using the hand held detectors however the portable gas chromatograph detected a number of compounds. In the upstairs bedroom methyl bromide, dimethyldisulphide, toluene, ethylacetate, and methyl isobutylketone were all detected while, in the lounge and kitchen, only methyl bromide, dimethyldisulphide and toluene were detected. The SUMMA canister sample collected in the lounge indicated there was > 0.1 ppm (V) of methyl bromide, 0.5 ppb (V) of toluene and 1.5 ppb (V) of dimethyl disulphide (relative to toluene).

The malodour was evidently dimethyldisulphide, most likely formed from reaction with sulphur in the poorly refined carpet underlay. The decision was made to remove and dispose of the carpet, underlay by deep burial. CCWA recommended vigorous airing of the house to remove the smell and that this should be done

prior to replacing the carpet because it could result in the malodour molecules dropping from the walls into the new carpet and tainting it as well.

Two 4 200 L/sec industrial fans were used to blow the smell out of the house by positioning one fan in a downstairs doorway blowing into the house and the other on an upstairs balcony blowing out. Doors and windows were adjusted to maximise the airflow through all rooms and the fans run for 12 hours. The malodour could be smelt 50 m into the street however in the house the odour, while not as bad, was still obvious.

In a further attempt to remove the smell an industrial ozone generator designed to cover a 1 000 square foot area, capable of producing 8 000mg/hr ozone was run upstairs for 12 hours. Fortunately, it almost completely removed the smell so a second unit was deployed and both were run continuously upstairs and downstairs for 24 hours. A follow up air measurement by CCWA showed the dimethyldisulphide was now down to 0.4 ppb.

However, the residents felt that the remaining smell left the house uninhabitable so all carpets and underlay were removed and buried at a toxic waste dump along with the plastic sheeting used to cover the house. All furniture was placed in the garage and a forensic cleaning company Grimescene Clean (http://www.grimescene.com.au) engaged to fog the inside of the house with alcohol-based products "GOE" Bio, "GOE" Washdown and Odor Eliminator "Cinnamon Spice". Areas treated were:

· Upstairs roof space, lifting insulation batts and delivering a fine mist of odour eliminating chemicals to entire area

· Main bedroom concrete floor pad, walk-in robe, including individually fogging numerous handbags, belts and items contained on the top shelf

· Main bathroom and toilet, including all shoes on shoe rack and the internal area of the bathroom cabinet

· Upstairs linen closet, including all shoes and shelving

· Bedroom two concrete floor pad, including the built-in robe and both suspended cloth storage items, including the built – in robe and suit bags

· Bedroom three concrete floor pad, including built-in robe and suit bags

· Entire staircase leading from ground to first floor

· Kitchen, including bench tops, inside kitchen cabinets, behind refrigerator and microwave oven recess

· Family room, tiled area including behind all existing furniture

· Dining room, tiled area including a small bookcase, wine rack and two camp chairs,

· Lounge room concrete floor pad

· Laundry, laundry cupboard, toilet and downstairs linen press

· Garage roof space, lifting insulation batts

· Double garage, including floor space and all items stored within the garage

· All tiled floor areas mopped twice with a bio-degradable disinfectant and all bench tops, doors and furniture wiped down.

While this was taking place the residents removed many items of clothing for washing or drycleaning. Some outfits required up to four washes to remove the smell that had impregnated the clothing.

The extensive post-fumigation treatment of the house had completely removed the smell and follow up CCWA SUMMA canister air sample showed that the dimethyldisulphide concentration was now below the level of detection of 0.3 ppb. The carpet was replaced and the tenants moved back in on June 9.

Debrief

Close cooperation between DAFWA, CBH Group, Health Department, local Shire, the property owner and tenants meant that the fumigation itself was completed in just over two weeks from the discovery of the pests. The aeration of the house took another week which is quite long and possibly due to the high concentrations of methyl bromide achieved during the two day exposure. The biggest delay in allowing the residents to return to a normal life was a result of prolonged efforts to remove the malodour which took three weeks and, combined with the cost of accommodation and cleaning, was as expensive as the actual fumigation.

We suspect that the post-fumigation steam cleaning of the carpet exacerbated the malodour problem by sucking the dimethyldisulphide molecules out of the carpet, combining it with water vapour facilitating adsorption to walls and fixtures. Treatments for insects like the khapra beetle where all belongings, fixtures and chattels must be exposed to the gas, we now recommend that, prior to the fumigation, carpets be

rolled up and placed so as to allow immediate removal and disposal once the house is cleared. This may have the added advantage of not confining the dimethyldisulphide close to the concrete floor as odour can penetrate several centimetres into the concrete to be slowly released over time. Odour from the carpet underlay in walk-in robes may also have heavily contaminated the hanging clothes with dimethyldisulphide. It is possible that the carpet itself (not the underlay) could be refitted if it was carefully rolled up by professional carpet layers thus saving on replacement of the most expensive component.

The aeration fans did not do as well as we had hoped but the ozone generators were very effective. Residual ozone smelled a little like chlorine but was no worse than an indoor pool and quickly dispersed when the house was aired.

Trace Back and Forward

This incursion is interesting in that it supports the assertion that cities are major freight endpoints and are often the first landfall for invasive species[9]. Further, cities contain a far greater diversity of plant, animal and environmental conditions than elsewhere thereby increasing the probability that an exotic pest will find favourable hosts/conditions in an "agriculturally un-important" situation.

Trace-back analysis to attempt to identify the source of the infestation and guard against it occurring again was carried out with the help of AQIS. The fact that live adult beetles, which only live for two weeks, were found in the infestation indicates there must have been a food source in the container. Previous interceptions of stored grain pests have been found in microwaveable therapeutic wheat heat bags, but while a cover was found in the house, there was no evidence of any grain.

The resident's personal effects were initially consolidated in a shipping container that arrived in Fremantle, Western Australia via Hamburg and Singapore. The container had previously been used for shipments of cotton textiles, then iron and steel articles, chemical products and lastly foodstuffs. The outgoing shipping container was intercepted on arrival in Norway by AQIS officials but no khapra beetle infestation or food residues could be found.

Surveillance Trapping

Monitoring and trapping activities over two years using Tr écéStorgard traps baited with kairamone lure to attract adult beetles and a ground raw wheat germ food source for larvae will be used. These traps have been placed in the treated residence (4 traps in the garage, garage roof cavity, pantry and upstairs roof cavity), 5 neighbouring properties (1 trap in the kitchen of each house), the shipping container receival facility (12 traps), a cardboard waste recycling facility (12 traps) and a waste transfer station (10 traps). These traps were checked weekly for the first month of the program then once a month during the winter months when insect activity is low. During the warmer months of September to April the traps are inspected fortnightly except for the private residences which are checked monthly.

At the time of writing, twelve months into the trapping schedule, over 750 trap inspections have not yielded any khapra beetle. This is expected to continue for another 12 months when the eradication program will be declared a complete success.

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0906

Advancement of Fumigation Technologies on Grain Storage in China

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Abstract: The application technologies of fumigants including choropicrin, methyl Bromide, dichloros, phosphine etc. and research progresses of fumigants such as sulfuryl fluoride, ethyl formate etc. in Chinese grain storage industries are discussed in this paper, with focus on the effective concentration of phosphine, fumigation technologies, application devices and so on. Accumulation of experience for application technologies has played an important role in controlling stored grain insect pests in China.

Key words: fumigants, stored grain insect pests, pest control

As most fumigants possess many advantages, including good diffusibility, permeability, easy gasification, effectiveness for controlling pests, low harmful residuals, low-cost, easy transportation, storage and management, and simple operation they have been regarded as the principal chemicals for controlling pests in grain storage and taken into applications and developments wildly in China. As early as the 11th century BC during the Western Zhou Dynasty, botanical insecticide fumigants have been applied for controlling pests in grain in China. Before the Peoples Republic of China was founded, few fumigants for grain storage had been used in China. Most of them were still in the process of experimentation. Since the 1950s, fumigants such as Choropicrin, Bisulfide Carbon, Methyl Bromide, Aluminum Phosphide, Calcium Phosphide, Cyanhydric Acid, Dichloroethane and Dichloros have been put into production and practice one after the other [1]. Actually, the industry of fumigant production has made rapid progress. Compared with the few dozen fumigant applications in the 1950s, three thousand fumigants are in practice for grain storage at present. Through technology research on fumigants for grain storage, such as fumigation technology utilizing phosphine, anthelminthic and synergism mechanisms, anti-blasting mechanisms, protective measures, equipment for fumigation and so on, much significant progress has been made and lots of experiments for application technologies have been accumulated. Meanwhile, the technical specifications for grain and oil storage and the technical rules for recirculation fumigation with phosphine have been drafted. So many advantages have been created for the proper application of fumigants in China [2].

1 Application Technology Research on Choropicrin

According to GBZ2 – 2002, China's national occupational health standards, the maximum acceptable concentration for Choropicrin is 1 mg/m^3. However, the application dosage usually reached 15 g/m^3 – 20 g/m^3 during fumigation, with fumigation temperatures more successful above 20℃. If Choropicrin was used as a bactericidal agent, the application dosage could take between 20mg/L and 30mg/L. Because of high absorbability to finished products and high residual traces, Choropicrin should not be allowed in fumigation of finished products. In addition, since it has a negative influence on germination rates of some crops, fumigation of grain seeds should also not be allowed. Being uneasy during gas exchange, Choropicrin is not suitable for fumigation in underground granaries. Some experiments carried out with mixture fumigants in China, such as Choropicrin and Phosphine, Choropicrin, dichloroethane and Phosphine, have shown that such general pests as *Sitophilus zeamais*, *Tribolium castaneum* could be controlled completely by fumigation with mixture chemicals. The same results occurred with *Rhizopertha dominica* even though there was high pesticide resistance [3].

However, CCl_3NO_2, inorganic nitrite and nitramine could be produced from chemical reactions in foods, which may have a carcinogenic effect. Because of many disadvantages, such as heavy workload for application, weak diffusibility, slow gas exchange and so on, Choropicrin is in few application at present and will begin to phase-out.

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2 Application Research and Phase-out on Methyl Bromide

Methyl Bromide (MB), one excellent fumigant, has many advantages such as: stable chemical characters, low boiling point, high vapor pressure, well diffusibility, little absorbability for grain, and ease in scattering poisonous gas after fumigation. Besides the above, MB could be used for fumigation at low temperature (beyond 6℃), and does not burn or explode at general concentrations. Gasiform MB does not leave negative effects on such materials as metal, cotton, gloria products, and wood and so on. Since the 1950s, MB had been used to control pests in agriculture, such as sterilizing in soil, plants and their products, and use in quarantine of plant products during international trade. It also has been a fumigant to control pests for all kinds of buildings such as: ancient buildings, granaries, cabins, vehicles, aircraft, and food processing plants and so on. Except some beans with high protein content, MB can be used in fumigation for all kinds of grains, finished products, oils and potato and so on. Generally, every cubic meter of grain needs 30 grams, and space needs 15 – 20 grams for 2 – 7 day fumigation. In 1999, an experiment on fumigation with a mixture of Phosphine and MB for grain storage was made by LanFang Jian. It showed that mixture fumigation could reach the same results in controlling pests as MB alone, but cost lower than MB fumigation alone [4]. In 2000, by fumigating grain bulks in carbin with a mixture of Phosphine and MB, KaiXiang Wang et al did indeed control pests to excellent effect [5].

However due to destruction of the ozone layer in the atmospheric stratosphere, and an overall negative effect on living conditions for all mankind on Earth, MB has been labeled an ozone layer deleting substance and is restricted or banned for application by United Nations Environment Program. The Chinese government has approved *the Copenhagen amendment* of *the Montreal Protocol*. According to the agreement, the consumption of MB should be decreased to 80% of the average consumption during the period of 1995 – 1998 in China before the year 2005. By 2015, MB should be phased-out completely except for use in fumigation for quarantine.

With the help of international assistance funds, provided by Multilateral Funds executive committee of *the Montreal Protocol* in July, 2004, the international assistant projects for the MB phase-out was launched officially in Chinese grain storage industries. By the current situation of application with MB in Chinese grain storage industries and granaries' conditions, the practice proposal for MB phase-out has been drafted, and alternative technologies for MB have been identified. The total goal of the proposal was that the consumption of MB would fall off by 50% in 2005 compared to 2003. That is: the amount of consumption would decrease to below 105 tons. Then, MB would fall off to 80% in 2006 of the consumption in 2003, or the amount of about 42 tons. From Dec 31st, 2006, MB was not allowed as a fumigant by any grain depots in Chinese grain storage industries. Until Jan, 2007, MB should be prohibited completely in Chinese grain storage industries, in order to make proper contribution for carrying out the amendment of *the Montreal Protocol* completely in China [6,7].

3 Dichloros and Its Application Research

With strong contact and stomach toxicity effects, good fumigation and to some extent lure effects, Dichloros (DDVP) was one kind of significant pesticide used in the control of grain storage pests in China. The daily allowed intake for mankind was between zero and 0.004 milligrams by the Food and Agriculture Organization and World Health Organization (1967). Because of weak diffusibility and easy adsorption to grain, DDVP has always made some application for fumigation on the surface of grain bulks, empty granary, procession plants, equipment for package, matting materials and so on. Reference data is as follows: a full storage granary could be fumigated with 80% emulsifiable DDVP at concentrations of 0.3 g/m^3 – 0.4 g/m^3, and an empty granary and devices at concentrations of 0.2 g/m^3 – 0.3 g/m^3. A seal should be kept for 2 – 5 days following application with DDVP, and *Sitophilus zeamais*, *Tribolium castaneum*, *Rhizopertha dominica*, all kinds of moth adults or larva and other grain storage pests can be controlled.

Much research on fumigation has laid a stable foundation for pest control in empty storage and grain bulk surfaces with DDVP. In 1964, the Grain Research and Scientific Institute in the GuangDong Province carried on research on pest control with DDVP in empty granaries [8]. In 1965, research on DDVP having a toxic influence on *Sitophilus zeamais*, *Oryzaephilus surinamensis* and *Tribolium castaneum* was carried out by the Research and De-

sign Institute of the grain ministry [9]. In 1966, DDVP was applied to control pests on the surface of grain bulks at the dosage of 0.2 mL/m^3 – 0.3 mL/m^3 by the Grain Research and Scientific Institute in ShangHai City [10]. In 1966, the Grain Research and Scientific Institute in ZheJiang Province carried out research on controlling grain storage pests with DDVP by different application methods. It is also worth mentioning that operating staff must be put on canister respirators and armors during the fumigation of the indoors and greenhouse, which should be available to filter poisonous gas.

4 Research on Phosphine Application Technology

It has been over 40 years since self – development, production and application of Phosphine in China occurred in 1965. Lots of researches on fumigation with Phosphine have been conducted, including effective concentration, pharmacodynamic impact factor, and fumigation technologies and devices for application. Their application and promotion has played a significant role on pest control in Chinese grain storage industries.

4.1 Research on Effective Concentration of Phosphine

During the early period of fumigation with phosphine in China, the dosage of Aluminum Phosphide was used to guide practice. Dosage of each unit or volume of grain and total fumigant consumption was considered alone during fumigation. Generally, 6 g/m^3 – 9 g/m^3 of fumigants were put into practice in full storage. However, many series of problems depended on experience and sensation to practice, such as whether the effective concentration could be reached in the granary or grain bulks after fumigation or not How much time does it take to reach the effective pesticide concentration Is the concentration too high, leading to and increase in pests with protective stupefaction or not Are there fumigation corners and partial districts of low concentration or not Will the effective concentration keep for enough time or not Without inspecting concentration, fumigation could not always get the results desired, and grain storage pests easily developed resistance to Phosphine increasingly. According to LS/T1201 – 2002, the rules on recirculation fumigation with Phosphine issued in 2002, the concentration of Phosphine and anthelminthic effects should be brought forward clearly to guide practice. By different pest rates and temperatures of grain, it was recommended that fumigation with a concentration of 100 mL/m^3 – 350 mL/m^3 over 14 – 28 days was necessary. However, to control some pests with high pesticide resistance, such as *Cryptolestes ferrugineus*, 350 mL/m^3 – 500 mL/m^3 concentrations should be reached during fumigation to control pests completely. From recent applications, reference data has successfully guided fumigation practice in Chinese grain storage industries [1].

4.2 Research on Application Technology with Phosphine

To assure effective and homogeneous distribution of phosphine concentration during fumigation, lots of research on application technologies with phosphine have been made by grain storage staff in China, including such more practical and feasible technologies as slow-release, phosphine generators outdoors, mixture gas in cylinder outdoor applications and so on. Furthermore, by recirculation technologies, phosphine could be distributed quickly and homogeneously into large grain bulks to assure the effect of controlling pests.

To keep an effective concentration to control pests at each stage of sensitiveness, it was by way of intermittent fumigation that pests at a sensitive stage could be controlled ahead, while pests at other stages with strong resistance became sensitive, and all of them could then be killed. The experiment on control pests by way of intermittent fumigation was carried out by ChangJin Zhou et al., and it showed that chemical consumption decreased and labor intensity lightened. What was more important was that lethal rates reached 100%. The whole operation procedure was as follows: during the first application in large granaries, the effective concentration of phosphine should be kept for 6 – 10 days, followed by an interval period of 6 – 10 days, and last, after replenishing for 1 – 2 days, the time for sealing should reach over 21 days [12]. For controlling the speed of phosphine release, Aluminum Phosphide was put into the polyethylene film, which could separate phosphine from moisture, thus slow-releasing fumigation could control the reaction speed of Aluminum Phosphide decomposition and the releasing speed of phosphine. The experiment on slow – releasing fumigation in wheat bulks was carried on by XiangGang Wang et al., it showed that slow-releasing could achieve excellent effects, and mixtures of combinations conventional with slow-releasing fumigation could reach better

effects on grain with a few beetles [13]. Application recirculation technologies could improve phosphine distribution homogeneously in large grain bulks, and so assure the effect of controlling pests. For outdoor fumigation, the difference between grain temperature and temperature at the outlet for mixture gas exchange was not over 5℃. What is most important is that the distance between grain bulks with the air outlet is close. It is also worth noting that if granaries have no better sealing performance, effective concentration for control pests in grain bulks could not be reached, so it would not only miss the purpose of controlling pests, but also could induce pesticide resistance of pests [14].

4.3 Application and promotion of Recirculation technology with phosphine

4.3.1 Research on recirculation technology with phosphine

Research and application of recirculation fumigation technologies with phosphine were carried out by LaiLin Zhang et al [15] in 1994, NaiQiang Sun [16] in 1995, Yifu Yu et al [17] in 1997, and Rong Zhang et al [18] in 1998 one after another. Meanwhile, such important devices for fumigation as outdoor phosphine generators, recirculation fumigation devices, sampling devices for phosphine indoors and phosphine inspectors have been researched and developed by research staff in China and implemented in industrialization production. These research results have widely laid down the foundation for promoting recirculation fumigation technologies with phosphine.

4.3.2 Application and promotion of recirculation fumigation technology with phosphine

As recirculation fumigation technologies have been promoted and applied in large warehouses and squat silos, built since 1998 [21~23], development of technologies for controlling grain pests have been promoted rapidly in China. During the actual application, these technologies have developed to such advanced levels as recirculation fumigation technologies with phosphine under film [24], recirculation fumigation technologies by dynamic and nature deliquescence [25], recirculation fumigation technologies by application of chemicals at combination grain surface with air outlet, partial recirculation fumigation technologies in grain bulks and so on [26]. Hundreds of papers on these fields have been published one after another, and rapid strides have been made in China in these fields.

4.4 Research on Combination Fumigation Technology with Phosphine

4.4.1 Fumigation technology by combination phosphine with carbon dioxide

The application of carbon dioxide has increased the phosphine diffusion binding and speed, decreased grain absorption consumption of phosphine and been in favor of its well – distribution. Furthermore, it could postpone the exposure time at maximum concentration than single fumigation. By stimulating the respiration of pests and increasing pesticide's consumption in pests, the lethal speed of pests has been raised [27,28]. It has been reported that by many experimental steps, such as 16% concentration of carbon dioxide for 24 hours, ventilation for 10 minutes, and then fumigation for 24 hours at 0.009 mg/m^3 of concentration, the mortality ratios of *Sitophilus granarius*, *Tribolium confusum* reached 88.3% and 98.0% respectively. When fumigating with a single kind of fumigant, both moralities of pests were below 44%.

