Susceptibility of *Lasioderma serricorne* (Coleoptera: Anobiidae) Life Stages to Elevated Temperatures Used During Structural Heat Treatments

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ABSTRACT Heat treatment of food-processing facilities involves using elevated temperatures (50–60°C for 24–36 h) for management of stored-product insects. Heat treatment is a viable alternative to the fumigant methyl bromide, which is phased out in the United States as of 2005 because of its adverse effects on the stratospheric ozone. Very little is known about responses of the cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae), a pest associated with food-processing facilities, to elevated temperatures. Responses of *L. serricorne* life stages to elevated temperatures were evaluated to identify the most heat-tolerant stage. Exposure of eggs, young larvae, old larvae, and adults during heat treatment of a food-processing facility did not clearly show a life stage to be heat tolerant. In the laboratory, exposure of eggs, young larvae, old larvae, pupae, and adults at fixed times to 46, 50, and 54°C and 22% RH indicated eggs to be the most heat-tolerant stage. Time–mortality responses at each of these three temperatures showed that the time for 99% mortality (LT_{99}) based on egg hatchability and egg-to-adult emergence was not significantly different from one another at each temperature. Egg hatchability alone can be used to determine susceptibility to elevated temperatures between 46 and 54°C. The LT_{99} based on egg hatchability and egg-to-adult emergence at 46°C was 605 and 598 min, respectively, and it decreased to 190 and 166 min at 50°C and 39 and 38 min at 54°C. An exponential decay equation best described LT_{99} as a function of temperature for pooled data based on egg hatchability and egg-to-adult emergence. Our results suggest that during structural heat treatments eggs should be used in bioassays for gauging heat treatment effectiveness, because treatments aimed at controlling eggs should be able to control all other *L. serricorne* life stages.

KEY WORDS methyl bromide alternative, heat treatment, heat tolerance

The use of elevated temperatures, also termed heat treatment, has long been documented as an effective approach for managing stored-product insects infesting food-processing facilities (Dean 1911, Fields and White 2002). It is becoming popular as a methyl bromide alternative because of the 2005 phase out of methyl bromide in the United States (Makhijani and Gurney 1995, Dosland et al. 2006). The mechanism of heat treatment is to raise the ambient temperature of the entire facility, or a portion of it, to 50–60°C, and hold these elevated temperatures for 24–36 h to facilitate heat distribution throughout the entire space of the facility for effective disinfestation (Mahroof et al. 2003a). Limited quantitative data are available on relative susceptibility of life stages and time–mortality relationships for the cigarette beetle, *Lasioderma sericorne* (F.) (Coleoptera: Anobiidae), an important pest associated with food-processing facilities (Sinha and Watters 1985), exposed to elevated temperatures. Conyers and Collins (2006) determined time–mortality responses of eggs and fourth instars of *L. serricorne* at 45 and 50°C and used both these stages because past literature has suggested these two stages to be heat-tolerant (Powell 1931). Therefore, this study was designed to determine responses of eggs, young larvae, old larvae, pupae, and adults of *L. serricorne* when exposed to three constant elevated temperatures within the range observed during facility heat treatments. Our objectives were to determine the relative susceptibility of *L. serricorne* life stages, develop time–mortality relationships, and compare lethal times required to kill 99% of most heat-tolerant life stage of *L. serricorne* with similar stages of other species exposed to elevated temperatures. Understanding relative heat susceptibility of insect life stages to elevated temperatures is important for identifying the most heat-tolerant life stage (Mahroof et al. 2003b). Heat treatments should target the most heat-tolerant life stage because this should ensure control of all other stages (Fields 1992). Furthermore, time–mortality responses

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data at constant temperatures can be used to develop dynamic thermal death kinetic models (Wang et al. 2002, Boina et al. 2008) for predicting mortality of the most heat-tolerant insect life stage during commercial facility heat treatments where temperatures are changing over time.

In the present investigation, eggs, young larvae, old larvae, pupae, and adults of *L. serricorne* were exposed to 46, 50, and 54°C. The elevated temperatures tested (≥46°C) were well above the upper limit for development and survival of *L. serricorne* (Powell 1931, Howe 1957). Although 50°C is the minimum temperature required for effective disinfestation (Wright et al. 2002, Roesli et al. 2003, Boina et al. 2008), vertical and horizontal stratification of temperatures during heat treatment may result in temperatures below or above 50°C in some portions of the facility (Dosland et al. 2006). Therefore, temperatures between 46 and 54°C were selected for this study.

