ENHANCED EFFECTIVENESS OF VACUUM OR CO₂ IN COMBINATION WITH INCREASED TEMPERATURES FOR CONTROL OF STORAGE INSECTS

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ABSTRACT

Laboratory studies were carried out to identify the combinations that enhance the effectiveness of insect control based on vacuum or CO₂ in combination with increased temperatures as a quarantine treatment of storage pests. The roles of the commodity moisture content and the partial pressure of oxygen on the effectiveness of vacuum were described. For T. castaneum, E. cautella and P. interpunctella the egg stage was the most resistant to low pressure, the times needed to obtain LT₉₉ were 22, 45 and 49 h, respectively. For T. castaneum the larva and adults were the most susceptible stages with an LT₉₉ of 7 h; for E. cautella and P. interpunctella the adult was the most susceptible stage with LT99 times of only 6 h and 5 h. The effect of CO₂ at 45°C on reducing the exposure time for diapausing larvae of T. granarium showed that by increasing the CO₂ concentration to 90% the LT₉₉ value decreased to about 10 h, whereas at 35°C the LT₉₉ value was 29 h. Ephestia cautella larvae were shown to be the most resistant stage to 90% CO₂ at 40°C, with an LT₉₉ value of only 6 h. For Oryzaephilus surinamensis under the same conditions, the LT₉₉ value was 9 h for the most resistant egg stage. These encouraging results led to the idea of developing a transportable flexible storage system to render the technology a practical tool for the control of insect pests. Experiments were carried out using a 15-m³ capacity plastic container termed the "Volcani CubeTM" or "GrainPro CocoonTM". Bioassays in field trials at 30°C demonstrated that complete mortality of test insects composed of all four developmental stages of E. cautella and Tribolium castaneum, was observed upon 3days exposure to vacuum maintained between 23 and 75 mm Hg.

Keywords: methyl bromide alternatives, vacuum, CO₂, storage insects, transportable systems

Introduction

A significant development that has occurred over the past 10 years in post-harvest technologies has been the fast approaching phase-out of methyl bromide in developed countries by the year 2005 and worldwide by 2015 under the terms of the Montreal Protocol (UNEP, 1998). This has resulted in a significant increase in the number of publications dealing with alternatives to methyl bromide (Bell *et al.*, 1996; Donahaye et al., 2001; International Conference on Alternatives to Methyl Bromide, 2002). In particular, the search for non-chemical methods of insect control has increased in intensity. Additionally, a public awareness has arisen with respect to pesticide residues in food and their harmful influence on the environment. Public pressure is increasingly being brought to bear on legislators to close every loophole that might enable the contamination of food with toxic materials. Consequently, future prospects for using new fumigants on stored food products remain very limited.

Many research groups are now in a "rethink" mode as a direct result of pressure from national and international legislative bodies and contract restrictions on grain purchase imposed by importing countries. These authorities are fast reducing the range of existing chemical options, while the development of new friendly chemicals, specifically for the stored product market has become prohibitively expensive. These

constraints have led to a realization that prevention is better than cure. And so, the emphasis is rapidly shifting to integrated pest management (IPM) with chemical means of control as a last resort. However, in practice chemical control still plays a dominant role, with phosphine fumigation as the mainstay of the grain storage industry, even though, - as with methyl bromide - its use may also become increasingly more restricted in the future.

Widespread experience has proven that chemicals applied at commercial levels have the characteristic of developing resistance by insects. The wisdom of relying upon a single chemical such as phosphine, with the hope that resistance does not develop has been invalidated (Winks, 1987; Zettler, 1993). PH₃ is a very useful fumigant but it is slow acting and insects in various countries have developed resistance (Winks, 1987; Zettler, 1993).

Controlled atmospheres (CAs) technology can fulfill a specific niche where use of other fumigants is unacceptable such as treating organic foods. CAs are limited by the long exposure times required to produce complete mortality (Navarro and Jay, 1987), and are similar to those required for phosphine (PH₃) fumigations (Navarro and Donahaye, 1990). In cases where rapid disinfestation of commodities is required, the possibility of using CO₂ at temperatures raised to levels that will not adversely affect the commodity should be considered. It has been recognized that insecticide treatments, particularly those affecting the respiratory system are more pronounced at higher temperatures (Navarro and Calderon, 1980). Respiratory metabolism has also been observed to increase with temperature up to a maximum and then sharply declines at the upper lethal temperature (Chapman, 1982). A number of laboratory studies on the combined effects of high CO₂ and optimal elevated temperatures against insects have shown a corresponding increase in insect mortality with increase in temperature (AliNiazee, 1971; Adler, 1994; 1997; Bell et al., 1980; Donahaye et al., 1996; Navarro and Jay, 1987; Soderstrom et al., 1991).