4.4.2 After recirculation fumigation by combination phosphine with DDVP, some difficulties on controlling pests in large granaries could be settled excellently. It had an especially complete effect on *Cryptolestes ferrugineus*, booklouse and grain storage mites with certain resistance, and made the idea of fumigation at low concentration one time every year possible [30-32]. The process of mixture fumigation using a combination of phosphine with DDVP could overcome some disadvantages of fumigation with DDVP simply, such as slow diffusion and weak penetration abilities. Depending on recirculation fumigation by combination of phosphine with DDVP, application alcohol accelerating diffusion in large bulks had been taken by XinHua Lai et al; it was shown that there were excellent practice effects.

5 Fumigants in Development and Research

As pesticide resistance development and environment impact from chemicals is increasing, many kinds of pesticide have been prohibited. Most experts agree on the development of new, more effective fumigants for controlling pests by fumigants other than phosphine. However, not only is there great cost for promotion of a new kind of chemical into markets and limited resource of material, but also excellent fumigants with bioactivity are rather rare. There is no optimistic prospect to research and develop

new fumigants.

5.1 Ethyl Formate(EtF)

EtF, one regularly practiced fumigant, has been used for the fumigation of dry fruit for the past many years. Many stored products produce natural EtF during storage, including many kinds of vegetables, fruits, corn and animal products. EtF degrades into formic acid and ethanol after fumigation, both substances existing naturally in many kinds of food, so it would not have a negative effect on grain qualities and germination after fumigation.

At ordinary temperatures, EtF decreases to original concentrations quickly, and has no residual effect. With Methyl Bromide phasing-out and phosphine resistance increasing recently, EtF, as one kind of environmentally-friendly fumigant, will arouse people's concern again.

PeiAn Tang et al, from Southwestern University, made systemic research on the lethal effect on such important grain storage pests as *Sitophilus oryzae* and *Tribolium castaneum* by fumigation with EtF at different temperatures, concentrations and fumigation times. It was shown that EtF had excellent immediate and lethal effect to both of these pests at their immature stages, and is better for the fumigation of the young larvae of *Sitophilus oryzae*. Pupa and egg both possess excellent resistance to pesticide. The best lethal effect of EtF against Tribolium castaneum was obtained in egg developmental period [35]. By fumigating wheat, maize, and paddy bulks at a concentration of 70g/m^3 in simulation granaries, it was shown that the best effect was observed during fumigation of wheat bulks with EtF. The lethal rates of four such kinds of test pests, *Sitophilus oryae*, *Tribolium castaneum*, *Rhizopertha dominica* and Liposcelis were at 100%. There was also excellent fumigation effect in maize bulks. However, the effect to paddy bulks was worse. It was found that there were strong penetration effects in maize and wheat bulks, thus having an excellent fumigation effect. Therefore, EtF has been regarded as one alternative fumigant for use in control of *Sitophilus oryze*, , *Tribolium castaneum*, *Rhizopertha dominica* and Liposcelis in wheat or maize granaries. Because of poor fumigation effect in paddy bulks, EtF could be used in combination with carbon dioxide to carry on mixture fumigation [36]. This research has supplied many pieces of scientific evidence for application of EtF during full storage.

5.2 Carbon Disulfide

Carbon Disulfide was tested and proven to have abilities to control all grain storage pests in 1958. With good penetration abilities and ease in evaporation in places with high temperature, Carbon Disulfide had more practical values. According to GBZ2 – 2002, national occupation health standards in China, many contact poison limitations were as follows: the Carbon Disulfide average allowed concentration time weighted was 5mg/m^3 in vitro skin permeation, contact allowed concentration in short time was 5mg/m^3 in vitro skin permeation [37].

The preliminary test on fumigation with carbon disulfide to control Sweet potato black rot and the experiment on controlling grain storage pests with carbon disulfide were carried out in 1966 and 1967 one after another. Meanwhile, the research on carbon disulfide residual standards allowed in grain was carried out as well. However, carbon disulfide has not been in practice for grain fumigation for many years. Recently it has been reappraised as a substitution for Methyl Bromide. With many advantages, such as convenience of use, fumigation at low temperatures of below 10℃, and low residuals there is the excellent desired effect of controlling pests by fumigation by combination carbon disulfide with carbon tetrachloride mixture at certain proportion, especially to *Rhyzopertha dominica* with strong phosphine resistance [38].

5.3 Carbonyl Oxysulfide

Carbonyl Oxysulfide, which is a chemical material that exists naturally in the atmosphere and has important ingredients for sulfur cycling in the earth, was used for synthesis of oxycarbide, sulfoacid, thiosulfate and thiazole in many industries. Therefore, it was regarded as a patent of one new fumigant by John Stooker, Australian. According to research made by XianChang Tan in 1994, paddy had more resistance than wheat and maize with 7 day fumigation at a temperature of 39℃ and concentration of 25 g/m^3 – 39 g/m^3. After fumigation at 25 g/m^3, prophase germination rate decreased to 6.29%, 28.4%, 20.3% respectively. With dosage acceleration, both prophase germination rate and germination ratio have been decreased accordingly.

The dosage for fumigating barley and oil seeds by Carbonyl Oxysulfide was recommended at 17 g/m^3, 15 g/m^3 respectively. During fumigation, gas is distributed equally and the concentration is decreased to 8% 6 hours later, with the average concentration in barley and

oilseed granary being 13 g/m^3 and 12 g/m^3 respectively 7 days later. After fumigation for 7 days, six kinds of test beetles, three kinds of booklouse, and one kind of moth and carpet beetle larvae were killed completely. By ventilation for 2 – 4 hours by 0.4kW ventilators after fumigation, the residual of Carbon Oxysulfide in grain was at maximum residual standards of 0.2 mg/kg, Australian ruled.

5.4 Ethylene Oxide

Ethylene Oxide has been taken into practice widely for cold sterilization of medical treatment materials and devices, as well as for prevention of food and flavoring from mold. It has always been used for paddy, miscellaneous grain crops and some plants products. At actual concentrations, it has homicidal poisoning abilities to many kinds of bacteria, fungi and viruses. However, it also had rank poison to plants and a negative effect on the germination of seeds. As it was easy to burn, in practice it was usually used in a combination with carbon dioxide. For hydrocarbylation and inducing mutation, it could also have potential carcinogenic effect. The toxicity against pests was about at a middle degree. In China, it has been allowed only for fumigation wheat with *Tilletia contraversa* Kuhn.

5.5 Sulfuryl Fluoride (SF)

Advantages of Sulfuryl Fluoride (SF) include excellent diffusion, broad-spectrum anthelminthic abilities, low drug consumption, low residual content, rapid speed of taking into practice, short time of diffusion, practical convenience at low temperatures, difficulty in burning and exploding, noncorrosive to metals in the gas stage, and no influence to germination ratios of seeds. As such it has been used for controlling pests or termites widely in some places, such as granaries, cargo boats, containers and buildings, water reservoirs dams, gardens and arbor vitae. SF has been regarded as an alternative substance to Methyl Bromide in some industries, such as plants quarantines, healthy quarantines, agriculture storage and building industries and so on.

Since the 1970s, after the project founded by Chinese agriculture ministry, SF has been developed by 21 units including the Plants Quarantine Institute. Application of SF in grain industries has been researched widely by such Chinese scholars as GuoGan Xu, WangChang Li, XianChang Tan et al [39]. At present, SF has been registered for fumigating such materials as wood, official files, books, embankments and buildings, and was already taken into application in more ranges of buildings and quarantine ministries.

Due to the notable pharmacodynamic action of SF, there was excellent effect on control of pests such as: bark beetles, longicorn, termite, *Trogoderma granarium*, *Sitophilus oryza*, *Sitophilus zeamais*, *Tribolium castaneum*, *Sitophilus granaries*, *Callosobruchus chinensis*, *and Lasioderma serricorne*. Pharmacodynamic tests against about 30 species of pests have been carried out on over 30 units including the Plants Quarantine Institute of Agriculture Ministry. These tests showed that the mortality effect against pests could reach 100% at concentrations of 20 g/m^3 – 60 g/m^3 and fumigations of 2 – 3 days, especially against pests at postembryonic period. It also has a shorter explosive time, lower chemical consumption, more rapid gas diffusion.

Research on acute and subacute inhalation toxicity has been carried out by the Healthy Institute of China Academy of Medical Science. They have shown that LC50 of SF against Mus musculus albus was 800 mL/m^3, and the lethal concentration against house rabbit was 3 250 mL/m^3. After narcotics tests against an albino rat for 2 hours at a concentration of 55.6 mL/m^3 by subacute tests, there was no obvious hurt to the important organs of the rat. There was also a lower toxicity against higher animals compared to others fumigants.

The temporary residual and permanent residual on SF has been researched by GuoGan Xu et al. The temporary residual could be disadsorbed by ventilation. Compared with fumigation of soya bean and maize with Methyl Bromide, the consumption of SF only was 36% of consumption of Methyl Bromide during fumigation of soya bean, and 20.3% during fumigation of maize. The speed of SF disadsorption was higher than Methyl Bromide after fumigation. There was also no residual poison inspected by gas chromatographic analysis during fumigation analysis of the granary after 8 hours, and no residual poison inspected by the gas detector during fumigation of cotton granaries in large shipments with diffusions of about 4 – 12 hours. However, the permanent residual could not be disadsorbed after chemical reactions with fumigants. The permanent residual of SF was lower than that of Methyl Bromide as well.

In the experiment on residual inspection after fumigation of grain with SF at high con-

centrations by GuoGan Xu and Guang Li et al., it was shown that the residual of SF would increase with increasing consumption, and the residual of fluorine in powder grain was higher than in original grain. According to GB2762 – 2005 *Maximum levels of contaminants in foods*, the maximum levels of fluorine in grain should be below 1.5 mg/kg, in rice and flour below 1.0 mg/kg, and in beans and their products below 3.0 mg/kg. Furthermore, according to GB5009.18 – 2003, *Fluorine inspection in foods*, the official main inspection used was colorimetry by diffusion-fluorine reagents, and the maximum level of fluorine inspection was 0.1 mg/kg.

Following the experiment on the fumigation of several dozen plants seeds such as bean, mung bean, black bean, cucumber, eggplant, Chinese cabbage and so on with SF at concentrations of 70 g/m^3 and 100 g/m^3 respectively, there were no evidence of SF influencing the germination ratios of these plants seeds.

At present, temporary registration certification for SF use has been approved to take into practice in grain.

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Evaluating Importance and Implementation of the Building Pressurization Test in Structural Fumigation Using Computer Simulations

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Abstract: Pressurization In a previous study, a validated Computational Fluid Dynamics (CFD) model of the structural fumigation process in a flour mill was utilized to evaluate the effect of multi-year weather conditions (1996-2006) on the half-loss time (HLT) and concentration × time (Ct) product, concluding that past fumigation data should not be the primary means for quantifying the effectiveness of temporary structural sealing. In the present study, using the same CFD model the standardized building pressurization test and superposition method commonly used for heating/cooling load and in-door air quality calculations in buildings were evaluated for prediction of HLT and Ct product. A simulated test was performed in order to determine the flour mill's effective leakage area. Then, the simulated mill was subjected to several fixed environmental conditions to determine the stack and wind coefficients which were necessary for the superposition calculation. The HLTs and Ct products generated by the 11-year fumigation simulations were compared with the corresponding values predicted based on the superposition method. The HLT and Ct product predictions were within ±20 and ±10% of the simulated values, respectively, except for one simulated fumigation. These results showed that the pressurization test and superposition method have potential application benefits for optimizing the structural fumigation process.

Key words: structural fumigation, half-loss time, computational fluid dynamics (CFD), building pressurization

Introduction

Optimizing fumigant usage for a structural fumigation requires that the fumigant leakage rate (i.e., half-loss time, HLT) be predicted so that the target concentration × time product (i.e., Ct product) is precisely reached at the end of the exposure period. HLT is influenced by weather conditions, especially wind and ambient temperature. However, since the HLT concept was introduced, the relationship between HLT and weather conditions has never been quantified. While fumigators typically rely on concentration data recorded from past fumigations to determine the HLT of a structure, a simulation study of fumigation in a flour mill[1] found that the HLT between fumigations could vary up to 100% due to the variation in weather conditions. In addition to weather conditions, the HLT is also affected by the air-tightness of the structure, which can vary due to aging and changes in sealing quality. As a result, the prediction of HLT based solely upon past fumigation data is subject to substantial uncertainty.

The standardized pressurization test[2], also known as the blower door test, and the superposition of the wind and stack effects have been used by the heating, ventilation, and air conditioning (HVAC) industry to quantify air infiltration into structures for energy saving and indoor air quality purposes[3]. This superposition was primarily developed for residential houses. However, the use of the pressurization test in commercial/industrial structures is not uncommon. Fumigant leakage is linked to the infiltration process. Thus, the pressurization test and superposition method could be applied for the prediction of HLT and Ct product in structural fumigation. The objective of this study was to utilize the validated Computational Fluid Dynamics (CFD) model developed by Chayaprasert et al.[4] to evaluate the pressurization test and superposition method for prediction of structural fumigation performance.

Materials and Method

Theoretical Calculations

One of the correlations that is most widely used to describe the relationship between the

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infiltration rate, Q (m^3/s), and the pressure difference across the building envelope, (p (Pa), is the power law equation:

$$Q = c(\Delta p) \qquad \text{Eq. 1}$$

where c is the flow coefficient ($m^3/s\ Pa^n$). The pressure exponent, n is dimensionless and has the limiting values of 0.5 and 1 for fully developed turbulent and laminar flow, respectively[5]. The characteristic constants, c and n, are different for different buildings. To conduct the pressurization test, one or more specifically calibrated fan(s) installed at the perimeter of the structure are used to induce a pressure difference across the building envelope. The airflow rate that is required to maintain this induced pressure difference is recorded. By testing at multiple pressure levels, the power law relationship of the building can be established. Natural infiltration is a non-linear and complex process. Most infiltration models rely on a simplification method called superposition in which the wind and stack effects are determined separately and then combined together based on a predefined correlation. One additive correlation is[3]:

$$Q = \frac{A_L}{1000}\sqrt{C_s\Delta t + C_w U^2} \qquad \text{Eq. 2}$$

where C_s is the stack coefficient ($(L/s)^2/cm^4 - K$), C_w is the wind coefficient ($(L/s)^2/cm^4 - (m/s)^2$), (t is the average indoor – outdoor temperature difference (K), and U is the average local wind speed (m/s) typically measured at a nearby weather station. The effective leakage area, A_L (cm^2), is calculated as:

$$A_L = 1000\ Q_r\sqrt{\frac{\rho/2\Delta p_r}{C_D}} \qquad \text{Eq. 3}$$

where ρ is the air density (kg/m^3), C_D is the dimensionless discharge coefficient, and Q_r is the infiltration rate (m^3/s) predicted at the reference pressure difference, Δp_r (Pa). Eq. 3 implies that all cracks and openings in the building are collectively represented as one equivalent leakage area and a corresponding discharge coefficient. Substituting Eq. 1 into Eq. 3 yields:

$$A_L = \frac{10000\ c}{C_D}\sqrt{\frac{\rho}{2}}\Delta p_r^{(n-0.5)} \qquad \text{Eq. 4}$$

While the flow coefficient, c, and the pressure exponent, n, can be determined from the pressurization test data, the reference pressure difference, Δp_r, and the discharge coefficient, C_D, are typically chosen by the user. Sherman and Grimrud[6] used $\Delta p_r = 4$ Pa and $C_D = 1$.

Simulation Setups

The CFD model used in this study was constructed based on a 28,317 m^3 flour mill and was described in detail by Chayaprasert et al.[4]. The pressurization test was simulated by performing eight steady – state flow simulations in which pressure differences of ±2.5, ±10, ±20 and ±50 Pa between the inside and outside of the mill were investigated. Note that both the positive and negative pressure ranges were included, representing both pressurization and de – pressurization (suction). The primary result obtained from the simulated pressurization test was the volume air flow rate through the leakage areas on the mill envelope for each simulation. By fitting this result with Eq. 1, the c and n constants were determined. Assuming $\Delta p_r = 4$ Pa and $C_D = 1$, the effective leakage area, A_L, (Eq. 4) was then calculated.

Assuming zero wind speed, Eq. 2 can be re – written as:

$$Q = \frac{A_L}{1000}\sqrt{C_s\Delta t} \qquad \text{Eq. 5}$$

Similarly, assuming zero temperature difference Eq. 2 can be re – written as:

$$Q = \frac{A_L}{1000}\sqrt{C_w U^2} \qquad \text{Eq. 6}$$

Once the correlated data points of Q and (t are obtained, C_s can be calculated by fitting these data points with Eq. 5. A similar approach can be applied to Eq. 6 for calculating C_w. The data points that were fitted with Eq. 5 were obtained from 16 simulations. Two indoor temperatures were selected, 25 and 30 ℃. At each indoor temperature, eight outdoor temperatures that yielded temperature differences of ±5, ±10, ±15 and ±20 ℃ were selected. Another simulation set was performed to acquire the Q – vs – U correlation in Eq. 6. The C_w value of a structure is unique for every wind direction, depending on the layout of surrounding area. It has been shown that wind direction has a considerable effect on the fumigant leakage rate[1,4]. However, it was not possible to determine a C_w value for every possible wind direction. In order to minimize the number of simulations, only the wind coefficients corresponding to eight wind directions (i. e., N, NE, E, SE, S, SW, W and NW) were determined. Four simulations each with different fixed wind velocities (i. e., 4, 8, 12 and 16 m/s) were performed for

each wind direction.

Data Processing

Chayaprasert et al.[1] utilized the CFD model to evaluate the effect of multi-year weather conditions on the HLT and Ct product. Eleven sulfuryl fluoride (SF) fumigation simulations were performed using hourly average historical weather data of the same time period between 1996 and 2006. It was assumed that for each year's simulation (1996, 2006) the fumigation started at 12:00pm on 4 July and lasted 24 hours. The HLT of each simulation was determined by first normalizing the average fumigant concentration curve by the initial concentration. Next, the normalized concentration curve was fitted with the following equation:

$$C_{norm} = \frac{1}{2^{\frac{t}{HLT_{sim}}}} \qquad \text{Eq. 7}$$

where C_{norm} is the normalized concentration (dimensionless) and t is the elapsed time (hr). The Ct product was effectively the area under the average concentration curve, which was calculated by integrating the non-normalized concentration curve.

In the present study, the HLT and Ct product determined from the average fumigant concentration curve were compared with the respective values predicted using the superposition method. The HLT prediction was calculated using the following equation:

$$HLT_{sup} = \frac{V}{Q}\frac{\ln(2)}{3600} \qquad \text{Eq. 8}$$

where V is the volume of the structure (m^3) and Q is the volumetric gas leakage rate (m^3/s) predicted by the superposition method. For each of the 11 – year fumigations, the gas leakage rate, Q, was calculated by substituting the average ambient temperature and wind speed into Eq. 2 and selecting the wind coefficient based on the most dominant wind direction. The V/Q term is the reciprocal of the air change rate. A different form of Eq. 8 is used by the tracer gas dilution standard test method[7] to describe the relationship between the tracer gas concentration decay and air change rate in a single volume. The Ct product was predicted as follows:

$$Ct_{sup} = \frac{-C_{i,sim}HLT_{sup}(2^{-\frac{t}{HLT_{sup}}}-1)}{\ln(2)} \qquad \text{Eq. 9}$$

where $C_{i,sim}$ is the initial gas concentration (g/m^3) determined from the simulation. This equation is essentially the integration of the non – normalized form of Eq. 7.

