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PROSPECTS OF LOW PRESSURE FOR USE IN THE DISINFESTATION OF STORED-PRODUCTS

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ABSTRACT

Prospects of low pressure, simulating vacuum, in disinfestation of stored-products were investigated in the laboratory by examining the mortality of developmental stages and adults of three stored-product insects, *Tribolium castaneum* (Herbst), *Plodia interpunctella* (Hubner) and *Rhyzopertha dominica* (F.) exposed to 32.5 mm Hg at 25, 33, 37 and 40°C; and the eggs of *T. castaneum*, *P. interpunctella, R. dominica* and *Ephestia cautella* (Walker) to 50, 75, 100, 200 and 300 mm Hg at 22.5, 30 and 37.5ºC. In preliminary studies, adults of the three species were found to be very susceptible to low pressure and complete mortality was obtained within 3 h exposure to low pressure at 25°C. The adults were therefore excluded from further investigation. Among the species investigated, developmental stages of *R. dominica* were found to be most tolerant to low pressure while developmental stages of *P. interpunctella* and *E. cautella* were the most susceptible. The egg stage was the most tolerant stage while larval stages were most sensitive to low pressure. The egg stage investigated at several low pressures showed that very low pressure (50, 75, and 100 mm Hg) produced mortality earlier than moderate pressures of 200 and 300 mm Hg. Higher temperatures of 30, 37.5 and 40ºC lowered LT_{99} for all the species and their life stages exposed to low pressure and this was most pronounced for developmental stages of *R. dominica* that were tolerant at the lowest exposure temperature of 25ºC. Sensitivity of eggs to low pressure was high when they were freshly laid, and up to a certain time-frame depending on species, and then increased again before hatch.

INTRODUCTION

Some stored-product insects, such as *Sitophilus oryzae* (L.), *Sitophilus granarius* (L.), *Trogoderma granarium* Everts, *Ephestia cautella* (Walker), *Tribolium castaneum* (Herbst), *Lasioderma serricorne* (F.), *Ephestia elutella* (Hubner) and *Callosobruchus maculatus* (F.) have been previously investigated for mortality under low pressure (Bare, 1948; El Nahal, 1953; Calderon and Navarro, 1968; Calderon and Leesch, 1983; Navarro and Donahaye, 1987; 1989; Locatelli and Daolio, 1993).

Most of these studies did not focus on identifying the least exposure periods required to cause complete mortality of insects and in some cases mortality was investigated under one set of pressure, temperature and exposure time. For instance, Bare (1948) recorded the mortality of adults and developmental stages of *E. elutella* and *L. cerricorne* exposed to a pressure of 28.7 in Hg (730 mm Hg) for 24 h, while Cline and Highland (1987) observed mortality of these insects at 48 mm Hg for 1 week at 27ºC.

Some stored-product insects were found to tolerate low pressure and survived long exposure periods. For instance, some *Trogoderma variabile* (Ballion) adults exposed to 48 mm Hg at 27ºC for 12 weeks survived. Higher temperatures may reduce the exposure time needed to produce complete mortality of stored-product insects under low pressure. Most previous investigators exposed the insects to low pressure at temperature range between 20 and 28ºC. There is need therefore to comprehensively investigate the effect of low pressure in combination with temperatures on the mortality of storage insects. This will provide information as to the suitability of low pressure for the management of insect pests in stored commodities.

MATERIALS AND METHODS

The insects used in the present study were raised in cultures in constant temperature chambers maintained 30±0.5°C and 67±9% r.h. *E. cautella,* the almond moth (AM) and *P. interpunctella,* the Indianmeal moth (IMM) were reared on a moth medium (Mbata, 1985) while *T. castaneum*, the red flour beetle (RFB) was reared on unbleached wheat flour containing 3% brewers yeast. *R. dominica*, the lesser grain borer (LGB) was reared on wheat kernels. Developmental stages – eggs, larvae, and pupae of IMM, RFB and LGB were investigated for mortality under low pressure at four temperatures: 25, 33, 37 and 40ºC.

