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APPLICATION OF VACUUM IN A TRANSPORTABLE SYSTEM FOR INSECT CONTROL

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ABSTRACT

It was demonstrated that the response of insects to low pressures is temperature and moisture dependent. *Trogoderma granarium* quiescent larvae were considered the most resistant storage insects tested, a 172-h exposure to 25 mm Hg being necessary for their control at 30ºC. To control them within a 145-h exposure time it was necessary to increase the temperature to 35ºC.

The encouraging reports led to the idea of developing a transportable system to render the technology a practical tool for the control of insect pests. Two sets of experiments were carried out using a 15 m^3 capacity plastic container termed the "GrainPro Cocoons™" or "Volcani Cube®". This container is made of a flexible liner and characterized by its transportability. The first test was carried out in Foxboro MA, USA using an oil-lubricated vacuum pump (3 hp) to reduce the pressure to 25-mm Hg within 25 min. Then the pressure was maintained between 25 and 29 mm Hg for 17 days. Three sets of bioassay replicates were retrieved on day 3, 10, and 17 of treatment. Complete mortality of test insects was observed after the 3-days exposure to vacuum. The second test using vacuum was carried out in Israel using a similar set-up for the vacuum pump and the Volcani Cube. The purpose of these tests was to evaluate the technology so as to contribute to improved performance. Vacuum was maintained within 22 and 75 mm Hg for over 25 days.

INTRODUCTION

A large variety of potential alternatives to methyl bromide (MB) for disinfestation of durable commodities have been suggested, though development of these alternatives is likely to be costly. Under present agreements of the Montreal protocol, there are exemptions for all countries from controls on MB when used for quarantine and preshipment fumigations, and for some critical agricultural uses, yet to be defined. However, the limitations on MB production levels are now reflected in rising prices over the past year, and the availability of this fumigant is becoming increasingly restricted. Under the terms of Montreal Protocol (UNEP, 1998) use and production of MB is scheduled to phase out in developed countries by the end of 2004 and worldwide by 2020.

Previous work has shown the effect of low oxygen (O_2) tensions (Navarro, 1978; Navarro and Donahaye, 1985; Navarro and Jay, 1987), and of low atmospheric pressures (Calderon *et al.,* 1966; Calderon and Navarro, 1968; Navarro, 1972), on stored-product insects. It has been reported also that lowering the relative humidity (r.h.) in exposure chambers enhances the lethal effect of low atmospheric pressures on insects (Navarro, 1974). In these cases, low r.h. resulted in desiccation of insects, as expressed in loss of weight reaching a critical level, to which insect mortality was attributed. At very low O_2 tensions, mortality occurred, even when desiccation was prevented by high r.h. (Navarro, 1974, 1975). Navarro and Calderon (1979) revealed the influence of low atmospheric pressures and low O_2 concentrations on *Ephestia cautella* pupae at 26ºC. Effects on respiration, insect mortality and loss in weight obtained were due to low O_2 tension only, at both normal and low atmospheric pressure.

Gas tight flexible structures equipped for quarantine infestation treatment using either modified atmospheres or 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 manufacture 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 Cubes®” or “GrainPro Cocoons™” (Navarro *et al.,* 1999) and have potential for use in small-scale applications, particularly for high-value crops such as cocoa, coffee, and spices.

In this project the authors planned to develop a pesticide-free treatment based on vacuum held in these flexible hermetic storage units. They also proposed that this work be made commercially more attractive by developing and testing a scheme to provide safe, integrated storage and transport for commodities stored in these pesticide-free environments, not only in the countries of origin, but also during transportation, and finally in the countries of destination. This work resulted in a preliminary design of the "PITS" (Pesticide-Free Integrated Transport and Storage) system, for which its inventors, employed by GrainPro, Haogenplast, and ARO, have filed a U.S. patent. Therefore the objective of this work was to provide experimental data to support the experimental vacuum work, using a number of insect species, at varying temperatures and levels of vacuum between 25 mm Hg and 100 mm Hg. The second objective was to demonstrate the feasibility of an integrated methyl bromide-free transportation and storage system termed "PITS".