Results and Discussion

By fitting the data points of the simulated pressurization test with Eq. 1, the flow coefficient, c, and the pressure exponent, n, were determined to be 0.293 and 0.5, respectively. The discharge coefficient, C_D, and air density were assumed equal to 1 and 1.18, respectively. Substituting these c, n, C_D and (values in Eq. 4 resulted in an effective leakage area, A_L, of:

$$A_L = \frac{10000 \times 0.293}{1}\sqrt{\frac{1.18}{2}}\,\Delta p_r^{(0.5-0.5)} = 2251\ cm^2$$

The resulting infiltration rates of the simulations performed for determining the stack coefficient yielded the following correlation between the temperature difference, (t, and the infiltration rate, Q:

$$Q = 0.0673 \times \Delta t^{0.5}$$

The stack coefficient was calculated by equating the above equation to Eq. 5:

$$\frac{A_L}{1000}\sqrt{C_s \Delta t} = 0.0673 \times \Delta t^{0.5}$$

$$\frac{2251}{1000}\sqrt{C_s} = 0.0673$$

$$C_s = 0.000894$$

The resulting infiltration rates of the simulations performed for determining the wind coefficients yielded the following linear correlation between the infiltration rate, Q, and wind velocity, U:

$$Q = au$$

where the slope a varied between 0.0125 0.0664 for different wind directions. The wind coefficient was calculated by equating the above equation to Eq. 6:

$$\frac{A_L}{1000}\sqrt{C_w U^2} = aU$$

$$\frac{2251}{1000}\sqrt{C_w} = a$$

$$C_w = 2.251 \times a$$

The resulting wind coefficients for all wind directions were between $0.308 \times 10^{-4} \times -8.697 \times 10^{-4}$. As previously mentioned, this characteristic difference was a result of the fact that the surrounding landscape around the flour mill is not the same in all directions. Grain bins and silos are located on the north end of the flour mill. These structures reduced the dynamic head of the wind from the north and north-west directions. The south side of the mill is not exposed to the external environment, but attached

to a grain bulk structure and a packaging building. Therefore, the wind coefficients of the mill for the north, north-west and south wind directions were noticeably lower than those for the other wind directions.

The historical weather data and fumigation results of the 11-year fumigation simulations[1] as well as the respective HLT and Ct product predictions by the superposition method are summarized in Table 1. Wind speed and ambient temperature are given in terms of average values and standard deviations. The average wind speeds and outdoor temperatures were between 1.5 and 5.1 m/s and 16.2 and 29.1℃, respectively. Wind direction is given in terms of the most dominant wind direction (i. e., the mode) and the numbers of hours during which the mode wind direction occurred. A greater number of hours of a particular mode wind direction indicated that the wind was relatively steady in terms of traveling direction. The initial concentrations were between 44.6 and 54.3 g/m^3. The simulated HLTs and Ct products ranged from 10.7 to 23.3 hours and from 476 to 840 g · h/m^3, respectively. Note that the superposition method assumed fixed weather conditions in predicting the HLT and Ct product while those used in the simulations did not remain constant during the fumigation period (i. e., outdoor temperature varied in a sinusoidal fashion, and both wind speed and direction randomly varied). The HLT and Ct product predictions were however relatively accurate. For all except one simulation (2005), the HLT and Ct product difference percentages were within (20 and 10%), respectively.

Table 1. The weather conditions and results of the 11-year fumigation simulations as well as the respective HLT and Ct product predictions by the superposition method.

Year	Outdoor Temp.[a] (℃)	Wind Spd.[a] (m/s)	Wind Dir.[a] (degree)	Simulation[a]			Superposition		% Difference	
	Avg. [S. D.]	Avg. [S. D.]	Mode [# of hrs]	Init. Conc. (g/m^3)	HLT (hr)	Ct (g · h/m^3)	HLT (hr)	Ct (g · h/m^3)	HLT	Ct
1996	20.9 [4.4]	1.5 [1.3]	0 [21]	54.3	23.3	840	19.2	773	18	8
1997	16.2 [3.5]	4 [1.6]	315 [16]	49.7	13.6	633	13.6	624	0	1
1998	23.3 [2.9]	4.4 [1.3]	0 [11]	53.4	18.2	757	18.9	757	−4	0
1999	28.3 [3.6]	4 [1.6]	225 [20]	49.6	13.2	598	14.6	641	−11	−7
2000	24.4 [2.5]	2.1 [1.9]	0 [10]	52.8	19.6	752	23.5	798	−20	−6
2001	22.5 [3.5]	3.5 [0.9]	270 [14]	52.5	15.5	696	13.4	654	14	6
2002	29.1 [3.3]	3.2 [0.8]	45 [8]	51.2	19.8	730	22.4	763	−13	−5
2003	25.2 [4.9]	5.1 [2.6]	225 [7]	48.5	12.5	571	11	552	12	3
2004	23.6 [3.7]	4.7 [2.4]	270 [14]	44.6	10.7	476	11.2	511	−4	−7
2005	25.5 [4.1]	4.1 [1.3]	0 [6]	49.7	15.9	658	22.1	738	−39	−12
2006	22.4 [3.1]	4.8 [1.4]	45 [10]	49.7	15.7	672	12.8	607	18	10

aData generated by Chayaprasert et al.[1]

Overall, the simulated pressurization test and superposition method yielded satisfactorily accurate predictions of HLT, suggesting their benefits to optimizing structural fumigation.

Nevertheless, due to the fact that this study was simulation – based, several simplifications were assumed. While it was utilized on a flour mill structure in this study, the superposition method was originally developed for application in residential houses. All results in this study were generated based on a CFD model which already includes a set of inherent assumptions such as pressure distribution on the external walls, leakage characteristic, and numerical rounding. Accuracy of the effective leakage area, A_L, can be affected by the choice of the discharge coefficient, C_D, which can be between 0.6 and 1 depending on the pressurization test standard. It is not possible to obtain the true values of the stack and wind coefficients, C_s and C_w. Thus, the accuracy of the superposition method will always be compromised by the estimation of their values. The accuracy of the Ct product prediction is affected not only by the HLT, but also the initial concentration. Unlike in this study, in practice the initial concentration has to be estimated by dividing the amount of initially released fumigant by the estimated structure volume before the fumigation starts. As can be seen in Eq. 9, the Ct product is directly proportional to the initial concentration. In other words, the error percentage in the initial concentration estimation yields the same error percentage in the Ct product prediction (e. g., 10% error in the structure volume estimation would result in 10% error in the Ct product prediction). As a result, the pressurization test and superposition method should be further evaluated experimentally. In addition, the costs versus benefits of the pressurization test should also be properly analyzed.

Conclusions

The application of the pressurization test and superposition method for prediction of structural fumigation performance were evaluated by CFD simulations with the CFD model developed by Chayaprasert et al.[4] A simulated pressurization test was performed to determine the structure's effective leakage area. Then, the simulated structure was subjected to several fixed environmental conditions to determine the stack and wind coefficients. Finally, the HLTs and Ct products generated by the 11 – year fumigation simulations[1] were compared with the values estimated based on the superposition method. The results showed that the pressurization test and superposition method have potential application benefits for optimizing the structural fumigation process. The HLT and Ct product predictions were within (20 and (10% of the simulated values, respectively, in 10 out of 11 years. However, additional experimental investigation is needed to verify their application in practice.

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Interests in the Mixture Ethyl Formate/Allyl Isothiocyanate for the Fumigation of Infested Wheat by the Rice Weevil: *Sitophilus oryzae* L. and the Granary Weevil: *Sitophilus granarius* L.

CIESLA Yann and DUCOM Patrick *

Abstract: Since several post – harvest insecticides like dichlorvos or methyl bromide are being phased out in the European Union, new alternatives have to be found. The volatile liquid mixture ethyl formate/allyl isothiocyanate (AITC) 95%/5% (w/w), was recently tested successfully and patented by the Australian Stored Grain Research Laboratory (SGRL). We have carried out trials with an application rate of 60 g/m^3 and an exposure time of 24 and 48 hours, and with an application rate of 120 g/m^3 and an exposure time of 24 hours against the two species: *Sitophilus oryzae* and *Sitophilus granarius*. Ethyl formate concentrations were measured by GC, FID. These trials were carried out with samples of 12 kilograms of infested wheat in 30 – litre gas – tight drums at about 20 C. Applications were made by introducing the mixture with a syringe through a septum on the grain in movement. With an application rate of 60 g/m^3, the mortality rate of granary weevil adults is about 42 % after 24 and 48 h, and respectively 80 and 92 % for the mortality rate of rice weevil adults after 24 and 48 h. The pupae and/or aged larvae emergence reduction is very low: between 0 to 40 % depending on the conditions. However, results of treatments with an application rate of 120 g/m^3 show respectively a control of 96.6 and 99.3 % of granary weevil and rice weevil adults. Hidden stages of granary weevils and rice weevils show respectively a emergence reduction of 97.4 and 92.5 % for pupae and aged larvae, 98.9 % for larvae of both species, 100 and 99.8 % for eggs. This efficacy is encouraging, but grain treatment with this application rate of 120 g/m^3 is higher than the lower explosive limit (85 g/m^3), so new ways of application must be found.

Key words: fumigation, *sitophilus oryzae*, *sitophilus granarius*, ethyl formate, allyl isothiocyanate

Introduction

In European Union, several grain insecticides were recently phased out methyl bromide, dichlorvos, malathion.. Besides some contact insecticides like chlopiriphos methyl pirimiphos methyl, deltamethrin, and phosphine, there is a need for new ways for grain disinfestations. It is the case with the mixture ethyl formate (EF)/ allyl isothiocyanate (AITC) which can be potentially a promising alternative. EF is an old fumigant which presents an interesting insecticide effect tested at the beginning of 20th century [1]. Lateron, many trials have been carried out on stored grain pests with success [2]. Currently, pure ethyl formate is used on dried fruits in Australia, and more recently it registered in mixture with CO_2 under the trade name "VAPORMATE". The Australian Stored Grain Research Laboratory (SGRL) have carried out several trials on wheat with pure EF [3] and with a mixture composed of 95% of EF and 5% (w/w) of a synergist: MITC (methyl isothiocyanate), an isothiocyanic ester similar to AITC[4]. These isothiocyanic esters are extracted from the plant family Cruciferae[5] and enhance the efficacy of EF. The main advantages of the mixture EF/AITC are that the insecticide efficacy is very quick compared with Phosphine, the fumigant registered worldwide and the EF residues decline after fumigation to natural levels without forced aeration[6]. In Australia, the MRL fixed for dried fruits is 1 mg/kg and the natural levels of EF are sometimes (few months after harvest) higher than just after fumigation[7]. Moreover, EF can be applied safely because its Threshold Limit Value (TLV) is 100 ppm and its toxicological classification Xn. The main inconvenient of EF is its very low flash point (–22℃) and a low flammability level at 85 g/m^3. The application rate should be below this limit [8] or 92.5 g/m^3[4] in accordance with temperature rate. The internal stages of *Sitophilus sp.* are difficult to control with a successful of 100%, and to obtain this result an application rate above the flammable level is necessary [3]. That's why AITC is involved in the reduction of concentration in EF necessary

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to control all stages of *Sitophilus sp.*

These trials were carried out to investigate the efficacy of the mixture with an application rate of 60 g/m^3 and a double dosage 120 g/m^3 on the rice weevil: *Sitophilus oryzae* and the granary weevil: *Sitophilus granarius* in wheat. The double dosage was tried to find a better efficacy but in practice the flammability problem will have to be solved. In the same time, this study allowed the observation of sorption of the mixture in infested wheat.

Materials and Methods

Rearing Technique

The insects used in these trials were reared on wheat in a rearing room at 25℃ (±1℃) and 60% r. h. (±5%). Two species were used in this study, the granary weevil: *Sitophilus granarius* L. and the rice weevil: *Sitophilus oryzae* L. When first adults emerged three kilograms of infested wheat were mixed with nine kilograms of non-infested wheat to avoid a too high infestation rate in 30 liters gastight drums and therefore anoxia during trials. The final infestation rate was checked by an X - Ray machine and was between 0.5% to 2% of the grains.

Pre-fumigation Procedure

To begin, samples of 12 kg of infested wheat were put into the drums. A temperature sensor (Captsystemes) was put in each drum and it was programmed to take a measure every 10 minutes. Every drum was equipped with a septum and two pipes, in the aim to respectively inject liquid mixture of EF/AITC and measure gas concentrations during the fumigation.

In the first series, the trials were carried out with the two species of weevils and with two exposure times, 24 and 48 hours. So, eight drums (four treated drums and four control drums) were placed in the same conditions of temperature: about 20℃. The application rate of the mixture was 60 g/m^3.

In the second series, a double dosage was applies: 120 g/m^3. The exposure time was just 24 hours and the sensibility of the two species was tested in the same conditions than in the first series.

Fumigation

The injection of the EF/AITC mixture was made with a syringe through a septum on the grain in movement. Drums were turned 5 minutes after injection in order to homogenize the insecticide. Drums were placed at about 20℃ during the fumigation. Ethyl formate concentrations were measured by Gas Chromatograph (GC), with Flame Ionization Detector (FID) (Varian Star, 3400 CX).

Post - fumigation Procedure

At the end of the exposure time, a measure of oxygen concentration was taken to be sure that insects in gas-tight drums were not in anoxia. The material used was an oxygenmeter HM16N (±1% error). The drums were opened just after the last gas concentration measurement. After fumigation and aeration, the moisture content of the grain was taken with a moisture meter (Chopin, Wile 55) in each drum.

Insect Efficacy of the EF/AITC Mixture

Just after the fumigation the wheat was sifted and all adult insects were observed to see if they were dead, alive or dying. For the controls a sample of only 2 kilograms was sifted. All dying adults were placed in the rearing room and 24 hours later they were observed once again and identified like dead or alive.

To determine the efficacy on hidden stages, a sample of 1 kilogram's of each drum was placed, after the first sifting and without adults, in the rearing room. Eleven days later, all samples were sifted compared with the controls in order to obtain the emergence reduction. This period corresponds to the emergence of pupae and aged larvae at the time of fumigation [9]. Thirty - two days after the end of fumigation, another sifting was made in the order to obtain emergence reduction of larvae (except the oldest larvae). To finish, a last sifting was made thirty - eight days after fumigation to obtain emergence reduction of eggs.

Results and Discussion

Fumigation with An Application Rate of 60 g/m^3

The first results of this study reveal that EF concentrations in drums declined very quickly after injection (Fig. 1). This graph shows that after 24 hours concentrations are below 1 g/m^3 in the two drums shown in this figure. So, ethyl formate was quickly sorbed after the beginning of the fumigation. In fact, one hour and thirty minutes after injection, concentrations are already reduced by half (between 28 and 29 g/m^3). In a previous study, AITC concentrations were measured by GC (TSD) and show the same rate of sorption [10]

Before aeration, oxygen concentration meas urements show that the concentrations in the headspace atmosphere of the drums were slightly lower than normal atmosphere (less than 3%).

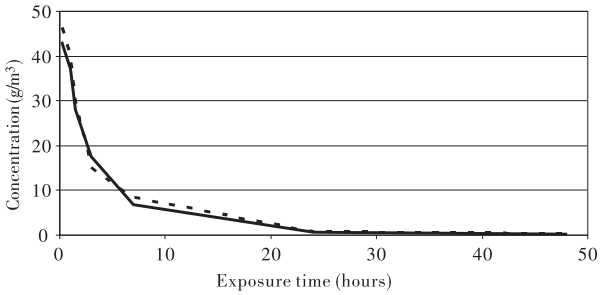

Fig. 1 Evolution of ethyl formate concentration measured by GC (FID) in the drums of *Sitophilus granarius* (solid line) and *Sitophilus oryzae* (broken line) fumigated at 60 g/m^3 with an exposure time of 48 hours.

But in every case, the oxygen percentage was lower in the control drum than in the treated drum (Table 1), as a result of more live insects in the controls. The grain moisture is between 14.3 and 15.2%.

Table 1. Oxygen concentrations in every drum at the end of fumigation

Species	Exposure time (hours)	O_2 concentration in treated drums (%)	O_2 concentration in control drum (%)
S. oryzae	24	20.2	19.5
S. granarius	24	20.3	19.7
S. oryzae	48	19.7	18.3
S. granarius	48	20.0	18.5

The first sifting after fumigation (Fig. 2) shows that less than half of granary weevil adults were dead (42%) and there were no differences between the two exposure times tested. On the other hand between 92% of rice weevil adults were killed in 24 hours fumigation and 80% after 48 hours fumigation. In the control, the mortality rate was between 0.5 and 1.5%, except in the control of the drum with granary weevils with an exposure time of 48 hours, the mortality rate of this control was about 7%. The fumigation seems to be more efficient on the rice weevil adults than on the granary weevil adults. So, after these results it's obvious that the exposure time of the fumigation didn't give better results with a period longer than 24 hours. After 24 hours, gas concentrations are very low, less than 1 g/m^3 and practically 0 g/m^3 after 48 hours. That confirms that the second day of fumigation is useless because it remains no gas and then it do not enhance the efficacy of the treatment.

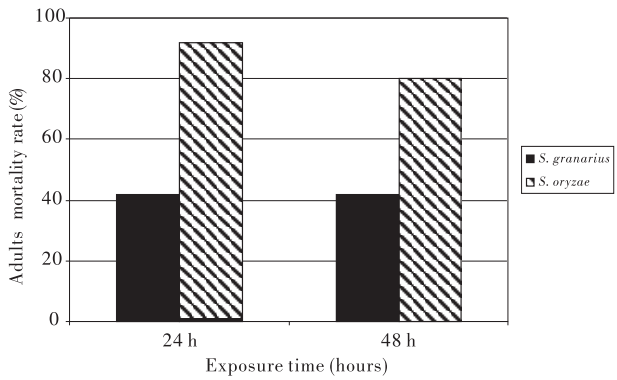

Fig. 2 Mortality rate of *S. granarius* and *S. oryzae* adults after fumigation with the mixture EF/AITC with exposure times of 24 and 48 hours and an application rate of 60 g/m^3

The results of emergence reduction (Table 2), corresponding to pupae and aged larvae during the fumigation, confirm the lake of efficacy of the treatment with this application rate. Indeed, there were not emergence reductions in the samples of rice weevils, very few for the granary weevil samples: 21% for the 24 h fumigation and less than 40% for the 48 h fumigation. These results show the ineffectiveness of EF/AITC mixture on hidden stages of *Sitophilus. spp*. That's why, after this ineffectiveness, the siftings were stopped and the trials with this application rate were considered uninteresting since the mixture was not efficient on the most resistant stages of these species, eggs and pupae[11].

Table 2. Emergence reduction of pupae and aged larvae of *Sitophilus granarius* and *Sitophilus oryzae* after fumigation with the mixture EF/AITC with an application rate of 60 g/m^3

Species	Exposure time (hours)	Number of insects emerged/kilogram's of wheat in treated samples	Number of insects emerged/kilogram's of wheat in control samples	Emergence reduction (%)
S. oryzae	24	47	37	0
S. granarius	24	23	29	20.7
S. oryzae	48	48	48	0
S. granarius	48	20	33	39.4

Fumigation with an Application Rate of 120 g/m^3

The concentration of EF in drums, after an injection of the mixture EF/AITC, decreases very quickly (Fig. 2) and after six hours of fumigation it remained less than 20 g/m^3 in the two drums. After an exposure time of 24 h, concentrations were between 1 and 2 g/m^3 of EF.

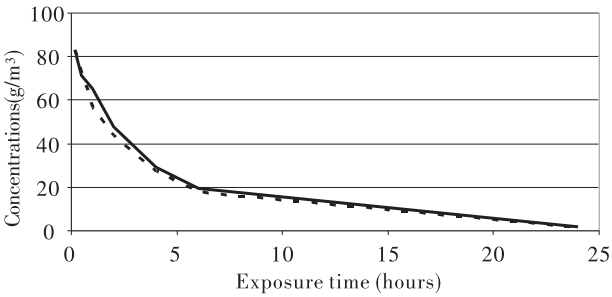

Fig. 3 Evolution of ethyl formate concentration measured by GC (FID) in the drums of *Sitophilus granarius* (solid line) and *Sitophilus oryzae* (broken line) fumigated at 120 g/m^3 with an exposure time of 24 hours.

The grain moisture was measured just after aeration and it was between 15.3% and 15.7%.

After fumigation, the first sifting (Fig. 4.) reveals that 99.3% of granary weevil adults were killed and 99.6% of rice weevil adults and less than 3% in the control (respectively 1.3% and 2.8%). So, when the dosage is twice more important, the efficacy is much better but all insect adults are not killed.

