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Susceptibility of *Plodia interpunctella* (Lepidoptera: Pyralidae) developmental stages to high temperatures used during structural heat treatments

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Abstract

Heating the ambient air of a whole, or a portion of a food-processing facility to 50 to 60°C and maintaining these elevated temperatures for 24 to 36 h, is an old technology, referred to as heat treatment. There is renewed interest in adopting heat treatments around the world as a viable insect control alternative to fumigation with methyl bromide. There is limited published information on responses of the Indian meal moth, *Plodia interpunctella* (Hübner), exposed to elevated temperatures typically used during heat treatments. Time-mortality relationships were determined for eggs, fifth-instars (wandering-phase larvae), pupae, and adults of *P. interpunctella* exposed to five constant temperatures between 44 and 52°C. Mortality of each stage increased with increasing temperature and exposure time. In general, fifth-instars were the most heat-tolerant stage at all temperatures tested. Exposure for a minimum of 34 min at 50°C was required to kill 99% of the fifth-instars. It is proposed that heat treatments aimed at controlling fifth-instars should be able to control all other stages of *P. interpunctella*.

Keywords: heat treatment, heat tolerance, Indian meal moth, *Plodia interpunctella*, methyl bromide alternative

Introduction

The production and use of methyl bromide, a space fumigant, is being phased out in the developing and developed countries since its identification as an ozone-depleting substance (Makhijani & Gurney, 1995; Fields & White, 2002). The use of elevated temperatures or heat treatments for managing stored-product insects associated with food-processing facilities and museums is becoming popular as an alternative method (Fields, 1992; Strang, 1992). Heat treatment of structures involves raising the ambient temperature of food-processing facilities to between 50 and 60°C, and holding these temperatures for at least 24 h (Imholte & Imholte-Tauscher, 1999; Mahroof *et al*., 2003a; Roesli *et al*., 2003). Although heat treatment of structures has been known since the early 1900s (Dean, 1911) very little quantitative data have been collected on the temperature–time mortality relationships of stored-product insects associated with food-processing facilities. An understanding of temperature–time mortality relationship of insects is important for determining minimum temperature–time combinations for killing 99 or 99.9% of a species and identifying the most heat-tolerant developmental stage. Wright *et al*. (2002) reported large larvae (6–7 mm in length) of the warehouse beetle, *Trogoderma variabile* (Ballion), to be the most heat-tolerant developmental stage at 50 to 56°C. Mahroof *et al*.

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(2003b) reported young larvae (0 to 1-day old) of the red flour beetle, *Tribolium castaneum* (Herbst), as the most heat-tolerant stage when compared with eggs, old larvae (22-day-old), pupae, and adults at 50 to 60°C. In the confused flour beetle, *Tribolium confusum* (Jacquelin du Val), however, old larvae (22 to 23-day-old) were found to be more heat tolerant than eggs, young larvae (2 to 3-day-old), pupae, and adults at 46 to 60°C (Boina & Subramanyam, 2004). These studies suggest that the relative susceptibility of various developmental stages to elevated temperatures differs among the species.

There is limited data on the susceptibility of the Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), a serious pest of raw and processed food products worldwide (Sedlacek *et al*., 1996; Doud & Phillips, 2000; Johnson *et al*., 2003; Campbell & Mullen, 2004) to temperatures used during heat treatments. Arbogast (1981) studied effects of 40, 45 and 50°C on the mortality and reproduction of *P. interpunctella* exposed to these temperatures as pupae. Pupae estimated to be within 1 or 2 days of becoming adults were placed in snap cap bottles and exposed for either 2 or 4 h in a hot water bath. All pupae were killed by a 2 h exposure to 45 and 50°C, whereas only 3% of the pupae were killed when exposed to 40°C for 2 h. Lewthwaite *et al*. (1998) did not find differences in susceptibility of 1-, 2- and 3-day-old eggs of *P. interpunctella* at 42, 46 or 48°C, but the time for 99% mortality (LT_{99}) decreased from 10.3 h at 42°C to 59 min at 46°C and 34 min at 48°C.