Preparation of IMM and LGB eggs for exposure to low pressure

Eggs of IMM or LGB laid within 24 h were arranged on a strip of double-coated transparent scotch tape, which had one surface attached to one side of a dark filter paper (1 x 3 cm). Twenty eggs were placed on each tape. The strips of filter paper were placed singly in glass vials (4.5 x 1.5 cm), which were subsequently covered with plastic caps that had the center section removed and replaced with muslin screen to allow for ventilation. Ten vials were set up for each species per trial.

Preparation of eggs of RFB

The eggs of RFB were obtained by placing 1,200 adults for 24 h in 200 mL unbleached wheat flour containing 5 mL of brewer's yeast. The adults were sieved off after 24 h and the flour was distributed into vials (4.5 x 1.5 cm), 2 mL in each

vial. The vials were covered with lids screened with muslin. Ten of these vials were used at each set of conditions investigated.

Preparation of larvae and pupae

The larvae and pupae of RFB were obtained from standing cultures. The larvae and pupae were sieved from the rearing medium and kept in separate petri dishes. Young (0-24 h old) pupae characterized by having white pupal skin that has not been darkened by keratin, and last instar larvae, were used for the study. The larvae and pupae of RFB were placed in separate vials, 20 individuals in each vial, containing rearing medium for RFB.

Last instar larvae of IMM were obtained from the cultures and were distributed 5 in each vial (7.5 x 2.5 cm) while the pupae, on corrugated cardboard strips, were placed 10 per vial. Ten vials were set up for each of the moth's developmental stages for each trial.

To obtain last instar larvae and pupae of LGB, cultures were set up with freshly laid eggs placed on wheat kernels in 250 mL jars and kept in a chamber maintained at 30±0.5ºC, 67±2.5% r.h. Wheat kernels were taken from the cultures and x-rayed to determine the exact stages of the developing individuals. The last larval stage was attained between 20 and 22 d while the pupal stage was attained between 24 and 27 d from the egg stage. The X-raying of kernels from cultures kept for the above durations helped in selecting the last instar larvae and pupae of LGB used for this study. Twenty larvae or pupae were placed in each vial (4.5 x 1.5 cm) and ten vials were set up for each batch of conditions.

Exposure of test insects to low pressure

These vials were distributed into 1-L Erlenmeyer flasks. Ten vials for each stage of each of the insects investigated were placed in the flasks. Flasks were prepared for 0, 0.5, 1, 2, 3, 6, 12, 24, 48, 72, 96, 120, and 144 h exposure periods at 25, 33, 37, and 40ºC. The wider outlet of the Erlenmeyer flask was fitted with a pressure gauge while the side outlet was connected to a Fishers Budget Dyna vacuum pump with gauge, *via* thick Fisher Tygon rubber tubing (S-50-HL) with internal diameter of 4.76 mm and wall thickness of 1.59 mm. The gauges used were graduated between 0–760 mm Hg. The air in the flasks was exhausted with the vacuum pump to 32.5 mm Hg. The rubber tubing to the side arm was shut off with a vacuum clip when the desired pressure was attained. The control was set up at normal pressure of 760 mm Hg. Thereafter, the flasks were transferred to control chambers set to desired temperatures. At the expiration of the exposure periods, the clips were loosened to ventilate flasks. Exposed eggs, larvae or pupae were incubated for upward of 48 h before evaluation of survival. The eggs and pupae of IMM were kept for a period of 8 d that is, twice the normal time required for emergence of first instar larvae or adults from eggs or pupae at 30ºC, respectively. Exposed eggs of LGB and RFB were kept for 2 weeks for the emergence of first instar larvae with respect to LGB, or emergence and development into second instar larvae in the case of RFB. The

internally developing larvae or pupae of LGB were incubated for adult emergence. Numbers of dead individuals were noted.