Materials and Methods

Insects and Experiments. Cultures of *L. serricorne* were reared on ground, pelleted feed (95% by weight) plus brewer's yeast (5% by weight) diet that was sifted through a sieve with 250-μm openings (Yu 2008). The pelleted feed (10% moisture) used is a standard diet for poultry (broiler grower feed). This poultry diet is produced on a regular basis by the Department of Grain Science and Industry's pilot feed mill (Manhattan, KS) for a specific client. The poultry diet is made up of major and minor ingredients and these include: ground corn (69.8% by weight), soybean meal (19.8%), poultry by-product meal (5%), poultry oil (3.1%), limestone (0.7%), deflourinated phosphate (0.8%), salt (0.4%), poultry vitamin premix NB 3000 (0.3%), D,L-methionine (0.1%), and L-lysine (0.06%). All ingredients were mixed for 6 min in a Forberg paddle mixer (Forberg International AS, Larvik, Norway) of 454-kg capacity. The mixture was thermally processed (85°C) and pelleted using a pellet mill (model HD, series 1000, CA Pellet Mill Company, Crawfordsville, IN) by using a die to achieve a pellet diameter of 4 mm. The pelleted feed obtained from the feed mill was frozen for 1 wk at −13°C to kill any live insects. The pellets were then thawed at room conditions. Pellets (10.1% moisture) and brewer's yeast were ground separately in a Stein laboratory mill (model M-1, Fred Stein Laboratories, Inc., Atchison, KS) for 1 min, after which they were sifted separately through a U.S. Standard Sieve No. 60 sieve with 250-μm openings (Thermo Fisher Scientific, Waltham, MA). The final *L. serricorne* rearing diet was a mixture of ground feed (95% by weight) and yeast (5% by weight). Approximately 50 g of the diet was placed in a 0.45-liter glass jar and seeded with 100 adults to start the cultures. Cultures were reared at 28 ± 0.5°C, 65 ± 5% RH, and a photoperiod of 14:10 (L:D) h in a growth chamber (model I-36 VL, Percival Scientific, Perry, IA).

Field and laboratory experiments were conducted using various life stages of *L. serricrone.* In the field experiment at a food-processing facility subjected to elevated temperatures, eggs (3–4 d after oviposition), young larvae (3–4 d from the time of eclosion from eggs), old larvae (20–21 d from the time of eclosion from eggs), and adults (3–4 d after eclosion from pupae) were used. Large numbers of pupae were unavailable for use in the field experiments. In two separate laboratory experiments at the three elevated temperatures, eggs, young larvae, old larvae, pupae (3–4 d after pupation), and adults were used. These two laboratory experiments were designed to identify the most heat-tolerant stage. In addition, time–mortality responses at the three elevated temperatures were conducted using the most heat-tolerant stage.

Exposure of Life Stages During a Facility Heat Treatment. Eggs and young larvae were separated from the rearing media by using a sieve with 250-μm openings (Thermo Fisher Scientific). Old larvae and adults were separated from rearing media using a sieve with 425-μm openings. For exposures, 20 individuals of a life stage were transferred to separate plastic test boxes (4.5 by 4.5 by 1.5 cm), each holding 100 mg of the rearing medium. Test boxes had perforated lids (3-cm-diameter perforation) covered with mesh (600-μm openings) for ventilation. All test boxes were placed on the warehouse floor of a food-processing facility subjected to heat treatment during 20–22 July 2007. This particular facility conducts heat treatments in several rooms on a monthly basis by using steam heaters. Five locations were selected to place test boxes: two locations were near the heater (distances were <1 m) and three locations were farther from the heater (distances ranged from 3 to 5 m). At each of the five locations, a HOBO data-logging unit (Onset Computer Corp., Pocasset, MA) was placed next to the boxes on the floor to record temperature at one minute intervals during the entire heat treatment period (33 h). Boxes with eggs, young larvae, old larvae, and adults were used in three of the five locations (locations 1–3), and only eggs were used in two other locations (locations 4 and 5). The use of certain stages was based on availability of insects from laboratory cultures. Test boxes were collected at ≈11, 24, and 33 h into the heat treatment. The pick-up times for the boxes varied because of time spent moving from location to location in this large facility. The number of test boxes collected at the specific time intervals varied from 1 to 12, because of the limitations of the total bioassay boxes available. Test boxes were brought back to the laboratory and the contents transferred to 150-ml round plastic containers with perforated lids each holding 10 g of *L. serricorne* rearing diet. The plastic containers were placed in a growth chamber at 28°C and 65% RH. After 72 h, the diet was sifted to count the number of live and dead adults. Adult mortality, expressed as a percentage, was determined based on number of dead adults out of the total exposed. Immature stages were reared to the adult stage in the plastic containers as described above, and the mortality of immature stages was based on number of adults that emerged out of the total exposed.

Laboratory Tests at Elevated Temperatures. Three growth chambers (model I-36 VL, Percival Scientific) were used for exposing life stages of *L. serricorne* to constant temperatures of 46, 50, and 54°C and 22% RH. A humidity of 22% was used, because during a commercial heat treatment, the humidity inside the facility quickly falls to 22–25% (Mahroof et al. 2003a, Roesli et al. 2003), and this rapid drop can occur within 0.5 to <4 h after starting a heat treatment (Mahroof et al. 2003a). A fourth growth chamber at 28°C and 65% RH served as the control treatment. The internal volume of growth chambers was 0.84 m^3 (29.5 $feet^3$). Air velocity, measured with an electronic wind speed indicator (Davis Instruments, San Leandro, CA), inside the growth chambers at 28, 46, 50, and 54°C, ranged from ≈0.6–1.2 m/s.

