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Effect of low pressures on the survival of three cocoa pests at 30°C

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Abstract

This study forms part of an effort to eliminate the need for methyl bromide fumigation to control insects in stored commodities, through development of a novel “vacuum-hermetic” technology. The effects of low pressures and exposure times on the mortality of insects in stored cocoa beans were studied at a temperature of 30°C in order to simulate cocoa bean storage conditions in tropical climates. Insects were exposed within test chambers containing the cocoa beans at a moisture content in equilibrium with 55% r.h. and at a constant temperature of 30°C. Three species of insects were used, all being major pests of cocoa beans in producer countries: *Ephestia cautella* (Walker), *Plodia interpunctella* (Hübner) and *Tribolium castaneum* (Herbst). At 50 ± 5 mmHg, the egg stage was the most resistant in all three species, times needed to obtain 99% mortality being 45, 49 and 22 h, respectively. Results show that low-pressure treatment can provide an additional and more effective option to the 5 days fumigation with phosphine used today in the replacement of methyl bromide. The use of low pressures allows the control of insect pests at shorter exposure times without the need for toxic chemicals with their environmental impact.

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1. Introduction

The demand by the international community to redress environmental imbalance and to refrain from the use of toxic chemicals is gaining more public attention. This demand was echoed in the decision of the Montreal Protocol to end the use and production of methyl bromide in developed countries by the end of 2004, and worldwide by 2015 ([United Nations Environment Programme](#),

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1998) due to its destructive effect on the ozone layer (Casanova, 2002). In response to this call we have considered and investigated several options as alternative methods to control post-harvest insect pests without the need for toxic chemicals, among them the use of modified atmospheres (Donahaye et al., 1997), hermetic storage (Navarro et al., 1994) and cooling (Navarro and Noyes, 2001). Furthermore, we have re-evaluated the use of low pressures for controlling insect infestations. The objective of this study was to demonstrate the use of low pressure alone as a control procedure for infested commodities and to determine its potential for quarantine uses. In this project we chose cocoa beans (*Theobroma cacao* L.) for study as being representative of a large number of commodities infested by stored-product insects, this commodity typifying many high-value products exported from tropical to temperate climates of the world. In their tropical countries of origin, the cocoa beans may be stored in sheds at the production locations during the peak season, and later at warehouses near the ports. In the Ivory Coast, every consignment is fumigated with methyl bromide or phosphine before being shipped to various ports in the northern temperate zones (Kisiedu and Ntifo, 1975). In the production area, our own temperature recordings of in-store cocoa beans in the Ivory Coast indicated a range of $30^{\circ} \pm 0.3^{\circ}\text{C}$ (Finkelman et al., 2003b), and this temperature was chosen as being typical of the season when the cocoa beans are first disinfested. Although the concept of using low pressures for post-harvest insect control is not new (Back and Cotton, 1925; Bare, 1948; Calderon et al., 1966) and has been recently revived (Finkelman et al., 2002, 2003a), none of these studies investigated the effects of low pressures on insect mortality at 30°C .

For the laboratory trials described here, three major pests of cocoa beans were chosen: the tropical warehouse moth, *Ephestia cautella* (Walker), the Indian meal moth *Plodia interpunctella* (Hübner) and the red flour beetle, *Tribolium castaneum* (Herbst).

2. Materials and methods

Laboratory cultures of the moths *E. cautella* and *P. interpunctella* and the beetle *T. castaneum* maintained in a rearing room at $28 \pm 2^{\circ}\text{C}$ 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. *Ephestia cautella* and *P. interpunctella* eggs were collected after 24 h from the bottom of 1-l oviposition jars containing 1–3-day-old adult moths. Eggs from *T. 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 US 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).