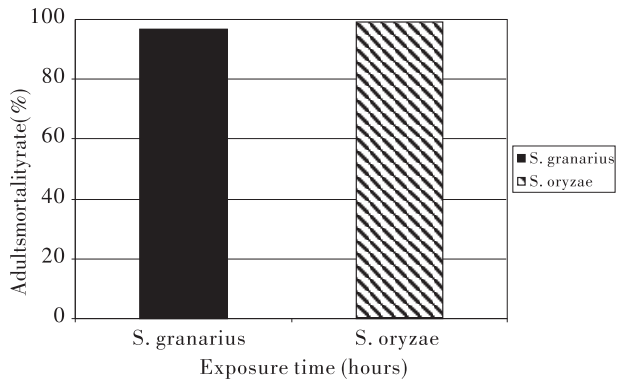

Fig. 4 Mortality rate of adults after fumigation with the mixture EF/AITC with an exposure time of 24 hours and an application rate of 120 g/m^3

The second sifting, eleven days after the end of fumigation, shows that the emergence reduction of pupae and aged larvae is better than in the first trials with an application rate of 60 g/m^3 (Table 3).

Table 3. Emergence reduction of pupae and aged larvae of *Sitophilus granarius* and *Sitophilus oryzae* after fumigation with the mixture EF/AITC with an application rate of 120 g/m^3

Species	Number of insects emerged/kilogram's of infested wheat in treated samples	Number of insects emerged/kilogram's of infested wheat in control samples	Emergence reduction (%)
S. oryzae	4	53	92.5
S. granarius	2	77	97.4

The third sifting, thirty – two days after the end of the fumigation, reveals that the emergence reduction of larvae was very important with 98.9% for the two species. But the total efficacy is not yet reached. Larvae stages are the most sensible hidden stages but the fumigation did not reach a 100% efficacy.

The fourth and last sifting, 38 days after the end of the fumigation shows that the emergence reduction of eggs is complete for the granary weevil sample with 100% of emergence reduction (Table 4). For the rice weevil sample, just three insects emerged and the total emergence reduction is not reached even if 99.8% of eggs were killed during the fumigation.

Table 4. Emergence reduction of eggs of *Sitophilus granarius* and *Sitophilus oryzae* after fumigation with the mixture EF/AITC with an application rate of 120 g/m^3

Species	Number of insects emerged/kilogram's of infested wheat in treated samples	Number of insects emerged/kilogram's of infested wheat in control samples	Emergence reduction (%)
S. oryzae	3	1564	99.8
S. granarius	0	1008	100

Conclusion

For grain disinfestations, the volatile liquid mixture ethyl formate/allyl isothiocyanate (AITC) 95 %/5 % (w/w), was tried with an application rate of 60 g/m^3 and an exposure time

of 24 and 48 hours, and with an application rate of 120 g/m^3 and an exposure time of 24 hours against the two species: *Sitophilus oryzae* and *Sitophilus granarius*. There are big differences of efficacy between the two fumigations with the two concentrations tested (60 and 120 g/m^3). However the control of the two stored products insects *Sitophilus granarius* and *Sitophilus oryzae* is not reached except for the eggs of *Sitophilus granarius*. It is then possible to think that a higher dosage could kill all stages of these two species of weevils. If the gas exposure time is longer, the efficacy of the fumigation will certainly not increase since the sorption of EF by wheat is very quick and after 24 hours the gas concentration is very low, below 2 g/m^3 of EF. But the main problem with this mixture Ethyl formate/Allyl isothiocyanate (95%/5%) is the flammable level. Fumigations with an application rate of 120 g/m^3 is already higher than this flammable level, and to be fully efficient the dosage should be still increased. We are far beyond the flammability level of 85g/m^3. The main factor which could help to maintain a high level of gas, would be to decrease the sorption. To face this problem, the technique of application or the formulation of the mixture should be adapted.

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0909

Fumigant Activity of Essential Oil from *Armoracia rusticana* (L.) against *Plodia interpunctella* (Lepidoptera: Pyralidae)

Chen Haoliang[1], Xiong Heming[2], Akinkurolere R. O.[1], Zou Jianghan[3], Xu Hebing[3] and Zhang Hongyu[1*]

Abstract: The fumigant toxicity of essential oil from Horseradish plant, *Armoracia rusticana* (L.) was assessed against the Indian meal moth, *Plodia interpunctella* (Hübner). Eggs, larvae, pupae and adults were exposed to different concentrations of 0, 2, 4, 8, 16 and 32 μL/L of essential oil. *A. rusticana* oil was found to significantly affect the egg hatch rate, pupal survival, larval and adult mortality of *P. interpunctella*. Results showed that the LC_{50} value for larvae was 15.53g/m^3, as well as 5.54g/m^3 for adults. Fumigation activity of the *A. rusticana* oil appeared to be dose dependent. An increase in treatment concentration led to reduced hatch rate in eggs, and an increase in mortalities of both larvae and adults. Although there was significant difference in survival between the pupae that received plant oil fumigation and those in control treatments, but it appears that pupal eclosion was generally high across treatments, since a high percentage (69%) of pupae eclosed as compared to 90% eclosion observed in the control. These results indicate that it may be possible to achieve toxicity levels similar to those of standard chemical fumigants through the applications essential oils from *A. rusticana*.

Key words: plant essential oil, *Plodia interpunctella*, fumigant activity

Introduction

Insect pests cause a great deal of losses of stored food products. The Indian meal moth, *Plodia interpunctella* (Hübner), is one of the major lepidopteran pests of stored products in China and around the world, causing serious losses in stored produce [1,2,3]. The complete development of this moth takes 27 days from egg to adult at optimal temperature of 30 ℃, 70% r. h. and controlled photoperiod of 16 h light and 8 h dark [4]. Agrochemicals are frequently being used to counter the attack by insect pests. However, these chemicals are faced with great criticisms for their non-environmentally friendly effects and high costs. The foregoing demerits of agrochemicals have lead to the quest for alternative control measure.

Horseradish, *Armoracia rusticana* (Linn.) is a perennial plant of the Brassicaceae family, which includes mustard and cabbages. The plant is probably native to southeastern Europe and western Asia, but is popular around the world today. It grows up to 1.5 metres (five feet) tall and is mainly cultivated for its large white, tapering root[5].

The root is the only part now used, and in the fresh state only. It is nearly cylindrical, except at the crown, where it is somewhat enlarged. It contains potassium, calcium, magnesium and phosphorus, the chief produce being the volatile oil called allylisothiocyanate (AITC), which is identical with that of Black Mustar, which is an antibiotic: protecting food against pathogens. This volatile oil, which is easily developed by scraping the root when in a fresh state, does not pre – exist in the root, the reaction not taking place in the root under normal conditions, because the Sinigrin and Myrosin exist in separate cells, and it is only the bruising of the cells that brings their contents together [6].

The oil is highly diffusible and pungent on account of the Myrosin contained, 1 drop being sufficient to odorize the atmosphere of a whole room. On exposure to the air, the root quickly turns colour and loses its volatile strength[7].

Materials and Methods

Insect Culture and Source

Plodia interpunctella (Hübner) used in this study were obtained from laboratory colony

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maintained on artificial diet consisting of cracked wheat (1000 g), wheat shorts (1000 g), wheat germ (100 g), brewer's yeast (80 g), sorbic acid (4 g), methyl – *p* – hydroxybenzoate (4 g), glycerine (240 mL), pure honey (240 mL) and 120 ml of water[8]. The insect was reared at 29 (±1)℃, 40 60% relative humidity (r. h.), with a 14:10 h light: dark regime.

Source of *Armoracia rusticana* and Extraction of Essential Oil

A. rusticana used in this investigation was obtained fresh during May 2007 from the Teaching and Research farm of Huazhong Agricultural University, Wuhan Hubei China.

Fumigation Bioassay

Fumigation bioassays were carried out in 500ml glass conical flask (fumigation chamber). Essential oil was applied onto a filter paper (1cm 4cm) and suspended vertically within the chamber by thread, together with insect cage. The fumigation chamber was covered with a rubber stopper and sealed with an adhesive tape. Twenty adult *P. interpunctella* (0 – 2 d old), were placed in the cylindrical cage (9cm 3cm), which was perforated with small holes to allow the penetration of the gas. *A. rusticana* oil was applied at the dose of 0 (control), 2, 4, 8, 16 and 32 g/m^3 and each treatment had 3 replicates. Controls received filter paper alone. Percentage insect mortality was recorded after 72 h of exposure to the essential oil gas.

Fumigation of Eggs with the Essential Oil of *A. rusticana*

Black cloth (12cm × 12cm) was placed in a jar, then 15 pairs of newly emerged (0 – 24 h old) adult of *P. interpunctella* were introduced into the jar and were allowed to oviposit on the cloth for 24 h. Afterwards, the cloth bearing the freshly laid eggs was removed and counted. Black cloth bearing 30 eggs was placed in a 500ml conical flask. The essential oil was introduced at different dose rates as described in the previous section. After fumigation, the gas was released from the chamber and eggs were held for 5 days at 29 ± 1℃, until mortality could be determined by the presence or absence of hatching in both treated and untreated (control) eggs.

Fumigation of Larva with the Essential oil of *A. rusticana*

Newly laid eggs were placed in glass together with small quantity of artificial diet, the bottle was covered with organdy screen and incubated at 29(±1)℃. The resulting 3rd instar larvae emerging 2 d after hatching were selected for this experiment. It should be noted that result from a preliminary culture showed that 3rd stadium of *P. interpunctella* were obtained 12 d after hatching. Thirty *P. interpunctella* larvae (3rd instar) were selected from the stock insect culture and placed in a cylindrical insect cage (4.5cm × 1.5cm), together with small amount of artificial diet (5g), then capped with rubber stopper, the larva were used for the bioassays. Thereafter, the essential oil together with the insect cage was introduced into the arena as described in the previous section. After fumigation, the gas was released and the larvae was held for 5 days at 29 ± 1℃, then larval mortality was counted in both treated and control arenas.

Fumigation of Pupa with the Essential Oil of *A. rusticana*

Thirty pupae (30) of *P. interpunctella* were placed in a perforated insect cage (4.5cm × 1.5cm), capped with rubber stopper, the cage and the essential oil were suspended into the fumigation chamber as earlier described. After fumigation, the gas was released from the chamber and held for 7 days at 29 ± 1℃, until mortality could be determined by the presence or absence of adult emergence in both treated and control pupae.

Statistical Analysis

Data from egg hatch, larval, pupal and adult mortality subjected to analysis of variance and where significant differences existed, means were compared using Tukey's b test. Because percentage hatch rate, larval and adult mortality were not normally distributed, data were first normalized by arcsine transformation before analysis. This consists of taking the arcsine of the square root of a number (y = sine (x) ^ – 1). After analysis, data were back – transformed by squaring the sine of the number[9].

Results

Effect of *A. rusticana* Oil on Eggs of *P. interpunctella*

The fumigant effect of differnrent concentrations of *A. rusticana* oil on eggs of *P. interpunctella* is shown in Fig. 1. *A. rusticana* oil affected percentage hatch rates significantly ($P < 0.001$). The fumigant activity of the oil on the eggs was dose dependent. At lowest tested dose (2 μL/L), there was high percentage hatchability and this was not significantly ($P < 0.05$) different from that observed in the control. Whereas significantly lower percentages

hatch rates were observed at higher doses. This is suggestive that *A. rusticana* had fumigant action against the eggs of *P. interpunctella*.

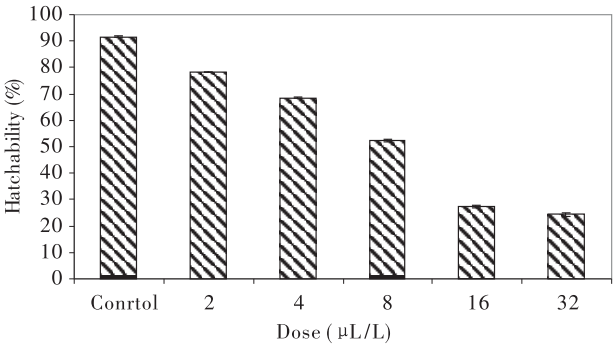

Fig. 1 Effect of fumigation with varying concentrations of *Armoracia rusticana* oil against eggs of *Plodia interpunctella*. Columns followed by the same alphabet(s) are not significantly different ($P < 0.001$) using Tukey's b test.

Effect of *A. rusticana* Oil on Larvae of *P. interpunctella*

The percentage mortality of *P. interpunctella* larvae after exposed to different doses of *A. rusticana* oil is represented in Fig. 2. Fumigation with *A. rusticana* oil had significant effect on larval mortality at all treatment levels in relation to the control ($P < 0.001$). As concentration increases, larval mortality increases. The highest mortality was observed among larvae groups that were exposed to 32 μL/L dose of *A. rusticana* oil.

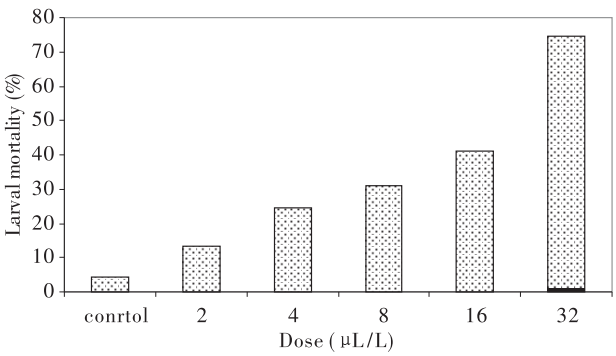

Fig. 2 Fumigant effect of *Armoracia rusticana* oil at different concentrations against larvae of *Plodia interpunctella*. Columns followed by the same alphabet(s) are not significantly different ($P < 0.001$) using Tukey's b test.

Effect of *A. rusticana* Oil on Pupae of *P. interpunctella*

After pupae of *P. interpunctella* were fumigated with oil of *A. rusticana*, there was significant ($P < 0.013$) difference in the number of pupa that became adult (eclosed) Fig. 3. It was generally observed that there was high percentage pupal survival across treatments. However, there was no significant ($P > 0.05$) difference in the percentage survival of among the pupae that were exposed to 2, 4 and 8 μL/L and between those exposed to 16 and 32 μL/L doses of plant oil.

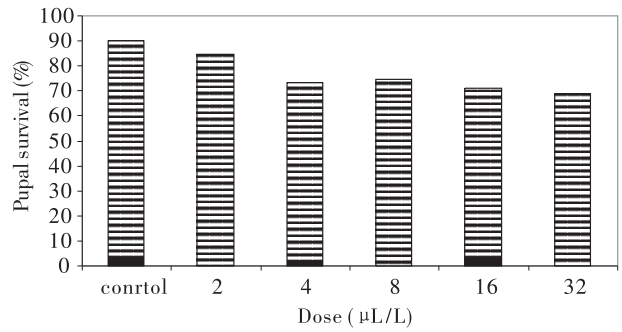

Fig. 3 Fumigant effect of *Armoracia rusticana* oil at different concentrations against pupae of *Plodia interpunctella*. Columns followed by the same alphabet(s) are not significantly different ($P < 0.013$) using Tukey's b test.

Effect of *A. rusticana* Oil on Adults of *P. interpunctella*

Fig. 4 shows the effect of fumigation with varying concentrations of *A. rusticana* oil against adults of *P. interpunctella*. *A. rusticana* oil had significant ($P < 0.001$) effect on *P. interpunctella* adult mortality. The fumigant activity of the oil on the adults was dose dependent, with percentage mortality increasing in relation to increase in treatment concentration. The highest mortality was recorded in insects exposed to 32 μL/L, however, this was not significantly ($P > 0.05$) different from mean percentage mortality of adults exposed to 16 μL/L of plant oil. This indicates that *A. rusticana* had fumigant activity against the adults of *P. interpunctella*.

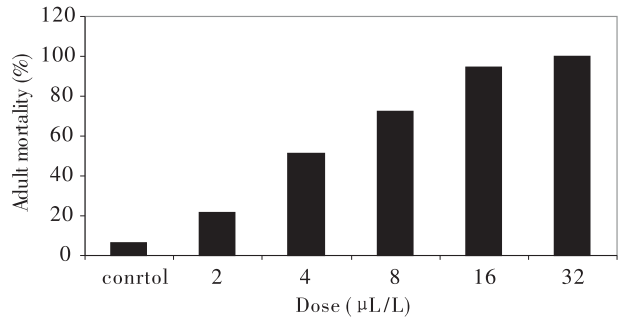

Fig. 4 Effect of fumigation with varying concentrations of *Armoracia rusticana* oil against adults of *Plodia interpunctella*. Columns followed by the same alphabet are not significantly different ($P < 0.001$) using Tukey's b test.

Discussions

The essential oil of *A. rusticana* was

shown here to possess fumigant bioactivity against *P. interpunctella* (adults, pupae, larvae and eggs). It caused high percentage mortality of larvae and adult insects exposed to 32 μL/L gas vapour of *A. rusticana*, and hatchability was also inhibited at this dose. The oil however, appeared not to have much impact on pupal eclosion, since a high percentage (69%) of pupae eclosed as compared to 90% eclosion observed in the control.

The reason for the high fumigant effect of *A. rusticana* oil on eggs, larvae and adults of *P. interpunctella*, could be attributed to its high pungent odour due to the presence of AITC in the volatile oil. It could be that the volatile oil is able to block the spiracles of the insects by impairing breathing and thereby chocking them to death[10,11]. Its relatively low fumigant effect on pupal survival might be that the gas vapour could not permeate through the thick wall of the pupal case.

A number of toxic chemicals produced by plants elicit pungent sensation in mammals [12,13]. The efficiency of the WasaOuro system, an insecticide based on AITC, the active component responsible for insecticidal action of horseradish and other brassicaceae family, was found to possess fumigant action against *Lasioderma serricorne* and *Tribolium confusum* by disrupting normal reproductive cycles of both insects, resulting in an insect population reduction in grain foods[14]. Natural toxin of isothiocyanates including AITC, has been shown to have insecticidal activities. AITC was reported to increase the production of carbon dioxide in the American cockroach [15]. Fumigant activities of horseradish and garlic oils against *Lycoriella ingenua* (Diptera: Sciaridae) have been reported[11].

Increasing problems concerning the use of modem synthetic chemical insecticides, such as persistence of residues, resistance, and damage to the environment and human health have generated interest in naturally occurring products. It should be noted that biologically active compounds of food plants are assumed to be environmentally more acceptable and less hazardous than others to humans. The results presented in this study suggest that *A. rusticana* oil or its major constituents could be efficient fumigants and also could be integrated with other pest management procedures. Further studies are needed to assess the fumigant activity of this essential oil and its constituents to other insects. Also, detailed mechanism of action of AITC in target pests could be an interesting area of research.

Acknowledgements

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0910

Commercializing a New Fumigant: the ProFume® Success Story

Ellen Thoms[1], John Busacca[2] and Suresh Prabhakaran[2]

Abstract: ProFume gas fumigant (99.8% sulfuryl fluoride) is a broad spectrum, non-ozone depleting fumigant developed and manufactured by Dow AgroSciences LLC for the control of rodent, insect and other invertebrate pests. This fumigant was developed in response to post-harvest industry requests for an alternative to methyl bromide. ProFume has been developed for use in food handling establishments (eg., pet food facilities, bakeries, food production facilities, mills, warehouses, etc.), stationary transportation vehicles (railcars, shipping containers, trucks, etc.), temporary and permanent fumigation chambers, and storage structures. ProFume is relatively non-reactive as a gas and does not cause off-flavors. It is an odorless, colorless inorganic gas, and as such, does not form unpleasant odors. In addition, due to its higher vapor pressure and lower sorption characteristics, ProFume® compared to methyl bromide penetrates commodities more effectively reaching target pests faster for optimum control. Globally, over 1 000 commercial fumigations have been completed with high level of customer satisfaction. Development and successful commercial use in many countries prove that ProFume is a technically and economically viable alternative to methyl bromide and can also be used to fumigate insects resistant to phosphine.

Introduction

Post-harvest insect pests that infest food commodities in mills, warehouses, food storage and processing facilities can cause substantial economic and quality losses. Localized treatment, sanitation, and other physical methods may not adequately control these pests if infestations are widespread or in inaccessible areas. In these situations, fumigation has been the preferred method of pest control. Methyl bromide, previously the fumigant of choice, has been identified as an ozone depleting chemical and is being phased out under an international agreement known as the Montreal Protocol. It is to be completely phased out in developed nations by 2005 (with some critical use exemptions) and by 2015 in developing countries.

With the adoption of the Montreal Protocol, the phase out of methyl bromide in developing countries started and the search for replacements began. About this time, several progressive food industries in the United States and Europe approached Dow AgroSciences to consider developing sulfuryl fluoride for food commodity use. As a result, Dow AgroSciences formed partnerships with leading stored product researchers, fumigators and food industries around the world and developed ProFume® gas fumigant as a successful post-harvest fumigant.