Except for Arbogast (1981) and Lewthwaite *et al*. (1998), data are lacking on minimum temperature–time requirements necessary to kill eggs, larvae, pupae, and adults of *P. interpunctella*. Therefore, the current study was designed to determine stage-specific susceptibility of *P. interpunctella* to five elevated temperatures experienced by insects during a typical facility heat treatment. The study will ultimately help to determine the minimum temperatures and time periods necessary to kill eggs, fifth-instars, pupae, and adults of *P. interpunctella* during a commercial heat treatment.

Materials and methods

Insect preparation

Cultures of *P. interpunctella* were reared on a poultry-mash diet (Subramanyam & Cutkomp, 1987). These cultures have been maintained in the Department of Grain Science and Industry's Stored-Product Entomology, Research and Education Laboratory since 1999. Eggs (0–1-day-old), fifth-instars (20–21-day-old), unsexed pupae (25-day-old), and unsexed adults (0–1-day-old) were used in experiments. Male and female moths of ≤ 24 h of age were introduced into 940-ml glass Mason jars. Mason jars had lids fitted with a wire mesh screen of 925-μm openings. Jars were inverted over 9-cm glass Petri dishes and placed in an environmental growth chamber (Model I-36 VL, Percival Scientific, Perry, Iowa) at 28°C, 65% relative humidity (RH), and 14:10 h L:D photoperiod (Carrillo *et al*., 2005). Eggs (≤ 24-h-old) that fell onto the Petri dishes through the wire mesh screen were collected daily and were used for maintaining colonies and for use in the experiments. Eggs were added to 250 g poultry-mash diet in a 940-ml glass jar. Jars were maintained in growth chambers at the same conditions as described before. When the larvae in the culture jars were 20–21 days-old, they were collected for experiments. The mean ± SE ($n = 20$) fresh weight of fifth-instars used in experiments was 21.3 ± 0.7 mg. Fifth-instars were used because they are relatively more heat tolerant than other instars (Johnson *et al*., 2003). To collect pupae, a corrugated paper spool was placed above the diet in each jar, to provide pupation sites for wandering-stage larvae. Pupae collected from spools were age-graded based on cuticle pigmentation (Arbogast, 1981), and only those that were tan and hard (25 days old since egg hatch and within 3 days before emergence as pharate adults) were used in the tests. Protective silken cocoons around the pupae were not removed during exposure to the high temperatures because we wanted to simulate natural conditions for pupal exposure. Insect colonies were checked daily and newly emerged moths (0–1-day-old) were used in experiments.

Determination of temperature and diet equilibration time

Insect developmental stages were exposed to high temperatures in square plastic boxes (4.5 × 4.5 × 1.5 cm) fitted with perforated lids (3-cm diameter perforation) for ventilation. Lid perforations were covered with 600-μm wire mesh screens to prevent insect escape. Each box held a mean ± SE ($n = 10$) of 3.3 ± 0.1 g of poultry-mash diet. Two HOBO® H8 RH Temp data-logging units (Onset Computer Corporation, Bourne, Massachusetts; temperature accuracy ± 0.7°C at 21°C) were used to measure actual temperatures inside diet of two of the many plastic boxes placed in growth chambers. The accuracy of the set chamber temperature was verified by comparing readings with mercury thermometers and HOBO® data-loggers. All test boxes were placed on the top shelf within the growth chamber.

The poultry-mash diet, when used in plastic test boxes for experiments, was at room temperature (24–26°C). Therefore, an experiment was carried out to determine the time required (time lag) for the diet and air temperatures inside boxes to equilibrate with the set chamber temperature. Three test boxes, each with 3.3 g of diet, were placed in growth chambers at constant temperatures of 44, 46, 48, 50 and 52°C. The relative humidity at each temperature was 20 to 22%, typical of humidity levels during structural heat treatments of food-processing facilities (Mahroof *et al*., 2003a,b; Roesli *et al*., 2003). Thermocouples of HOBO® data-logging units were inserted into the diet in test boxes to record the time taken for the diet to reach the set chamber temperature. A linear regression model was fitted to these data to describe the relationship between temperature and equilibration time.

