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Some Research Progresses of Stored-grain Protection in China

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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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