An experiment was conducted with test boxes containing only 100 mg of ground, pelleted feed diet to verify whether insects within boxes would be exposed to the set chamber temperature and humidity levels (46, 50, or 54°C and humidity 22%). The test boxes were placed in the four corners and center of the top shelf of the growth chamber, a procedure we have followed previously (Mahroof et al. 2003b, Boina and Subramanyam 2004). This experiment was replicated three times. The air temperature and relative humidity inside growth chambers and inside test boxes containing *L. serricorne* diet were measured every minute using HOBO data-logging units. To measure temperature and humidity inside growth chambers at each of the three temperature treatments, a HOBO data-logging unit was placed in each of the four corners and the center of the top shelf. To measure temperature and humidity inside a test box, the thermocouple wire of a HOBO data-logging unit was taped to the floor of the test box (not the sensor head), and 100 mg of insect diet was added to cover the thermocouple sensor head. The accuracy of each HOBO data-logging unit is periodically verified in the laboratory by conducting calibration tests with a mercury thermometer at 45, 50, 55, and 60°C and was found to be within ± 0.1°C of the mercury thermometer.

Diet Equilibration Time. The diet used in test boxes was kept at 28°C and 65% RH before adding insects for elevated temperature exposure. Three test boxes with 100 mg of the diet were placed in the top shelf of growth chambers set at 46, 50, or 54°C and 22% RH. Thermocouples of HOBO data-logging units were glued to the bottom of test boxes before adding the diet as explained above. The time taken for the diet to reach the set chamber temperature was recorded. Each experiment was replicated three times.

Fixed Time Responses of Life Stages at Three Elevated Temperatures. To determine the most heat-tolerant stage, test boxes each with 50 individuals of a life stage (eggs, young larvae, old larvae, pupae, and adults) of *L. serricorne* were exposed to 46, 50, and 54°C at 22% RH. In the first experiment, the exposure time for all stages at 46, 50, and 54°C were fixed at 300, 90, and 40 min, respectively. The exposure times selected resulted in 100% mortality of all postembryonic stages at 50°C and 100% mortality of all life stages at 54°C. Therefore, in the second experiment at 46, 50, and 54°C exposure times of 240, 60, and 30 min, respectively, were selected. Each of these experiments was replicated three times. The control treatment, also replicated three times, consisted of life stages placed separately in test boxes with diet at 28°C and 65% RH to assess mortality at the longest exposure time (300 min).

Time–Mortality Responses of Eggs at Three Elevated Temperatures. Fixed time responses indicated eggs to be the most heat-tolerant stage. Test boxes, each with 50 eggs of *L. serricorne* and 100 mg of diet, were exposed in growth chambers set at 46, 50, and 54°C and 22% RH. At 46°C, eggs in test boxes were exposed for 240, 280, 300, 320, 360, 400,420, 460, 500, and 600 min; at 50°C eggs were exposed for 20, 40, 60, 80, 90, 100, 110, 120, 140, 150, 160, and 180 min; and at 54°C eggs were exposed for 5, 10, 15, 18, 20, 22, 25, 28, 30, and 35 min. Natural (control) mortality of eggs was determined by placing eight boxes, each with 50–100 eggs and 100 mg of *L. serricorne* diet at 28°C and 65% RH for the maximum duration corresponding to each elevated temperature treatment. At each temperature and exposure time, one to two boxes were removed from the growth chamber to determine egg mortality. Egg mortality was estimated by two methods. In the first method, egg mortality was assessed by examining the number of eggs that hatched out of the total exposed. For this assessment, the test boxes removed from the elevated temperature treatments were placed in the control growth chamber (28°C and 65% RH) for 1 wk before examining each box for egg hatchability. In the second method, test boxes with eggs removed at specific observation times were immediately transferred to 150-ml plastic containers with 10 g of *L. serricorne* diet. These containers were placed at 28°C and 65% RH until emergence of adults, and the mortality of eggs was based on number of adults that emerged out of the total eggs exposed. Each temperature-time combination for both methods was replicated three times, and the replications were blocked by time.

Data Analysis. The temperature data from HOBO data-logging units at each of the five locations in the food processing facility were used to determine the starting temperature, number of hours required to reach 50°C, number of hours temperatures were maintained above 50°C, and the maximum temperature. Heating rate at each location was calculated as follows: ([50°C – starting temperature in °C]/h to 50°C). The mortality of *L. serricorne* life stages at each location was determined along with corresponding temperature at ≈11, 24, and 33 h into the heat treatment.

Mortality data of *L. serricorne* life stages in experiments at fixed exposure times at the three constant temperatures were corrected for natural mortality by using formula of Abbott (1925). Corrected mortality data at each temperature were transformed to angular values (Zar 1984) and subjected to one-way analysis of variance (ANOVA) and Fisher protected least significant difference (LSD) test at $\alpha = 0.05$ level to

Table 1. Temperature data at five locations where test boxes with *L. serricorne* life stages were placed during heat treatment of a food-processing facility

Location	Insect stage[a]	Time to 50°C (h)	Rate to 50°C (°C/h)[b]	Time above 50°C (h)	Max temp (°C)
1	E, YL, OL, A	12.8	1.3	20.6	54.7
2	E, YL, OL, A	6.4	2.5	27.4	65.8
3	E, YL, OL, A	10.7	1.5	22.7	54.1
4	E	6.6	2.4	27.1	59.9
5	E	11.9	1.4	21.3	56.0

The starting temperature at all locations was 34°C.
[a] E, eggs, YL, young larvae, OL, old larvae, and A, adults.
[b] Rate = (50°C–34°C)/time to 50°C.

determine significant differences among stages by using the GLM procedure of SAS (SAS Institute 2003).