A second set of experiments investigated eggs of AM, IMM, LGB and RFB at 50, 75, 100, 200 and 300 mm Hg, at temperatures of 22.5, 30 and 37.5°C. The exposure times for these studies were 0, 12, 24, 48, 72, 120, and 168 h. Preparation of eggs for exposure to low pressure was as described above.

Age-based sensitivity of eggs to low pressure

Eggs of IMM and LGB were used for this study. Preparation of eggs was the same as described above except for the fact that eggs were collected earlier than they were in previous experiments. Many jars containing adults were set up and the eggs were collected 1 h later for IMM and after 6 h for LGB. The eggs used were aged 12, 24, 48, 96 and 120 h for LGB and 3, 6, 12, 24, 36 and 48 h for IMM.

Statistical Analysis

Data redaction was carried out using Probit analysis to determine exposure periods required to obtain 50 and 99% mortality at each set of conditions of pressure and temperature.

RESULTS

Figures 1-3 give the exposure time in h, required to obtain 99% mortality (LT_{99}) for eggs, larvae and pupae of IMM, RFB and LGB. Among the three species, all the developmental stages of LGB exhibited the most tolerance to low pressure, and required significantly longer exposure periods to obtain 99% mortality than the development stages of other species ($P < 0.05$). The developmental stages of IMM with exception of pupae (Fig. 1) were more tolerant to low pressure than those of RFB (Fig. 2) at low temperature of 25ºC ($P < .05$). The eggs were the most tolerant stage to low pressure and the exposure period required to obtain 99% mortality of eggs of the three species were significantly higher than those for larvae and pupae ($P < 0.05$). The larvae were the most susceptible to low pressure, having in most cases, LT_{99} less than half those for eggs.

Temperature had a tremendous effect on the mortality of all stages of the insects under low pressure (Figs. 1-3). As the temperature of exposure increased, the exposure period required to obtain 99% mortality decreased. Exposure times required to obtain LT_{99} were about 2 and 12 h for all stages of IMM and LGB, respectively at 40ºC. Life stages of species such as the eggs of LGB that required long exposure periods to obtain 99% mortality at 25ºC had their LT_{99} significantly shortened by elevated temperatures (Fig. 3). This was more pronounced than those that had small LT_{99} values at 25ºC, such as larvae of IMM (Fig. 1).

