Integrated storage pest control methods using vacuum or CO_2 in transportable systems

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Abstract: The suggested potential alternatives to MB (methyl bromide) for disinfestation of durable commodities are likely to be costly compared to the use of MB. In addition, very few of the suggested treatments have the effectiveness of short exposure time comparable to MB. The objective of our investigation was to identify the combinations that enhance the effectiveness of the treatments based on vacuum or a combination of heat and CO_2.

Tests of the influence of CO_2 at 45°C on reducing the exposure time expressed as LT_{99} values for diapausing larvae of *Trogoderma granarium* showed that by increasing the CO_2 concentration to 90% the exposure time decreased to about 10 h, whereas at 35ºC the LT_{99} value was 29 h. *Ephestia cautella* pupae were shown to be the most resistant stage to the same treatment with an LT_{99} value of only 3 h. For *Oryzaephilus surinamensis* under the same conditions, it was 9 h for the most resistant egg stage.
In laboratory studies with *Lasioderma serricorne* exposed to low pressures at 30ºC, LT_{99} value for adults was 15 h when exposed to 25 mm Hg. *Trogoderma granarium* larvae were the most resistant species, whereby 172 h exposure was necessary under the same conditions.

These encouraging reports led to the idea of developing a transportable 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 Cube™" or "GrainPro Cocoon®". The pressure was maintained between 25 to 29 mm Hg for 17 days. Bioassay 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 on the 3-days exposure to vacuum.

Key words: IPM, storage pests control, vacuum, CO_2, transportable systems, methyl bromide alternatives, *Oryzaephilus surinamensis, Trogoderma granarium, Ephestia cautella, Plodia interpunctella, Tribolium castaneum, Lasioderma serricorne*

Introduction

There are only two universally available fumigants remaining for disinfestation of durable commodities, namely, methyl bromide (MB) and phosphine (PH_3). Each of these has its own limitations. MB is facing a phase-out in developed countries by the year 2005 and worldwide by the year 2020 under the terms of the Montreal Protocol (UNEP, 1998). Under present agreements of the Montreal Protocol, there are exemptions for all countries from controls on MB when used for quarantine and pre-shipment fumigations, and for some critical agricultural uses, yet to be defined. PH_3 is a very useful fumigant but it is slow acting and insects in various countries have developed resistance (Winks, 1987; Zettler, 1993).

Since the announcement that MB would be phased out due to its role as an atmospheric ozone-depleter, research has focused on finding alternatives that can be registered as effective and safe.

Although there is a large number of suggested potential chemical and non-chemical alternatives to MB, each has limitations that prevent it from being a direct replacement for methyl bromide in all its current uses (Bell *et al*., 1996). Controlled atmospheres (CAs) have been one of the considered alternatives to MB. This technology can fulfill a specific niche where use of other fumigants is unacceptable such as treating organic foods. The use of controlled atmospheres as alternative to MB at normal ambient temperatures is limited by the long exposure times required to produce complete mortality (Navarro and Jay, 1987). These periods are similar to those required for PH_3 fumigations (Navarro and Donahaye, 1990). . Insect development and metabolism are positively correlated with temperature (Donahaye *et al*., 1996), and it has long been recognized that insecticide treatments, particularly those affecting the respiratory system are more pronounced at higher temperatures. In cases where rapid disinfestation of commodities is required, the possibility of using CO_2 at temperatures raised to levels that will not adversely affect the commodity should be considered.

The possibility of using low pressures in post-harvest storage was first explored by Back and Cotton (1925), Bare (1948), and later on by Calderon *et al*. (1966), 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). The effects of vacuum in combination with elevated temperatures were not considered in some studies (Back and Cotton 1925; Bare 1948; Calderon *et al*., 1966; Calderon and Navarro 1968).

To achieve the extremely low pressures for insect mortality a prohibitively expensive investment might be required in massive vacuum chambers. In a first attempt to use low pressures to store cacao beans, Challot and Vincent (1977) used polyethylene bags to apply and maintain a low pressure of 600 mmHg in order to preserve cacao beans quality. Although 600 mmHg 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; 1994; Navarro *et al.,* 1990; Silberstein *et al.,* 1998). These structures consist of plastic chambers with manufacturers specifications to a gas tightness level that will enable treatment without significant modified atmosphere or fumigant gas loss and within exposure times of no longer than 24 hours (Navarro *et al.,* 1995). They are termed "Volcani Cube™" or "GrainPro Cocoon®" (Navarro *et al.,* 1999) and have potential for use in small-scale applications, particularly for high-value crops such as cocoa, coffee, and spices. The use of these flexible storage facilities to maintain low pressures was reported in two recent works (Phillips *et al*., 2000; Navarro *et al*., 2001). In these structures, low pressures of 25-30 mmHg were achieved and cacao beans were preserved for over two months.

The objective of this paper was to report on the effects of exposure time and treatment temperature on mortality of different life stages of stored product insect pests exposed to elevated temperatures and a CO_2 enriched atmosphere or under a constant low pressure.

