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Effect of low pressures on the survival of cocoa pests at 18°C

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Abstract

This study forms part of an effort to eliminate the need for fumigation with methyl bromide to control insect infestations in stored cocoa beans, through development of novel alternative vacuum-hermetic technology. In this communication, the effects of low pressures and exposure time were studied on the mortality of insects at a temperature of 18°C, chosen to simulate cocoa bean storage conditions in temperate climates.

Three insect species were used, two of which are major pests of cocoa beans in producer countries, *Ephestia cautella* (Walker), and *Tribolium castaneum* (Herbst), while the third, *Oryzaephilus surinamensis* (L.), is a potential storage pest in temperate climates. For *T. castaneum* and *E. cautella* the egg stage was the most resistant to 55 ± 10 mm Hg at 18°C, the times needed to obtain 99% egg mortality were 96 and 149 h, respectively. For *O. surinamensis*, the adult stage was the most resistant with 164 h being required to obtain 99% mortality.

Keywords: Cocoa; Methyl bromide alternatives; Low pressure; *Ephestia cautella*; *Tribolium castaneum*; *Oryzaephilus surinamensis*

1. Introduction

Cocoa beans are stored in burlap sacks after harvest in producing countries, and are subsequently shipped from the tropics to various ports in the Northern temperate zone from where they are transported to storage sites prior to arrival at the processing plants. During that time, insects that survived earlier chemical treatments or those introduced into the shipments by "cross-infestation" in infested ships or warehouses, are able to build up considerable populations that damage the beans and reduce their market value (Kisiedu and Ntifo, 1975). During a 6 year

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study, Moermans et al. (1998) observed 26 different species infesting cocoa beans, though only five of those recorded, namely *Ahasverus advena* (Waltl), *Carpophilus obsoletus* (Erichson), *Cryptolestes ferrugineus* (Stephens), *Ephestia cautella* (Walker) and *Tribolium castaneum* (Herbst) represented 92% of the total population. In Ghana, the most common insects infesting stored cocoa were *E. cautella*, *Lasioderma serricorne* (F.), *Araecerus fasciculatus* (De Geer) and *T. castaneum* (Moermans et al., 1998). At Norfolk, VA, USA, imported cocoa beans were also infested with many insect species with the most common being *A. advena* and *C. ferrugineus*, *T. castaneum* and *Oryzaephilus mercator* (Fauvel) (Bullington and Pienkowski, 1993).

In order to prevent insect infestations, cocoa beans are treated at three main points in the "pipeline". In countries of origin during the peak season, cocoa beans are stored in sheds at the harvesting locations, and may be fogged with pyrethrum. Later at the ports, every consignment is fumigated with methyl bromide before being shipped (Kisiedu and Ntifo, 1975). At destination ports such as Norfolk, VA, USA, a major port through which cocoa beans are imported into the United States, the beans are stored in warehouses that are subjected to space treatments with dichlorvos vapors from impregnated resin strips or using ultra-low volume application of dichlorvos with a fogger (Bullington and Pienkowski, 1993). In response to the international resolution under the terms of the Montreal Protocol, the use and production of methyl bromide in the developed countries will be phased out by the year 2005 and worldwide by 2015 (United Nations Environment Programme, 1998). Intensive work has been undertaken in various countries (MBAO, 1998, 1999, 2000). In this project we have studied an alternative for treatment of cocoa beans (*Theobroma cacao* L.).

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). Calderon et al. (1966) studied the effect of 10–12 to 16–20 mm Hg on larvae and adults of six stored-products insects at 18°C and 25°C. They reported that *E. cautella* adults were the most susceptible stage, followed by *Oryzaephilus surinamensis* adults, *T. castaneum* adults, *Callosobruchus maculatus* (F.) adults, *T. castaneum* larvae, *Trogoderma granarium* Everts larvae, *Sitophilus oryzae* (L.) adults, *C. maculatus* larvae and *S. oryzae* larvae. All reached 100% mortality after 120 h except *C. maculatus* and *S. oryzae* larvae (Calderon et al., 1966).