Corrected time–mortality response data for eggs at 46, 50, and 54°C based on egg hatchability and egg-to-adult emergence were fit to the complementary log-log (CLL) model (Robertson and Preisler 1992) by using the PROBIT procedure (SAS Institute 2003) to estimate the time required to kill 50% (LT_{50}) and 99% (LT_{99}) of the exposed eggs. The goodness-of-fit of the CLL model to the data were compared using a chi-square statistic (SAS Institute 2003).

All pairwise comparisons of LT_{99} values for eggs based on egg hatchability and egg-to-adult emergence at the elevated temperatures were made using the

Table 2. Mortality of *L. serricorne* life stages in test boxes at five locations sampled at three different time intervals during a facility heat treatment

Location	Stage[a]	Sample collection time (h)	Temp at sample collection (°C)	No. test boxes	No. dead insects/total	Mortality (%)
1	E	11.2	48.5	6	80/120	66.7
		24.1	52.4	6	119/120	99.2
		33.3	54.7	3	60/60	100.0
	YL	11.2	48.5	2	2/40	5.0
		24.1	52.4	2	40/40	100.0
		33.3	54.7	3	60/60	100.0
	OL	11.2	48.5	2	40/40	100.0
		24.1	52.4	2	40/40	100.0
		33.3	54.7	1	20/20	100.0
	A	11.2	48.5	2	3/40	7.5
		24.1	52.4	2	40/40	100.0
		33.3	54.7	3	60/60	100.0
2	E	11.5	55.4	8	144/160	90.0
		24.4	64.2	8	160/160	100.0
		33.7	64.2	4	80/80	100.0
	YL	11.5	55.4	2	40/40	100.0
		24.4	64.2	2	40/40	100.0
		33.7	64.2	1	20/20	100.0
	OL	11.5	55.4	2	40/40	100.0
		24.4	64.2	2	40/40	100.0
		33.7	64.2	1	20/20	100.0
	A	11.5	55.4	2	40/40	100.0
		24.4	64.2	2	40/40	100.0
3	E	11.2	50.1	12	114/240	47.5
		24.1	51.2	12	236/240	98.3
		33.3	51.8	6	120/120	100.0
	YL	11.2	50.1	2	10/40	25.0
		24.1	51.2	2	40/40	100.0
		33.3	51.8	1	20/20	100.0
	OL	11.2	50.1	2	40/40	100.0
		24.1	51.2	2	40/40	100.0
		33.3	51.8	1	20/20	100.0
	A	11.2	50.1	2	24/40	60.0
		24.1	51.1	2	40/40	100.0
		33.3	51.8	1	20/20	100.0
4	E	11.6	56.0	6	119/120	99.2
		24.4	57.9	6	120/120	100.0
		33.7	57.2	3	60/60	100.0
5	E	11.0	49.0	8	99/160	61.9
		23.9	54.7	8	160/160	100.0
		33.2	55.4	4	80/80	100.0

[a] E, eggs, YL, young larvae, OL, old larvae, and A, adults.

lethal time ratio test (Robertson and Preisler 1992). The two LT_{99} values being compared were considered significantly different ($P < 0.05$) from one another if the 95% confidence limit (CL) for the ratio does not include 1 (Robertson and Preisler 1992).

Results

Temperature Profiles and Responses of Life Stages During a Facility Heat Treatment. The starting temperature at all five locations of the warehouse was 34°C, and the time required to reach 50°C among the locations varied from 6.4 to 12.8 h (Table 1). Despite slow heating rates (1.3–2.5°C/h), temperatures above 50°C were maintained for 21–27 h. Except for one location, the maximum temperature did not exceed 60°C.

In locations 1, 2, and 3, 98–100% of all exposed life stages in test boxes died 24 h into the heat treatment, and all life stages died at the end of the heat treatment (Table 2). At these three locations, 100% of only the old larvae died 11 h into the heat treatment, whereas some survival of eggs, young larvae, and adults was observed at this time. In locations 2 and 4, egg survival was 10 and 0.8%, respectively at 11 h into the heat treatment, compared with locations 1, 3, and 5 where it ranged from 33.7 to 52.5%. The low egg survival in locations 2 and 4 could be due to higher heating rates, maximum temperatures, and temperatures being held above 50°C for longer times compared with locations 1, 3, and 5 (Table 2). The lack of consistent trends in stage-specific susceptibility made it impossible to determine a heat-tolerant stage based on the field test. Therefore, laboratory experiments were needed to determine relative susceptibilities of *L. serricorne* life stages at elevated temperatures.

Temperature and Humidity Measurements in Growth Chambers. The temperatures and relative humidities recorded by HOBO data-logging units on the top shelf of growth chambers and inside test boxes with 100 mg of *L. serricorne* diet were similar to the set chamber temperature and humidity levels (Table 3). This indicated that the insects were exposed to the predetermined treatment and control temperatures and humidity levels.

