APPLICATION OF VACUUM TO SEALED FLEXIBLE CONTAINERS: A VIABLE ALTERNATIVE TO DISINFESTATION OF DURABLE COMMODITIES WITH METHYL BROMIDE

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ABSTRACT

This study forms part of a project aimed at eliminating the need for fumigation with methyl bromide (MB) to control stored product insects through the development of novel, vacuum-hermetic technology. First objective was to study the effects of low air pressures, temperature and exposure time on insect mortality in stored cocoa beans. A second was to study the potential of an innovative, transportable, sealed storage system as a practical tool for controlling insect pests at low air pressures. Two insects, both major pests of cacao beans in producer countries were used: *Ephestia cautella*, and *Tribolium castaneum*. The experiments conducted in the laboratory showed that the eggs were the most resistant stage to storage under a low air pressure of 55 ± 10 mm Hg at the two studied temperatures of 18°C and 30°C. Times needed to obtain 99% mortality of *T. castaneum* eggs at 18°C and 30°C were 96 and 53 hours respectively. For *E. cautella* eggs 99% mortality at 18°C and 30°C was obtained after 149 and 41 hours respectively. Two experiments were carried out in the field trial, each using 15 m^3 capacity plastic containers termed the “GrainPro Cocoon™” or “Volcani Cubes™”, specially adapted to facilitate low pressure ("vacuum cube"). The pressure in the vacuum cube was established within the range of 23 to 75 mm Hg. In one cube, the low pressure was held for 3 days and in the second, for 7 days. In both cubes 100% mortality of all test insects was obtained.

Keywords: vacuum-hermetic technology, cacao, low pressure, *Ephestia cautella, Tribolium castaneum,* storage.

INTRODUCTION

Back and Cotton (1925) were the first to explore the possibility of using low pressures in post-harvest storage, followed by Bare (1948), and later by Calderon *et al.* (1966). However, to achieve the extremely low pressures necessary to obtain insect mortality, a prohibitively expensive investment in massively constructed vacuum chambers was required. The new terms of the Montreal Protocol, ending the use and production of methyl bromide (MB) in developed countries by the year 2005 and worldwide by 2015 (UNEP 1998), prompted us to re-examine the possibilities of using low pressures as a fumigation replacement to control stored-product insect infestations.

In a recent study by Navarro *et al.* (2001), a PVC-based, sealed, flexible storage system was developed to maintain low pressures. In this storage structure, low pressures of 25-30 mm Hg were achieved and maintained continuously for more than two months. These gas-tight, flexible structures were originally developed for long-term hermetic storage of grain, particularly for storage in developing countries, but they were also shown to be suitable for quarantine treatments using either modified atmospheres or the hermetic storage method. They are now in use on a commercial scale in several countries (Navarro *et al.* 1988, 1994; Navarro *et al.* 1990). The structures are made from plastic liners manufactured to specifications that provide a level of gas tightness that precludes significant loss of modified atmosphere or fumigant (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 our field trial we used two of these structures, each of 15 m^3 capacity.

For our study, cacao beans were the chosen commodity and two insect species that are among the major pests of cacao beans: the tropical warehouse moth, *Ephestia cautella* (Walk.), and the red flour beetle, *Tribolium castaneum* (Herbst) were used for bioassay.

MATERIALS AND METHODS

Laboratory trials

Exposure of insects to low air pressure and temperature combinations: The sensitivities of the test insects were tested in chambers consisting of 3-liter desiccators filled with 1 kg cacao beans, and connected to a vacuum pump. The test chambers were held in an incubator kept either at 18°C or 30°C. Air pressures ranged between 45 mm Hg and 65 mm Hg (55 ± 10 mmHg) at a constant relative humidity of 55 ± 3%.

Test insects: Laboratory colonies of the moth *E. cautella* and the beetles *T. castaneum* were maintained in a rearing room at 28 ± 2°C and 70 ± 5% relative humidity.