The five constant temperatures used in the present tests were slightly different from the temperature ranges used by Mahroof *et al*. (2003b). In the previous experiment, *T. castaneum* developmental stages were exposed to temperatures between 42 and 60°C. In the current study however, preliminary trials showed that *P. interpunctella* developmental stages were relatively more susceptible to high temperature than *T. castaneum* developmental stages. For example, 100% mortality was observed for all *P. interpunctella* developmental stages in less than 20 min at 54°C (data not shown). Therefore, a temperature range of 44 to 52°C was selected, which was above the upper physiological limit for *P. interpunctella* and not conducive for development or

survival. It was also a typical temperature range observed during a commercial heat treatment (Mahroof *et al*., 2003a). A similar range of temperatures (44–52°C) was also used by Johnson *et al*. (2003) for studying the thermal death kinetics of fifth-instar *P. interpunctella*.

Insect exposure

Ten individuals of a *P. interpunctella* developmental stage were transferred to separate plastic boxes. Boxes with insects were exposed in an environmental growth chamber set at one of the five constant temperatures. Descriptions and specifications for the environmental growth chamber were similar to those described by Mahroof *et al*. (2003b). At each temperature, one plastic box with a particular developmental stage was removed at fixed time intervals, after accounting for the time required for both diet and air temperatures to equilibrate with the set chamber temperature. At each temperature, insects were removed for a total of 18 time intervals (18 boxes; 180 individual insects). Each temperature–time combination treatment for a given developmental stage was replicated three times.

There was a separate control treatment that corresponded with each temperature treatment for each developmental stage. The control treatment consisted of boxes with a *P. interpunctella* developmental stage transferred to 3.3 g of poultry-mash diet and kept in a chamber at 28°C, 65% RH, and 14:10 h L:D photoperiod. Boxes in the control treatments were used to determine the natural mortality of insects and were removed twice from the control chamber, once at the beginning and another at the end of the time interval used for those in temperature treatments. Mortality observed at the beginning and end of exposure was averaged and considered as one replication. Similar to temperature treatments, control was replicated three times. A separate study was carried out to determine *P. interpunctella* egg hatchability. Egg hatchability of *P. interpunctella* ranged from 98 to 100%, with a mean ($\pm$SE) of $99.33 \pm 0.67\%$ ($n = 3$). The study proved that natural egg hatchability of *P. interpunctella* is considerably high and therefore will not influence treatment outcomes.

Determining insect mortality

Boxes with adult moths, removed at different time periods from growth chambers, were held for an additional 8 h at room temperature (28°C) and RH (65%) before assessing mortality. Moths that did not move when stimulated with a camel hair brush were considered dead. Mortality of adult moths was based on number dead out of the total exposed. Plastic boxes with treated pupae along with diet were transferred to 450-ml glass jars and maintained in the environmental growth chamber for one week at 28°C, 65% RH and 14:10 h L:D photoperiod until adult emergence. Treated eggs and fifth-instars in each of the plastic boxes were transferred to individual 450-ml glass jars, each containing mean $\pm$ SE ($n = 30$) 20.1 ± 0.1 g of poultry-mash diet. The glass jars were placed in an environmental growth chamber maintained under the same conditions as described for pupae, and held until adult emergence. Mortality of eggs, fifth-instars and pupae was based on those that failed to emerge into adults out of the total exposed.