Diet Equilibration Time. The mean ± SE time for the diet to equilibrate from 28°C to the set chamber temperatures of 46, 50, and 54°C was 7.8 ± 0.2, 7.0 ± 0.2, and 6.2 ± 0.3 min, respectively. These times were subtracted in experiments at each of the temperatures where the life stages were exposed for a fixed or variable time.

Table 4. Corrected mortality (% mean ± SE; $n = 3$) of *L. serricorne* life stages exposed for fixed time periods at three elevated temperatures (test I)

Stage	46°C (300 min)	50°C (90 min)[a]	54°C (40 min)
Eggs	13.6 ± 1.4e	79.2 ± 1.5	100.0 ± 0.0
Young larvae	24.9 ± 1.7d	100.0 ± 0.0	100.0 ± 0.0
Old larvae	46.2 ± 1.0c	100.0 ± 0.0	100.0 ± 0.0
Pupae	68.4 ± 2.8b	100.0 ± 0.0	100.0 ± 0.0
Adults	83.7 ± 1.8a	100.0 ± 0.0	100.0 ± 0.0

The mean ± SE ($n = 3$) natural (control) mortality of eggs, young larvae, old larvae, pupae, and adults was 20.0 ± 2.9, 13.3 ± 1.3, 6.3 ± 0.3, 15.0 ± 1.0, and 0%, respectively. Means among stages at 46°C followed by different letters are significantly different from one another ($P < 0.05$; Fisher's protected LSD test).

[a] One-way ANOVA: $F = 666.29$; df = 4, 10; $P < 0.0001$.

Fixed Time–Mortality Responses of Life Stages. The natural (control) mortality among the stages ranged from 0 to 20%. In the first experiment, a 40-min exposure at 54°C resulted in 100% mortality of all life stages (Table 4). Similarly, a 90-min exposure at 50°C resulted in 100% mortality of all stages except for the egg stage ($F = 666.29$; df = 4, 10; $P < 0.0001$). There were significant differences in susceptibility among the stages at 46°C ($F = 215.24$, df = 4, 10; $P < 0.0001$), and all stages were significantly different from one another. The data at 50 and 46°C showed eggs to be the most heat-tolerant stage.

In the second experiment, exposure times that were 10–60 min shorter than the first experiment were used to ensure survival of all life stages, especially at 54 and 50°C. There were significant differences in the mortality of life stages at 54°C ($F = 59.37$, df = 4, 10, $P < 0.0001$), 50°C ($F = 51.56$, df = 4, 10, $P < 0.0001$), and 46°C ($F = 323.04$, df = 4, 10, $P < 0.0001$). At 54 and 50°C, differences among certain postembryonic life stages were not apparent, because of increased susceptibility at these high temperatures. However, clear cut susceptibility differences among stages were observed at 46°C (Table 5). Eggs were always significantly ($P < 0.05$) less susceptible compared with other stages at each of the three temperatures. These experiments also confirmed eggs to be the most heat-tolerant stage.

Table 3. Comparison of temp and humidity levels measured by HOBO data-logging units inside and outside test boxes versus set chamber conditions

Chamber		Top shelf of growth chamber		Inside test boxes	
Temp (°C)	RH (%)	Temp (°C)	RH (%)	Temp (°C)	RH (%)
46	22	45.9 ± 0.1 (198)[a]	21.9 ± 0.02 (198)	45.9 ± 0.03 (198)	21.8 ± 0.02 (198)
50	22	50.1 ± 0.1 (207)	21.5 ± 0.03 (207)	50.1 ± 0.1 (207)	21.6 ± 0.04 (207)
54	22	54.1 ± 0.1(216)	21.3 ± 0.07 (216)	54.1 ± 0.1 (216)	21.3 ± 0.04 (216)
28[b]	65	28.3 ± 0.5 (270)	64.1 ± 1.3 (270)	28.3 ± 0.2 (270)	63.9 ± 1.3 (270)

[a] Numbers in parenthesis represent the number of pooled replicate data observations collected over time used for computing means and associated SEs.

[b] Control growth chamber.

Table 5. Corrected mortality (% mean ± SE; $n = 3$) of *L. serricorne* life stages exposed for fixed time periods at three elevated temperatures (test II)

Stage	46°C (240 min)	50°C (60 min)	54°C (30 min)
Eggs	5.4 ± 1.1e	36.3 ± 0.7d	79.5 ± 1.5c
Young larvae	16.5 ± 1.0d	56.4 ± 2.3c	97.4 ± 1.0b
Old larvae	48.1 ± 1.4c	79.8 ± 0.7b	100.0 ± 0.0a
Pupae	59.8 ± 1.8b	83.9 ± 1.4b	99.0 ± 0.6b
Adults	68.0 ± 1.5a	96.3 ± 2.3a	100.0 ± 0.0a

See footnote to Table 4 for control mortality. Means among stages followed by different letters are significantly different from one another ($P < 0.05$; Fisher's protected LSD test).