Investigations on effects of low pressures on storage insects were carried out by Back and Cotton (1925), Bare (1948), and later on by Calderon *et al.* (1966), Calderon and Navarro (1968), and Navarro and Calderon (1969; 1972a; 1972b). Recently Mbata and Phillips (2001) investigated the effects of temperature and exposure time on mortality of three stored product insects exposed to low pressure. Insect mortality under low pressure is predominantly a result of oxygen deficit and not due to physical pressure effects (Navarro and Calderon 1979).

In a first attempt to use low pressures to preserve cacao beans quality, Challot and Vincent (1977) used polyethylene bags applying a low pressure of 600 mm Hg. Although 600 mm Hg may be effective in maintaining the product quality and prevent ingress of insects, storage insects can tolerate this pressure. For mortality of storage insects, low pressures below 100 mm Hg are required.

Gas tight flexible structures using the hermetic storage method have been developed and are in use on an industrial scale (Navarro *et al.*, 1988; 1990; 1994; Silberstein et al., 1998). These structures enable treatment with modified atmospheres or fumigation (Navarro *et al.*, 1995), and they are termed "Volcani CubesTM" or "GrainPro CocoonsTM" (Navarro *et al.*, 1999). The use of these flexible storage facilities to maintain low pressures of 25-30 mm Hg was reported in two recent works (Phillips *et al.*, 2000; Navarro *et al.*, 2001).

The objective of this paper is to report on the mortality of different life stages of stored product insect pests exposed to increased temperatures and a constant low pressure or under CO₂ enriched atmospheres, and on the application of transportable systems for the environmentally friendly treatment of storage pests.

MATERIALS AND METHODS

Temperature, CO₂, and low-pressure combinations

For CO₂ treatments, concentrations varying from 60% to 90% of CO₂ in air at temperatures ranging from 30° to 45°C were tested. For low-pressure treatments, an absolute pressure of 50 (in the range 45 and 55) mm Hg at a temperature of 30°C was tested.

Test insects

Diapausing larvae of Khapra beetle (*Trogoderma granarium*) were obtained by removing active larvae from cultures and placing them in groups of several hundred without food for one month at 28°C (Lindgren and Vincent, 1960). Adults of *Tribolium castaneum*, *Oryzaephilus surinamensis*, *Ephestia cautella* and *Plodia interpunctella* were taken from laboratory cultures maintained at the Department of Stored Products, Volcani Center, Agricultural Research Organization, Bet Dagan, and mass reared on a standard artificial diet. Eggs, pupae and adults (1-2 days old) and larvae (4-15 days old) were taken from the same batch.

Eggs of tested species were used within 0-2 days of oviposition. The eggs were obtained by placing 500-1000 adults beetles in 500 g of wheat flour containing 5 g of brewers' yeasts. To obtain eggs from moths, *E. cautella* and *P. interpunctella* were placed on a mesh covered inverted jar, overnight and the females laid the eggs in a Petri dish. Two Perspex slides each with 50-drilled "wells" were used to individually place 100 eggs from each of the studied species. The slides were then covered with a cover glass to retain the eggs (Navarro and Gonen, 1970).

Exposure of insects to low pressures

Treatment chambers consisted of 3-L desiccators filled with 1 kg cocoa beans stabilized at an equilibrium relative humidity of 55 r.h. at 30°C. For exposure, sets of 50 insects were confined in cages of 15-mm diameter and 50 mm length made of 100 mesh stainless steel.

Post fumigation procedures

Following treatment, larvae, pupae and adults were transferred to small jars (50 ml) and maintained at 28±1°C and 65±5% R.H. The larvae were provided with food. The eggs were transferred to watch glasses and incubated under the same conditions as the other developmental stages. Mortality counts for larvae were carried out after two weeks of exposure; for pupae after one week, for adults after one day, and for eggs after 4-5 days of exposure. Mortality for larvae was based on those that failed to pupate, for pupae, those that failed to emerge as adults, for adults, those that were dead or moribund, and for eggs, those that failed completely to hatch.

RESULTS AND DISCUSSION

Effects of CO_2 and temperature

Table 1 shows the influence of CO₂ concentrations at different temperatures as expressed in LT₉₉ mortality values for diapausing larvae of *T. granarium*. At 45°C, by increasing the CO₂ concentration to 90% the LT₉₉ value decreased to 10 h, whereas at 35°C the LT₉₉ value was 29 h.