Sulfuryl fluoride is fully oxidized and does not interact with or contribute to local ozone formation. It contains no chlorine or bromine and does not contribute to stratospheric ozone depletion[1,2]. Sulfuryl fluoride is broken down mainly through hydrolysis to release fluoride and fluorosulphate ions[1]. In 2002, Dow AgroSciences was awarded the Stratospheric Ozone Protection Award by the U. S. A. Environmental Protection Agency (EPA) for the development of ProFume gas fumigant. This award recognizes global, extraordinary achievements in international leadership and innovation in preserving the Earth's protective stratospheric ozone layer. Nominated winners have demonstrated a commitment to environmental stewardship through their precedent-setting innovation and leadership. In 2007, Dow AgroSciences was named a winner of the United Nations Montreal Protocol Innovators Award at the meeting commemorating the 20th anniversary of the Montreal Protocol. At that meeting, Dow AgroSciences was also named a winner of the EPA's "Best of the Best" Ozone Protection Award which honors an elite group of companies, organizations and individuals who have demonstrated long-term excellence in efforts to protect the stratospheric ozone layer.

History

Dow AgroSciences has registered and marketed sulfuryl fluoride as Vikane gas fumigant

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since 1961. It has been successfully used to fumigate more than one million structures including homes, museums, cathedrals, historical landmarks, rare book libraries, and scientific and medical research laboratories to eradicate termites and wood boring beetles. In Europe it was first introduced in the early 1990s in Germany to eliminate wood destroying beetles, such as *Anobium punctatum* and *Ptilinus pectinicornis*, from structures. In Sweden, sulfuryl fluoride is used in shipping containers and for the disinfestations of homes and wooden artifacts.

Physical Properties of Sulfuryl Fluoride

Sulfuryl fluoride has many physical properties that make it suitable for commodity fumigation. Sulfuryl fluoride is an inorganic, non-flammable, odorless and colorless gas. Because of its low boiling point and high vapor pressure, sulfuryl fluoride readily vaporizes under normal fumigation conditions, thus allowing rapid dispersion after gas introduction. Sulfuryl fluoride is noncorrosive, an important characteristic for a fumigant used in environments where sensitive equipment and electronic devices are employed. It does not react with materials to form unpleasant odors. However, heaters and open flames must be extinguished, because temperatures over 400℃ will cause decomposition products. Because of its low sorption characteristics, sulfuryl fluoride rapidly aerates from structures and commodities. Penetration in material and commodities is also fast. A study has shown that 60% of initial sulfuryl fluoride concentration could be reached at a depth of 30 cm of wheat flour in less than three hours[3].

Sulfuryl fluoride residues are transient in fumigated commodities. Sulfuryl fluoride rapidly dissipates following proper aeration procedures. The common residue following fumigation is fluoride. An extensive program of food quality studies have been conducted on a variety of dried fruits and tree nuts in cooperation with the Dried Fruit and Tree Nut Association (DFA) of California and other commodity groups. Similar studies on cereal grains, flour, and other key commodities have been conducted with food science experts. These research studies confirmed lack of adverse quality effects on cereal grains, dried fruits and tree nuts. The National Association of British and Irish Millers (NABIM) also evaluated these studies and is satisfied by the results.

Efficacy of ProFume

Efficacy research has been conducted in the laboratory and in the field to define dosages and treatment practices to optimize the control of key post-harvest insect pests. Laboratory efficacy studies have been conducted in cooperation with the USDA-ARS in Fresno, California; DFA of California, Central Science Laboratory (CSL) in the UK, Julius Kuehn Institute (formerly the Federal Biological Research Center for Agriculture and Forestry) in Germany, the University of Milan in Italy, and Laboratoire National des Denr es Stock es in France to define the dosages required to control all the life stages of target pests under a range of fumigation conditions. These studies[4-7] have confirmed the effectiveness of sulfuryl fluoride on all life stages of a wide range of post harvest insect pests including the important pest species of Coleoptera and Lepidoptera. These data have been used to develop the dosage calculations for the ProFume Fumiguide™, described below.

In addition to extensive laboratory and field efficacy trials with ProFume gas fumigant, population rebound studies have been undertaken in Europe[8,9] and the USA[10,11] to demonstrate effectiveness of ProFume in controlling stored product pest populations. The objective of these studies was to compare the impact of ProFume and methyl bromide fumigations upon populations of the red flour beetle (*Tribolium castaneum*), the confused flour beetle (*Tribolium confusum*), the Mediterranean flour moth (*Ephestia kuehniella*) and the Indian meal moth (*Plodia interpunctella*). Calculated percentage reduction in insects trapped per day during the post-fumigation monitoring period clearly indicated that ProFume has good efficacy and compares very favorably with the efficacy of methyl bromide. Variations in the long – term level of control following fumigation were attributed more to the level of hygiene and sanitation in the structure than to the product used, methyl bromide or sulfuryl fluoride[8].

ProFume® Fumiguide™

ProFume has been developed with an emphasis on Precision Fumigation™, defined as optimizing fumigant use to maximize efficiency and minimize risk. An important tool in Precision Fumigation is the ProFume Fumiguide, proprietary software developed by Dow AgroSciences. The Fumiguide integrates factors such as target pest, temperature, half loss time (HLT) and exposure period to accurately determine the fumigant dose and accumulated concentration x time (CT) CT dosage required for the space or commodity. When monitoring data are entered into the Fumiguide, the program will calculate

the actual HLT and accumulated CT dosage, predict the CT dosage outcome for the planned exposure period, and update instructions on exposure time (on target, shorten or lengthen) and fumigant concentration ("on target" or "add more").

The Fumiguide software continues to be enhanced. The most recent edition was released in January, 2008. It includes new pests, additional temperature calculations for selected key pests, one step report generation, and commodity sorption adjustments. The Fumiguide has been programmed to meet the needs of a global fumigation market; its calculations can be converted to English or metric units and the program is available in multiple languages.

The Fumiguide has been an invaluable aid in enabling fumigators to adapt ProFume fumigations to meet the needs of their customers. Fumigators can easily run multiple fumigation scenarios for a site to determine which combination of factors best meets the customer needs. The ability to accurately determine the fumigation dose when changing fumigant exposure time, temperature, or confinement is a great asset which has not been available for methyl bromide or phosphine. In addition, the Fumiguide provides a written record of each job, including the fumigation plan (pest, temperature, volume, etc.), monitoring data, and achieved CT dosage. The reports can be customized to meet customer requests and regulatory requirements.

Global Registration Status of ProFume

ProFume® gas fumigant received its first global registration in Switzerland for use in flour mills in 2003. Since then, ProFume has been registered in an additional 13 countries (Table 1). Registration activities have been started in Spain (flour mills), Greece, (flour mills, dried fruit, tree nuts), Turkey (dried fruit, tree nuts), and Thailand. The European Union t – MRLs (Annex III of 396/2005/EC) are anticipated in September, 2008 to set the MRLs for sulfuryl fluoride and fluoride in various commodities. Registration feasibility assessments for ProFume are underway in Asia, Latin America, Africa and the Middle East. .

Commercial Acceptance of ProFume®

Over 1 000 commercial fumigations have been completed using ProFume with high levels of customer satisfaction. These fumigations were conducted in many different geographies at different times of the year in a wide range of environmental conditions. The pest control ratings and satisfaction levels expressed by users of ProFume gas fumigant clearly demonstrate its technical and economical viability.

Table 1. Global registration status of ProFume as of June 2008

Year	Geography	Registration
2003	Switzerland	Flour mills
2004	USA	Dried fruit, tree nuts, cereal grain storage, milling, and processing
2004	Italy	Emptied flour mills/pasta factories; food processing facilities
2004	UK	Emptied flour mills
2004	Germany	Flour mills, dried fruit and tree nuts
2005	Puerto Rico	Dried fruit, tree nuts, cereal grain storage, milling, and processing
2005	USA	Expanded food tolerances and use patterns
2005	Belgium	Emptied flour mills
2006	Canada	Emptied flour mills and food processing facilities
2006	France	Emptied flour mills
2006	CODEX Alimentarius Commission approved MRLs for sulfuryl fluoride for international trade	
2007	European Union Annex I listing of Directive 98/8/EC (Biocide) of the active ingredient sulfuryl fluoride	
2007	Ireland	Emptied flour mills
2007	Spain	Food processing facilities
2007	Trinidad and Tobago	Dried fruit, tree nuts, cereal grain storage, milling, and processing
2007	Mexico	Cereals and beans
2007	Australia	Dried fruit, tree nuts, cereal grain storage, seed, hay
2007	Mauritius	Cereal grains, flour mills and processing
2008	European Union	Harmonized MRL (maximum residue level) of sulfuryl fluoride (Directive 396/2005/EC)

In North America, Dow AgroSciences has verified using Fumiguide data that at least 455 individual structures at more than 182 locations in 25 states, two Canadian Territories, and Puerto Rico have been fumigated using ProFume. About 32% of the structures have been fumigated 2 – 7 times with ProFume over successive

years, indicating customer satisfaction and adoption. Rice mills representing 50% of the USA rice processing have converted to fumigating with ProFume.

A survey of ProFume® gas fumigant users in the USA revealed that 96% of the survey respondents would use ProFume again and 4% remained undecided. ProFume fumigator satisfaction ratings averaged 4.4 out of 5 and miller satisfaction ratings (at 60 days post-fumigation) averaged 4.5 out of 5.

In Europe, Dow AgroSciences has documented at least 361 commercial structural fumigations with ProFume from 2003 – 2007 (Table 2). The total number of fumigation conducted in 2007 doubled compared to those conducted in 2006, indicating commercial acceptance.

Table 2. Commercial Structural Fumigations Conducted with ProFume in Europe, 2003 – 2007.

Country	2003	2004	2005	2006	2007	Total
Belgium	0	0	0	0	8	8
France	0	0	0	0	30	30
Germany	0	3	16	30	60	109
Italy	0	2	30	40	80	152
Spain	0	0	0	0	1	1
Switzerland	1	7	8	15	15	46
UK	0	1	4	3	7	15
Total	1	13	58	88	201	361

Additional ProFume fumigations have been conducted in Australia, Trinidad, Mexico, and Mauritius. A ProFume fumigation conducted on Dec. 31 – Jan 2, 2008 of a flour mill in Mauritius was funded from the Multilateral Fund (Montreal) and implemented through GTZ (Windhoek) under the direction of the Mauritius Ministry of Environment. Representatives from Insects Limited, Inc., Dow AgroSciences, Hardy Henry Services, and Rentokil International assisted. The mill was fumigated in the past with phosphine as a methyl bromide alternative; however, even with careful sealing of the electronic panels and equipment, these components suffered serious damage during phosphine fumigations. For the ProFume fumigation, the mill had a mean HLT of 12.9 and a mean accumulated CT dosage of 855 mg · h/L. The target pests were successfully controlled with no damage to electrical equipment.

Case Studies

Cocoa

In the USA, fumigation of cocoa beans is now being conducted with ProFume® gas fumigant. This is the result of a collaborative research effort between Dow AgroSciences, cocoa fumigators, and the Chocolate Manufacturer's Association (CMA). Cocoa beans and fractions (cocoa butter, cake and liquor) fumigated at maximum CT dosage rate (1 500 mg · h/L) with ProFume had a marginal increase in F-residues that was much lower than the MRLs granted by the USA-EPA. The CMA conducted sensory evaluation using ProFume-fumigated cocoa and concluded that the results are satisfactory. ProFume is more economical than methyl bromide for this use pattern. This is due in part to the sorption of sulfuryl fluoride into cocoa beans is much lower than that of methyl bromide under identical conditions, as confirmed by research conducted by Phillips et al., Oklahoma State University (unpublished data). A commercial efficacy trial demonstrated that ProFume killed the target pests of cocoa beans at low temperatures. Pallets of bagged cocoa beans were stored in two refrigerated trucks for two days at 7.2℃, then one trailer was fumigated with ProFume for 20 h at an accumulated CT dosage of 750 mg · h/L. All insects in bioassays in the fumigated trailer died, including eggs of the Indian meal moth, compared to the moderate to high survival rates of insects in bioassays in the non-fumigated trailer. The demonstrated technical and economic viability of ProFume for cocoa bean fumigations resulted in the withdrawal of the methyl bromide critical use nomination for this use pattern.

Seeds

Dow AgroSciences conducted extensive research for three years evaluating seed of grass, wheat, corn, cotton, soybean, and canola in collaboration with three major seed companies. Comparative tests were conducted between phosphine and ProFume gas fumigant, under varying exposure periods and temperatures. The results concluded that fumigating all tested seed types at 750 mg · h/L did not negatively impact germination or interact with seed treatments, and compared well with phosphine regarding germ impact. As a result, major seed companies in the USA are now adopting ProFume for their seed fumigation. ProFume is used for insect and rodent control in climate controlled and non-climate controlled seed warehouses for production export, farm returns, and commercial seed storage. ProFume offers flexibility compared to phosphine in reducing the fumigant exposure time, an important attribute when seed warehouses are on tight time schedules to fumigate

seeds prior to international shipment. In addition, all areas of a seed production facility can be fumigated with ProFume, including those with valuable electronic equipment; i. e. sizing towers, packaging lines, etc. that would be susceptible to damage from phosphine fumigations. ProFume, when applied according to label directions, can be used to fumigate sensitive electrical equipment without damage.

Grain Fumigation of Phosphine Resistant Insects

Studies testing sulfuryl fluoride efficacy on strains of phosphine-resistant red flour beetle indicated no cross – resistance. Resistance issues with sulfuryl fluoride are not anticipated because of use patterns, unique mode of action, and lack of known cross – resistance to other fumigants. Phosphine resistant lesser grain borer, *Rhyzopertha dominica*, is wide spread globally and well documented[12,13]. In the USA, phosphine-resistant lesser grain borer occurs in California and grain fumigations, primarily for rice and some corn, in this geography have successfully converted from phosphine to ProFume gas fumigant. Fumigators will typically install a J fan at the base of the bin to recirculate air, and introduce ProFume in the top of the sealed bin. Using this method, ProFume has been recorded dispersing to the base of the bin within about three hours, even in bins up to 36 m in height. ProFume exposure times average 48 – 72 h, and fumigations are monitored to confirm sufficient dosage accumulation for successful control of phosphine-resistant lesser grain borer and confused and red flour beetles. Farmers appreciate the flexibility to rapidly fumigate and aerate grain immediately prior to shipment, the reliability of control, and the absence of particulate residues that were left in the bottom of the bins following fumigation with aluminum phosphide (Jim Garret, Fume Tech Inc., West Sacramento, California, personal communication).

Conclusion

All efforts of quality and productivity of growers could be ruined after harvest without proper desinfestation of stored product insects in commodities. Fumigants are the preferred solution for a fast and thorough treatment. With methyl bromide being phased out and resistance issues with phosphine increasing, ProFume offers a solution. Sulfuryl fluoride, recognized as an excellent wood fumigant for nearly 50 years, has been developed by Dow AgroSciences for commodity fumigation. Studies conducted both in Europe and the USA have shown this molecule fits the needs of agriculture and food industry for fast, and effective fumigation of commodities, food storage, mills and food processing plants without adverse effect on equipment, food quality and the environment when used according to label. Development and commercial launch success in many countries prove that ProFume is technically and economically viable alternative to methyl bromide, and to phosphine where resistance, damage, or time constraints are issues.

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Effects of Outside Air Temperature on Movement of Phosphine Gas in Concrete Elevator Bins

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Abstract: Studies that measured the movement and concentration of phosphine gas in upright concrete bins over time indicated that fumigant movement was dictated by air currents, which in turn, were a function of the difference between the average grain temperature and the average outside air temperature during the two weeks following application of the aluminum phosphide pellets. When the grain was warmer than the average outside air temperature during these two weeks, the phosphine gas would move upward through the grain mass. When the grain was cooler than the average outside air during these two weeks, the fumigant moved downward. Because insect problems normally occur in the top of the grain mass early in the storage season (June-August), a uniform application of fumigant pellets was more effective when the grain and outside air temperatures were similar during the two weeks following application of the fumigant. When grain was much warmer than the average outside air temperature during these two weeks, applying more fumigant pellets into the bottom half of the grain mass was the most effective strategy. If the grain temperature is expected to be cooler than the average outside air temperature during the two weeks following application of the fumigant, applying more pellets to the upper half of the grain mass would be the best strategy.

Introduction

Phosphine fumigation failures in concrete bins are common when a lethal concentration (200 – 300 ppm) of phosphine is not maintained for a sufficient duration (normally 3 – 5 days). Factors that affect the phosphine concentration and duration are grain temperature and air-tightness of the bin. Other factors such as convective air movement in the grain mass can also impact phosphine dispersion in the grain mass. Tall concrete bins (26 – 37 meters tall) are particularly susceptible to "chimney" effects. These effects should be strongest when there is a large difference between the outside air temperature and the grain temperature. In temperate climates, this often would occur in the fall, winter and spring. Winks and Russell demonstrated this chimney effect in bins monitored for temperature and pressure differential at the base of concrete bins[1]. Insect density is often highest in the top third of the grain mass[2]. Because of the vents in concrete grain bins, it is often difficult to maintain fumigant concentrations at high levels for sufficient time to kill all stages of the insects that are present. This problem can be exacerbated by cooler temperatures during the fall and winter.

Elevator grain managers have used various phosphine application strategies when fumigating grain. These can be characterized as uniform and non-uniform applications. The fumigant tablets or pellets are typically added to the grain stream as the grain is transferred from a full bin to an empty bin. For a uniform application, the operator adds the pellets at regular intervals throughout the grain transport period. For non-uniform applications, the operator adds all of the pellets to a certain portion of the grain; for example, to the bottom half of the grain mass. The reason why a grain manager may use one application strategy over another varies greatly. Some answers given by grain managers are that they often have insect hotspots in the bin bottoms; other managers prefer an even distribution because they often find more insect problems in the top of the grain.

The objective of the fumigation study was to characterize the movement of phosphine gas in grain stored in concrete elevator bins during the two weeks following application of the fumigant pellets. We wanted to investigate how cold and warm season fumigations affected gas movement, and whether uniform or non-uniform applications would be best for cold or warm season fumigations.

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This paper reports the results of research only. Mention of a proprietary product or trade name does not constitute a recommendation or endorsement by the US Department of Agriculture or Kansas State University.

Methods

This study was conducted at three different commercial elevators over a 3yr period in Kansas, USA. Gas sampling equipment consisted of 3.2 mm nylon tubes attached with plastic heat-shrink tubing to a 6 mm diameter steel cable. The nylon tubes were cut to different lengths so that the end of a single nylon tube protruded from the heat shrink at 3 – meter intervals. The bins were either 26 or 37 meter – tall, so a gas-sampling cable typically had 8 to 12 gas sampling tubes. In addition, three thermocouple wires were attached to the steel cable so that grain temperatures in the top, middle and bottom of the grain could be measured. Three gas – sampling cables were suspended from the roof of each bin, one at the center, and the other two at opposite directions about 60 cm from the bin wall. We used a PortaSens II gas meter with a 10 – 2000 ppm phosphine sensor to measure gas concentrations. A small electric vacuum pump (Cole-Parmer L79200) was used to pump gas from the gas sampling tubes to the PortaSens II gas detector at a flow rate of 0.4 – 0.5 liters/minute. Because of the amount of time needed to measure phosphine for each bin, and the expense and time needed to manufacture cables, only two bins per trial were sampled.

The cables were installed in two bins, then grain was turned into the bins and fumigant was applied either as a bottom application (all of the fumigant applied to the bottom half of the grain mass) or uniformly applied throughout the grain mass. The same dose of phosphine (300 pellets/27.2 tonnes) was used for all experiments. We sampled gas concentrations every 1 – 2 days, usually at noon. Data was analyzed by using contour plots (Surfer, Golden Software 1999) to provide a visual image of the change in the phosphine concentrations over time in each bin.

Results and Discussion

Phosphine gas has a specific gravity that is almost the same as air (air = 1.0, PH_3 = 1.17). Thus, if the air in the bin is not moving, the phosphine gas will diffuse very slowly in the grain mass and remain near the highest concentrations of pellets. If there is convective air movement in the grain mass, the phosphine gas will move with the air. For the first case that we investigated, during the two weeks following application of the fumigant pellets, the grain and outside air temperatures were similar, averaging 9℃ and 5℃, respectively. The fumigant pellets were applied uniformly to all layers of the grain in this 26 meter-tall bin.