Data analyses

Control mortality data for each *P. interpunctella* life stage collected in parallel with temperature treatments were subjected to one-way ANOVA by using SAS (SAS Institute, 2000). Before data analysis, control mortality data were transformed using the $\log(X + 1)$ to satisfy the assumptions of normality and homogeneity of variance (Zar, 1984). The time–mortality data of each *P. interpunctella* stage at an elevated temperature were subjected to probit analysis using the complementary log-log (CLL) regression model (Robertson & Preisler, 1992; Mahroof *et al*., 2003b) for estimating the time required to kill 99% (LT_{99}) of the exposed insects. In the CLL model, percentage mortality (y) is transformed to $\log_e(-\log_e[1 - y/100])$ scale, and exposure time (x) is transformed to $\log_{10}$ scale. The goodness-of-fit of the CLL model to the data was compared by using a χ^2 statistic.

In addition, the LT_{99} values of any two *P. interpunctella* developmental stages at each temperature were compared by using lethal time ratios as described by Robertson & Preisler (1992). Lethal time ratios make no assumption about the parallelism of the regression lines being compared; thus, two different lethal time values could be directly compared. At each temperature, there were six pair-wise comparisons among the five stages tested. If the 95% confidence limit (CL) for the ratio includes 1, then the LT_{99} values are not significantly different from one another ($P > 0.05$).

The change in LT_{99} with temperature for each stage was described by using equation 1 (Jandel Scientific, 1994):

$$\ln y = a + bx \quad (1)$$

where y is the LT_{99} in min, x is the temperature in °C between 50 and 60, and, a and b are constants estimated from the LT_{99}-temperature data.

Results

Natural mortality of P. interpunctella

The natural mortality (mean $\pm$ SE) of eggs, fifth-instars, pupae and adults in the control treatment at 28°C was 2.0 ± 1.1, 0.7 ± 0.7, 4.6 ± 1.6, and 0%, respectively. Natural mortality of eggs in control treatments corresponding to the different temperature treatments (44, 46, 48, 50 and 52°C) was not significantly different ($F = 2.00$; df = 4, 10; $P = 0.17$). Natural mortality corresponding to the different temperature treatments for fifth-instars ($F = 1.00$; df = 4, 10; $P = 0.45$), and pupae ($F = 1.80$; df = 4, 10; $P = 0.21$) was also not significantly different. Natural mortality of adults was not subjected to ANOVA because all control mortality was zero. In the overall study natural mortality was $< 7\%$, therefore, mortality of each developmental stage exposed to elevated temperatures was not corrected for control mortality (Robertson & Preisler, 1992).

Temperature measurements and diet equilibration time

Temperatures measured inside the boxes were similar to the set chamber temperatures and mercury temperatures. At the set chamber temperature of 44, 46, 48, 50 and 52°C, the mean $\pm$ SE ($n = 2$) diet temperature inside test boxes was 44.3 ± 0.1, 46.2 ± 0.2, 48.4 ± 0.1, 50.1 ± 0.2, and 52.0 ± 0.3°C,

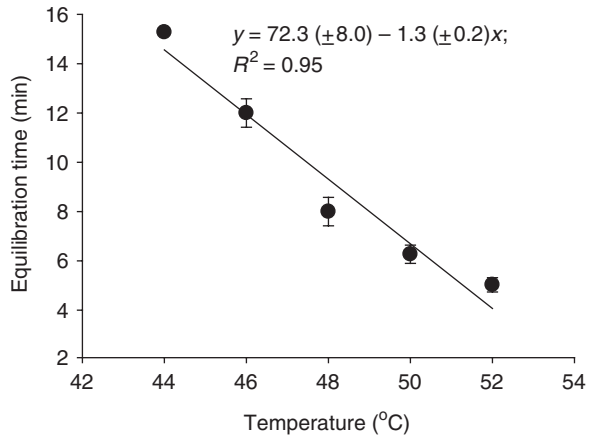

Fig. 1. The equilibration time required for the diet in the test boxes to reach the set chamber temperature at 44 to 52°C.

respectively. This indicated that our HOBO® data-loggers accurately represented the set chamber temperatures. At chamber temperatures of 44, 46, 48, 50 and 58°C, it took 15.3 ± 0.1, 12.0 ± 0.6, 8.0 ± 0.6, 6.3 ± 0.4 and 5.0 ± 0.1 min, respectively, for the diet and air temperatures inside boxes to equilibrate with the set chamber temperatures. The time required for the diet in test boxes to attain the set chamber temperature decreased with an increase in temperature. The linear regression showed that the equilibration time decreased by 62 s for every 1°C rise in temperature between 44 and 52°C (fig. 1).