MATERIALS AND METHODS

1. Laboratory trials

Temperature and low pressure combinations

Temperatures for testing the sensitivity of the insects were 25, 30 and 35ºC in combination with absolute pressures of 25, 50 and 100 mm Hg.

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).

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 saturated solution of sodium nitrite imbibed onto a wad of folded filter paper. Exposure temperatures were 25, 30 and 35ºC. Prior to each test, sets of 50 larvae were confined in cages of 15-mm diameter and 50 mm length made of 100 mesh stainless steel.

Post fumigation procedures

After exposure, larvae were transferred to small jars (50 mL) and maintained at 27±1ºC and 65±5% r.h. The larvae were provided with food. Mortality counts were made after 2 weeks. Mortality was based on those larvae that failed to pupate.

Statistical analysis

Probit analysis (Daum, 1979) of log concentration against mortality of the treatments was carried out. Where a significant probit line was not obtained, the experiments were repeated.

2. Field trial

Background

The field trial was conducted in 1999 in Boston, MA, in collaboration of GrainPro, ARO, Haogenplast, the Organic Commodity Project and the Mesoamerican Development Institute. A GrainPro Cocoon™ (Volcani Cube©) (vacuum cube) of 15 m^3 capacity was equipped with inlet and exhaust ports. The cocoon was loaded with 107 jute bags each weighing 75-kg (total 8,035 kg) of cocoa beans (Figs. 1 and 2). Prior to closure of the cocoon, polypropylene sheets were placed along the stack side to provide a uniform surface beneath the zipper that joins the lower and the upper sections of the cocoon (Fig. 3). A second stack of untreated-bagged cocoa beans was designated as control, and was not contained within a flexible structure.

Bioassay

Insect bioassay receptacles were prepared and placed among the bags inside the flexible storage structure at the start of the experiment (Fig. 4). Bioassay receptacles consisted of ventilated vials and jars containing insect pests that infest cocoa beans: almond moth pupae, *Ephestia cautella;* eggs and pupae of the Indian meal moth; *Plodia interpunctella*; and mixed eggs and larvae of the red flour beetle, *Tribolium castaneum*. A data logger recording temperature and r.h. at 1-hour intervals was placed in the center of each stack. Bioassay receptacles were placed at three locations within each stack, and 3 receptacles were inserted at each location to allow for removal after 3, 10 and 17 days from the start of the test. Survival of these insects was compared with those of insects held in the untreated bagged cocoa beans stack.

Vacuum Treatment

A rotary vane oil-lubricated vacuum pump was used 3 hp Becker model U 4.70, Germany with a suction capacity of 1.274 m^3/min at normal atmospheric pressure, and 0.048 m^3/min at maximum vacuum. A programmable vacuum sensor installed on the cocoon exhaust port cover controlled the pump. The pressure in the cocoon was reduced from 758-mm Hg (normal atmospheric at Foxboro, MA) to 25-mm Hg absolute pressure within 30 min. At 25-mm Hg pressure, the vacuum controller triggers a solenoid valve to open, allowing additional air to pass through a needle valve and into the pump. At 29-mm Hg pressure, the vacuum controller triggers the solenoid valve to close, thus the absolute pressure within the cocoon was maintained throughout the exposure within a range of 25-mm to 29-mm Hg.

Fig. 1. Preparing cocoon for loading with cocoa bean bags.

Fig. 2. Loading cocoon with aid of fork lift.

Fig. 3. Installing bioassay caged insects.

Fig. 4. Zipper support in place on cocoon (vacuum cube)..

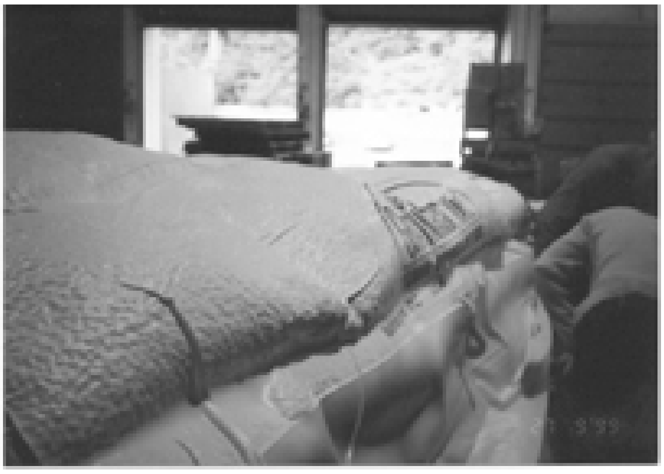

Fig. 5. Cocoon under vacuum.