To achieve the extremely low pressures for complete mortality of such tolerant species a prohibitively expensive investment in massive vacuum chambers may be required. However, in the first attempt to use low pressures to store cocoa beans, Challot and Vincent (1977) used polyethylene bags to apply and maintain a low pressure of 600 mm Hg in order to preserve cocoa bean quality. Recently, use of a flexible storage facility to maintain low pressures in a PVC based sealed storage system was reported (Navarro et al., 2001). In this structure low pressures of 25–30 mm Hg were achieved to preserve cocoa beans for a storage period of over 2 months.

The objective of this study was to demonstrate the use of low pressure alone as a control procedure for infested cocoa at 18°C. This temperature was selected to reflect local ambient conditions when the cocoa beans are treated using methyl bromide at the import facilities in North America or Europe during the autumn.

For our laboratory trials, three insect species were chosen. Two were among the main pests of cocoa beans: the tropical warehouse moth, *E. cautella*, and the red flour beetle, *T. castaneum.* The third was the saw-toothed grain beetle, *O. surinamensis*, a potential storage pest that may infest cocoa beans in the destination countries.

2. Materials and methods

Laboratory colonies of all three insect species maintained in a rearing room at $28 \pm 2^{\circ}C$ and $65 \pm 5\%$ relative humidity (r.h.) were used for these experiments.

Eggs of each species were used within 0–2 days of oviposition. *E. cautella* eggs were collected from jars containing 1–3 day-old adult moths. Eggs from *T. castaneum* and *O. surinamensis* were obtained by placing 500–1000 adults beetles in 500 g of wheat flour containing 5 g of brewers' yeasts. After 24 h the eggs were separated from the flour and the adults by sieving: for *T. castaneum* using US standard sieves mesh #25 and #70, and for *O. surinamensis* using sieves #35 and #70. Two Perspex slides each with 50-drilled "wells" were used to house 100 eggs individually from each of the studied species; eggs were then enclosed with a cover glass (Navarro and Gonen, 1970).

Larvae of these species were separated from their cultures at days 14–15, 18–19, and 15–16 after hatching for *E. cautella*, *T. castaneum* and *O. surinamensis*, respectively (Table 1). To obtain *E. cautella* pupae, polyethylene transparent tubing 2.0–2.5 mm i.d. was cut into sections 7 mm long and placed in the rearing jar when the larvae begin to wander. Wandering larvae tend to enter and pupate inside these tubes (Navarro and Gonen, 1970). Daily checking of the tubes indicated the exact date of the pupation and 1–2 day-old pupae were collected and placed with the tubes in the glass vials. A daily check of the beetle cultures revealed the first day when pupae appeared, and then 0–1 day-old pupae were removed from their cultures and placed in glass vials.

Test chambers consisted of nine 3-l desiccators filled with 1 kg cocoa beans from the Ivory Coast. The test chambers were placed in an incubator kept at $18^{\circ}C$.

In each test chamber 50 individuals (eggs—2 chambers, larvae, pupae or adults) from each of the species studied were placed in a small glass vial (4 ml), covered with paper and placed in the test chambers with the cocoa beans.

Air pressure in each test chamber was reduced to between 45 and 50 mm Hg using a vacuum pump ("STAG" ST-92, Italy). Pressure was monitored for 3 min to ensure that it was stabilized, and then the chamber was sealed with a screw-type tube-clamp. During the experiments air pressures in the test chambers were examined daily and if a pressure increase was detected the vacuum was restored to 45 mm Hg. If the pressure within a test chamber exceeded 65 mm Hg within 24 h the chamber was rejected. Consequently, low pressures ranged between 45 and 65 mm Hg (55 ± 10 mm Hg). Two additional test chambers with insects were kept at normal atmospheric pressure (760 mm Hg) as controls. Insects were exposed in the test chambers with cocoa beans, which stabilized the r.h. at $55 \pm 3\%$.