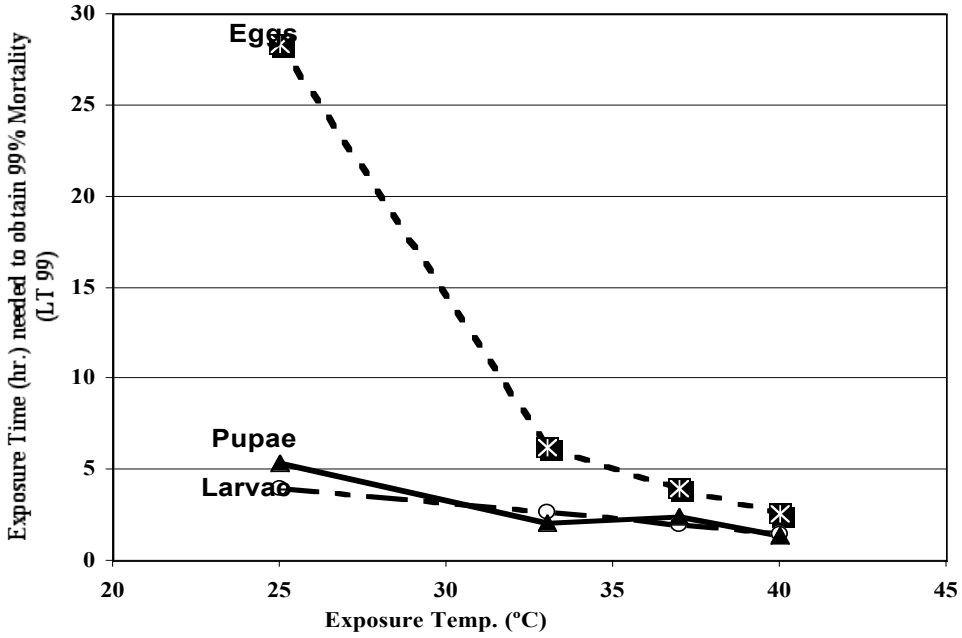

Fig. 1. Exposure time needed to obtain 99 % Mortality of developmental stages of *P. interpunctella* exposed to low pressure of 32.5 mm Hg at different temperatures.

Effect of different low pressures on eggs

The exposure needed to obtain 99% mortality of eggs was shortest at low pressures (50–100 mm Hg) but increased with increase in pressure (Figs. 4–7). Eggs of LGB required the longest exposure periods at all pressures investigated (Fig. 7). High temperatures of 30 and 37.5ºC substantially reduced the exposure time needed to obtain LT_{99}. However, the reduction of lethal time was not significant for AM and IMM when the temperature was raised from 30 to 37.5ºC.

Effect of age of eggs on their sensitivity to low pressure

The sensitivities of eggs of IMM and LGB to low pressure are shown in Figs. 8 and 9. The sensitivities of eggs of IMM and LGB were very high when the eggs were 6-h old or less, and 12-h old or less, respectively. As the eggs aged, their sensitivity to low pressure increased again.

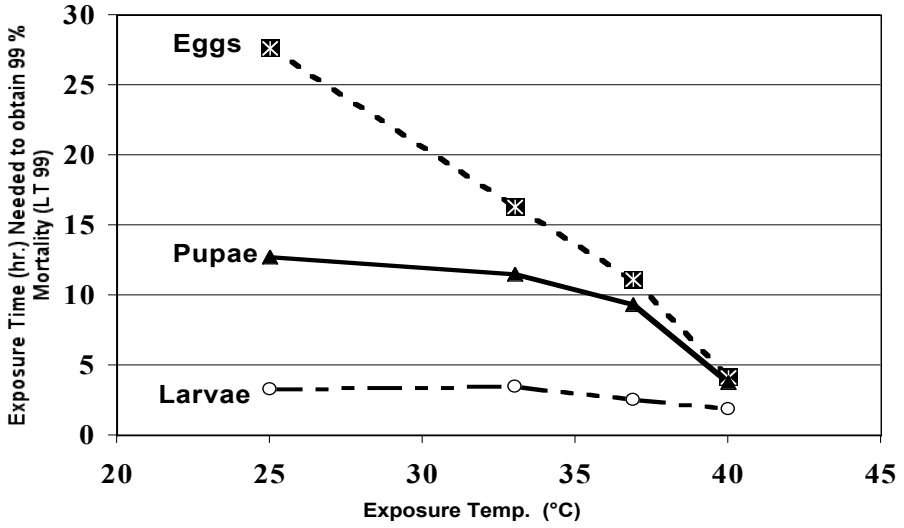

Fig. 2. Exposure time needed to obtain 99 % Mortality of developmental stages of *T. castaneum* exposed to low pressure of 32.5 mm Hg at different temperatures.