Bioassay: Insects for the bioassay were chosen as follow: Eggs from each species were used within 0-2 days of oviposition. Larvae of *E. cautella* were 14-15 days old and *T. castaneum* larvae were 18-19 days old. Pupae of *E. cautella* were 1-2 days old and pupae of *T. castaneum* were 0-1 days old. *T. castaneum* adults were 30-31 days old and *E. cautella* adults were 1-2 days old. Each Bioassay contained two Perspex slides each holding 50-drilled "wells" that were used to place 100 eggs individually from each of the studied species, and were then covered with a cover glass to retain the eggs (Navarro and Gonen, 1970). In addition, 50 individuals (larvae, pupae or adults) from each of the studied species were placed in a small glass vial (4 ml), covered with paper or metal mesh and placed in each of the test chambers with the cacao beans.

Post fumigation procedures: Mortality of the test insects was determined as failure to reach the next developmental stage. Eggs of the two species were held in the rearing room for 10 days, after which the hatched larvae and un-hatched eggs were counted. Larvae and pupae were held for 2-3 weeks and observed three times each week. Survival of adult beetles was determined after 15 days, and of *E. cautella* 4 days after exposure, since their life expectancy is only about 4-6 days at 28ºC.

Statistical analysis: Probit analysis of log concentration against mortality of the treatments was carried out (Daum 1979). Where a significant probit line was not obtained, the shortest exposed time needed to achieve 100% was used.

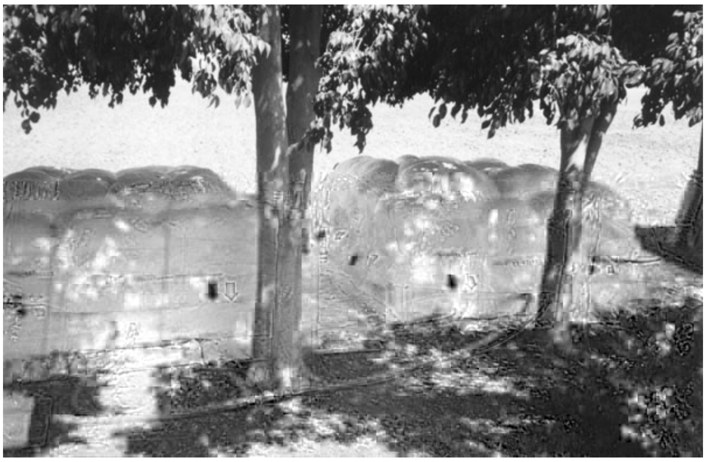
Field trial

Background: The field trial was conducted on March 2001 in Israel, at the Agricultural Research Organization (ARO) campus. Two vacuum cubes of 15 m^3 capacity, adapted to facilitate low pressure, were used. The pressure in the cubes was established using a rotary-vane, oil-lubricated vacuum pump (3 hp Becker model U 4.70, Germany) to within the range of 23 and 75 mm Hg for duration of 3 days in one cube and 7 days in the other. The commodity was cacao beans originating from the Ivory Coast (previously fumigated with methyl bromide). Each cube contained 100 jute bags each weighing 65-kg (total 13,000 kg per cube). Each cube was loaded manually and stacked with six layers of bags (Figure 1).

The vacuum cube system: In order to adapt the standard cubes to low pressure use, a quick-release hose and one-directional valve were incorporated. In addition, the system was connected to the pump using flexible 1.5” connecting tubes. The system was designed to be modular enabling the user to connect several cubes to the same vacuum pump, or to disconnect one of the cubes without changing the pressure in the other connected cubes.

Bioassay: Five sets of bioassay replicates were placed in each of the cubes, each set containing all life stages of *E. cautella* and *T. castaneum*. Four of the bioassay sets were located, one on each side of the four cube walls at mid-center height, and one at the top-center. The control bioassay was placed on the top, above the liner of the 7-day cube in an open plastic container filled with cacao beans. Temperatures at the top and at the four side faces of the cubes were recorded during the trials using data-loggers (HOBO Pro Series).