Responses of P. interpunctella *developmental stages to elevated temperatures*

Mortality of *P. interpunctella* developmental stages increased with an increase in temperature. The mortality of each stage also increased at a given temperature with an increase in exposure time. The χ^2 values were not significant ($P > 0.05$) for 18 of the 20 time–mortality regressions, indicating good fit of data to the CLL model (table 1). However, significant χ^2 values ($P < 0.05$) for the remaining two time–mortality curves indicated that the responses of *P. interpunctella* fifth-instars were heterogeneous. The CLL regression estimates (intercepts, slopes, and LT_{99} values) were inconsistent among the four life stages at each temperature or among the different temperatures for a given stage (table 1). In general, the LT_{99} values decreased with an increase in temperature. The time required for 99% mortality of *P. interpunctella* developmental stages exposed at 44°C ranged from 86 to 314 min, whereas 99% mortality of all developmental stages at 52°C took less than 34 min.

The ratio tests indicated that the LT_{99} value for adults at 44°C was significantly different ($P < 0.05$) from those of eggs, fifth-instars, and pupae (table 2). The LT_{99} value at 44°C was not significantly ($P > 0.05$) different between fifth-instars and eggs, or between fifth-instars and pupae, but the LT_{99} value of eggs and pupae were significantly different ($P < 0.05$) from each other. Of the six comparisons at 46°C, three pair-wise comparisons showed significant differences from each other. The LT_{99} value of fifth-instars was significantly different ($P < 0.05$) than that of eggs or adults, and the LT_{99} value for pupae at 46°C was significantly different ($P < 0.05$) from that of adults. At 48°C, the LT_{99} value of fifth-instars was significantly different ($P < 0.05$) from all other stages. At the same temperature, the LT_{99} values of eggs and pupae were

Table 1. Time-mortality regression estimates for *Plodia interpunctella* developmental stages exposed to five constant temperatures.

Temperature (°C)	Stage[a]	Intercept ± SE	Slope ± SE	LT_{99} (95% CL) (min)[b]	χ^2 (*df*)[c]
44	Eggs	−6.23 ± 0.88	3.52 ± 0.49	161 (124–255)	25.96 (16)
	Fifth-instars	−12.09 ± 2.47	5.45 ± 1.35	314 (187–1379)	15.88 (16)
	Pupae	−6.28 ± 0.84	3.15 ± 0.47	299 (204–595)	11.00 (16)
	Adults	−26.55 ± 2.35	14.53 ± 1.28	86 (82–90)	18.54 (16)
46	Eggs	−8.03 ± 0.76	5.46 ± 0.50	56 (51–64)	19.90 (16)
	Fifth-instars	−12.48 ± 1.22	7.62 ± 0.77	69 (62–80)	20.31 (16)
	Pupae	−8.15 ± 1.00	5.34 ± 0.65	65 (56–82)	24.93 (16)
	Adults	−17.99 ± 1.5	11.31 ± 0.95	53 (50–57)	14.38 (16)
48	Eggs	−5.22 ± 0.58	4.25 ± 0.44	39 (34–46)	15.70 (16)
	Fifth-instars	7.17 ± 0.90	4.81 ± 0.66	64 (51–94)	14.59 (16)
	Pupae	−9.09 ± 0.81	7.00 ± 0.61	33 (31–36)	20.22 (16)
	Adults	−16.16 ± 1.35	12.31 ± 1.01	27 (26–29)	13.65 (16)
50	Eggs	−4.74 ± 0.52	4.29 ± 0.42	29 (26–34)	20.62 (16)
	Fifth-instars	−10.78 ± 1.59	8.07 ± 1.22	34 (29–43)	41.89 (16)*
	Pupae	−6.05 ± 0.61	5.06 ± 0.49	31 (28–36)	17.85 (16)
	Adults	−7.62 ± 0.68	6.65 ± 0.56	24 (22–26)	18.58 (16)
52	Eggs	−6.39 ± 0.53	6.09 ± 0.47	20 (19–22)	16.37 (16)
	Fifth-instars	−6.54 ± 1.34	5.28 ± 1.13	34 (26–67)	71.89 (16)*
	Pupae	−4.74 ± 0.46	4.40 ± 0.40	27 (24–31)	23.49 (16)
	Adults	−9.18 ± 0.79	8.68 ± 0.73	17 (16–19)	3.18 (16)