Larvae of each species were separated from their cultures at days 14–15 after hatching for *E. cautella* and *P. interpunctella*, and 18–19 days after hatching for *T. castaneum* (Table 1). To obtain *E. cautella* and *P. interpunctella* pupae, transparent polyethylene tubing 2.0–2.5 mm i.d. was cut into sections 7 mm long and placed in the rearing jar when the larvae were beginning to wander. Wandering larvae tend to enter and pupate inside these tubes (Navarro and Gonen, 1970). Daily checks of the tubes were carried out to determine the exact date of pupation, and 1–2-day-old pupae were collected and placed, within their tubes, in glass exposure vials. Daily checks of the

Table 1
Ages^a of the various test insects at time of treatment

<i>E. cautella</i>	<i>P. interpunctella</i>	<i>T. castaneum</i>
Eggs: 0–1 d	Eggs: 0–1 d	Eggs: 0–1 d
Last instar larva: 14–15 d	Last instar larva: 14–15 d	Last instar larva: 18–19 d
Pupae: 1–2 d	Pupae: 1–2 d	Pupae: 0–1 d
Adults: 1–2 d	Adults: 1–2 d	Adults: 30–31 d

^a Ages were as follows: eggs–days after oviposition; larvae–days after eclosion; pupae–days after pupation; adults–days after emergence.

T. castaneum cultures revealed the exact first day when pupae appeared, and then 0–1-day-old pupae were removed from their cultures and placed in glass exposure vials. Pupae of moths and beetles, were examined daily and emerging adults were removed. Adult moths were exposed to treatment after 0–1 d due to their short adult life, whereas the *T. castaneum* adults were exposed 30–31 d after emergence.

Treatment chambers consisted of nine 3-l desiccators filled with 1 kg cocoa beans from the Ivory Coast which were stabilized at an equilibrium r.h. of $55 \pm 3\%$, 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 was calculated as 6.3%, using an equilibrium r.h./moisture content conversion table (Hall, 1960).

For each species, glass vials of 4 ml capacity, each containing 50 individual larvae or pupae or adults, were placed in each treatment chamber containing the cocoa beans, and food was added to the vials where required. The vials were sealed with plastic caps topped with a fine brass-mesh screen. Sets of 100 eggs were exposed by placing two previously prepared Perspex slides inside the treatment chambers. All test insect stages were exposed together in one test chamber apart from those stages for which it had been determined that complete mortality had already been achieved at the specified exposure period.

Each treatment chamber was connected to a central laboratory vacuum system that maintained a low pressure of between 45 and 55 mmHg. If the initial pressure of 50 mmHg reached 55 mmHg, vacuum was restored to 45 mmHg to compensate for leakage of the test chamber. The treatment chambers were placed in an incubator held at 30°C, together with the control chamber. The exposure times were determined for each developmental stage within a range of 1–62 h. At the end of each exposure period the vials 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 next developmental stage. Eggs of all three species were held in the rearing room for 10 days, after which the hatched larvae and infertile eggs were counted. Larvae and pupae were held in the rearing room for 2–3 weeks and observed 3 times per week. Adult survival of *T. castaneum* was determined after 15 days while for *E. cautella* and *P. interpunctella*, because of their short adult life, mortality was determined 24 h after the end of the exposure period. The final numbers of dead and live insects were subjected to probit analysis as described by Daum (1970). The calculated mortality curves, based on a minimum of 3 degrees of freedom, together with actual mortalities were plotted to facilitate comparisons.

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 *E. cautella* (Table 2), *P. interpunctella* (Table 3) and *T. castaneum* (Table 4) the egg stage was the most resistant to low pressure, the times needed to obtain 99% kill being 44.8, 49.0 and 22.2 h, respectively. For *T. castaneum* the larva was the most susceptible stage with an LT₉₉ of 6.5 h (Table 4; Fig. 3); for *E. cautella* and *P. interpunctella* the adult was the most susceptible stage with only 6.0 h (Table 2; Fig. 1) and 5.3 h (Table 3; Fig. 2), respectively, being the times required for 99% mortality.

Differences in heterogeneity of response among the tested life stages of the populations were revealed by the slopes of the probit lines (Tables 2–4). Larvae, pupae and adults of *T. castaneum*

Table 2

The effect of low pressure (50±5 mmHg) on mortality as expressed in LT values (hours to obtain mortality) for developmental stages of *E. cautella* at 30°C

Developmental stage	Slope	Slope SE	LT ₅₀ (fiducial limits) ^a	LT ₉₉ (fiducial limits) ^a
Eggs	8.18	1.01	23.3(21.70–24.78)	44.8 (39.27–54.99)
Larvae	7.34	1.19	4.9 (4.28–5.65)	10.3 (8.37–14.65)
Pupae	7.79	1.51	3.3 (2.70–3.73)	6.6 (5.359–9.01)
Adults	8.84	2.01	3.3 (2.76–3.59)	6.0 (5.05–8.89)

^a Fiducial limits were calculated at $P \leq 0.05$ level.

