# QUARANTINE TREATMENT OF STORAGE INSECT PESTS UNDER VACUUM OR $CO_2$ IN TRANSPORTABLE SYSTEMS

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## ABSTRACT
The objective of our investigation was to identify the combinations that enhance the effectiveness of insect control based on vacuum or $CO_2$ in combination with increased temperatures as quarantine treatment of storage pests. 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* diapausing larvae were most resistant species, whereby 172 h exposure was necessary under the same conditions. Effect of $CO_2$ at 45°C on reducing the exposure time for diapausing larvae of *T. granarium* showed that by increasing the $CO_2$ concentration to 90% the $LT_{99}$ value decreased to about 10 h, whereas at 35ºC the $LT_{99}$ value was 29 h. *Ephestia cautella* larvae were shown to be the most resistant stage to 90% $CO_2$ at 40°C, with an $LT_{99}$ value of only 6 h. For *Oryzaephilus surinamensis* under the same conditions, the $LT_{99}$ value was 9 h for the most resistant egg stage. These encouraging reports 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 Cube™" or "GrainPro Cocoon™". Bioassay in field trials demonstrated that complete mortality of test insects composed of all four developmental stages of *E. cautella* and *Tribolium castaneum* was observed upon 3-days exposure to vacuum.

**Keywords:** quarantine treatment, methyl bromide alternatives, vacuum, $CO_2$, storage insects, transportable systems, *Trogoderma granarium.*

## INTRODUCTION
Although there are a 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) 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 ($PH_3$) fumigations (Navarro and Donahaye, 1990). 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.

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), 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; 1994; Navarro *et al.,* 1990). These structures enable treatment of modified atmosphere or fumigation (Navarro *et al.,* 1995), and they are termed "Volcani Cubes™" or "GrainPro Cocoons™" (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 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 increased temperatures and a constant low pressure or under a $CO_2$ enriched atmosphere, and to report on the application of transportable systems that use these combinations for quarantine treatments.

## MATERIALS AND METHODS

### Temperature, low pressure and $CO_2$ combinations

For low-pressure treatments, absolute pressures of 25, 50 and 100 mm Hg at temperatures varying from 18° to 35ºC were tested. 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.

### Test insects

Diapausing larvae of Khapra beetle *(Trogoderma granarium)* were obtained by holding active larvae 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 reared on standard artificial diet. Eggs (0-2 days old), pupae and adults (1-2 days old) and larvae (4-15 days old) were taken from the same batch. Two Perspex slides each with 50-drilled "wells" were used to individually place 100 eggs from each of the studied species (Navarro and Gonen, 1970). Following treatment, larvae, pupae and adults were transferred to small jars (50 ml) and maintained at 28±1°C and 65±5% r.h. Adults and larvae were provided with food.

### 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.

### Application of the technology

The tested transportable system was made of flexible PVC, which has been in use commercially for hermetic storage of commodities to control insect disinfestation by 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 and bioassay consisted of all four developmental stages of *E. cautella* and *T. castaneum*.

## RESULTS AND DISCUSSION

### Effects of low pressures and increased temperatures

Table 1 shows partial results obtained on three developmental stages of *L. serricorne*. 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. Eggs exposed to 25 mm Hg even at $30^0$C 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. Mortality values for diapausing larvae of *Trogoderma granarium* are 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 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.

### Effects of $CO_2$ and increased temperatures

Table 2 shows the effectiveness of the combination of $CO_2$ at temperatures in the range of 35ºC to 45ºC on *Ephestia cautella.* 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. Results on *O surinamensis* development stages shows that 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. Mortality values for diapausing larvae of *T. granarium* at $45^o$C shows that 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 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. Results shown in Table 2 may serve as guidelines to the possibility of applying increased temperatures for the quarantine treatment of the most resistant diapausing larvae of *T. granarium.*

**TABLE 1: Effects of temperature and low-pressures on $LT_{99}$ (hours to obtain 99% mortality) values for *Lasioderma serricorne* at various development stages and for *Trogoderma granarium* diapausing larvae.**

| Pressure (mmHg) | Temp.(ºC) | *Lasioderma serricorne* | | | *T. granarium* |
|---|---|---|---|---|---|
| | | Eggs | Larvae | Adults | Diapausing larvae |
| 25 | 18 | -[1] | - | 47 | - |
| | 25 | - | - | 26 | >360 |
| | 30 | 75 | - | 15 | 172 |
| | 35 | - | - | | 146 |
| 50 | 18 | - | | 157 | - |
| | 25 | - | 191 | 43 | >360 |
| | 30 | - | 49 | 15 | 260 |
| | 35 | | | | >360 |
| 100 | 18 | 136 | - | - | - |
| | 25 | 75 | - | 75 | >360 |
| | 30 | 40 | - | - | >360 |
| | 35 | - | - | - | >360 |

[1] Data not available

**TABLE 2: Effects of temperature and $CO_2$ concentrations in air expressed in $LT_{99}$ (hours to obtain 99% mortality) values for *Ephestia cautella* and *Oryzaephilus surinamensis* at various development stages and for *Trogoderma granarium* diapausing larvae.**

| Insect species | Temp. (°C) | 30 | | | | 35 | | | | 40 | | | | 45 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $CO_2$ (%) | 60 | 70 | 80 | 90 | 60 | 70 | 80 | 90 | 60 | 70 | 80 | 90 | 60 | 70 | 80 | 90 |
| *Ephestia cautella* | Eggs | -[1] | - | - | - | 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 |
| *Oryzaephilus surinamensis* | Eggs | - | - | 38 | 22 | 29 | 25 | 22 | 9 | 15 | 7 | 6 | 6 | - | - | - | - |
| | Larvae | - | - | - | - | - | - | - | - | 8 | | 2 | 2 | - | - | - | - |
| | Pupae | - | - | - | - | - | - | - | - | - | - | - | 5 | - | - | - | - |
| | Adults | 21 | | 22 | 9 | 26 | 11 | 8 | 4 | 12 | 11 | 6 | 3 | - | - | - | - |
| *T.granarium* | Diapausing larvae | - | - | - | - | 38 | 29 | - | - | 24 | 28 | 20 | - | 15 | 17 | 15 | 10 |

[1] Data not available

Rigid metal chambers have been in use for the implementation of vacuum fumigation in agricultural commodities (Bond, 1984). These structures are expensive and are not easily transportable. 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). Bioassay in field trials at 22$^{o}$ to 25$^{o}$C demonstrated that complete mortality of test insects composed of mixed ages of *E. cautella*, and *T. castaneum* was observed following the 3-days exposure to vacuum. For quarantine treatment of durable commodities, these flexible storage containers can be considered for applying vacuum or $CO_2$ as alternative methods to methyl bromide or other toxic fumigants.


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