[a]Total number of insects tested for each stage for a given temperature is 540.
[b]The two decimal points for LT_{99} values were rounded off to the nearest integer.
[c]χ^2 values for goodness-of-fit of regression model to data.
*Significant ($P < 0.05$).

Table 2. Pairwise comparisons of LT_{99} values among *Plodia interpunctella* developmental stages at 44–52°C.

Temperature (°C)	Stages compared	LT_{99} ratio (95% CL)[a]
44	Eggs vs. **fifth-instars**[b]	1.96 (0.84–4.54)
	Eggs vs. **pupae**	1.86 (1.03–3.35)*
	Eggs vs. adults	1.87 (1.37–2.57)*
	Fifth-instars vs. pupae	1.05 (0.42–2.65)
	Fifth-instars vs. adults	3.67 (1.68–8.03)*
	Pupae vs. adults	3.50 (2.12–5.77)*
46	Eggs vs. **fifth-instars**	1.23 (1.03–1.45)*
	Eggs vs. **pupae**	1.15 (0.94–1.41)
	Eggs vs. adults	1.05 (0.93–1.19)
	Fifth-instars vs. pupae	1.07 (0.86–1.32)
	Fifth-instars vs. adults	1.29 (1.13–1.49)*
	Pupae vs. adults	1.22 (1.02–1.46)*
48	Eggs vs. **fifth-instars**	1.66 (1.20–2.29)*
	Eggs vs. pupae	1.18 (0.99–1.39)
	Eggs vs. adults	1.41 (1.21–1.64)*
	Fifth-instars vs. pupae	1.95 (1.44–2.64)*
	Fifth-instars vs. adults	2.94 (2.06–4.20)*
	Pupae vs. adults	1.20 (1.09–1.32)*
50	Eggs vs. **fifth-instars**	1.16 (0.94–1.43)
	Eggs vs. pupae	1.08 (1.05–1.42)*
	Eggs vs. adults	1.22 (0.8–1.4)
	Fifth-instars vs. pupae	1.07 (0.86–1.32)
	Fifth-instars vs. adults	1.42 (1.18–1.71)*
	Pupae vs. adults	1.33 (1.14–1.54)*
52	Eggs vs. **fifth-instars**	1.69 (1.16–2.46)*
	Eggs vs. **pupae**	1.33 (1.13–1.56)*
	Eggs vs. adults	1.17 (1.05–1.31)*
	Fifth-instars vs. pupae	1.27 (0.86–1.88)
	Fifth-instars vs. adults	1.98 (1.36–2.87)*
	Pupae vs. adults	1.55 (1.33–1.82)*

[a]Ratio = larger LT_{99}/smaller LT_{99}. The LT_{99} between the two stages are significantly different ($P < 0.05$) if the ratio does not include 1 (Robertson & Preisler, 1992).
[b]Stage in bold letters has the higher LT_{99} in the pair being compared.
*Significant ($P < 0.05$).

not statistically significant ($P > 0.05$). At 50°C, of the six possible pair-wise comparisons, three were significant ($P < 0.05$). However, when the temperature was increased to 52°C, five out six comparisons were significantly different from each other ($P < 0.05$).