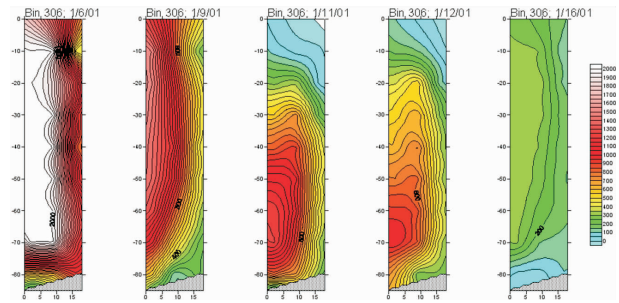

Fig. 1

Fig. 1. Phosphine concentration (ppm) in an upright concrete bin. Cold grain fumigated during January in Kansas. Fumigant pellets were added uniformly to the grain. The average grain and outside air temperatures were similar during the two weeks following application of the fumigant pellets, were 9℃ and 5℃, respectively.

Figure 1 shows that gas concentrations were fairly even in the grain mass, and did not move much either up or down during the 14-day fumigation. Because the grain and outside air temperatures were similar during this two week period, strong convective air movement inside the grain mass (chimney effect) would not be expected.

For the second case, we measured phosphine concentrations in wheat fumigated in November; in this case the grain was much warmer than the outside air temperature during the two weeks following addition of the fumigant (Fig. 2). Pellets were added to the bottom half of the grain in the 37 meter-tall bin. The grain and outside air temperatures during the two weeks following application of the fumigant pellets averaged 27℃ and 5℃, respectively. Because the grain was warmer than the outside air temperature during these two weeks, convective air movement within the bin caused the fumigant to move upwards.

Figure 2. Warm grain fumigated in November in Kansas. Fumigant pellets were added to the bottom half of the grain mass. The average grain and outside air temperatures during the two weeks following application of the fumigant pellets were 27℃ and 5℃, respectively.

For the third case, fumigant pellets were added uniformly to the wheat as it was moved to a 26-meter-tall bin in June, (Fig. 3). The average grain temperature was 18℃ and the average

outside air temperature was 26℃ during the two week period following application of the fumigant pellets.

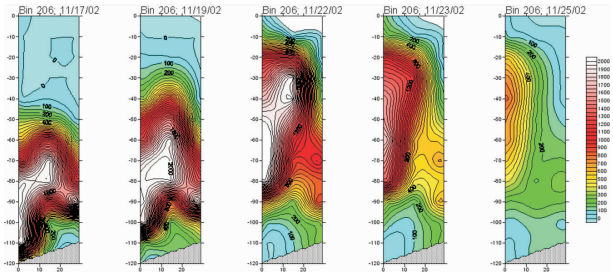

Fig. 2

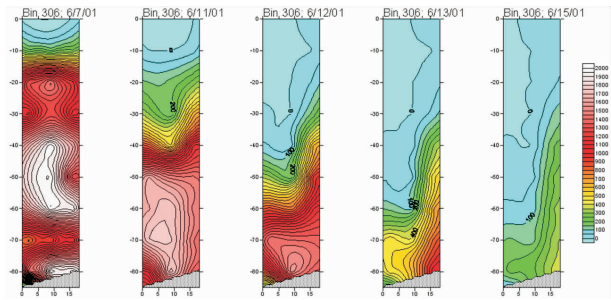

Fig. 3

Figure 3. Cool grain fumigated in June in Kansas. Aluminum phosphide tablets were added uniformly to the grain. The average grain and outside air temperatures during the two weeks following application of the fumigant pellets were 18℃ and 26℃, respectively.

On the first day after the fumigant pellets were added to the grain (7 June), an even distribution of phosphine gas was evident throughout the grain except near the grain surface. By the fifth day, the phosphine gas had moved to the lower half of the bin. On the ninth day, only a small amount of fumigant was present in the bottom of the bin. Because the grain temperature was cooler than the outside air, the air in the grain would tend to move down in the bin because it was denser than the outside air. This last case shows how difficult it is to treat the top layer of grain when the grain is cooler than the outside air. It's similar to trying to hold water in a glass that has a small hole in the bottom. Because air was moving down in the grain mass, it is obvious that if there were insects in upper layers of the grain mass, the worst application method would be to apply pellets to only the bottom half of the grain mass.

Because most concrete elevators have vents (both inter-bin and outside bin vents) in the walls just beneath the roof, it is difficult to hold lethal concentrations of phosphine gas for 3 – 5 days in the upper layers of the grain mass in most upright concrete grain bins. Lower gas concentrations, combined with the fact that insect populations in newly-stored grain often start in the upper layers of the grain mass, increase the probability of fumigation failures in the top of the bin.

When the average outside air temperature during the two weeks following application of the fumigant pellets is expected to be similar to the grain temperature, in most situations, applying pellets evenly to all of the grain would result in the best fumigation. When the average outside air temperature during the two weeks following application of the fumigant pellets is expected to be cooler than the grain temperature, it may be best to apply most of the fumigant to the bottom half of the grain mass. This would ensure adequate phosphine concentration and duration in cases where insect populations existed throughout the grain mass. Finally, when the average outside air temperature during the two weeks following application of the fumigant pellets is expected to be warmer than the grain temperature, it is probably best to apply most of the fumigant to the top half of the grain mass; however, it may still be difficult to get an effective fumigation under these conditions.

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0912

Controlled Atmosphere and Fumigation in India a Professional Pest Managers View Point

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Abstract: In India use of Controlled Atmosphere (CA) is limited at the present as compared to methyl bromide or phosphine. This paper discusses current applications for CAs and the potential For future applications. Compared to CAs, fumigants are much more extensively used. Use of Methyl Bromide in India is subject to control by the Montreal Protocol with An eventual phaseout for all uses other than quarantine & pre shipment applications by January 2015. The only other fumigant currently registered for use in India is Phosphine, which is extensively used for disinfestation of stored grain. Resistance to phosphine is a matter of serious concern and requires for extended exposure periods which are not always achieved. Potential uses of CAs as a Substitute to fumigants are discussed.

Key words: controlled atmosphere, fumigation, methyl bromide, phosphine, professional pest manager, India.

Introduction

This paper reports on use of fumigants and controlled atmospheres (CA) from the viewpoint of professional pest managers. Our business, Pest Control M. Walshe, was established 52 years ago in Mumbai. Since then our fumigation activities have extended from fumigation of stored bagged grain, to treatment of containerised cargoes, structures, and ships carrying bulk grains. This work may involve curative instorage, pre-shipment, and quarantine fumigation treatments.

The only fumigants currently registered for use in India are methyl bromide and phosphine. Use of the former is restricted to licensed fumigators, while phosphine is freely available to farmers and others, who grow and store food grains as well as licensed fumigators. Ethylene oxide, while not registered as a fumigant, is used commercially to sterilise a range of products and commodities.

All non quarantine and pre-shipment uses of methyl bromide in India are scheduled to be phased out by 1 January 2015, which will leave phosphine as the only fumigant available for infestation control in stored food and feed grains, and other commodities. This is likely to present a serious challenge to pest management professionals because of the high levels of resistance that have been reported in some strains of stored product insects commonly found in India.

While controlled or modified atmospheres have been used in India on a relatively smallscale with high value commodities for some 30 years, we have not used them on a commercial basis.

Fumigants-Methyl Bromide

Methyl bromide has primarily been used by commercial pest management businesses. Recently a number of state owned business enterprises including the Central Warehousing Corporation (CWC) and some State Warehousing Corporations (SWC) have been using methyl bromide.

We use this methyl bromide to treat:

· bagged stored grains enclosed under gas-tight enclosures

· wood packaging materials including pallets, dunnage in accordance with the requirements of ISPM 15

· machinery

· the crew accommodation and ship′s stores of sea going vessels

· cargoes in ships-for in-ship, in-transit disinifestation of commodities, particularly rice.

In-ship, in-transit disinifestation is required by quarantine authorities in some of the countries, to which such cargoes are exported. The effectiveness of such treatments is very much dependant on the use of effective recirculation systems that ensure the fumigant is evenly distributed so it can penetrate the entire grain bulk.

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Fumigants-Phosphine

The principal application for phosphine is disinifestation of stored food and feed grains and their products. Almost all of this work is undertaken by the Food Corporation of India and the Central and State warehousing corporations or on their behalf by pest management businesses.

We use phosphine to disinfest:

· cargoes in ships, mainly wheat, rice, maize, and animal feeds manufactured from these grains

· food grain under gas-tight enclosures

· bulk food grains in silos

· high value commodities (e. g., nuts-almonds, cashews; dried fruit-dates, figs) under gas-tight enclosures

Most of the phosphine that we use is generated from aluminium phosphide preparations. However, two types of generators manufactured by United Phosphorus Ltd are now commercially available in India. In addition to providing an instant' source of phosphine, these devices also have the advantage of eliminating the possibility of contaminating the commodity fumigated with spent residues of aluminium phosphide.

Fumigants-Ethylene Oxide

Ethylene oxide finds application in India a sterilizing agent. It is specifically used to sterilise spices and medical equipment in purpose built vacuum fumigation chambers.

Discussion

As mentioned above phosphine is the most extensively used fumigant in India. It's free availability to any person who chooses to purchase aluminium phosphide preparations and the widespread failure to understand how it should be used effectively has lead to the development of high levels of resistance. This should be a matter of concern to all people involved with infestation control in India because phosphine is now the only fumigant available for this purpose. We perceive the problem of resistance as the greatest challenge to for professional pest managers in India.

How has it come to this situation In terms of fumigation practice, we are all aware that it due to the abuse of fumigants. We define abuse in terms of the following fumigation malpractices:

· failure to apply the correct dose of phosphine

· failure to use the correct exposure period

· failure to ensure that phosphine is used in well sealed enclosures.

What factors have led to this situation We feel that free access to aluminium phosphide preparations by people untrained in its proper use has contributed heavily to this situation. In addition, a number of social' issues and economic pressures frequently affect the manner in which phosphine is used, as described above. So what is happening are the farmers to blame because they are not taught how to use phosphine correctly at the farm level Then, even if they were informed', do they choose to underdose because they choose to save money or do not have enough.

Their harvest is then moved to the warehouse level owned/managed either by private traders or the government. Are the malpractices listed above responsible for the development of resistance to phosphine.

What then is the role of pest managers? Do they, despite their training, concede to pressures' to the point where it becomes economically impossible to perform the fumigation treatment effectively, and respond to suggestions that the exposure period can be reduced.

The clients-well they always want the cheapest treatment possible and play off the pest managers against each other. Who is responsible here. Do professional pest managers spend any time educating' their customers in the effective use of phosphine, and work to the standards that they were trained to use.

If professional pest managers and their clients ignore the fact that in-the not very long-term-they might lose their ability to use phosphine then what will they use to save their goods when they are infested. Ignoring the fact that the costs of the raw materials required to manufacture aluminium phosphide are set to increase dramatically-what alternatives are there.

It is our opinion that the main contributor to the rise in resistance has been economic in nature because everyone wants the cheapest job done, notwithstanding the consequences.

It has very often been explained to clients that in terms of value for money that the cost of effective fumigation is literally pennies. This may be illustrated by the following example with rice.

Selecting one of the higher charges for fumigation, the tables below provides an idea of the cost of fumigation.

Example – using a recirculation system	
Shipload of 20 000 tons rice costed @ approx us $ 1 000.00 ton	US $ 20 000 000.00
Cost of fumigation using phosphine @ US $ 1.25/ton	US $ 25 000.00
Percentage cost of fumigation to value of cargo	0.001 25 %
Cost of a proper fumigation.	Minimum cost to be paid in case of refumigation.
US $ 25 000.00	Vessel standing charges Vessel shifting/port charges, etc not taken into account Min 5 days@ $ 10 000/day $ 50 000.00 Re – fumigation charges $ 20 000.00
TOTAL US $ 25 000.00	Total minimum cost to be paid $ 70 000.00

Similarly for wheat – selecting one of the higher treatment charges for fumigation, the table below provides an idea of the cost of fumigation.

Example – using the re – circulation system of fumigation	
Shipload of 20 000 tons wheat @ approx US $ 380.00/ton	US $ 7 600 000.00
Cost of fumigation using phosphine@ US $ 1.25/ton	US $ 25 000.00
Percentage cost of fumigation to value of cargo	0.003 %

In addition to this the professional pest manager has to endure a lack of effective infrastructure. All procedures, rules and regulations are in place for the fumigants currently in use require, for example, proper flooring at work sites, adequate space in which to treat containers, and proper security.

In India fumigation is quality controlled by two Indian standards for Fumigation (NSPM 11 & 12), and through its participation in the Australian Fumigation Accreditation Scheme (AFAS). Thus treatments with methyl bromide are monitored in accordance with these standards, e. g. at least twice during a 24 hour exposure period, and at least three times during a 48 hour exposure period.

We frequently face severe infrastructure constraints when carrying out fumigations. For example, the fumigation floor is very clearly not gastight as required by the standards, which leads to delays in carrying out the job as measures have to be taken o ensure that such sites comply with the standards-to ensure that the fumigation treatment can be carried out effectively.

We are always under pressure from clients, who want a job done in short time without giving consideration to the full exposure period as required by the standards. While previously, it was possible double up the dosage of methyl bromide and half the exposure period, this is no longer permitted by the standards.

At the end of the exposure period we are frequently under pressure to release goods immediately after the enclosure has been opened-without the full ventilation and clearance process being carried out. It is very difficult to convince clients that this practice is no longer permitted. The willingness to comply with these and other shortcuts demanded by clients make all too often decide a contract.

For treatments carried out with phosphine there are no specific guidelines. However, we monitor fumigant concentrations in accordance with internationally norms. In this respect the use of a phosphine generator is of immense help to us as it allows us to safely top up the gas concentration to the required level without the OH&S hazards of having to enter' an enclosure.

Customer awareness/understanding of the modern requirements for effective fumigation, and the dangers associated with fumigation is something that needs to be enhanced.

We are aware of cases when customers have removed fumigation sheets from goods under being fumigated without any PPE because they required the goods for either production or for shipment. In such cases not only has the customer risked put his employees at risk by degassing an enclosure without adequate protection but has also then shipped the goods inadequately fumigated which may cause a problem at the destination because of infestation being found-and a fumigation failure is always the responsibility of the fumigator.

Commercial considerations unfortunately lead to many corners being cut, which can be very dangerous. We are under constant pressure to reduce our prices. However, when it is explained that is very dangerous, such advise is

often disregarded. We very often are forced to let go of jobs as we are unwilling to take such risks.

Controlled or Modified Atmospheres

The use of controlled or modified atmospheres (MA) in India to control infestation is extremely limited. There is history dating back to the 1970s of small-scale use with a number of high value commodities such as cashew nuts packed in tins for export. However, the technology for these methods do not yet really exist in India, and as a consequence there is limited awareness or understanding of it amongst professional pest managers and their clients.

A few techniques such as the grain bags using vacuum to reduce oxygen content have had limited success in India. In the latter case, the manufacturers claimed that a hard to kill species such as *Rhyzopertha* spp. can be killed in 72 hours. Validation has been difficult, and we as Pest Managers have yet to be convinced that this treatment regime is effective before we start to sell' it. Added to this is the ever present client aversion to increased cost that mitigates against adoption of new techniques.

It appears that controlled or modified atmospheres, and similar disinifestation techniques will be much more expensive than the current use of phosphine and we have not yet started to seriously investigate their application to our requirements in India. If controlled or modified atmospheres treatments are to adopted in India there is need for the technology to be cheaper before it will be accepted in India. However, we believe that such disinifestation techniques hold good potential for use in India, specially in view of the fact that Methyl Bromide will be phased out by 2015. This opportunity may have led a Netherlands based company that provides disinifestation services using CAs to establish a branch in India.

There is a possibility that methyl bromide usage, whether for disinifestation up to 2015 or for quarantine and pre-shipment treatments thereafter will be reduced as a result of end user demands in our markets in the industrialised world. This may result in a requirement for safer' treatments, which may be satisfied by application of controlled or modified atmosphere technology. None the less the long exposure periods of such treatments will not be readily accepted by our clients.

Summary

The future for controlled or modified atmosphere disinifestation techniques, including heat, appears to us to be bright in a world that will have limited access to methyl bromide provided this technology is cost effective.

Companies researching/selling such technologies should look to sacrifice some of their profits for the good of mankind as use of this technology will surely save the ozone layer and the scarce resource called food.

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Some Research Progresses of Stored-grain Protection in China

Li Xingjun and Luan Xia

Abstract: The use of aeration, fumigation, and natural plant products in grain management programs was reviewed for the past 11 years in China. The methods and technologies for improved efficacy of phosphine fumigation have been well-documented in *China Grain Storage*, a journal sponsored by the storage branch of the Chinese cereals and oil association, but the alternative fumigant is limited to stored products. Natural plant products such as essential oil have long been used in Chinese grain storage despite their high molecular weight and boiling points.

Key words: grain storage, fumigation, insect pest management

Introduction

The mechanization of grain storage has always been paid great attention to by the Chinese government. In contrast with the developed countries, China stores grain for 3 – 4 years and tries to prevent the stored grain from quantity loss and quality decay. In recent years there have been a lot of research achievements, which have been turned into various products and widely used in the grain depots. In this mini-review, the advance in aeration, controlled atmosphere, natural plant products for grain storage in China, and the related technology in other countries is outlined. The purpose of this review is to demonstrate that natural plant extracts, especially seed coat components, are potentially useful in Chinese gain storage.

Use of Aeration and Controlled Atmosphere in Grain Management

Refrigerated aeration systems provide chilled air and force this air through the storage system, decreasing the temperature of the grain. Mechanical aeration systems are commonly used in Chinese grain storage. Most aeration studies have been conducted on wheat, which is usually binned and stored during the summer. Cooling stored wheat by low-volume aeration with axial type ventilation is used 3 – 4 times during the first year of storage. In most aeration programs the grain is initially cooled to seed dry-bulb temperatures of 15 – 18℃. These are the approximate lower developmental temperatures for most stored-product insects. While the surrounding air temperature is low during the winter season, two times usages of the small capacity ventilation for reduction of temperature of the stored cereal grains is good for cooling the grains, retaining its quality and preventing the growth of mold and pests. As the moisture content of the grain decreases, the threshold temperatures required to limit population growth increase. This would allow increased utilization of aeration in warm dry climates.

CO_2 controlled atmosphere storage was used in southern China since the 1970s. During the past 11 years 15 articles published in *China Grain Storage*, a journal sponsored by the storage branch of the Chinese cereals and oil association, were concerned with the efficacy of CO_2 controlled air storage on insect [1-3] and fungi[4] control, as well as the effect on the quality of rice and wheat [5-6].

One barrier to the inclusion of chilled aeration in management programs is the capital expense required for the equipment and the apparent cost of treatment. In the developed regions and cities, economic and social conditions justify the cost and chilled aeration with high concentrations of CO_2 and N_2 could replace protectants in some situations.

Phosphine Fumigation

Phosphine is a primary fumigant used to control insects in on-farm and commercial storages throughout the world, and much of the current research involves new methods and technologies for improved efficacy. Throughout the past 11 years 129 articles that deal with the new techniques and modifications including the improved methods for sealing, recirculation and

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recycling systems for improved distribution and emission control, and new formulations for the controlled release of phosphine, were published in *Grain Storage*. Wang and Bian[7] summarized the usage methods of phosphine fumigation in China grain storage. The usage of phosphine includes low airflow fumigation release, preceded by a single phosphine-producing apparatus outside of a warehouse, and mixing of phosphine and other protectants outside of a warehouse. For a fast and uniform distribution, the recirculation systems were modified from the external mobile or fixed systems to internal systems under the cover film. The recirculation systems were fit for big warehouse, middle to small bins, and external batch storage. The release of phosphine was changed from an external gas fumigant into a combination of release from aluminum phosphine tablets or pellets followed by recirculation. Chen and Cao[8] reviewed the action mechanism of phosphine. Phosphine enters the insect body from the body wall and spiracles with the participation of oxygen, and may interact with peroxidase, cytochrome oxidase and other enzymes.

