SUSCEPTIBILITY OF LASIODERMA SERRICORNE (F.) LIFE STAGES EXPOSED TO ELEVATED TEMPERATURES

by

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Abstract

Heat treatment of food-processing facilities involves using elevated temperatures (46 to 60^{o}C for 24 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. However, very little is known about responses of the cigarette beetle, *Lasioderma serricorne* (F.), a pest associated with food-processing facilities, to elevated temperatures. The main objective of my research was to evaluate stage-specific susceptibility of *L. serricorne* to elevated temperatures to identify the most heat tolerant stage. In the laboratory, I found *L. serricorne* was able to develop on ground, pelleted feed at 28^{o}C and 65% RH; however, there is no data on the biology of this species on this diet. Therefore, several life history parameters of *L. serricorne* were studied on ground, pelleted feed at 28°C and 65% RH, to facilitate harvesting stages of specific ages in large numbers for assays with elevated temperatures. The mean duration for eggs was 8.1 d, and the mean egg survivorship was 92.0%. There were four discrete instars, and the mean durations of first, second, third, and fourth instars were 4.7, 4.5, 4.7, 11.8 d, respectively. The survivorship of first through third instars was about 99%, whereas that of fourth instars was 85%. The mean pupal duration was 4.6 d, and pupal survivorship was 98%. Newly eclosed unmated female adults lived 5 d longer than unmated males (29 d), whereas, mated males lived 6 d longer than mated females (17 d). Mated females started laying eggs on the third day after emergence and continued this activity for an additional six to eight days. Females, on average, laid 105 eggs with a mean daily output of 12 eggs. The data reported here provide new information on the biology of *L. serricorne* on ground, pelleted feed, which appears to be an optimal diet for mass rearing this species.

Exposure of eggs, young larvae (3 to 4- July 2007 did not clearly show which of the life stages was heat- tolerant. However, exposure of all life stages to fixed times at 46, 50 and 54^{o}C and 25% RH in the laboratory indicated eggs to be the most heat-tolerant stage. Time-mortality responses, at each of these three d old), old larvae (20 to 21-d old), and adults during heat treatment of a food-processing facility in 20-22 temperatures,

showed that the time for 99% mortality (LT_{99}) based on egg hatchability and egg-to-adult emergence was not significantly different at each temperature. The LT_{99} based on egg hatchability at 46^{o}C was 605 min and it decreased to 190 min at 50^{o}C and 39 min at 54^{o}C. Therefore, during structural heat treatments eggs should be used in bioassays for gauging heat treatment effectiveness, because treatments aimed at controlling the egg stage should control all other life stages of *L. serricorne*.

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CHAPTER 1 - NOTES ON THE BIOLOGY AND ECOLOGY OF LASIODERMA SERRICORNE (F.)

The Insect. The cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae), is a major pest of stored products. This species was first described in North America by Fabricius in 1792 (Powell 1931), and was first found infesting tobacco in Paris in 1848 (Runner 1919), and hence the name. *Lasioderma serricorne* is distributed throughout the tropical and subtropical parts of the world. In addition to tobacco, this species has been reported infesting a wide variety of stored products. Although adults have been reported as an occasional feeder, most of the damage to stored products is caused by larvae.

Food Materials Attacked. Plant materials infested by *L. serricorne* include cocoa beans, coffee beans, grains, oilseeds, dried fruit, processed foods, spices, yeast, and herbarium specimens. Animal products infested by this species include dried fish, dried insects, and fishmeal. Interestingly, it has been reported attacking leather, upholstery, paper, books, and furniture. It was mentioned that maize meal plus yeast powder is the best food known (Howe 1957).

Damage Caused by the Insect. Damage to stored products caused by *L. serricorne* usually results in loss of weight and decrease in quality. A single insect only causes a few milligrams of weight loss, whereas populations measured by millions of *L. serricorne* individuals can bring considerable weight loss. Stored products are holed and contaminated with cocoons and frass when infested by *L. serricorne.* In cigar and cigarettes the holes destroy the product, and holes spoil the sack or package. Infestation of cereal grains and of seeds of beans and other plants could adversely affect germination as the germ is attacked (Howe 1957).