Table 3

The effect of low pressure (50±5 mmHg) on mortality as expressed in LT values (hours to obtain mortality) for developmental stages of *P. interpunctella* at 30°C

Developmental stage	Slope	Slope SE	LT ₅₀ (fiducial limits) ^a	LT ₉₉ (fiducial limits) ^a
Eggs	3.91	0.29	12.5 (11.39–13.63)	49.0 (40.41–63.15)
Larvae	7.74	1.20	6.0 (5.17–6.67)	11.9 (10.22–15.49)
Pupae	8.44	1.29	5.2 (4.66–5.64)	9.8 (8.41–12.63)
Adults	8.94	2.42	2.9 (2.24–3.29)	5.3 (4.50–8.65)

^a Fiducial limits were calculated at $P \leq 0.05$ level.

Table 4

The effect of low pressure (50±5 mmHg) on mortality as expressed in LT values (hours to obtain mortality) for developmental stages of *T. castaneum* at 30°C

Developmental Stage	Slope	Slope SE	LT ₅₀ (fiducial limits) ^a	LT ₉₉ (fiducial limits) ^a
Eggs	4.12	0.56	6.0 (5.63–6.49)	22.2 (16.69–35.96)
Larvae	28.56	3.49	5.4 (5.21–5.47)	6.5 (6.23–6.81)
Pupae	11.16	1.44	7.9 (7.28–8.48)	12.8 (11.58–14.87)
Adults	21.97	1.86	5.7 (5.52–5.77)	7.2 (6.94–7.58)

^a Fiducial limits were calculated at $P \leq 0.05$ level.

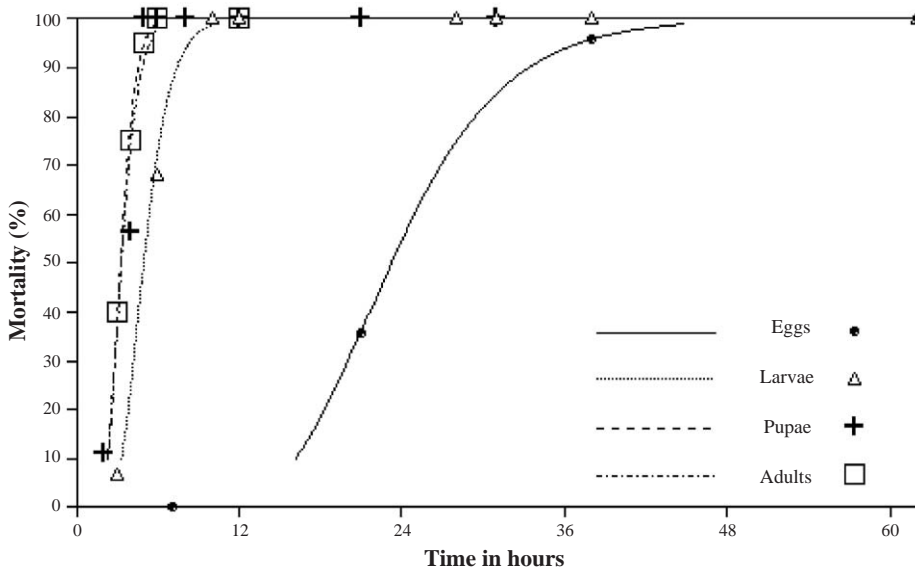


Fig. 1. Experimental results and calculated lines representing the effect of low pressure (50 ± 5 mmHg) at various exposure times on the mortality of four developmental stages of *E. cautella* at 30°C and $55 \pm 3\%$ r.h.