Table 1. The number of articles concerned with CO_2 controlled air storage, phosphine fumigation, and plant extracts, published in *China Grain Storage* during the past 11 years

Year	CO_2 controlled atmosphere storage	Phosphine fumigation	Plant extracts
1996	0	10	1
1997	2	8	1
1998	2	7	0
1999	1	8	1
2000	0	9	1
2001	0	21	3
2002	2	23	0
2003	2	18	1
2004	4	9	2
2005	0	6	2
2006	2	5	2
2007	0	5	4
Total	15	129	18

The effectiveness of phosphine is increased by its low molecular weight and low boiling point characteristics that promote its rapid diffusion and penetration into grain. Although highly toxic to many insects, it is markedly less so to certain stages of some species. For example, phosphine at 10 mg/h per liter is sufficient to control adult *Sitophilus spp*, but control of young pupae of these species requires 300 mg/h per liter [9]. The addition of carbon dioxide or nitrogen can enhance the toxicity of phosphine, and improve penetration within the grain mass [10] along with retarding the deterioration of grain quality. The hazards of using phosphine as a fumigant are relatively low because of its slow release following the exposure of the solid formulations to moisture. Phosphine is degraded into phosphine oxides with small environmental problems. Like phosphine, ethyl formate is highly toxic to insects, and easy to degrade into formate and ethanol, as an alternative fumigant displacing methyl bromide. The sociological problems associated with pesticide residues may be reduced by substituting fumigants or controlled atmosphere treatments for grain protectants.

Biopesticide Control

A. Bt Product

Biopesticides can include viral and fungal pathogens, insect growth regulators and natural plant products. In general there is considerable potential for the development of microbial products and the expansion of biopesticides for stored grain in most developed countries. Currently the majority of biopesticide sales are those products which contain *Bacillus thuringiensis* (Bt) as the active agent. For example one Bt formulation controls *Rhizopertha dominica* pests with LC_{50} 1.5 mg/kg, and 91.4% mortality at a concentration of 4.5 mg/kg [11]. Another formulation used in the USA controlled *lepidopteran* pests of stored grains but there were no products to control coleopteran pests in the same environment. This limits the use of Bt products because of the significance of beetle pests in stored grains. In addition, some moth species including *Plodia interpunctella* can develop resistance to Bt.

B. Insect Growth Regulators

There are many reports concerning the efficacy of insect growth regulators as grain protectants. Methoprene is primarily used in tobacco storages and its formulations are available for grains and oilseeds, but are considerably more expensive than conventional protectants. In Australia methoprene is used at a reduced rate in combination with organophosphorus compounds to control strains of *Rhyzopertha dominica* [12]. Hydroprene is a juvenile hormone analogue used in urban and stored insect control

programs in developed countries [13].

C. Natural Plant Extracts

Natural plant extracts have been used to control insect pests in small-farm in China for many years. Within the past 11 years, there were 18 articles about natural plant extracts published in *China Grain Storage*. Yao et al [14] classified the biopesticides from plant extracts into 4 groups: crude extracts, active components, essential oils, and mixture applications (Table 2).

Plant essential oils may control insects by poisoning, trapping, baiting, growth inhibition, and so on[15]。 They act on enzymes such as cytochrome P450 monooxygenase, acetylcholinesterase and aldrin expoxidase. Natural products can be extracted from local plants. These products neither contaminate grain and environment, nor induce the resistance of pests. With high molecular weight and high boiling point characteristics, these plant products are not likely to replace existing protectants in developed countries, but there may be increased opportunities for using natural products for specific small markets.

It was previously known that the citral from *Litsea cubeba* oil has an antibacterial effect on *Aspergillus flavus*, but the action mode of citral had not been demonstrated. Recently, the ultrastructure of spore and mycelium of *A. flavus* was investigated after being poisoned by either the liquid or gaseous solution of either isomer of citral, geranial and neral [16]. The changes of cell membrane were measured by transmission electron microscope, multiplex microanalysis and co – focus laser Raman microanalysis. The use of either citral isomer in liquid or gaseous state was effective to inhibit the growth of *A. flavous*. The synergistic inhibition was also observed in the mixture form of the two citral isomers. It was found that the two isomers might exert their antibacterial action by destroying the ultrastructure of *A. flavous* and the function of cell membrane.

D. Isoflavonoid and Protein

Proanthocyanins, isoflavonoid and glycitin contribute to resistance to legume weevils. However, legume seed resistance to pests and pathogens may also involve factors other than the phenolics [17].

Table 2. The classification of plant extracts

Classification	Main components	Prevented insects
Crude extracts	Chinese *stellera root juice*	*Sitophilus oryzae*, *Rhizopertha dominica*, *Sitophilus oryzae*
	Paeonol	*Sitophilus oryzae*, *Rhizopertha dominica*, *Tribolium confusum*
	Acetone – soluble *Kaempferia galange*	*Callosobruchus chinensis*
Active extracts	Cinnamic aldehyde, Capillarisin, camphor oil	*Sitophilus zeamais*, *Tribolium confusum*, *Rhizopertha dominica*, *Oryzaephilus surinamensis*
Essential oil	Isoquinoline, cassiaoil, star anise oil	*Sitophilus zeamais*, *Rhizopertha dominica*, *Tribolium confusum*
	Litsea cubeba, cooking oil, red – peperoil, cotton seed	*Callsobruchus maculates*
Mixture of plant extracts and chemical pesticides	Hesperetin, cinnamic acid, *Alpinia officinarum* oil, *Artemesia annua* oil and other insecticide	*Rhizopertha dominica*, *Sitophilus zeamais*, *Cryptolestes ferrugineus*

In the seed coat of the common bean (*Phaseolus vulgaris*), neither thickness nor the levels of phenolic compounds such as tannins and tannic acids alone were significant for resistance. Vicilin-like 7S storage globulins, such as canavalin, concanavalin A, canatoxin and phaseolin, reported in Jack bean (*Canavalia ensiformis*), lima bean (*Phaseolus lunatus*) and common bean have been implicated. Canatoxin was shown to be toxic to some insects and plant pathogenic fungi. Canavalin inhibits spore germination of several fungi. Furthermore, both

phaseolin and canavalin have detrimental effects on larval development in bruchids.

The insecticidal properties of the pea albumin 1b peptides have opened new possibilities for seed protection against cereal weevils [18]. Although the mechanism of action of this toxin is still unknown, binding to insect protein extracts occurs. This variation of albumin is the first entomotoxic cystine-knot peptide identified. It might belong to a multi-gene family, as at least five isoforms of the peptide exist within a single pea genotype. The cystine-knot structural motif is present in peptides and proteins from a variety of species and appears to be a highly efficient motif for structure stabilization.

Gijzen et al. [19] isolated a class I chitinase from the soybean seed coat. Although chitin is absent in plants, it is a mojor component of fungal cell walls. Therefore, chitinase may play a role in plant defense against pathogens. The seed coat chitinase is expressed late in seed development, with particularly high expression levels in the seed coat. Moreover, expression is associated with senescence, ripening and response to pathogen infection.

E. Polysaccharides

A polysaccharide fraction isolated from *P. vulgaris* seeds, present at a level of *c*. 1% dry weight, increases larval mortality and reduces rate of larval development [20]. Gatehouse et al. [21] also observed that the carbohydrates from *P. vulgaris* seeds reduced *Acanthoscelides obtectus* adult emergence, and this activity was due, at least in part, to the presence of a heteropolysaccharide which has an unusually high content of arabinose and fructose. Oliveira et al. ® indicated the presence of the polysaccharides galactorhannan in the innermost cell layer of the seed coat and also in the cotyledons of the Jack bean [22]. The concentration of this polysaccharide in the seed coat (*C*. 2%) is sufficient by itself to protect the seeds from attack by *Callosobruchus maculates*.

In summary, natural plant products such as essential oil have long been used in Chinese grain storage despite of their high molecular weight and boiling points. With the development of seed coat biotechnology, new grain protectants derivated from polysaccharides and protein in seed coats will arise in the future.

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SESSION 10

TECHNOLOGY TRANSFER AND INTERNATIONAL COOPERATION IN CA AND FUMIGATION

Chairpersons:
Niu Xinghe, China
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Use of Computer-assisted Learning in Training on Grain Quality Management

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Abstract: Computer – assisted learning (CAL) incorporates elements of computer technology into traditional learning, The latter includes pictorial material such as graphs, still photographs, videos but also accommodates animations, sound and computer simulations, The training modules can be recorded on CDs or DVDs reducing their cost below that of printed materials.

A series of tutorials related to grain storage management have been developed over a number of years by an international group of grain scientists from Australia, China, Indonesia, Thailand and Vietnam, The topics comprised in the tutorials are based on the research results of the grain scientists from the collaborating countries covering a period of over twenty years and on the industrial practice, Tutorials include: integrated commodity management; biology, ecology and identification of grain pests; moulds and mycotoxins; pest control options and temperature and moisture manipulation of grain in storage.

The tutorials have originally been developed in English and then translated into Bahasa Indonesia, Chinese mandarin, Thai and Vietnamese, A series of training courses have been conducted in collaboration with research and extension organisations and grain industry in China, Indonesia, Thailand and Vietnam, The paper describes the lessons learnt from the use of CAL as a training tool aiming at improving grain quality in storage.

Key words: computer – assisted learning, multilingual tutorials, integrated commodity management

Introduction

Grains represent an important proportion of stored produce, Given the current price increases of food worldwide, it is obvious that reducing the loss of grain in storage should have a high priority, Preserving grain quality in storage requires a good understanding of the mechanisms leading to losses and knowledge of ways to prevent them.

An understanding of the diverse factors involved in preserving grain quality, and the sometimes complex interactions between them, is essential for efficient management of grain postharvest operations, These factors include environmental conditions, mainly temperature and relative humidity, physico-chemical and biological properties of grains, pests and moulds, physical and chemical treatments, storage structures and handling.

As grains are traded internationally, it is also important to know the market requirements for acceptable treatments, especially with regard to the types of chemicals used and acceptable grain residue levels, Furthermore, there may be restrictions regarding pests with quarantine status, Such pests, if positively identified, will require a specific, often costly, treatment. However, if not properly identified, they may enter a country where they are not present and cause significant economic damage, with likely adverse consequences for the importer and/or exporter,

In practice, there are often various existing methods for grain quality preservation during storage but they may not be known to the majority of potential users or not used properly, The reason for these shortcomings is that technical, managerial and economic disciplines related to grain preservation may not be integrated in the production system, Moreover, there is often insufficient awareness in the industry about the results from the recent scientific research in grain preservation conducted by universities or research organisations, Dissemination of knowledge related to various aspects of grain quality preservation is therefore a key to the successful implementation of preventive and curative measures leading to postharvest loss reduction,

Another issue is the fact that training should be provided to those that are actively involved in the work on the ground, i. e. , in the grain depots or quality control laboratories, The staff in such places may be technically qualified

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and experienced but not sufficiently proficient in English to take advantage of a training tutorial in that language. In order to make the training more effective for this important audience the training materials it is preferable to conduct the training in their own language, Multimedia, multilingual computer-assisted learning (CAL) systems have the potential to address these issues and convey the appropriate technical concepts and practices to the target audiences in an effective manner.

Computer Assisted Learning

Systematic research into various disciplines related to stored grain protection has been carried out by a number of research organisations in leading grain-producing countries, This involved various disciplines such as entomology, fumigation, modified atmospheres and other pest control measures, storage engineering, integrated commodity management, mycology and mycotoxins and grain drying.

Since many of the grain protection measures depend on moisture content control, climatic conditions, grain thermophysical properties have been determined and computer – based heat and mass transfer simulations have been developed. As a result, sophisticated research tools have been developed and extensively tested in developing safe drying systems throughout South – East Asia, selected locations in China and Australia.

Over the past two decades, many collaborative research projects on grain storage have been conducted by Australian research organisations and research organisations throughout Asia, This work has resulted in numerous publications, industrial applications and training courses, Most of these collaborative projects have been funded by research grants from the Australian Centre for International Agricultural Research (ACIAR) and training grants jointly provided by ACIAR and AusAID.

Integration of various elements of required for management of grain quality in storage can be achieved through integrated commodity management (ICM) [1]. The aim of ICM is to use the resources available at a storage complex in rational way to create a dynamic combination of practices designed and implemented to protect stored grain. The key characteristics of successful ICM are a suite of effective and economic storage options, good stock management, and a monitoring and hygiene program that is both regular and efficient to cope with any storage or processing situation. Moreover, ICM provides a systems approach that can be used to introduce new techniques into a storage system in an effective and methodical way.

Decision tools can assist in the implementation of ICM by identifying, the key issues implicated in a particular storage situation through explicit and rigorous analysis of the underlying problems and facilitating interactions between various management levels through improved training[2].

CAL has the potential to make a significant contribution to the training of quality management staff and to the transfer of technology from the laboratory to the field, CAL systems, which utilise the latest multimedia technologies, are inherently interactive and allow self – paced learning and more effective communication of complex concepts to trainees. They can be structured to allow students to explore alternative management options and learn the consequences of their actions. This can happen in individually or in small groups[3].

Embracing these principles, a series of projects lead to the development of decision-support information systems and training tools to facilitate the rational and sustainable management of quality of grain in storage.

A Multilingual, Multimedia CAL System for Managing Grain Quality Postharvest

Grain Storage Tutor[4] has been developed in several phases. The initial project began in Indonesia where the national central grain storage system relies on the combined use of fumigant and spraying of bag-stack surfaces and the fabric of storage structures with contact insecticides, BULOG, the agency responsible for rice marketing and storage in Indonesia, needed to improve cost-efficiency and reduce the pesticide residues in grain, The initial objective of developing Grain Storage Tutor was to achieve more effective integration of quality management strategies, with an emphasis on pest biology and control procedures, such as fumigation, modified atmospheres and use of insecticides. The package was developed initially in English and later translated into Bahasa Indonesia, to provide a better understanding of issues involved in making quality management decisions and facilitate improved decisions-making. Training courses were conducted in Bahasa Indonesia and the feedback from participants used to enhance the package and translate the material into other Asian languages.

As a result, new tutorials were written in-

cluding the identification of moulds and detection of mycotoxins and also on principles and applications of grain drying. The latter, based on several years of collaborative research conducted in the region, included a number of unique features such as thermophysical data of the main grain varieties grown in the region, several years of weather data of principal grain growing locations and also drying simulation models. The drying simulations models included several grain quality models so that various drying scenarios could be tested for given crop under a range of climatic conditions. The results outputs of the simulations could be used by the users to choose the most appropriate scenario in terms of drying time, cost and grain quality. The existing tutorials were expanded, particularly in the area of grain quality management and pest control options by including the ASEAN Fumigation Manuals, An interactive insect determination system was also added.

All tutorials were produced in English and then translated into Bahasa Indonesia, Chinese Mandarin, Thai and Vietnamese. The translations included captions of the graphs, sound tracks of the videos and most of the features of the simulations.

Main Features of Grain Storage Tutor

The examples of the start – up and of the contents pages are given in Figures 1 – 3.

Fig. 1 The start – up page of Grain Storage Tutor with instructions in five languages

Some country-specific content was added, with translation into all four languages. Examples include outdoor storage practiced in the Philippines and flat-bed dryers used in Vietnam, Hyperlinks within text and a sophisticated menu system facilitate navigation between modules and access to pictures, graphs, simulation models and videos. All the tutorials are indexed making searches very easy. There is a

Fig. 2 The general entry page of Grain Storage Tutor in English and the front pages in five languages

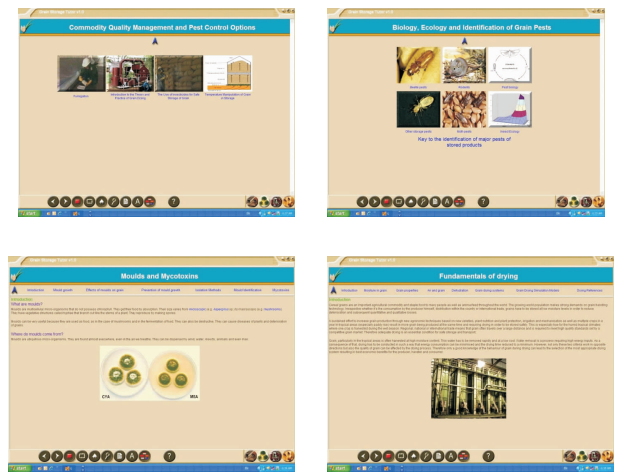

Fig. 3 The four main modules of Grain Storage Tutor in the English version

possibility of switching between languages by clicking on an icon at the bottom of the screen.

Use of Grain Storage Tutor for Training

Grain Storage Tutor has been tested in several countries. There have been international training courses using the English version as well as courses in the Philippines (in English), in Indonesia (both in English and in Bahasa Indonesia), in Thailand (in Thai) in China (in Chinese Mandarin) and in Vietnam (in

Vietnamese). A training facility established at the Post-Harvest Institute (PHTI) in Ho Chi Minh City in Vietnam allowed a thorough testing in the presence from a number of representatives from various sectors of the food industry, Both general and specialised training courses were conducted in Ho Chi Minh City, Hanoi, the Mekong Delta and Dak Lak, in the coffee-producing Highlands of central Vietnam.

The training courses involved the demonstration of the software, hands-on exercises in the utilisation of the package, especially with regard to the use of computer simulations, and practicals in laboratories or warehouses. There were two options, a general training, involving all modules and taking 3 – 4 days or a specialist training, e. g, for fumigators taking 1 or 2 days. At the end of every training course, course contents and mode of delivery were assessed by the participants. The results of the assessment were incorporated into the subsequent improved pre-release version of the package. Despite initial teething problems, mainly due to lack of adequate computer hardware, the training courses were successful and well received by the participants. The collaborating Vietnamese agencies, namely PHTI and the Plant Protection Department (PPD), based in Ho Chi Minh City, have shown an enthusiastic support for this approach of training and organised a number of additional courses. As a result, in excess of 600 personnel have been trained.

Following completion of the various trial training courses in Vietnam, Indonesia, Thailand, China and the Philippines, a revised version of Grain Storage Tutor was released in early 2005.

Since then, further training courses have been conducted in various countries, often organised by the agencies involved in the initial training courses. There were three training courses using Grain Storage Tutor in Thailand (Bangkok, Chiang Mai and Bangkok) involving a total of over 100 participants. The majority of the participants came from the industry.

A series of courses were conducted in various regions of China. There were courses in Harbin and Mishan (Heilongjiang province in Northeast China), Beijing (at the China Agricultural University), two in Chengdu, Sichuan province in Southwest China (organised by Chengdu Grain Storage Research Institute) and in Guiyang, Guizhou Province, (organised by Guizhou Institute of Mountain Environment & Climate), Like in Thailand, most of the participants came from the industry.

Grain Storage Tutor, developed with funding from Australian Centre for International Agricultural Research (ACIAR) and AusAID, is becoming a widely-used tool for training throughout Southeast and East Asia.

There are obviously other training packages using multimedia techniques that have been developed in the last decade. One of them is the Canadian Grain Storage CD – ROM' developed by the University of Manitoba. It is focusing on the Canadian grain storage conditions and includes mainly topics such as common names of insects and mites, methods to control stored grain insects, phosphine corrosion calculator and a computer program for stored grain management,

Another package is the Managing On-farm Grain Storage CD developed by Quality Wheat CRC in Australia. It is focusing on wheat and includes wide range of topics related to quality such as standards and inspection, structures and equipment, grain hygiene, farm safety, economics, HACCP planning of pre and post – harvest operations, a comprehensive glossary of terms and an interactive part including problem solving in questions related to grain management.

There is also a number of internet based training resources such as Grain Elevators and Processing Society in the USA (http://www.geaps.com/) or the Purdue University based archives:

The CD-based training resources have the advantage of being capable of accommodating a number of features such videos, animations and computer simulations which are not possible with printed materials. Moreover, the upgrades are significantly less expensive than those of printed media, Whilst internet-based resources can be updated frequently, limited internet bandwidth is often a severe constraint for materials such as videos.

Conclusions

Grain Storage Tutor, has been shown to be a flexible and comprehensive tool for dissemination of knowledge required to improve grain quality in storage. Although not the only resource of its type, it has been thoroughly tested by a large and diverse range of users, whose feedback has facilitated the refinement of the system into its current form. The ability to communicate information in the native language of the countries in which the training will be delivered makes the package particularly attractive

in comparison to similar, but less comprehensive training packages.