Description of Life Stages. The body of adults is uniformly dull reddish in color and is 2.2 to 3 mm long. The head is broad and the eyes are small. The fourth to tenth antennal segments are serrate (Runner 1919). The adult beetles are capable of flight (Ashworth 1993). The adults live in dark or semi-dark places, often in crevices. They avoid light during the daytime but are attracted to artificial light at night. They are very active before the evening, usually at sunset, and continue their activity throughout the night. Adults are inactive at temperatures below 17ºC. In general, the adults of *L. serricorne* do not feed (Howe 1957), but Lefkovitch and Currie (1963) reported that adults do feed and feeding affects female fecundity. For example, unfed mated females

lay fewer eggs than fed counterparts. Weight of the adults is affected by the quality of larval food (Jones 1913). Females weigh more than males, and this difference in weight initially occurs during the third instar stage (Lefkovitch and Currie 1963). The overall sex ratio of adults (male:female) is about 1:1 (Ashworth 1993).

The eggs of *L. serricorne* are white in color, 0.4 to 0.5 mm long and 0.2 mm wide (Jones 1913). Each egg weighs approximately 8.4 µg and has a waxy shell which protects the egg from desiccation. The larva eats the egg shell at the time of hatching (Ashworth 1993). The eggs become dull in color before hatching. Surface of the egg is smooth, without sculpture except at the end portion of egg from which the larva emerges (Runner 1919).

Ashworth (1993) stated that the first instar is less than 1 mm long and covered with fine hairs. The larvae go through four larval instars before pupation, and the weight ranges from 2.5 to 5.0 mg. Runner (1919) reported that the first instar is 0.55 to1.4 mm long and yellowish white in color. The second instar is about 3 mm long and yellowish white, and the last instar is 4 mm long, and body is yellowish white, set entirely with long, yellowish brown hairs. Newly hatched larvae move away from light and are extremely active (Ashworth 1993). These tiny larvae are able to infest packaged food by entering through small holes (Runner 1919). The older larvae are less active but are still capable of considerable wandering and remain negatively phototropic. The larvae stop feeding and build cell when they are fully grown, and the formation of this cell is influenced by the food substrate. Disturbance may cause old larvae to give up a partly-made cell and build new cells or even cause them to form naked pupae (Howe 1957). They tend to penetrate deeply into loosely packed commodities. Insect activity ceases when the temperature falls below 19.5ºC (Runner 1919) and the beetle overwinters in the larval stage (Ashworth 1993). Development of larvae stops when the temperature falls below 17ºC or above 42ºC (Howe 1957).

Pupa is uniformly white when first formed, and is 3.5 mm long and 1.7 mm wide. Tips of elytra reach the fourth segment of the abdomen. Metathoracic legs are formed under the elytra. The head is curved beneath pronotum. The ultimate portion of the abdomen is paired with lateral protuberances (Runner 1919).

Effect of Temperature and Humidity on Immature Development and Survival. Temperature is the single most important factor affecting development and survival of *L. serricorne.* Howe (1957) reported that development and hatching of eggs to occur between 20 and 34 ºC. The optimum temperature for rapid larval development is 32.5ºC and development slows down at 20ºC. Development of pupae takes the shortest time at 32.5-35.0ºC. Development of *L. serricorne* cannot be completed at 17.5 ºC or 40 ºC.

The optimum humidity for larval development is 70-80%. Larvae fail to pupate at 90% humidity. Humidity does not appear to have any marked effect on the duration eggs and pupae except at the lower limits of humidity tolerance (Howe 1957). Powell (1931) indicated that the egg and larvae stages are prolonged at lower humidity levels (45-60%), and no adults emerged at any temperature at 30% humidity.