Table 1
The age of the various test insects at the time of the treatment

E. cautella	*T. castaneum*	*O. surinamensis*
Eggs, 0–1 day	Eggs, 0–1 day	Eggs, 0–1 day
Last instar larvae, 14–15 day	Last instar larvae, 18–19 day	Last instar larvae, 15–16 day
Pupae, 1–2 day	Pupae, 0–1 day	Pupae, 0–1 day
Adults, 1–2 day	Adults, 30–31 day	Adults, 20–22 day

The exposure times to low pressures were 8, 14, 16, 48, 72, 96, 119, 144, and 168 h, and control chambers were opened at 8, 16, 24, 48, 96, 144, and 168 h. At the end of each treatment, food was added to the containers and the insects were placed in the rearing room for observation. Mortality of the test and control insects was determined as failure to reach the next developmental stage. Eggs of all three species were held in the rearing room for 10 days, after which the hatched larvae

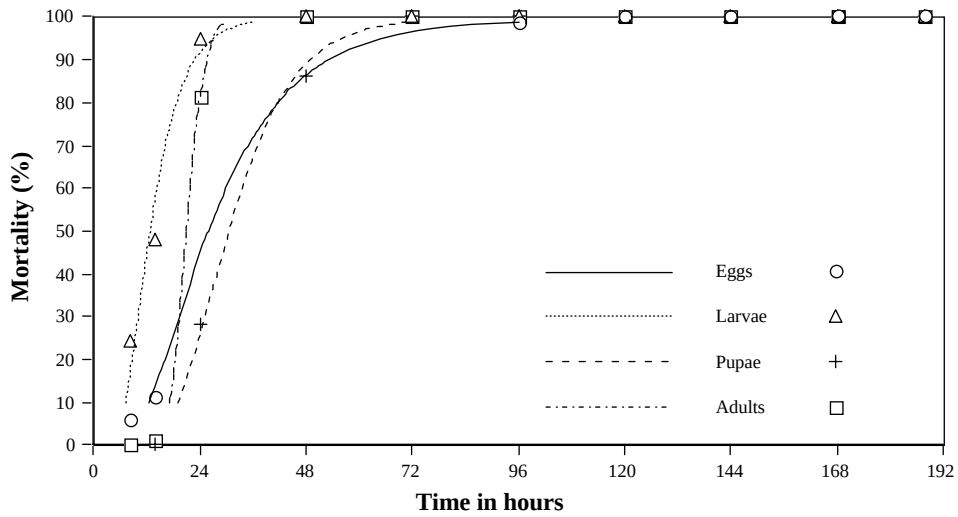

Fig. 1. Experimental results and the calculated lines representing the effect of low pressures (55 ± 10 mm Hg) at various exposure times on mortality (%) of four developmental stages of *T. castaneum* at 18°C and 55% ± 3 r.h.

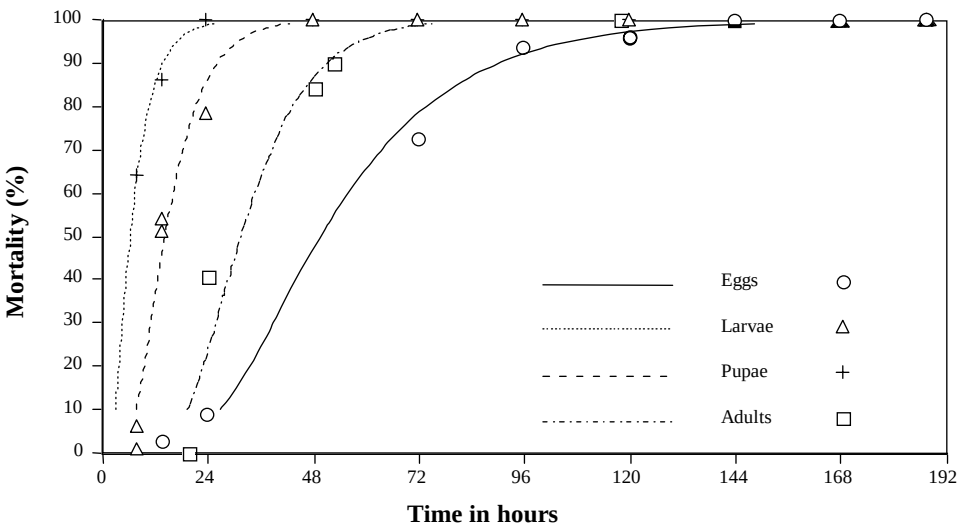

Fig. 2. Experimental results and the calculated lines representing the effect of low pressures (55 ± 10 mm Hg) at various exposure times on mortality (%) of four developmental stages of *E. cautella* at 18°C and 55% ± 3 r.h.