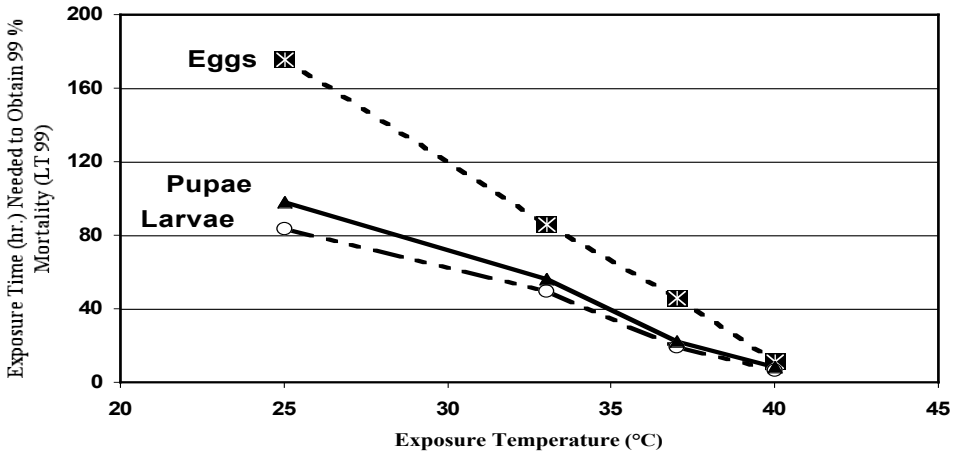

Fig. 3. Exposure time needed to obtain 99 % Mortality of developmental stages of *R. dominica* exposed to low pressure of 32.5 mm Hg at different temperatures.

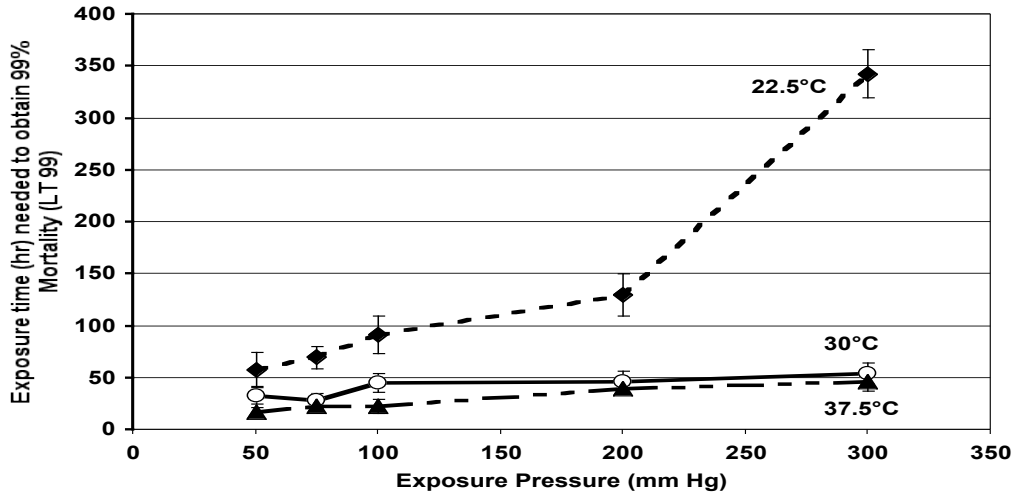

Fig. 4. Exposure time needed to obtain 99 % Mortality of eggs of *P. interpunctella* exposed to different pressures at different temperatures.