FIGURE 1: the two cubes (3 days exposures on the right and 7 day exposures on the left) connected together to the pump at the trial site under a pressure of 50 mm Hg.



RESULTS AND DISCUSSION

Laboratory trials

Cacao-beans are stored in burlap backs in the production countries of the tropics, and are subsequently shipped from the tropics to various ports in the Northern Temperate Zone from were they are transported to the processing plants. During the interim, the cacao beans are treated against insect infestation in both climatic zones and are exposed to different ambient conditions. In the tropics the commodity temperature fluctuates at around 30°C, while in the Northern Temperate Zone the commodity temperature can drop to below 20°C. It was therefore deemed necessary to study the influence of low pressures on insect mortality at both these temperature ranges. The exposure periods of low pressure required to control the life stages of *E. cautella,* as expressed in LT_{99} mortality values at 18°C and 30°C are presented in Table 1 and for *T. castaneum* in Table 2.

Table 1: The effect of low pressure (55 ± 10 mm Hg) on mortality as expressed in LT99 (hours to obtain 99% mortality) values for the developmental stages of *Ephestia cautella* at 18°C and 30°C

Developmental Stage	LT_{99}[1] at 30°C (range)	LT_{99}[1] at 18°C (range)
Egg	40.7 (36.16 - 50.34)	148.8 (172.22 - 133.23)
Larva	< 28	43.6 (76.63 - 32.33)
Pupa	< 8	26.2 (139.87 - 17.48)
Adult	< 10	76.7 (180.35 - 54.88)

[1] Numbers in brackets are the 95% confidence limits.

TABLE 2: The effect of low pressure (55 ± 10 mmHg) on mortality as expressed in LT99 (hours to obtain 99% mortality) values for the developmental stages of *Tribolium castaneum* at 18°C and 30°C

Developmental Stage	LT_{99}[1] at 30°C (range)	LT_{99}[1] at 18°C (range)
Egg	53.0 (46.51 - 63.98)	96.3 (139.8 - 73.29)
Larva	< 28	36.8 (58.08 - 28.69)
Pupa	< 38	71.8 (102.70 - 58.53)
Adult	< 28	29.9 (151.67 - 26.62)

[1] Numbers in brackets are the 95% confidence limits.

The results show that for both species, the egg was the most resistant stage at both 18°C and 30°C. For *E. cautella* the time needed to obtain 99% egg mortality at 18°C was 149 hours and at 30°C it was 41 hours. The times needed to obtain 99% mortality of *T. castaneum* eggs were 96 hours and 53 hours at 18°C and 30°C, respectively. The influence of temperature on mortality was more dramatic for *E. cautella,* with a one third reduction in the time needed for control of all life stages at 30ºC. At 18°C, *E. cautella* showed a higher resistance to low pressure then *T. castaneum* while at 30°C this tendency was reversed and *T. castaneum* was more resistant to the treatment at all life stages.

Field trial

Pressure within the two test cubes was regulated at of 23-75 mm Hg for the two time durations. Subsequent bioassays revealed complete mortality within three days of exposure for all life stages of the two insect pest species, *E. cautella* and *T. castaneum.*

The pump required 55 minutes to reduce the pressure in the two cubes to 23mm Hg. The suspension time between pumping was 10 min at the first day of the trial. For the three-day exposure cube, temperature at the top of the cube was 28.0 ± 0.5^0C and the relative humidity stabilized at 65%. At the northern cube-wall face the temperature was 27.9 ± 1^0C and the relative humidity stabilized at 69.5%. For the seven-day exposure cube, the temperature at the top was 27.9 ± 0.5^0C and the relative humidity stabilized at 69.9 ± 0.5% at the top and the northern cube-wall face of the cube.

In conclusion, the low-pressure/vacuum treatment was successful in providing total mortality of the insects pests and in protecting the commodity from re-infestation. Furthermore the cube provided protection for the cocoa beans from loss or increase in moisture during storage. These results indicate that effective control can be obtained in less than three days.

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