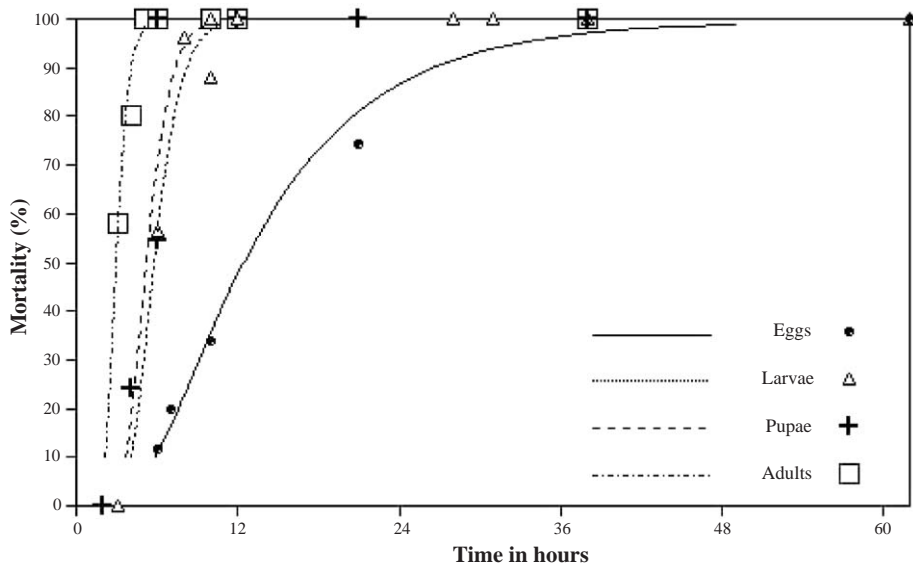


Fig. 2. Experimental results and calculated lines representing the effect of low pressure (50 ± 5 mmHg) at various exposure times on the mortality of four developmental stages of *P. interpunctella* at 30°C and $55 \pm 3\%$ r. h.

had a probit-mortality slope of above 11 (Fig. 3). For the larvae, pupae and adults of *P. interpunctella* and all of the developmental stages of *E. cautella* the slope was between 7 and 9 (Tables 2–3 and Figs. 1–2). The probit-mortality lines for eggs of *P. interpunctella* and *T. castaneum* had slopes of about 4 (Tables 3 and 4 and Figs. 2 and 3).

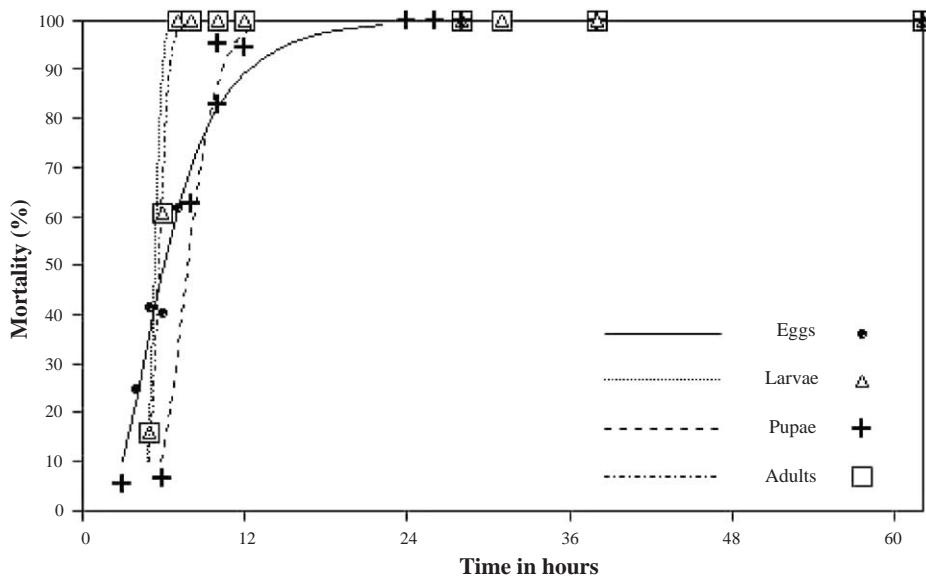


Fig. 3. Experimental results and calculated lines representing the effect of low pressure (50 ± 5 mmHg) at various exposure times on the mortality of four developmental stages of *T. castaneum* at 30°C and $55 \pm 3\%$ r.h.