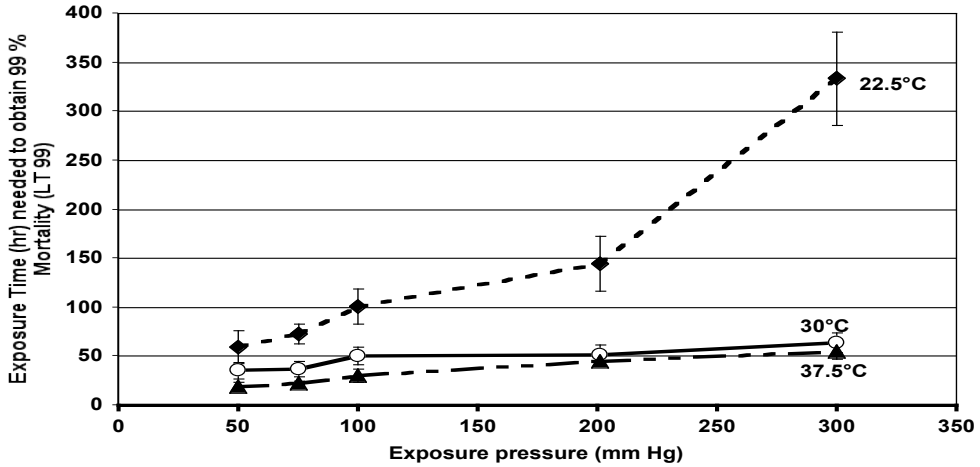

Fig. 5. Exposure time needed to obtain 99 % Mortality of eggs of *E. cautella* exposed to different pressures at different temperatures.

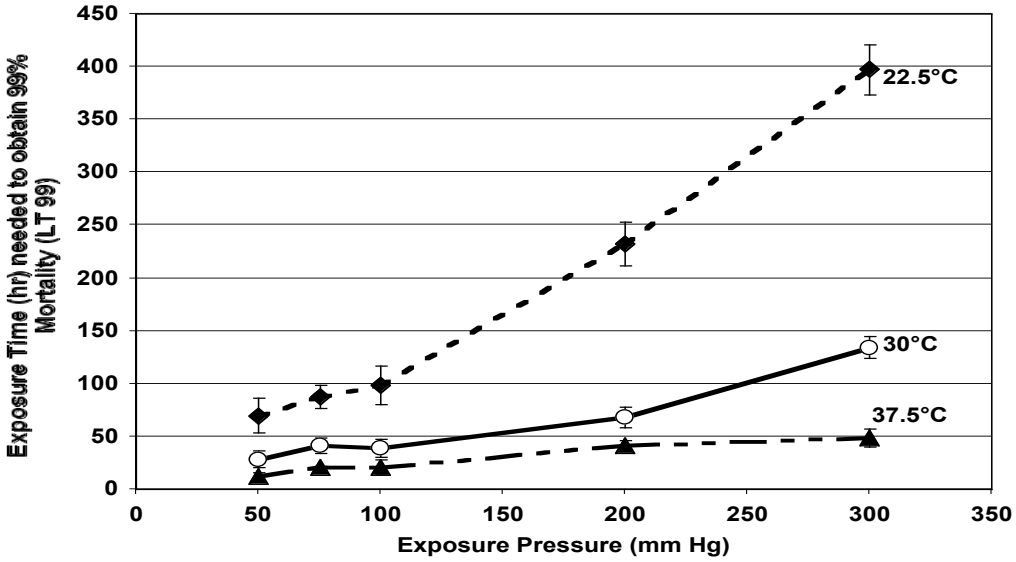

Fig. 6. Exposure time needed to obtain 99 % Mortality of eggs of *T. castaneum* exposed to different pressures at different temperatures.