RESULTS AND DISCUSSION

1. Laboratory trials

The influence of low pressures at different exposure times and temperatures as expressed in LT_{99} mortality values for diapausing larvae of *Trogoderma granarium* larvae is shown in Table 1. When the pressure was decreased to 25 mm Hg and the temperature raised to 35°C, the LT_{99} value was 145 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 (1966), on non-diapausing larvae at 25ºC and 65% r.h., where complete mortality was obtained within 120 h on exposure to 20 mm Hg. Clearly, not all fumigation treatments are required to control *T. granarium.* Many commodities may be infested with species less resistant than *T. granarium.*

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. Its present distribution includes Western Africa through the Northern Indian subcontinent (Cuperus *et. al.,* 1992).

TABLE 1
The influence of reduced pressures on exposure time as expressed in LT_{99} (hours to obtain 99% mortality) values for *Trogoderma granarium* diapause larvae at three different temperatures (figures in brackets indicate fiducial limits at 0.05 significance level)

Temperature	Pressures (mm Hg) / exposure times (h)		
ºC	25	50	100
25	>360	>360	>360
30	172 (101-1205)	261 (166-1889)	>360
35	145 (81-727)	153 (121-212)	>360

2. Field trials

Percentage mortalities of the three stored-product insects shown in Table 2 were derived from the numbers that survived in the treated vials relative to those surviving in the untreated control vials. End-points for scoring survival of treatments were when individuals survived to the next life stage. Thus, when eggs were treated, surviving larvae were counted two weeks later; for pupae, emerging adults were scored. In the case of *Tribolium castaneum*, flour was infested with 10 adult beetles one week prior to the trial. These adults laid eggs up to the time of the treatment, and presumably some of those eggs hatched into larvae prior to the test. Thus, eggs and larvae were treated, and the end-point scored was the total number of adults after 6 weeks minus the original 10 from the starting date. An entry of "NA" means the data are not available, either due to the vials being lost or destroyed, or if there was no survival in the controls. All mean mortality values are based on 2 or 3 replicate vials.

The average temperature for the first three days of treatment, during which nearly all of the mortality occurred, was 21.3ºC, with a high of 23.5ºC, and a low of 19.4ºC. The average relative humidity for this period was 57.5% r.h., with a high of 61.8% r.h., and a low of 47.5% r.h.

The three insect species tested can infest stored cocoa. But, particularly *Ephestia cautella* is a well-known cocoa bean pest. None of the eggs of this pest survived the bioassays (treatment or control), so we could not evaluate effects of the treatments, though we did achieve the results above from treated pupae. The vacuum was nearly 100 percent effective at three days. A second application of two or three days could be timed to attack the red flour beetle, or other resistant pest, when it is at a more vulnerable stage of development. The cube offers protection from moisture loss from cocoa beans during storage. This trial results invite further examination of the effectiveness of the vacuum system application for periods less than 3 days.

TABLE 2

Effects of vacuum (expressed in percent mortality relative to controls) on pupae of *Ephestia cautella,* mixed ages of *Tribolium castaneum,* and eggs and pupae of *Plodia interpunctella* exposed to vacuum in bag stacks of cocoa

Insect species	Life stage	Days exposure	Mortality (%)
Ephestia cautella	Pupae	3	100
		10	NA
		17	100
Tribolium castaneum	Mixed age	3	75.5
		10	99.0
		17	NA
Plodia interpunctella	Eggs	3	100
		10	100
		17	100
	Pupae	3	100
		10	NA
		17	100

The second vacuum trial was carried out in Israel using a similar set up for the vacuum pump and the vacuum cube. The purpose of these tests was to evaluate the technology that would contribute to improved performance. Vacuum was maintained within 22 and 75 mm Hg for over 25 days.

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