Equation 1 satisfactorily described the decrease in LT_{99} values for each *P. interpunctella* developmental stage as a function of temperature (fig. 2), because the R^2 values for eggs, fifth-instars, pupae, and adults were 0.94, 0.89, 0.92 and 0.98 respectively. Fifth-instars had larger LT_{99} values than other stages at all the temperatures tested. In general, fifth-instars of *P. interpunctella* were relatively more heat tolerant than eggs, pupae, and adults. The observed LT_{99} values for fifth-instars decreased approximately four-fold as the temperature increased from 44 to 46°C. Beyond 46°C, the decrease in LT_{99} was less than one-fold.

Discussion

Facility heat treatments are distinctly different from heat treatments for fresh commodities. In heat treatments for

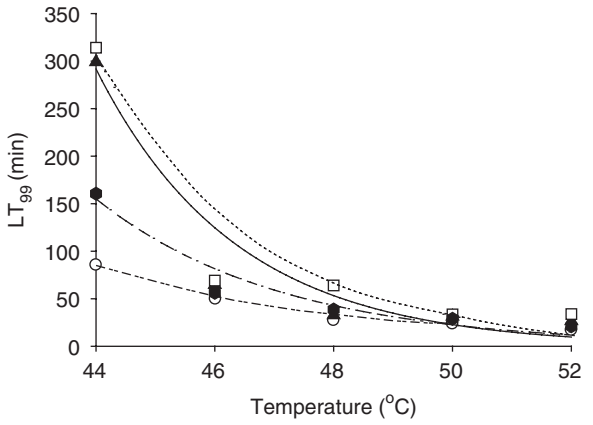

Fig. 2. Observed and fitted lines (equation 1) describing LT_{99} of *Plodia interpunctella* developmental stages as a function of temperature. Regression equations for the stages are: eggs (---), $\ln y = 19.19 - 0.32x$; fifth-instars (· · ·), $\ln y = 22.33 - 0.38x$; pupae (—), $\ln y = 24.38 - 0.43x$; and adults (- · · -), $\ln y = 14.38 - 0.23x$.

fresh commodities, products are exposed to about 60 to 85°C for several minutes with a heating rate that can range from 10 to 15°C min^{-1} (Dermott & Evans, 1978; Evans, 1981; Wang *et al*., 2002). In facility heat treatments, gas, electric or steam heaters are used to slowly heat the ambient air. Typical heating rates observed during facility heat treatments range from 0.3 to 13.7°C h^{-1} (Mahroof *et al*., 2003a; Roesli *et al*., 2003). The 24 to 36 h duration for the heat treatment is necessary for the heat to penetrate wall voids and pieces of equipment to kill insects harbouring in them. The work reported here mainly focused on killing *P. interpunctella* harbouring in cracks and crevices of the facility or inside equipment.

Johnson *et al*. (2003) used a heating block system to determine the mortality of fifth-instar *P. interpunctella*. The LT_{99} values for fifth instars calculated based on a 0.5th order kinetic model at 44, 46, 48, 50 and 52°C were 121.4, 27.5, 8.8, 2.5 and 1.0 min, respectively. In the present study, fifth-instar exposures at 44, 46, 48, 50 and 52°C resulted in LT_{99} values of 314.1, 69.1, 63.9, 33.5 and 33.8 min, respectively. Differences in LT_{99} values between these two studies may be due to differences in heating conditions (heating block system vs. environmental growth chamber), heating rates, or presence or absence of medium for the exposed insects. Johnson *et al*. (2003) used a rapid heating rate of 18°C min^{-1}, whereas we exposed *P. interpunctella* developmental stages to constant elevated temperatures at variable and slow heating rates (≈1.5°C min^{-1} at 44°C and 3.2°C min^{-1} at 52°C). In addition, Johnson *et al*. (2003) did not use any medium to expose fifth-instars. Insects were placed directly in the heated block chamber. In the present study, insects were transferred to plastic boxes containing diet before placing them in a heated growth chamber. We have also taken into account the diet equilibration time when determining time–mortality relationships for various *P. interpunctella* developmental stages. The variation in the equilibration time, if unaccounted, could lead to errors in accurately estimating time–mortality relationships of insects exposed to elevated temperatures. One or many of these explained variations may be accountable for the differences