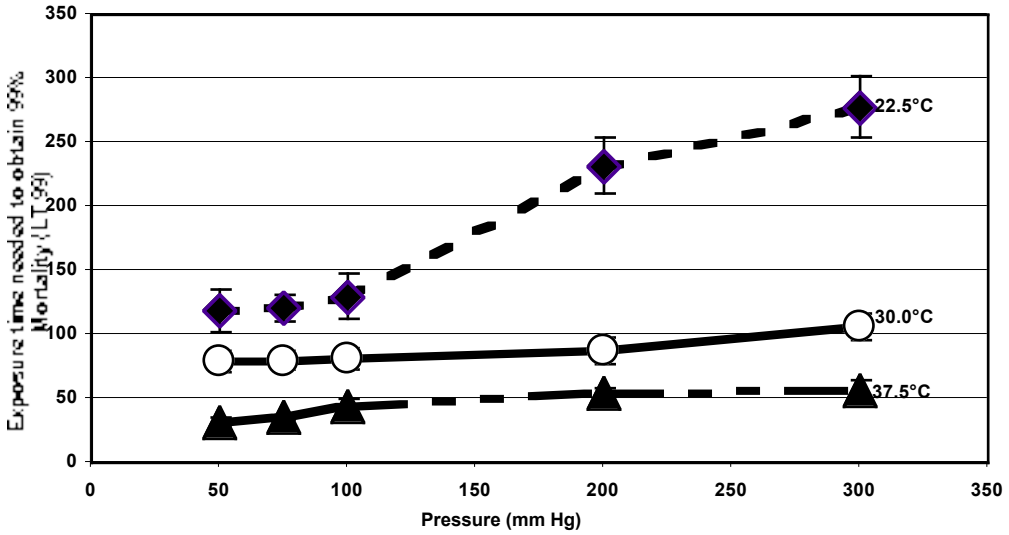

Fig. 7. Exposure time needed to obtain 99 % Mortality of eggs of *R. dominica* exposed to different pressures at different temperatures.

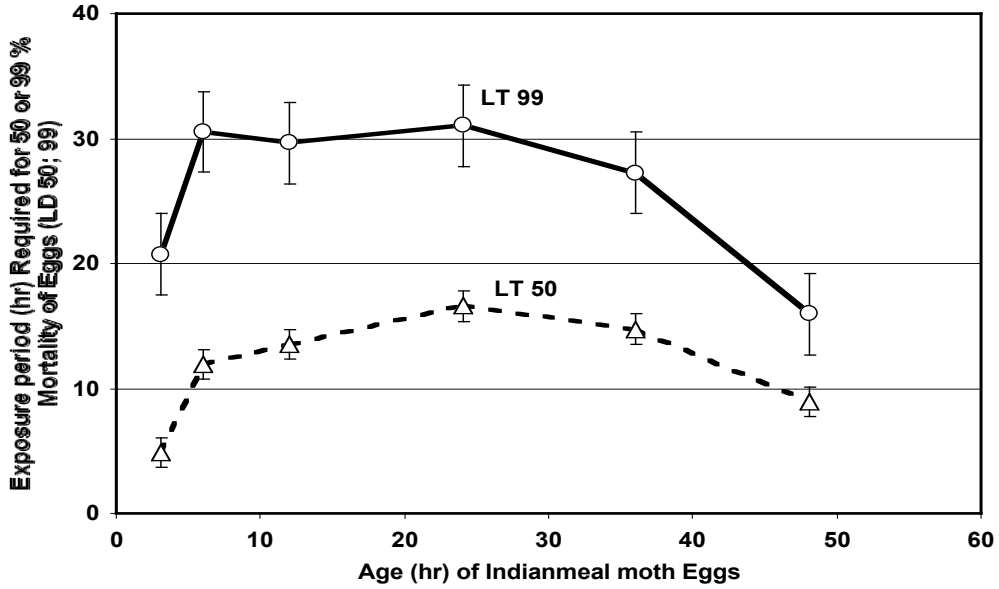

Fig. 8. Effect of age on the sensitivity of eggs of *P. interpunctella* to low pressure of 50mm Hg at 30ºC.