Time–Mortality Responses of Eggs at Elevated Temperatures. The natural (control) mortality at the three constant temperatures based on egg hatchability and egg-to-adult emergence was 17–18 and 14–15%, respectively. In general, the intercepts, slopes, and lethal time estimates at each of the three temperatures based on the two methods for egg mortality assessment were quite similar (Table 6). In general, lethal time estimates decreased with an increase in temperature. Irrespective of the mortality assessment method used, the lethal time estimates (LT_{50} or LT_{99}) were three- to six-fold lower at 50°C compared with 46°C, and four- to five-fold lower at 54°C compared with 50°C. Lethal ratio tests indicated that differences between the LT_{99} values based on egg hatchability and egg-to-adult emergence at each of the elevated temperatures were not significantly different from one another ($P > 0.05$) (Table 7), but the LT_{99} values between any two elevated temperatures based on egg hatchability or egg-to-adult emergence were significantly different from one another ($P < 0.05$). Approximately 10 h were needed to kill 99% of *L. serricorne* eggs at 46°C, 3 h at 50°C and 0.6 h at 54°C.

Because no significant differences in LT_{99} (y) were observed at each of the three temperatures (x) based on egg hatch or egg-to-adult emergence, the LT_{99} data at these temperatures was pooled ($n = 6$) and an exponential decay model, $y = a(\exp^{-bx})$, was fitted to these data. The model fit the data well ($r^2 = 0.9991$) (Fig. 1), and the mean ± SE values for parameters a and b were 1129062767.07 ± 602597279.82 and 0.31 ± 0.01, respectively. The above-mentioned values can be used in the exponential decay equation to predict LT_{99} of *L. serricorne* eggs between 46 and 54°C.

Table 7. Pairwise comparisons of LT_{99} values for *L. serricorne* eggs based on egg hatchability and egg-to-adult emergence

LT_{99} values compared[a]	LT_{99} ratio (95% CL)[a]
Egg hatchability vs. adult emergence at 46°C	1.01 (0.95–1.08)
Egg hatchability vs. adult emergence at 50°C	1.15 (0.98–1.33)
Egg hatchability vs. adult emergence at 54°C	1.03 (0.92–1.16)
Egg hatchability, 46°C vs. 50°C	3.19 (2.81–3.61)*
Egg hatchability, 46°C vs. 54°C	15.51 (14.13–17.04)*
Egg hatchability, 50°C vs. 54°C	4.86 (4.21–5.61)*
Egg-to-adult emergence, 46°C vs. 50°C	3.61 (3.26–4.01)*
Egg-to-adult emergence, 46°C vs. 54°C	15.80 (14.30–17.44)*
Egg-to-adult emergence, 50°C vs. 54°C	4.37 (3.86–4.95)*

*, $P < 0.05$.

[a] LT_{99} values of a pair being compared are significantly different ($P < 0.05$) from one another if the 95% CL for the ratio does not include 1 (Robertson and Preisler 1992).

Discussion

The exposure of life stages of *L. serricorne* during a facility heat treatment failed to identify a heat-tolerant stage. Mahroof et al. (2003a) exposed eggs, young larvae, old larvae, pupae, and adults of the red flour beetle, *Tribolium castaneum* (Herbst), during heat treatment of a flour mill and observed pupae to be the most heat-tolerant stage. However, laboratory tests using the same life stages at six constant elevated temperatures between 42 and 60°C showed young larvae to be the most heat-tolerant stage (Mahroof et al. 2003b). During facility heat treatments, the rate of heating to 50°C from the ambient can range from 0.3 to 14.0°C/h, because of horizontal and vertical stratification of temperatures, despite use of fans to facilitate uniform heating (Mahroof et al. 2003a, Roesli et al. 2003). It is plausible that heating rates may have an impact on which stages develop heat tolerance during facility heat treatments. Heating rates were shown to determine which stage will become heat-tolerant, based on studies with elevated temperatures (50–60°C) when disinfesting grain (Beckett and Morton

Table 6. Time-mortality probit regression estimates (mean ± SE) and lethal time values for eggs of *L. serricorne* exposed to three elevated temperatures

Temp (°C)	Mortality assessment[a]	N[b]	Intercept ± SE	Slope ± SE	LT_{50} (95% CL) (min)	LT_{99} (95% CL) (min)	χ^2 (df)[c]	P value
46	EH	1,950	−28.3 ± 1.5	10.7 ± 0.6	403.0 (394.4–411.2)	605.6 (582.9–634.2)	15.54 (11)	0.159
	EA	1,500	−27.3 ± 1.7	10.4 ± 0.6	393.0 (383.5–402.3)	598.1 (571.2–633.1)	11.49 (8)	0.175
50	EH	2,250	−8.3 ± 0.7	4.3 ± 0.3	68.7 (61.9–74.6)	189.7 (169.9–219.6)	29.90 (13)	0.005*
	EA	1,500	−8.1 ± 0.6	4.4 ± 0.3	60.8 (56.3–64.9)	165.5 (152.6–182.8)	8.00 (8)	0.434
54	EH	1,500	−6.9 ± 0.5	5.3 ± 0.3	17.2 (16.2–18.0)	39.0 (36.3–42.8)	11.46 (8)	0.177
	EA	1,500	−6.5 ± 0.4	5.1 ± 0.3	16.0 (15.1–16.9)	37.9 (35.1–41.6)	6.43 (8)	0.600

The mean ± SE ($n = 8$) natural (control) mortality based on egg hatchability at 46, 50, and 54°C was 17.8 ± 2.1, 17.5 ± 2.2, and 16.6 ± 1.9%, respectively. The corresponding control mortality ($n = 3$) based on egg-to-adult emergence was 14.9 ± 1.0, 14.9 ± 0.2, and 14.2 ± 0.6%, respectively.