T. granarium is one of the most serious pests of stored cereal grains and oil seeds, and is subject to strict quarantine regulations in the US, Australia and several other countries. It is a member of the dermestid family and is a voracious feeder of grain products. The larvae can hide in cracks of the storage structure in a state of facultative diapause and can remain in this condition for years. It is particularly difficult to control with insecticides. Consequently, many quarantine treatments are mandatory when products such as rugs, spices and cereal products are imported from infested countries. In such situations, MB is still the only effective fumigant against this pest. Present distribution of T. granarium includes Western Africa through the Northern Indian subcontinent (Cuperus et. al., 1992). Results shown in Table 1 may serve as guidelines to the possibility of applying slightly elevated temperatures for control of the most resistant diapausing larvae of T. granarium.

Table 1 - Influence of CO_2 concentrations expressed in LT_{99} (hours to obtain 99% mortality) values for *Trogoderma granarium* diapausing larvae at three different temperatures.

Temperature (°C)		CO ₂ concentration (%)					
	60	70	80	90			
35	38	29	-	29			
40	24	28	20	-			
45	15	17	15	10			

A similar approach of applying various CO_2 concentrations at different temperatures was investigated for four developmental stages of *E. cautella*. Results in Table 2 summarize the effectiveness of the combination of CO_2 at temperatures in the range of 35°C to 45°C. Tests with *Ephestia cautella* showed that the pupa was the most resistant stage when exposed to 90% CO_2 with an LT_{99} value of 17 h at 35°C, and only 3 h when exposed at 45°C. The adult was the most sensitive stage of E. cautella requiring only 4 h of exposure to 90% CO_2 at 35°C.

Results on the influence of various CO_2 concentrations at different temperatures on O surinamensis development stages are shown in Table 3. For this species as well, an increase in CO_2 concentration resulted in a decrease in the LT_{99} value. Generally, the eggs were the most resistant stage; at 40°C and 90% CO_2 a six h exposure was required to obtain an LT_{99} value.

Table 2 - Influence of CO_2 concentrations expressed in LT_{99} (hours to obtain 99% mortality) values for *Ephestia cautella* various development stages exposed to CO_2 concentrations in air at three different temperatures.

Temp. (°C)	35			40			45					
CO ₂ (%)	60	70	80	90	60	70	80	90	60	70	80	90
Eggs	23	23	17	9	16	12	8	5	9	5	3	2
Larvae	60	27	20	12	17	9	6	6	5	4	2	2
Pupae	56	37	17	17	36	10	8	4	7	4	4	3
Adults	20	14	6	4	6	5	3	2	3	2	2	2

Table 3 - Influence of CO₂ concentrations expressed in LT₉₉ (hours to obtain 99% mortality) values for *Oryzaephilus surinamensis* various development stages exposed to CO₂ concentrations in air at three different temperatures.

Temp. (°C)	Life Stage	CO ₂ concentrations (%)						
		60	70	80	90			
20	Eggs	-	-	38	22			
30	Adults	21	-	22	9			
35	Eggs	29	25	21	9			
	Adults	26	11	8	4			
40	Eggs	15	7	6	6			
	Larvae	8		2	2			
	Pupae	-	-	-	5			
	Adults	12	11	6	3			

Effects of low pressures and temperature

The response of four developmental stages of three storage insects to 55 mm Hg is shown in Table 4. For T. castaneum, E. cautella and P. interpunctella the egg stage was the most resistant to low pressure, the times needed to obtain LT_{99} being 22, 45 and 49 h, respectively. For T. castaneum, the larva and adults were the most susceptible stages with an LT_{99} of 7 h. For E. cautella and P. interpunctella the adult was the most susceptible stage with only 6 h and 5 h.

Temperature plays an important role in the exposure time needed to achieve mortality of insects. At 18°C and at 55 mm Hg times needed to achieve LT₉₉ for eggs, larvae, pupae and adults of *E. cautella* were 149, 44, 26 and 77 h, respectively (Finkelman et al., 2002), while at 30°C these times were shortened to 45, 10, 7 and 6 h (Table 4). For *T. castaneum* the temperature effect was even more marked; at 18°C the times needed to achieve LT₉₉ were 93, 37, 72 and 30 h, respectively, while at 30°C the times were shortened to 22, 7, 13 and 7 h.

The only other information available on the effects of low pressure on *E. cautella*, and *T. castaneum* is that provided by Calderon et al. (1966) who reported that at 10-12 and 16-20 mm Hg and at 25°C, adults of *E. cautella* were very sensitive, and less than 1 h exposure was required to obtain 99% mortality, while for *T. castaneum* adults 2.7 h were necessary. Mbata and Phillips (2001) reported on the eggs of *P. interpunctella* as the most resistant stage compared to the larvae and the pupa, with LT₉₉ values for eggs exposed to 32.5 mm Hg at 25°C as 28 h. An increase in temperature to 33°C resulted in a decrease in LT₉₉ for eggs to an exposure time to 6 h.