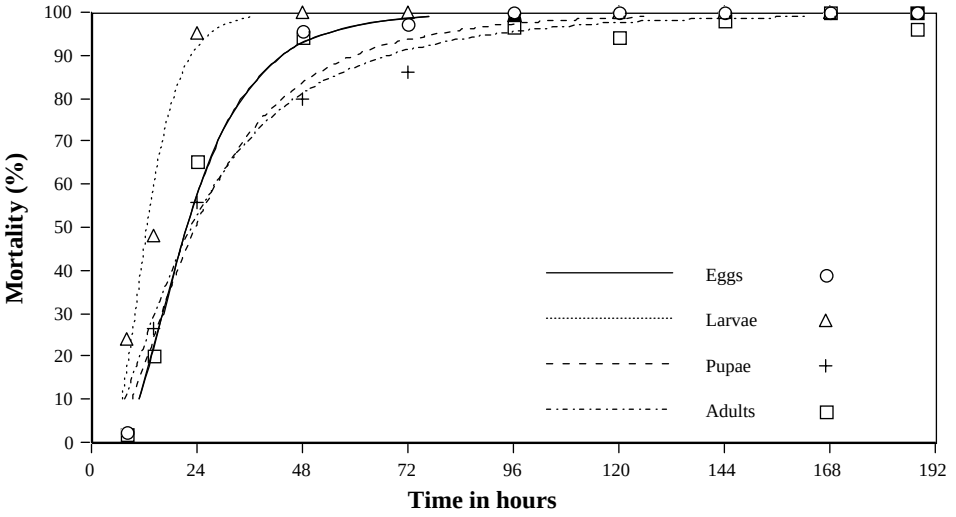

Fig. 3. Experimental results and the calculated lines representing the effect of low pressures (55 ± 10 mm Hg) at various exposure times on mortality (%) of four developmental stages of *O. surinamensis* at 18°C and $55\% \pm 3$ r.h.

and unhatched eggs were counted. Larvae and pupae were held in the rearing room for 2–3 weeks and observed three times each week. Adult survival of beetles was determined after 15 days and *E. cautella* during the fourth day after exposure, since they have a life expectancy of only 4–6 days at rearing room temperature. The final numbers of "dead" and live insects were subjected to probit analysis (Daum, 1970). Actual average mortality data were plotted together with the calculated curves as shown in Figs. 1–3.

3. Results

The various sensitivities of the different life stages of the three test insects are given in Tables 2–4, and Figs. 1–3. For both *T. castaneum* (Table 2) and *E. cautella* (Table 3) the egg stage was the most resistant to low pressure, the times needed to obtain 99% mortality being 96.3 and 148.8 h, respectively. For *T. castaneum* adults were the most susceptible stage with an LT_{99} of 29.9 h (Table 1, Fig. 2), while for *E. cautella* pupae were the most susceptible stage with only 26.2 h being required for 99% mortality (Table 3, Fig. 2).

The response of *O. surinamensis* was different compared to that of *T. castaneum* and *E. cautella*. Adults of *O. surinamensis* are the most tolerant stage, and more then 164.1 h were required to achieve 99% mortality. The pupa also showed a high tolerance and required 128.2 h, while the larva was the most susceptible stage to the treatment with an LD_{99} of 36.8 h.