in LT_{99} values observed in the present study and Johnson *et al*. (2003).

Lewthwaite *et al*. (1998) reported a time period of 34 min to kill 99% of 1 to 3 day-old *P. interpunctella* eggs at 48°C, which was similar to the LT_{99} value reported in our study for eggs at 48°C (38 min, 95% of confidence limit 34–46 min). In a related study, Arbogast (1981) observed that the LT_{50} value for both male and female pupae was approximately 22 min at 45°C. In our study, the LT_{50} value for pupae exposed at 46°C was 28 min (95% CL of 25–30 min). Slightly larger values in LT_{50} values in the present study may be attributed to additional protection provided by the silken cocoon. The pupal cocoon was not removed before exposing insects to elevated temperatures. We treated pupae with their cocoons to simulate natural conditions in which *P. interpunctella* pupae are found within their cocoons in cracks and crevices of food-processing facilities.

In all elevated temperatures tested for all developmental stages, adults of *P. interpunctella* seemed to be the least heat-tolerant developmental stage. Fifth-instars had larger LT_{99} values than those of other stages at all the temperatures tested. Thus, it seems that fifth-instars of *P. interpunctella* were the most heat-tolerant stage. Johnson *et al*. (2003) also reported that the fifth-instars of *P. interpunctella* were the most heat tolerant stage. But fifth-instars (20 to 21-day-old) of *P. interpunctella* are more susceptible than heat tolerant stages of beetles. For example to achieve 99% mortality at 50°C, young larvae of *T. castaneum* (0–1-day-old) required an exposure time of 433 min (Mahroof *et al*., 2003b) and old larvae of *T. confusum* (22–23-day-old) required an exposure time of 90 min (Boina & Subramanyam, 2004) as opposed to the 34 min for *P. interpunctella* fifth-instars. Heat tolerance, or the ability to withstand elevated temperatures in insects varies among different species (Hallman & Denlinger, 1999), within a species among developmental stages (Wright *et al*., 2002; Mahroof *et al*., 2003b; Boina & Subramanyam, 2004), and within a developmental stage among different ages (Mahroof *et al*., 2003b; Boina & Subramanyam, 2004). Comparisons made among available data on *T. variabile*, *T. castaneum*, *T. confusum*, and *P. interpunctella*, indicated that *P. interpunctella* is the most susceptible of the four species to elevated temperatures. All other species compared being beetles and *P. interpunctella* being a moth also may have contributed to the variation in susceptibility. The susceptibility of *P. interpunctella* to elevated temperatures during actual facility heat treatment was also inferred by Roesli *et al*. (2003). Trap catches of *P. interpunctella* adults before and after heat treatment in a pilot feed mill showed that *P. interpunctella* population rebound after heat treatment was significantly reduced. Following the heat treatment, *P. interpunctella* were caught in traps after a month, and these catches were mainly due to the re-introduction of this species through infested raw ingredients. However, in the same feed mill *T. castaneum* were caught in traps relatively earlier than *P. interpunctella*.

In summary, the present study shows that heat treatments aimed at controlling fifth-instars of *P. interpunctella* should be able to control all other developmental stages. We recommend the use of fifth-instars as test insects to gauge the effectiveness of heat treatments against *P. interpunctella* in food-processing facilities. The information presented in this paper provides a quantitative basis for successful use of heat treatments to manage *P. interpunctella* in food-processing facilities.

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