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DEVELOPMENT OF A METHYL BROMIDE ALTERNATIVE FOR THE CONTROL OF STORED PRODUCT INSECTS USING A VACUUM TECHNOLOGY

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ABSTRACT

Laboratory studies were carried out on the effect of 50 mm Hg on six important stored-product pests: *Trogoderma granarium*, *Lasioderma serricorne*, *Oryzaephilus surinamensis*, *Tribolium castaneum*, *Ephestia cautella* and *Plodia interpunctella*. At 30°C and a relative humidity of 55% the egg stage was found to be the most resistant stage in all species, the times needed to obtain 99% mortality being 46, 91, 32, 22, 45 and 49 h, respectively. Additional studies indicated that at lower temperatures or at higher relative humidities the times needed to achieve mortality were prolonged. Treatment time must be based on the sensitivity of the most resistant stage of the most resistant species. However, no less important are temperature and the relative humidity in the chamber, both these parameters being determined by the condition of the commodity. Methods of application of low pressures in commercial flexible chambers named GrainPro Cocoons™ previously known as Volcani Cubes™ made of welded PVC liners were studied in semi-commercial pilot plant trials. Commodities tested were; nuts, chick peas, sunflower seeds, semolina, wheat and corn flour, cereals, oatmeal, spices, coffee and cocoa beans. Under vacuum, the chamber wall shrinks over the commodity indicating retention of vacuum. Field studies revealed that an absolute pressure of 50 mm Hg is an achievable practical vacuum level.

INTRODUCTION

A significant development that has occurred over the past 10 years in post-harvest technologies has been caused by the 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 considerable 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.

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 preventing 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 "GrainPro Cocoons™" previously known as Volcani Cubes™ (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).

Previous information on the effect of low pressures on these species is fragmented. Bare (1948) reported on the effect of low pressures on *L. serricorne* at 21ºC within bales of tobacco. A period of approximately 10 days was needed to achieve 100% mortality of all life stages at a pressure of 29 inch (equivalent to 23 mm Hg absolute pressure). Calderon et al. (1966) reported that at 10-12 and 16-20 mm Hg and at 25ºC, an exposure of 3.5 h was required to obtain 99% mortality of *O. surinamensis* adults, while for *T. granarium* larvae 91 h were necessary. At 18ºC, 148 h was required to obtain 99% mortality of *T. granarium* larvae at the same pressure range. At the same temperature of 18ºC but at pressures of 55 mm Hg and 55 % r.h. Finkelman et al. (2003) found that eggs, larvae, pupae and adults of *O. surinamensis* required 77, 37, 128 and 164 h respectively to obtain 99% mortality. In previous field studies using flexible containers it was observed that a pressure of 50 mm Hg could be maintained at reasonable operating conditions of the vacuum pump (Finkelman et al. 2002a). Therefore the pressure chosen in this study is within the range shown to be feasible using the present technology as demonstrated in published field studies (Finkelman et al. 2002a; 2002b). Our previous studies and this one were carried out using cocoa beans as a representative commodity that provides a stable

ambient relative humidity for the insects since humidity is one of the principal factors determining insect sensitivity to low-pressure (Navarro and Calderon 1974; Navarro, 1978).

The objective of this paper is to report on the mortality of the most resistant stages of six stored product insect pests exposed to a constant low pressure to enable the application of low pressure in transportable systems for the environmentally friendly treatment of storage pests.

MATERIALS AND METHODS

Laboratory cultures of the beetles *L. serricorne* and *O. surinamensis* maintained in a rearing room at 28 ± 2°C and 65 ± 5% relative humidity (r.h.), and cultures of *T. granarium* maintained in an incubator at 30 ± 2°C and 60 ± 5% r.h., were used for these experiments.

Eggs of each species were used within 24 h after oviposition. Eggs from *O. surinamensis* were obtained by placing 500-1000 adult beetles in 500 g of wheat flour containing 5 g of brewers' yeast. After 24 h the eggs were separated from the flour and the adults by sieving through U.S. standard sieves of # 25 and # 70 mesh respectively. Eggs from *T. granarium* and *L. serricorne* were obtained by placing 500-1000 adults' beetles on a U.S. standard sieve # 25 without food for 24 h to separate the eggs from the adults.

Ephestia cautella and *Plodia interpunctella* eggs were collected after 24 h from the bottom of 1-L oviposition jars containing 1-3 day old adult moths. Eggs from *Tribolium castaneum* were obtained by placing 500-1000 adult beetles in 500 g of wheat flour containing 5 g of brewers' yeast. After 24 h the eggs were separated from the flour and the adults by sieving through U.S. standard sieves of # 25 and # 70 mesh respectively. For each of the tested species 100 eggs were placed individually into Perspex slides each with 50 drilled wells and were confined in the wells with a glass cover (Navarro and Gonen, 1970).

Treatment chambers consisted of nine 3-L desiccators filled with 1 kg cocoa beans stabilized at an equilibrium relative humidity of 55 ± 3% r.h., typical of in-store humidities. The moisture content of cocoa bean samples was determined by testing their equilibrium r.h. using a water activity-measuring instrument (Defensor® Novasina model ms1, Switzerland). The moisture content of cocoa beans was calculated as 6.3%, using an equilibrium r.h./moisture content conversion table, equivalent to an equilibrium r.h. of cocoa beans at 55 ± 3% (Hall, 1960).