Differences in diversity of response to treatment among the tested life stages can be revealed from the slopes of the probit lines (Tables 2–4). All of the life stages of *T. castaneum* and *E. cautella*, except for adults of *T. castaneum*, yielded slopes of 4–6, which indicate some heterogeneity within the insect population in response to the treatment. Adults of *T. castaneum*, however, yielded a slope of 15.06 indicating a relatively homogenous response of the population

Table 2
The effect of low pressures (55 ± 10 mm Hg) on mortality as expressed in LT (hours to obtain % of mortality) values for *Tribolium castaneum* developmental stages at 18°C

Developmental stage	Slope	Slope SE	LT_{50} (Fiducial limits)*	LT_{99} (Fiducial limits)*
Eggs	4.07	0.38	25.8 (21.88–30.84)	96.3 (73.29–139.8)
Larvae	5.03	0.82	12.7 (10.74–14.48)	36.8 (28.69–58.08)
Pupae	6.26	1.02	30.5 (25.60–34.93)	71.8 (58.53–102.70)
Adults	15.06	6.73	20.9 (8.28–22.57)	29.9 (26.62–151.67)

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

Table 2

Table 3
The effect of low pressures (55 ± 10 mm Hg) on mortality as expressed in LT (hours to obtain % of mortality) values for *Ephestia cautella* developmental stages at 18°C

Developmental stage	Slope	Slope SE	LT_{50} (Fiducial limits)*	LT_{99} (Fiducial limits)*
Eggs	4.91	0.46	50.0 (42.78–56.11)	148.8 (133.23–172.22)
Larvae	4.89	0.83	14.6 (12.55–16.93)	43.6 (32.33–76.63)
Pupae	3.92	1.28	6.7 (2.77–8.69)	26.2 (17.48–139.87)
Adults	6.09	1.04	31.8 (25.14–40.39)	76.7 (54.88–180.35)

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

Table 3

Table 4
The effect of low pressures (55 ± 10 mm Hg) on mortality as expressed in LT (hours to obtain % of mortality) values for *Oryzaephilus surinamensis* developmental stages at 18°C

Developmental stage	Slope	Slope SE	LT_{50} (Fiducial limits)*	LT_{99} (Fiducial limits)*
Eggs	4.24	0.57	21.7 (15.35–27.29)	76.9 (63.84–99.29)
Larvae	5.03	0.82	12.7 (10.74–14.48)	36.8 (28.69–58.08)
Pupae	3.16	0.61	23.6 (14.44–31.58)	128.2 (87.76–269.14)
Adults	2.71	0.27	22.7 (18.25–27.29)	164.1 (121.06–251.97)

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

Table 4

to the treatment, which within 12 h of the start of insect deaths resulted in 99% mortality (Fig. 1). The probit analysis of adults of *O. surinamensis* gave a slope of 2.71 indicating a greater heterogeneity within the population in response to the treatment. To achieve 90% mortality only 67 h were needed, but in order to obtain 99% mortality an additional 97 h were needed (Fig. 3).

4. Discussion

Cocoa beans are a high-value commodity that forms the raw material of luxury products of the chocolate industry. In today's markets, directed mainly towards more affluent countries, there is an increasing consumer demand that these products be manufactured in an environmentally

friendly manner, and be free of toxic residues. The proposed vacuum-hermetic technology by which the product is held under low pressure in order to eliminate insect infestations as well as providing an after-treatment storage to prevent re-infestation, offers a sound solution to the above demands.

Furthermore, storage under low pressure can prevent oxidization processes within the commodity and consequent loss of aroma and flavor of the beans, as was indicated by the studies of Challot and Vincent (1977). In their laboratory tests they observed total mortality of the test insects, but they failed to report the pressures they used. Their trial was also marred by other constraints such as limited capacity and fragility of the bags under field conditions.

A preliminary study conducted in our laboratory also indicates the effectiveness of vacuum-hermetic technology. Four metric tonnes of cocoa beans packed in 72 burlap sacks were stored in the PVC enclosure (''Volcani cube™'' or ''GrainPro cocoon™'') of 7 m^3 capacity that was maintained for 37 days continuously at a pressure of 38–60 mm Hg. This preliminary test indicated that cocoa beans could be stored commercially under low pressure. Furthermore, moisture content of cocoa beans samples was determined by testing their equilibrium r.h. using a water activity test instrument (Defensor® Novasina model ms1, Switzerland). The moisture content was determined as 6.3%, equivalent to equilibrium r.h. of cocoa beans at $55 \pm 10\%$. This moisture content value was obtained using the equilibrium r.h./moisture content conversion table of Hall (1960).