4. Discussion

Previously, the use of low pressure in post-harvest situations could not be realized since it demanded massive, rigid and expensive treatment chambers. A flexible envelope for commodity preservation of rice, cocoa beans and other durable commodities has now been developed (Challot and Vincent, 1977). This installation was intended for generating a low pressure of 500–700 mmHg for quality preservation but was not sufficient for insect control. Recently a new option of employing flexible PVC storage enclosures termed “Volcani cubes™” or “GrainPro cocoons™”, enabled convenient application of prolonged low-pressures, and has permitted reconsideration of the use of low pressures by enabling application of a pressure as low as 50 mmHg for insect control (Navarro et al., 2001). Should the technical and commercial criteria be satisfied, the use of low pressures in the post-harvest sector could provide effective insect control for quarantine or in-transit storage. Furthermore, storage under low pressure can prevent oxidization processes within stored commodities and the consequent loss of aroma and flavor, as indicated by the studies of Challot and Vincent (1977) for cocoa beans.

The results presented in this study show that storage for 3 days under 50 mmHg at 30°C would eliminate the danger of infestation from the insects under study. The eggs of all three species were the most resistant developmental stage to low pressure, as has been shown previously for *Lasioderma serricorne* (F.) (Bare, 1948) and *Trogoderma variabile* Ballion (Cline and Highland, 1987).

Temperature plays an important role in the exposure time needed to obtain mortality. Finkelman et al. (2002, 2003a) exposed all developmental stages of *E. cautella* and *T. castaneum* to 18°C at 55 ± 10 mmHg and $55 \pm 3\%$ r.h. This is an overlapping range of pressure and the same

humidity with that used in this work. The times needed to achieve LT_{99} for eggs, larvae, pupae and adults of *E. cautella* were 148.8, 43.6, 26.2 and 76.7 h, respectively (Finkelman et al., 2002, 2003a), while in the current tests at 30°C these times were shortened to 44.8, 10.3, 6.6 and 6.0 h. For *T. castaneum* the temperature effect was even more pronounced. At 18°C the times needed to achieve LT_{99} values were 93.3, 36.8, 71.8 and 29.9, respectively (Finkelman, 2002, 2003a), while at 30°C the times were shortened to 22.2, 6.5, 12.8 and 7.2 h. Furthermore, population response to the treatment was also affected by temperature, as demonstrated by probit line slopes. Slopes in mortality curves obtained at 30°C were steeper than those at 18°C under the same experimental conditions (Finkelman et al., 2002, 2003a), indicating that the response of the insect population was more homogenous at higher temperatures for low-pressure treatment at 50 mmHg. At 18°C the slopes for eggs, larvae, pupae and adults of *E. cautella* were approximately 5, 5, 4 and 6, respectively, while at 30°C they were approximately 8, 7, 8 and 9. For *T. castaneum* the effect was even more marked for the larvae, pupae and adults but there was no effect on the eggs. At 18°C the slopes were 4, 5, 6 and 15, respectively, and at 30°C the slopes were 4, 29, 11 and 22 (Finkelman et al., 2002, 2003a).

The only other information available on the effects of low pressure on *E. cautella*, and *T. castaneum* is that provided by Calderon et al. (1966) who reported that at 10–12 and 16–20 mmHg and at 25°C, adults of *E. cautella* were very sensitive, and less than 1 h exposure was required to obtain 99% mortality, while for *T. castaneum* adults 2.7 h were necessary. However, such low pressures are not feasible in practice using flexible plastic liners. Data on the effect of low pressure on *P. interpunctella* are not available in the literature.

Navarro and Calderon (1979) compared the influence of low pressure on *E. cautella* pupae with that of low oxygen concentrations, and deduced that the partial pressure of oxygen has a decisive effect on insect mortality, while no significant function could be attributed to the low pressure itself. At 50 mmHg the partial pressure of oxygen is equivalent to 1.4%, this being similar to the target oxygen concentration under a modified atmosphere obtained by nitrogen flushing.

Today cocoa beans are treated in the Ivory Coast with phosphine for 5 days and this treatment has replaced the conventional methyl bromide fumigation. The option of low pressure therefore is a promising solution that can provide the required insect control in less time and without the need to use potentially harmful chemicals.

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