Materials and methods

Temperature, CO_2, and low-pressure combinations

For CO_2 treatments, concentrations varying from 60% to 90% of CO_2 in air at temperatures varying from 30° to 45°C were tested. For low pressure treatments, absolute pressures of 25, 50 and 100 mm Hg at temperatures varying from 18° to 35ºC were 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 28ºC (Lindgren and Vincent, 1960). Adults of *Oryzaephilus surinamensis, E. cautella* and *Lasioderma serricorne* were taken from laboratory cultures maintained at the Department of Stored Products, Volcani Center, Agricultural Research Organization, Bet Dagan, and mass reared on 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* 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

For all treatments, the r.h. in the exposure chamber consisting of vacuum flasks of 0.5-L was maintained at above 70% using a wad of folded filter paper imbibed with a saturated solution of sodium nitrite. Exposure temperatures were 25, 30 and 35ºC. 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±1ºC 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.

Statistical analysis

To determine the lethal time to obtain 99% mortality (LT_{99}) data were subjected to probit analysis (Daum, 1979). Results in this paper are presented without detailed statistical analysis to show the ranges of exposure times needed to control the test insects.

Results and discussion

Effects of CO_2 and temperature

Table 1 shows the influence of CO_2 concentrations at different temperatures as expressed in LT_{99} mortality values for diapausing larvae of *T. granarium*. At 45°C, increasing the CO_2 concentration to 90% the LT_{99} value decreased to 10 h, whereas at 35ºC the LT_{99} 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 the 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 (ºC)	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 35ºC to 45ºC. 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 35ºC, and only 3 h when exposed at 45ºC. The adult was the most sensitive stage of E. cautella requiring only 4 h of exposure to 90% CO_2 at 35ºC.

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

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, increasing the CO_2 concentration resulted in decreasing the LT_{99} value. Generally, the eggs were the most resistant stage; at 40ºC and 90% CO_2 a six h exposure was required for an LT_{99} value.

Table 3 - Influence of CO_2 concentrations expressed in LT_{99} (hours to obtain 99% mortality) values for *Oryzaephilus surinamensis* various development stages exposed to CO_2 concentrations in air at three different temperatures.

Temp. (°C)	Life Stage	CO_2 concentrations (%)			
		60	**70**	**80**	**90**
30	Eggs	-	-	38	22
	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

Table 4 shows partial results obtained on three developmental stages of *L. serricorne* exposed to low absolute pressures of 25, 50 and 100 mmHg at 18^0, 25^0, and 30^0C. Although the LT_{99} value for *L. serricorne* adults exposed to 25 mm Hg at 30ºC was 15 h, there is an apparent resistance of this species to low pressures. For example, eggs exposed to 25 mmHg even at 30^0C needed 75 h exposure to attain LT_{99} value. Bare (1948) also observed greater tolerance of *L. serricorne* eggs compared with other stages exposed to low pressure. More work is required to reveal the resistance of this species to low pressures.

Table 4 - Effects of temperature and low-pressures on LT_{99} (hours to obtain 99% mortality) of *Lasioderma serricorne* various development stages.

Pressure (mmHg)	Temp.(ºC)	Eggs	Larvae	Adults
25	**18**	-	-	47
	25	-		26
	30	75		15
50	**18**	-		157
	25	-	191	43
	30	-	49	15
100	**18**	136	-	-
	25	75	-	75
	30	40	-	-

The influence of low pressures at different exposure times and temperatures as expressed in LT_{99} mortality values for diapausing larvae of *Trogoderma granarium* is shown in Table 5. When the pressure was decreased to 25 mm Hg and the temperature raised to 35°C, the LT_{99} value was 146 h; at 30°C under the same pressure, it was 172 h. These lengthy exposures are comparable with 6 and 7-day exposures required for phosphine fumigation (Navarro and Donahaye, 1990). These findings may also be compared to those of Calderon and Navarro (1968), on non-diapausing larvae at 25ºC and 65% r.h., where complete mortality was obtained within 120 h exposure to 20 mm Hg. Clearly, not all fumigation treatments are addressed to the control of *T. granarium*. Many commodities may be infested with species less resistant than *T. granarium*.

Table 5- The influence of reduced pressures on exposure time expressed in LT_{99} (hours to obtain 99% mortality) values for *Trogoderma granarium* diapause larvae at three different temperatures.

Temperature (°C)	Pressures in mmHg		
	25	**50**	**100**
25	>360	>360	>360
30	172	260	>360
35	146	153	>360

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 CO_2 treatments also makes use of rigid chambers (Adler *et al*, 2000). These structures are expensive and lack of transportability, which restricts the implementation of environmentally sound methods. Based on the encouraging data herein obtained, in order to render the technology a practical tool, the possibility was recently investigated of using CO_2 or low pressures to control storage insects in a transportable system (Phillips *et al*., 2000). 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 Cube™" or "GrainPro Cocoon®". The pressure was maintained between 25 to 29 mm Hg for 17 days. Bioassay 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.

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