Last but not least, despite its success, developments in the grain preservation techniques continue and thus it will be necessary to update the content in the near future.

Acknowledgements

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Sino-Australia Cooperation in Grain Storage Technology ——an Example of Successful Institutional Cooperation

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Abstract: The paper summarises nearly two decades of collaborative research on grain postharvest between Chinese and Australian scientists and engineers. The four major projects conducted during that time were focusing on grain quality management through application of efficient pest control measures and drying. The joint work of the researchers from both countries resulted in the adoption of various techniques by the grain industry in China. A list of lessons learnt from the development and implementation of these four projects summarises the experience that both sides gained from several years of collaboration.

Key words: grain storage, grain protectants, phosphine fumigation, in – store drying

Introduction

Institutional cooperation is playing a very important part in international technology transfer and cooperation, from traditional area such as irrigation to high-tech field like aerospace research. Among them, technical cooperation aiming at overcoming poverty, feeding people with enough food in developing countries by improving farming and minimising postharvest losses have recently become of increasing concern for the UN and many of its member countries.

China is the world's largest country in terms of population and also the largest grain producer. Sustainable grain production and food safety are possibly of more significance than in any other country in the world. Great efforts towards enhancing agricultural technology cooperation with developed countries have been invested in both increasing grain production and reducing postharvest losses since the end of the 20th century by the Chinese government.

Australia is one of the important wheat producers and exporters, as well as important significant hard wheat trading partner of China. It is also one of the countries leading in grain storage especially in stored grain pest control.

Technological cooperation between SAG (State Administration of Grain of China) and ACIAR (Australian Centre for International Agricultural Research) has existed for nearly 20 years. The cooperation focused on grain storage and especially on pest control and in-store drying, Tangible results were obtained in both areas, leading to significant benefits to the grain industry in China but also to some extent in Australia.

Overview of Grain Storage Technology Cooperation between ACIAR and SAG.

Grain storage technology cooperation between SAG and ACIAR dates back to 1990s starting with the project Integrating Grain Protectants into Storage Pest Management (ACIAR PN:9035)'. This project was followed by a series of ACIAR-SAG collaborative projects, namely Phosphine Resistance in Insect Pests of Stored Grain (ACIAR PN:9415)', In – store Drying of Grain in China (ACIAR PN 9437)' and Integrating Effective Phosphine Fumigation Practices into Grain Storage Systems in China, Vietnam and Australia (ACIAR PN 98137).

All the above projects were proposed by Australian scientists with the assistance of Chinese scientists and mainly funded by ACIAR, Chinese relevant governmental organisation, SAG, formerly part of the Ministry of Internal Trade, was ACIAR's counterpart for those projects in China, and also provided financial supports, experimental sites and technical staff for the projects.

The list of institutions from both Australia and China involved in the projects is shown in Table 1. The objectives of these projects are shown in Table 2.

1. *COFCO Science and Research Institate*
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Table 1. Institutions involved in the four collaborative projects

Project	Australia	ChinaACIAR
ACIAR PN 9035	Queensland Department of Primary Industries; CSIRO Entomology.	The State Administration of Grain; Chengdu Grain Storage Research Institute.
ACIAR PN 9415	Queensland Department of Primary Industries	The State Administration of Grain; Chengdu Grain Storage Research Institute; Henan University of Technology; Guangdong Provincial Grain Research Institute.
ACIAR PN 9437	The University of New South Wales.	The State Administration of Grain; Chengdu Grain Storage Research Institute; Heilongjiang Provincial Grain Bureau; Sci. & Tech. Co. ,China Grains & Oils Group
ACIAR PN 98137	The Agricultural Production Systems Research Unit; Grainco Australia Pty Limited.	The State Administration of Grain; Henan University of Technology; Chengdu Grain Storage Research Institute; Guangdong Provincial Grain Research Institute.

Table 2. Objectives of the four collaborative projects

Project	Objectives
ACIAR PN 9035	To promote integration of grain protectants into storage pest management in P. R. China.
ACIAR PN 9415	To determinate resistance of main stored grain pests to phosphine in China and to search for corresponding strategies.
ACIAR PN 9437	To develop a technically and economically attractive drying and storage system for maize in Northeast China and to adapt existing in – store dryers for paddy to the subtropical conditions of Southern China.
ACIAR PN 98137	To prolong the service life of phosphine as a major fumigant and to enhance the effect of application of phosphine in the fumigation of stored grain

All of the projects were based in China and Australia. Most of the project activities took place in China in labs of universities and research institutes, as well as grain stores,

As an example, activities of ACIAR PN 9035 project as described by Chudleigh[1] were:

In Australia: the further enhancement of an expert system that had been developed by CSIRO.

In China:

■ Assessing the extent of resistance to the most commonly used grain protectant, malthion;

■ Determining the rates of fenitrothion and deltamethrin that might be used under Chinese storage conditions in order to improve the range of protectants available for Chinese grain storage;

■ Adaptation to Chinese conditions and extension of an Australian expert system in China.

For each project, ACIAR nominated a Project Coordinator, a Project Leader, and Project Scientists from one or two Australian research institutions or universities, SAG nominated a Project Officer, a Project Coordinator and a Project Team normally coming from different research institutes and universities.

Within each project, one or two technologies were transferred from Australia to China through initial training or demonstration tests. Then further research work, especially experiments were carried out mostly by the Chinese teams headed or supervised by Australian scientists. Once the technologies were verified under Chinese conditions or modified to fit the Chinese conditions, relevant technologies were distributed to Chinese grain storage industry through larger scale training, demonstration or even by incorporating into Chinese regulations or standards.

Duration of the implementation of projects was normally three to five years; an additional one to two years extension was allowed in case of failing to achieve the predicted results or delays in project equipment supplies etc.

Outputs of Grain Storage Technology Cooperation between ACIAR and SAG

All of the four projects have been successfully implemented and reached or exceeded their initial objectives, through the efforts of Australian and Chinese scientists under the leadership of ACIAR and SAG. The main achievements of the four projects can be de-

scribed as follows:

ACIAR PN 9035 Project

■ Data on major pest problems, the pesticide resistance status of individual pests, and the grain protectants most effective against them were acquired;

■ As a result of the study, application of protectants such as fenitrothion, deltamethrin and malathion has been greatly increased and applied to 10% of stored grains, Dosage of malathion application has been decreased by 60% (from 30ppm down to 10ppm) in China, by mixing malathion with deltamethrin;

■ On the basis of CSIRO's expert system, a Chinese version of stored grain pests control expert system was developed and widely distributed in the Chinese grain storage industry.

ACIAR PN 9415 Project

■ After a series of tests, 70 ppm was found to be the minimum concentration for effective phosphine fumigation under Chinese conditions, This finding was adopted by the Chinese Regulations for Re-circulating Fumigation;

■ It was found that the resistance of major stored grain pests such as *Rhizopertha dominica* (Fabricius), *Sitophilus oryzae* (Linnaeus) and *Cryptolestes ferrugineus* (Stephens) to fenitrothion was independant from that of phosphine, As a result, the application of phosphine and grain protectants would be helpful to slow down the development of resistance of stored grain pests to fenitrothion under appropriate conditions;

■ The inheritance of resistance of stored grain pests to phosphine was controlled by two or more genes, which behaved as incompletely dominant and noninterlocked, Results of this study were of much significance to fully understanding of resistance and increasing the service life of phosphine.

ACIAR PN 9437 Project

■ Thermo-physical properties of the most common maize variety in northern China and the hybrid japonica rice varieties in southern China were determined; climatic data from the regions where the project was implemented were recorded. The thermo-physical properties of maize and rice and the climatic data were incorporated into an existing drying simulation model to make it suitable for drying simulation under Chinese conditions;

■ Two automatic controllers for in-store grain drying in China were developed and installed; existing grain storage facilities were modified and in-store grain drying experiments in northern and southern China were conducted in continuous three or four years[2];

■ A rapid test for determining ergosterol levels in stored rice grain was developed; Chinese language training courses in grain drying were conducted six times in China and once in Australia;

■ As a result of the project activities, a portable in-store drying ventilation equipment with automatic control system for large scale flat warehouses was developed by the project team from Chengdu and the innovative ventilation systems were put into use in some grain depots (see Fig, 1). Meanwhile, a radial ventilation system for in-store drying in large-size silos was developed by the project team from Heilongjiang.

Fig 1 Photographs of portable ventilation system for in – store drying

ACIAR PN 98137 Project [3]

■ On the basis of achievements of ACIAR PN 9415 project, resistance to phosphine in major stored grain pests was further studied;

■ Chinese National Standard for Phosphine Fumigation was developed taking advantage of the project outputs;

■ Tests on re-circulating fumigation in well sealed large flat stores with a mixture of phosphine and 5% carbon dioxide were conducted and pest killing data was recorded;

■ Re-circulating fumigation in bulk grain covered with plastic film was studied, and this technique has been widely used in many grain depots in southern China;

■ Effects of phosphine adsorption of wheat, paddy and maize on phosphine concentration were studied. The results of the study were very helpful for the determination of minimum dosage of phosphine application in large-scale grain stores.

In general, both sides, but especially China, have benefited significantly through nearly eighteen years technical cooperation.

■ Chinese grain storage technologies including integrated management of stored grain pests and in-store drying have been improved through the application of achievements gained from these projects;

■ Experience that Australian scientists gained from project activities in China, has also enriched their knowledge on grain storage science and might be used in Australia as well as in other collaborating countries;

■ Technology transfer and communications in the field of grain storage between the two countries have been enhanced, which also helps for better mutual; understanding of each other's grain industry and for the promotion of cooperation in grain trade business.

Experience gained from Implementation of the Projects

Through implementation of the four projects, considerable experience was gained on how to conduct a successful project, The main points can be summarised as follows;

■ In order to properly select project topics, preliminary feasibility studies and sufficient exchanging of ideas between partners are necessary, which set the basis for the successful implementation of the project、Before proposing each project, ACIAR had sent Australian scientists to China to get to know the situation of Chinese grain storage technologies and found that integrating storage pest management, better understanding of phosphine resistance in insect pests of stored grain, and preserving quality during drying were priorities in the further development of Chinese grain storage and drying technologies;

■ Training is very helpful for the better understanding of project objectives, methods of research, and thereafter dissemination of projects achievements for the partners in developing countries;

■ Periodical discussions through seminars, meetings during the implementation of projects are more important than just issuing reports. Face to face discussion provides a good platform for exchange of ideas and helps to find out what has been achieved and what has to be improved;

■ Modification or improvement of transferred technologies to fit to the conditions in developing countries is also important. The development of portable in-store drying ventilation equipment with automatic control system for large scale flat warehouses was a good example;

■ Governmental organisations or institutions play a very important part in coordinating project activities by providing financial supports, experimental conditions, as well as dissemination of project achievements etc;

■ Postharvest operations like drying and fumigation are carried out seasonally, project activities must be well planned in order not to miss the most appropriate time for experiments;

■ Procurement, manufacturing and long distance shipping of project equipment are time consuming. Purchase and/or manufacture equipment locally when applicable will cut the project budget and supply the equipment in time.

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ABSTRACT SESSION

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The Effect of Grain Temperature on the Toxicity of Phosphine against Phosphine – Resistant Insect Pests of Stored Grain

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Abstract: The Australian grain industry relies principally on phosphine fumigation to eliminate insect infestations that threaten grain integrity and market access. Currently there is no viable alternative to phosphine and it is likely that the industry will continue to rely on this fumigant at least for the medium term. A significant threat, however, to the on-going use of phosphine has been the development of resistance in target pests.

Our challenge is to effectively manage the threat of resistance until alternative strategies can be researched and implemented. It is well known that concentration and time are important dosage parameters for phosphine, however, little is known of the significance of a third variable, grain temperature, despite anecdotal evidence that it has an effect on the outcome of fumigations. The aim of our work was to quantify the effect of temperature on the toxicity of phosphine and to use this information in the development of fumigation protocols.

Mixed-age cultures (including all life-stages) of purified resistant strains of *Rhyzopertha dominica*, *Sitophilus oryzae* and *Liposcelis bostrychophila* were exposed to phosphine at a range of grain temperatures in the laboratory. Once dosage protocols were developed, these were tested in field trials under commercial conditions in bulk grain.

Toxicity of phosphine was strongly influenced by fumigation temperature. With *R. domincia*, there was no simple relationship between toxity and temp at most concentrations. For example, at 0. 17 mg L^{-1}, time to population extinction was longer at 30℃ than at 35 but it was 10 days shorter at 20 and 25. In contrast, toxicity against *S. oryzae* increased with temperature and this species was comparatively susceptible at higher temperatures. Similarly, increasing temprature had a marked effect on the toxicity of phosphine against populations of *L. bostrichophila*.

Key words: phosphine, resistance, temperature, stored grain, insects

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Study on the Sorption, Desorption and Accumulation of Phosphine Residue on Multi – fumigated Grains

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Abstract: The sorption of phosphine on hard wheat decreased with an increase in the number of fumigation cycles at 11. 3% – 12. 1% moisture content. For example, after the initial physical sorption of phosphine (which can be removed by aeration) the sorption of phosphine decreased by 10% for the second fumigation cycle, and by 20% for the third fumigation cycle. Theoretically, this result shows that there are a limited number of matrices in hard wheat which could react and/or chemically bind phosphine (200 ppm). There was no major difference in the sorption of phosphine with single and multi-fumigation on soft wheat, barley and field peas. This result indicates that there are certain levels of matrices in soft wheat, barley and field peas which could react and/or chemically bind phosphine. After 14 days exposure, 50% – 60% phosphine was absorbed by soft wheat, 75% – 80% by barley and 90% – 95% by field peas. Therefore, in comparison with wheat, the application dose for the fumigation of barley and field peas should increase by 20% and 30% respectively.

Desorption rate of phosphine from wheat (hard and soft), barley and field peas was affected by the number of fumigation cycles. The desorption rate increased with increasing number of fumigation cycles, eg. 5% – 10% more phosphine was desorbed with each successive fumigation cycle. The first

day aeration removed 85% – 95% of the phosphine from wheat (hard and soft) and 65% – 75% from barley and field peas. Therefore, in comparison with wheat, for barley and field peas a 20% longer period of aeration is required.

The levels of phosphine in wheat (hard and soft), barley and field peas was affected by the number of fumigation cycles. The residue levels increased with increasing number of fumigation cycles, eg. 10% – 30% more phosphine residues were present with increasing fumigation cycles. The first 2 days aeration removed 85% – 95% of the phosphine from wheat (hard and soft) and 65% – 75% from barley and field peas. This result is consistent with desorption. Therefore, in comparison with wheat, for barley and field peas a 20% longer period of aeration is required.

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Ethanedinitrile (C_2N_2) is a Potential Fumigant for Grain, Timber and Soil

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Abstract: Cyanogen (C_2N_2) is a new potential fumigant to replace methyl bromide for certain applications. It is highly toxic to insect pests. Cyanogen was evaluated for its potential as a grain and timber fumigant, where rapid action is important. The most of stored product insects (all stages) can be killed at 1 or 5mg L^{-1} for 6 or 3 hours exposure, e. g. the adult stage of *R. dominica* was completely killed at 1.0mg L^{-1}. Exposure for 6 hours to C_2N_2 at 21 – 25℃, all the larval stages of *A. glabripennis* were completely killed at 11mg L^{-1}, workers of *C. acinaciformis*, *C. brevis*, *M. darwiniensis* and *Reticulitermes speratus* were completely killed at 1.61mg L^{-1}, 3.0mg L^{-1} and 2.3mg L^{-1} respectively. In general, C_2N_2 showed high toxicity to all immature and adult stages tested and in this respect is more toxic than methyl bromide. The efficacy of C_2N_2 to other wood related pests shown that for 6hr exposure at 21 ±2℃, the LD_{99} value was 0.65, 4.64 and 0.63mg L^{-1} against *R. speratus*, *T. piniperda* and *H. cunea* adult, respectively. Also, C_2N_2 was highly effective (>95%) to a pinewood nematode (*Bursapelenchus xylophilus*) when applied 97g m^{-3}. The highest dose of C_2N_2 (148g m^{-3}) in the trials showed highest nematocidal activity but didn't achieve 100% mortality. The penetration of C_2N_2 into the Pinewood (Oregon, *Pseudotsuga menziesii*) blocks (10 cm (10 cm (30 cm) with 0.44 – 0.45 g cm^{-3} of density and a 7.8% moisture content was much better than the methyl bromide.

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Implementing a No Entry Phosphine Fumigation Strategy

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Abstract: The CBH Group, the central grain storage and handling organisation in Western Australia, has been trialling Vaporphos® as a no entry phosphine fumigation technique. These trials have seen the successful application of phosphine in storages as large as 280 000 tonnes.

As a result of this success the CBH Group has implemented a "Zero Harm" fumigation strategy to minimise potential staff exposure to phosphine during the application phase of the fumigation. The implementation of this strategy involved the adaptation of storage infrastructure, review of fumigation protocols, establishment of rapid fumigation business rules, and inclusion of these rules into the existing Grain Protection integrated pest management program.

This presentation gives an overview of the strategy and outlines the benefits associated with adopting this new approach to using phosphine.

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Managing Resistance in a Single Fumigant Environment – An Industry Perspective

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Abstract: The CBH Group, the central grain storage and handling organisation in Western Australia, has been totally reliant on phosphine as its primary insect control strategy for over the last 20 years. This reliance has put the CBH Group in a precarious position should phosphine resistance develop to levels where control failures occur.

As a result the CBH Group, in collaboration with other industry and government bodies, developed a phosphine resistance management strategy to combat resistance development and extend the life of phosphine in Western Australia. This strategy can be broken down into a number of critical components including the development of fumigation protocols, resistance monitoring, implementation of the Phosure extension program, Research and Development, and a $5 million Sealed Storage maintenance program. Furthermore a rigorous data capture, auditing and monitoring system has been implemented to measure the ongoing success of the strategy and compliance to the critical components.

This strategy has seen the maintenance of phosphine resistance at low levels within Western Australia.

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Recent Developments in the Application of CYTEC Cylinderized Phosphine Fumigants for Timber, Logs and Horticultural Produce

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Abstract: ECO_2FUME (2% phosphine and 98% carbon dioxide by weight) and $VAPORPH_3OS$ (99.3% phosphine) are two cylinderized phosphine fumigants which are currently used in many countries for the treatment of cereal grains, nuts and beans, dried fruits, tobacco, processed feeds and sructural fumigation. Being cylinderized formulation of fumigants, they have the distinct advantages over the solid metal phosphides of being faster, safer and greener.

With the increased pressure to find alternatives to methyl bromide, CYTEC in recent years h s embarked on expanding the applications of ECO_2FUME and $VAPORPH_3OS$ to timber, logs and horticultural produce. Small scale and commercial scale trials were conducted on the treatment of major export commodities from New Zealand such as sawn timber, raw logs, kiwi fruit and apples. This paper describes the results of the trials in terms of efficacy, residue and quality. Fumigation protocols were established as optimum treatment for sawn timber, raw logs and kiwi fruit and apples and achieved a 100% efficacy for the specific target insect pests. There was no significant changes in the quality of the treated fruits. Residue levels of phosphine in the kiwi fruit and apples at 48 hours after trreatment were far below the maximum residue limit of 0.01 mg/kg.

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Control of Mites and Insects in Pet Food Packages Using Controlled Atmospheres

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Insect infestations in packaged pet food products can be costly because of product returns and loss of customer goodwill. The reasons for infestation in packaged products could be due to poor seals, ability of insects to penetrate packages, poor handling practices that result in damage to packages, or presence of insects/mites at the time of packaging. Infestation of packaged products costs manufacturer's millions of dollars annually, and more research is needed to identify factors contributing to infestations in packages and methods to mitigate losses in packaged finished products. During 2007 – 2008, we examined the ability of selected insects and a mite species to infest two different packaged products-one a corn-based cat litter and the other, a pet food for dogs. Extensive laboratory tests were conducted to determine suitability of the litter to sustain insect infestation and effects of various combinations of oxygen, nitrogen, and carbon dioxide at different temperatures on various life stages of insects. The use of low oxygen concentrations inside packages of dog food on mite survival over time were studied, along with some preliminary tests on the use of ascorbic acid as an oxygen scavenger to reduce oxygen levels inside packages. These laboratory and field tests showed promising results on the use of modified atmospheres in protecting packaged foods from insect/mite infestations.

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