For each species, sets of 100 eggs were exposed by placing two previously prepared Perspex slides inside the treatment chambers. Each treatment chamber was connected to a central laboratory vacuum system that maintained a low pressure of between 45 and 55 mm Hg. If the initial pressure of 50 mm Hg reached 55 mm Hg, vacuum was restored to 45 mm Hg to compensate for leakage of the test chamber. The treatment chambers were placed in an incubator held at 18°C and at 30°C,

together with the control chamber. Mortalities were determined within a range of 1 h to 120 h exposure time. At the end of each exposure time the Perspex slides were removed from the treatment chambers and placed in a rearing room at a constant temperature of 28°C and at 65% r.h. Mortality of the test and control insects was defined as failure to reach the larval stage. Eggs of all the tested species were held in the rearing room for 10 days, after which time the hatched larvae and unhatched eggs were counted. The final numbers of "dead" and "live" insects were subjected to probit analysis described by Daum (1970).

RESULTS AND DISCUSSION

The various sensitivities of the egg stage of the test insects at 18°C and at 30°C are given in Table 1. At 18°C, the times needed to obtain 99% kill of *T. castaneum, O. surinamensis, E. cautella,* being 96.3 h, 76.9 h, 148.8 h, respectively. At 30°C, the times needed to obtain 99% kill of *T. castaneum, O. surinamensis, E. cautella, T. granarium*, *P. interpunctella* and *L. serricorne* being 22 h, 32.4 h, 44.8 h, 46.1 h, 49.0 h, and 91.1 h, respectively.

TABLE 1
The time in hours needed to obtain mortality of the most resistant stage (egg) of six storage insects as expressed in LT_{99} values at low pressure of 50 mm Hg, at 18°C and at 30ºC and 55 ± 3% r.h.

Specie of storage pest	18ºC	30ºC
	LT_{99} (Fiducial limits)*	LT_{99} (Fiducial limits) *
Tribolium castaneum	96.3 (73.29–139.8)	22.2 (16.69-35.96)
Oryzaephilus surinamensis	76.9 (63.84–99.29)	32.4 (25.45-47.50)
Ephestia cautella	148.8 (133.23–172.22)	44.8 (39.27-54.99)
Trogoderma granarium	-	46.1 (33.88-72.25)
Plodia interpunctella	-	49.0 (40.41-63.15)
Lasioderma serricorne	-	91.1 (125.64-72.01)

*Fiducial limits were calculated at $p \leq 0.05$ level.

From the data published on the use of low pressure to control stored-product insect species, the parameters affecting their sensitivities have been clearly defined, namely: partial pressure of oxygen, temperature, and relative humidity as influenced by the moisture content of the commodity (Calderon *et al.* 1966; Finkelman *et. al* 2003, 2004; Mbata *et al.* 2001; Navarro *et al.* 1974; 1978). As with fumigation, treatment schedules must be developed for low-pressure treatments by establishing a database on the relative susceptibilities of different insect species at all their stages that are liable to infest the commodity. However, in contrast to fumigations where schedules are provided by defining dosages to be applied for a predetermined time, at a set temperature range, low pressure treatment schedules must be presented as exposure

times at both a temperature range and relative humidity in equilibrium with the commodity moisture content. Our field studies using low pressures inside flexible liners have clearly demonstrated the many advantages of using this technology for low-pressure treatments (Navarro *et al*. 2001). In the course of these studies it appeared that it is not a practical approach to attempt to hold a pressure of below 45 mm Hg because of the energy required for prolonged operation of the pump. Conversely, pressures above 55 mm Hg prolong the time to achieve kill. Unfortunately most of the early studies do not fall within these parameters and their results cannot be added to the data base initiated in our earlier studies and complemented by the present one.

Results on sensitivities of the six tested species presented in this paper and from published information under similar treatment conditions, on *Tribolium castaneum, Ephestia cautella* and *Plodia interpunctella* (Finkelman *et. al* 2004), show that the egg is the most resistant stage. Comparative sensitivity of the effect of 50 mm Hg, 30°C and 55% r.h. on the egg stage of six storage insects are shown in Table 1. From this table it is clear that the egg stage of *L. serricorne* is the most resistant stage and this is critical where this species is likely to infest the commodity. To summarize: at the present stage we can already provide a tentative treatment schedule for exposure to low pressure treatments to achieve LT_{99} at 18°C and at 30ºC and 55 ± 3% r.h, as presented in Table 1. Although the lethal times given in this table are those for achieving 99% kill of eggs, to achieve probit 9 much longer exposure times are necessary. Probit 9 was suggested as a standard to provide adequate quarantine security for the highest risk commodities. To meet the standard of probit 9, vacuum treatment must kill 99.9968% of the pests in a test of at least 100,000 individual pests (Finney and Tattersfield, 1947). However, the probit 9 standard does not consider the level of infestation, the survival and reproductive capacity of the pest, and the ability of the pest to survive and establish itself. Also no consideration is given to packaging and shipping conditions or to the season of shipment that is particularly relevant for stored product commodities.

For many commodities, a less severe treatment is more appropriate than that of the probit 9 standard. Treatments may range in severity depending on the risk of allowing extended use of low pressures, controlled atmospheres, systems approaches, and other treatments which have not, in the past, been tested to the standard of probit 9 requirements. A less severe treatment would also result in a commercially acceptable commodity protection for the food industry.

The advantage of the approach of applying vacuum is that no toxic chemicals are employed. In comparison with phosphine, exposure times to provide kill are shorter, and fall within a range suitable for quarantine treatments. Where the commodity can be placed in flexible liners, and packed in a manner that can withstand the needed low pressure, this vacuum treatment can provide a good quarantine solution.

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