[a] EH, egg hatchability; EA, egg-to-adult emergence.

[b] N, total number of insects exposed.

[c] Chi-square values for goodness-of-fit of the CLL regression model to the observed mortality data. *, $P < 0.05$.

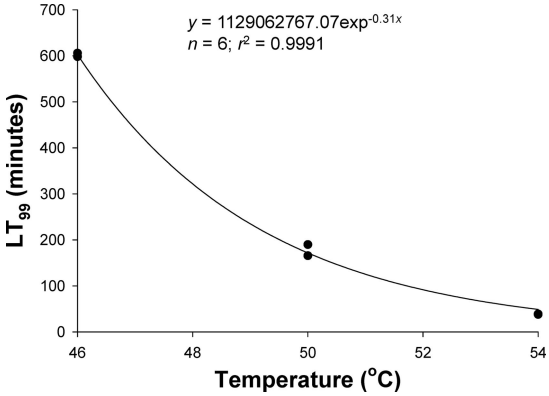

Fig. 1. A two-parameter exponential decay model describing time in minutes for 99% mortality (LT_{99}) of *L. serricorne* eggs at 46–54°C and 22% RH.

2003). When disinfesting grain elevated temperatures are used for a period of 0.01 to <16 h (high temperature short time), whereas during facility heat treatments, elevated temperatures are used for 24–36 h (high temperature long time). Therefore, data from responses of insects exposed to high temperatures for a short time and those exposed to high temperatures for a long time cannot be directly compared (Boina et al. 2008). In a recent article, Beckett et al. (2007) acknowledged that there are limited data to make inferences about heating rates and heat tolerance among insect stages exposed to facility heat treatments.

The fixed time mortality experiments showed eggs to be the most heat-tolerant stage of *L. serricorne.* A comparison of LT_{99} values for *L. serricorne* eggs at 50 and 54°C with that of other species showed *L. serricorne* eggs to be more heat tolerant. For example, at 50°C the LT_{99} values for eggs of *L. serricorne* (this study); *T. castaneum* (Mahroof et al. 2003b); the confused flour beetle, *Tribolium confusum* (Jacquelin du Val) (Boina and Subramanyam 2004); and the Indianmeal moth, *Plodia interpunctella* (Hübner) (Mahroof and Subramanyam 2006) were 165, 105, 41, and 29 min, respectively. At 54°C, the LT_{99} values for *L. serricorne*, *T. castaneum*, and *T. confusum* eggs were 38, 37, and 16 min, respectively. However, at 46°C, *T. castaneum* eggs were more heat tolerant than eggs of *L. serricorne*, *T. confusum*, and *P. interpunctella.* Unlike *L. serricorne*, the most heat-tolerant stage of *T. castaneum*, *T. confusum*, and *P. interpunctella* at elevated temperatures were observed to be young larvae, old larvae, and old larvae (wandering stage), respectively (Mahroof et al. 2003b, Boina and Subramanyam 2004, Mahroof and Subramanyam 2006). These studies indicate that stage-specific susceptibility to elevated temperatures varies among species. Within a given species heat tolerance among life stages may vary based on heating rates or temperature. The limited data of Conyers and Collins (2006) also reported eggs of *L. serricorne* to be heat tolerant, although the method of insect exposure used was different than the methods we used. The LT_{99} value of 8–9 h observed by Conyers and Collins (2006) for 2–4 d-old eggs of *L. serricorne* at 50°C was ≈5 h longer than what we observed in this study. Conyers and Collins (2006) exposed eggs along with 10 g of tobacco diet, whereas we used 100 mg of the ground, pelleted feed diet, and this may explain the difference in the reported lethal times.

The rapid drop in lethal time estimates at 50 and 54°C compared with estimates at 46°C is due to increased susceptibility of eggs at these temperatures. Similar increased susceptibilities of life stages of *T. castaneum*, *T. confusum*, and *P. interpunctella* at temperatures ≥50°C compared with temperatures below 50°C were reported by Mahroof et al. (2003b), Boina and Subramanyam (2004), and Mahroof and Subramanyam (2006). Past research and the current study reconfirm earlier findings that during structural heat treatments, the minimum temperature for effective disinfestations should be at least 50°C.

A comparison of LT_{99} values based on egg hatchability and egg-to-adult emergence yielded similar results at the three elevated temperatures. Therefore, either method can be used for assessing egg mortality. The exponential decay equation can be used to predicted LT_{99} for eggs of *L. serricorne* between 46 and 54°C. Rearing eggs to adulthood is time-consuming and can take at least a month at 28°C and 65% RH. Assessing egg mortality based on number of eggs that hatched out of the total exposed only takes a week.

In summary, eggs of *L. serricorne* were consistently the most heat tolerant of all stages tested at 46–54°C. Therefore, eggs should be used in evaluating heat treatment effectiveness because heat treatment designed to control eggs should be able to control all other *L. serricorne* life stages. The information presented in this article provides a quantitative basis for successful use of elevated temperatures for managing *L. serricorne* life stages associated with food-processing facilities.