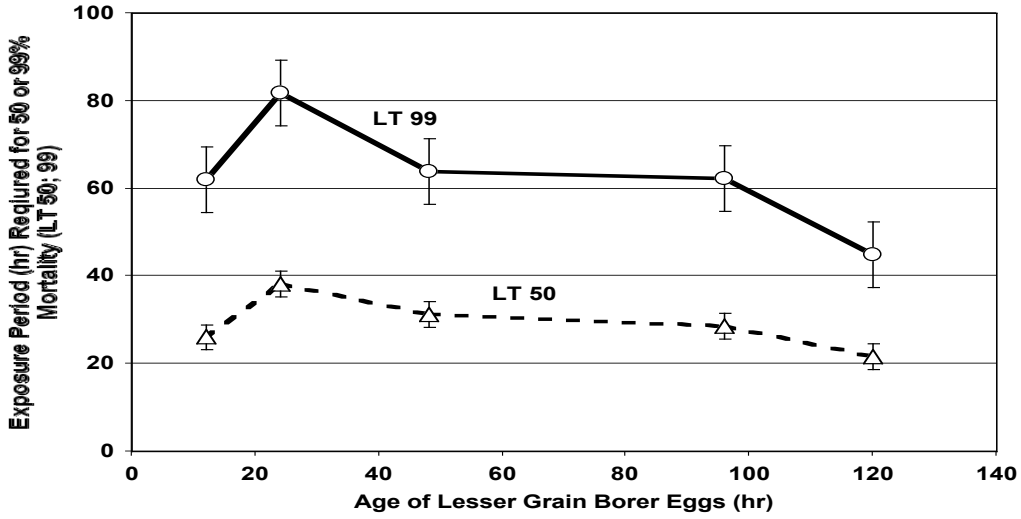

Fig. 9. Effect of age on the sensitivity of eggs of *R. dominica* to low pressure of 50mm Hg at 30ºC.

DISCUSSION

Exposure of developmental stages of *T castaneum*, *P. interpunctella* and *R. dominica* to low pressure of 32.5 mm Hg was able to cause complete mortality of these insects. This has been demonstrated for some other stored-product insects such as *T. castaneum*, *S. oryzae*, *S. granarius*, *L. serricorne*, *E. elutella*, *T. variable* and *C. maculatus* (Locatelli and Daolio, 1993; Navarro and Donahaye, 1987; 1989; Calderon and Navarro, 1968; Calderon and Leesch, 1983; Bare, 1948; and, El Nahal, 1953). However, a comparison of the mortality of several life stages of stored-product insects under low pressure at different temperatures and exposure periods indicates that the insects at their different life stages exhibited different sensitivities to low pressure. For instance, while exposure of *T. castaneum* to 32.5 mm Hg at 25ºC for 27.6 h caused complete mortality of all stages, an exposure period of 176.5 h was required to obtain complete mortality of all developmental stages of *R. dominica*. In a related study, Cline and Highland (1987) found survivals among *T. variable* adults exposed to 48 mm Hg at 27ºC for 12 weeks while one week exposure of *E. cautella* and *L. serricorne* to the same conditions caused complete mortality. Similarly, the larvae were generally the most susceptible developmental stage while the eggs were the most tolerant to low pressure. Bare (1948) also observed greater tolerance of *L. serricorne* eggs than other stages to low pressure. No one has investigated the causes of tolerance to low pressure in insects though it may be related to the number of spiracles and mode of ventilation in these insects and their life stages.

Pressures of 200 and 300 mm Hg required high exposure periods to produce 99% mortality of eggs of *P. interpunctella, E. cautella*, *T. castaneum*, and *R. dominica*. Exposure periods required to obtain 99% mortality was longer than the incubation period of these eggs at moderate pressures of 200 and 300 mm Hg at 22.5ºC. This explains why some eggs hatched at these moderate pressures. This implies that the eggs of these storage insects are able to respire at pressures of 200 and 300 mm Hg where the partial pressure of oxygen is about 62.85 mm Hg.

High temperature shortened the exposure period required to obtain complete mortality of these insects. High temperature is known to increase metabolic rate and may act by increasing the O_2 demand by insect tissue, especially since Navarro and Calderon (1979), observed that insect mortality at low pressure was due to low oxygen tension in the tissues of the exposed insects.

The sensitivities of eggs of *P. interpunctella* and *R. dominica* to low pressure (50 mm Hg) were high when the eggs were 5 and 10 h old respectively, and also when the eggs were close to hatching. The high sensitivity of older eggs to low pressure may be attributed to the high metabolic rate of mature embryos, while that of young eggs may be attributed to highly porous chorion.

ACKNOWLEDGEMENT

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