The results presented in this study show that the storage of cocoa beans for a week under 55 mm Hg at 18°C would eliminate the danger of infestation by the above insect species. It has been shown that mortality of insects under low pressures is caused mainly by the low partial pressure of oxygen resulting in hypoxia (Adler et al., 2000; Navarro and Calderon, 1979) and also dehydration due to removal of water vapor under vacuum (Jay et al., 1971; Navarro, 1978). Therefore, in our tests, this effect of low pressure resulting in low oxygen level is probably the major cause of insect mortality, since the r.h. within the experimental and control chambers was equivalent. Our experiments resulted in a reduced atmospheric partial pressure equivalent to an oxygen content of 1.3–1.8%. This oxygen level is close to the critical level needed for insect disinfestation by displacement of air with nitrogen (Adler et al., 2000; Donahaye, 1992). Little information about the response of insects to low pressure at 18°C is available. Data from Calderon et al. (1966) on *S. oryzae* adults and *T. granarium* larvae at 18°C at a range of pressures from 10 to 20 mm Hg shows the relative resistance of these two species when the equilibrium r.h. was about 66%. Under these conditions, for 99% mortality of *S. oryzae* adults and *T. granarium* larvae exposures of 132 and 145 h, respectively, were required. For the same insects exposed under the same conditions but at 25°C, the 99% mortality exposure was 91.2 h for *S. oryzae* adults and 89 h for *T. granarium* larvae. In contrast *E. cautella* adults were found to be very sensitive, less than 1 h exposure being required to obtain 99% mortality. For *O. surinamensis* and *T. castaneum* adults, 3.5 and 2.7 h, respectively, were required for 99% kill. In our experiments, when insects were exposed to 18°C and 55 ± 10 mm Hg, for *E. cautella*, *O. surinamensis*, and *T. castaneum* adults, exposures of 76.7, 164.1, and 29.9 h, respectively, were necessary to obtain 99% mortality.

Insect responses to low pressure have shown that life stages within a species can vary in susceptibility. The egg stage of *L. serricorne* (Bare, 1948) and *T. variabile* Baillion (Cline and Highland, 1987) was the most tolerant life stage when exposed to low pressures. In the current work, the egg stage of the two insects *E. cautella* and *T. castaneum* was also found to be the most

tolerant stage. In contrast, adults and pupae of *O. surinamensis* were much more tolerant to the treatment than the egg stage.

The slopes of probit mortality lines obtained with *O. surinamensis* showed that the populations of pupae and adults gave a more heterogeneous response to the treatment than those of all the other insect life stages studied. The most homogenous response to the treatment was that of the *T. castaneum* adult population. The heterogeneous response by the pupae and adults of *O. surinamensis* was due to a small portion of the population sample (1–5% of the adults). These results showed that in order to achieve 90% mortality only 67 h were needed, but an additional 97 h were needed to obtain 99% mortality. Cline and Highland (1987) also observed that a small proportion of *T. variabile* adults survived a low pressure exposure, in this case 12 weeks of exposure to 48 mm Hg at 27°C. There is a need for better understanding of the mode of action of low pressure treatment, and more work on factors affecting insect responses will be necessary, such as on the effect of temperature on insect metabolism at low pressures.

A cooperative research and development project is at present being undertaken to develop a novel "vacuum-hermetic" technology in order to eliminate the use of methyl bromide or other pesticides to control insect infestations in high-value commodities. These flexible PVC cubes are capable of serving as storage chambers that can retain low pressure for prolonged periods and can serve as a physical barrier to prevent insect infestation. Low-pressure storage in PVC cubes provides an economically feasible and promising strategy for high-value commodities such as cocoa and coffee beans, at the same time being an environmentally friendly and non-chemical technology.

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