Navarro and Calderon (1979) compared the influence of low pressure on Ephestia cautella pupae with that of low oxygen concentrations, and deduced that the partial pressure of oxygen has a decisive effect on insect mortality, while no significant function could be attributed to the low pressure itself. At 50 mm Hg the partial pressure of oxygen is equivalent to 1.4%, this being similar to the target oxygen concentration under a modified atmosphere obtained by nitrogen flushing. Table 5 shows the various units used in the literature for describing the equivalent values of various low pressures and equivalents in oxygen % at normal temperature and pressure of the atmosphere. In the process of evacuation, the main significant effect is the reduction in the partial pressure of the oxygen. The drop in the partial pressure of oxygen concentration is proportional to the atmospheric pressure and is almost linear. However, the commodity moisture largely dictates the humidity within the treated enclosure. Humidity response is linear if the treated enclosure is empty and no other gas then air is present in the enclosure. To demonstrate the effect of commodity moisture, Fig. 1 was prepared and tested for cocoa beans of 6.2% moisture content. According to Gough (1975), the equilibrium relative humidity (ERH) of cocoa beans is 60%. Although an evacuation process may reduce the humidity of the enclosure for a short time, the commodity gives off moisture to the interstitial space until the commodity moisture equilibrates with the humidity of the atmosphere of the interstitial space. For cocoa beans with a fill ratio of 63% in desiccators, this equilibration process lasted less than one hour at 30°C.

Rigid metal chambers have been in use for the implementation of vacuum fumigation in agricultural commodities (Bond, 1984). A recently developed technology based on high-pressure CO₂ treatments also makes use of rigid chambers (Adler *et al*, 2000). However, these structures are expensive and their lack of transportability h restricts the implementation of this environmentally sound method. Based on the encouraging data obtained herein, to render the technology a practical tool, the possibility was recently investigated of using CO₂ or low pressures to control storage insects in a transportable system (Phillips *et al.*, 2000).

Table 4 - The influence of 55 mm Hg on exposure time expressed in LT_{99} (hours to obtain 99% mortality) values of three storage insect pests at 30° C.

Developmental stage	Tribolium	Ephestia	Plodia
	castaneum	cautella	interpunctella
Eggs	22	45	49
Larvae	7	10	12
Pupae	13	7	10
Adults	7	6	5

Table 5 - Units used to express atmospheric pressure and their equivalent partial pressure of oxygen expressed in mmHg and in percentage.

mmHg (≈torr)	atm	kg/cm ²	inches Hg	kPa	mbar	mm Hg oxygen	% oxygen
760	1.00	1.03	29.92	101,325	1,013	159	20.9
600	0.79	0.82	23.62	79,993	800	125	16.5
500	0.66	0.68	19.68	66,661	667	105	13.8
400	0.53	0.54	15.75	53,329	533	84	11.0
300	0.39	0.41	11.81	39,997	400	63	8.3
200	0.26	0.27	7.87	26,664	267	42	5.5
100	0.13	0.14	3.94	13,332	133	21	2.8
50	0.07	0.07	1.97	6,666	67	11	1.4
0	0.00	0.00	0.00	0	0	0	0.0

Fig. 1 - Linear relationship between atmospheric pressure (mm Hg) and air humidity $(g/kg) 30^{\circ}C$.

Interstitial 60% ERH at any low pressures of cocoa beans at 6.2% moisture content Air humidity in empty chamber 25 Air humidity (g water/kg air) 20 15 -10 -5 100 200 400 500 600 300 700

mm Hg

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 (Navarro *et al.*, 1999). Experiments were carried out using a 15-m3 capacity plastic container termed the "Volcani CubeTM" or "GrainPro Cocoon[®]". The pressure was maintained between 25 and 29 mm Hg for 17 days. Bioassays in field trials demonstrated that complete mortality of test insects composed of mixed ages of *E. cautella Plodia interpunctella*, and larvae of *T. castaneum*, was observed following the 3-days exposure to vacuum (Phillips et al. 2000). For the disinfestation of durable commodities, these flexible storage containers can be considered as an alternative to treatments with methyl bromide and other toxic fumigants. Currently, cocoa beans are treated in the Ivory Coast with phosphine for five days and this treatment has replaced the conventional methyl bromide fumigation (Finkelman et al., 2002). The option of low pressure therefore is a promising solution that can provide the required insect control in less time and without the need to use environmentally harmful chemicals.

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