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References Cited

Abbott, W. S. 1925. A method for computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Beckett, S. J., and R. Morton. 2003. Mortality of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) at grain temperatures ranging from 50°C to 60°C obtained at different heating rates in a spouted bed. J. Stored Prod. Res. 39: 313–332.

Beckett, S. J., P. G. Fields, and Bh. Subramanyam. 2007. Disinfestation of stored products and associated structures using heat, pp. 182–236. *In* J. Tang, E. Mitcham, S. Wang, and S. Lurie [eds.], Heat treatments for postharvest pest control: theory and practice. CAB International, Oxon, United Kingdom.

Boina, D., and Bh. Subramanyam. 2004. Relative susceptibility of *Tribolium confusum* life stages exposed to elevated temperatures. J. Econ. Entomol. 97: 2168–2173.

Boina, D., Bh. Subramanyam, and S. Alavi. 2008. Dynamic model for predicting survival of mature larvae of *Tribolium confusum* during facility heat treatments. J. Econ. Entomol. 101: 989–997.

Conyers, S. T., and D. A. Collins. 2006. The effect of high temperature on the mortality of *Lasioderma serricorne* (F.), pp. 843–848. *In* I. Lorini,, B. Bacaltchuk, H. Beckel, D. Deckers, E. Sundfeld, J. P. dos Santos, J. D. Biagi, J. C. Celaro, L. R. D'A. Faroni, L. de O. F. Bortolini, et al. [eds.], Proceedings of the 9th international working conference on stored-product protection, 15–18 October 2006, Campinas, Brazil. Brazilian Post-harvest Association, Campinas, Brazil.

Dean, G. A. 1911. Heat as a means of controlling mill insects. J. Econ. Entomol. 4: 142–158.

Dosland, O., Bh. Subramanyam, G. Sheppard, and R. Mahroof. 2006. Temperature modification for insect control, pp. 89–103. *In* J. Heaps [ed.], Insect management for food storage and processing, 2nd ed. American Association of Cereal Chemists, St. Paul, MN.

Fields, P. G. 1992. The control of stored-product insects and mites with extreme temperatures. J. Stored Prod. Res. 28: 89–118.

Fields, P. G., and N.D.G. White. 2002. Alternatives to methyl bromide treatments for stored-product and quarantine insects. Annu. Rev. Entomol. 47: 331–359.

Howe, R. W. 1957. A laboratory study of the cigarette beetle, *Lasioderma serricorne*, with a critical review of the literature on its biology. Bull. Entomol. Res. 48: 9–56.

Mahroof, R. and Bh. Subramanyam. 2006. Susceptibility of *Plodia interpunctella* (Lepidoptera: Pyralidae) developmental stages to high temperatures used during structural heat treatments. Bull. Entomol. Res. 96: 539–545.

Mahroof, R., Bh. Subramanyam., J. E. Throne, and D. Eustace. 2003a. Temperature and relative humidity profiles during heat treatment of mills and its efficacy against *Tribolium castaneum* (Herbst) life stages. J. Stored Prod. Res. 39: 555–569.

Mahroof, R., Bh. Subramanyam., J. E. Throne, and A. Menon. 2003b. Time-mortality relationships for *Tribolium castaneum* (Herbst) life stages exposed to elevated temperatures. J. Econ. Entomol. 96: 1345–1351.

Makhijani, A., and K. R. Gurney. 1995. Mending the ozone hole: science, technology, and policy. The Massachusetts Institute of Technology Press, Cambridge, MA.

Powell, T. E. 1931. An ecological study of the tobacco beetle, *Lasioderma serricorne*, with special reference to its life history and control. Ecol. Monogr. 1: 334–393.

Robertson, J. L., and H. K. Preisler. 1992. Pesticide bioassays with arthropods. CRC, Boca Raton, FL.

Roesli, R., Bh. Subramanyam, F. J. Fairchild, and K. C. Behnke. 2003. Trap catches of stored product insects before and after heat treatment in a pilot feed mill. J. Stored Prod. Res. 39: 521–540.

SAS Institute. 2003. Statistical Analysis System, version 8, for Windows. SAS Institute, Cary, NC.

Sinha, R. N., and F. L. Watters. 1985. Insect pests of flour mills, grain elevators, and feed mills and their control. Agriculture Canada Publication 1776. Canadian Government Publishing Centre, Ottawa, ON, Canada.

Wang, S., J. Tang, J. A. Johnson, and J. D. Hansen. 2002. Thermal-death kinetics of fifth-instar *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae). J. Stored Prod. Res. 38: 427–440.

Wright, E. J., E. A. Sinclair, and P. C. Annis. 2002. Laboratory determination of the requirements for control of *Trogoderma variabile* (Coleoptera: Dermestidae) by heat. J. Stored Prod. Res. 38: 147–155.

Yu, C. 2008. Susceptibility of *Lasioderma serricorne* (F.) life stages exposed to elevated temperatures. M.S. thesis, Kansas State University, Manhattan.

Zar, J. H. 1984. Biostatistical analysis, 2nd ed. Prentice Hall, Saddle River, NJ.

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