Food Preferences. Kohno et al. (1983) tested the olfactory response of *L. serricorne* to various host foods. They found that only females were strongly attracted to cured tobacco leaves, and toasted coffee meal was the most attractive among the items of host food rather than cured tobacco leaves. Ashworth (1993) stated that *L. serricorne* prefers tobacco with high sugar content and low nicotine content.

The insect can grow well on a low-carbohydrate diet, irrespective of whether it is of animal or vegetable origin, but does not grow well on a dried fruit diet. Less than 16 mg of food per larva leads to a decrease in larval populations, and prolongs developmental time and increases mortality (Lefkovitch and Currie 1963).

The endosymbiotes in mycetomes are an integral part of the beetle's physiology, and produce several important substances which allow the insect to survive on foods deficient in certain nutrients (Ashworth 1993). Shortage of food prolongs developmental time and reduces survival of the immature stages, and also reduces the weight of the resulting adults (Lefkovitch and Currie 1963).

Cannibalism. Powell (1931) and Lefkovitch and Currie (1963) stated that adults do not eat eggs in the presence or absence of food. No evidence of damage to the eggs is caused by larvae in the presence of food. However, larvae eat eggs and pupae only in the complete absence of food, but larvae do not eat one another. Dead larvae do not seem an

attractive food material for other larvae even when no other food is available (Lefkovitch and Currie 1963).

Sex Pheromone, Mating, and Reproduction. Pheromones are chemical messengers that influence the behavior or physiology of *L. serricorne*. In general, pheromones produced by the female are attractive to male adults of the same spices, while male pheromones are aphrodisiacs and are not attractive to females. Female sex pheromones are active over longer distances than those of the male. The pheromone activity on male cigarette beetles was observed as several responses, such as elevation of the antennae and the pro- and meso-thoracic legs, rapid zigzag movement towards the pheromone source, and the sex simulating activity (Ashworth 1993).

Sexual maturity is attained during the pupal stage, and mating occurs within 2-3 d after adult emergence. Males remain responsive to females after previous matings. Multiple mating occurs with over 90% of females mating at least twice and over 90% of males mating at least six times. Lefkovitch and Currie (1963) did not find a relationship between female age and the subsequent performance of the offspring.

Runner (1919) stated that female's oviposition is stimulated by smell; a large proportion of the females lay eggs on the second and third day after mating. Females lay eggs singly in crevices, folds or depressions in the food (Howe 1957). Powell (1931) found that unfertilized females do not lay eggs. Females may lay eggs in the medium without food, such as sawdust, sand, or sacking surface, but does not lay eggs in empty jars. After females are fertilized, presence or absence of males does not affect oviposition. Ashworth (1993) stated that female leaves a chemical on the oviposition site after laying eggs. Other females can recognize the chemical and avoid lay eggs at the same site.

The Lethal Effects of Low and High temperatures. Larvae of *L. serricorne* are very resistant to low temperatures. At a low temperature (below 17ºC), the development of *L. serricorne* is not completed and adult activity ceases (Howe 1957). Runner (1919) stated that the larvae become dormant and do not damage at low temperature but can survive long enough to pass the winter. Childs et al. (1970) found that at 4.4ºC the third and fourth instars die in three weeks; at 7.2ºC third instars die in three weeks and fourth instars in 5 weeks. At 10ºC, 60% of third instars and 20% of fourth instars die in 11

weeks. There is limited information on the effects of high (elevated) temperatures on the survival of life stages of *L. serricorne*. Therefore, I conducted field and laboratory experiments, reported in this thesis, to determine the impact of elevated temperatures on the survival of *L. serricorne* life stages. The first part of my research involved a careful study of the biology of *L. serricorne* on ground, pelleted feed at 28^{o}C and 65% relative humidity to enable me to harvest specific stages for exposure to elevated temperatures. The second part of my research involved exposure of eggs, young larvae, old larvae, pupae, and adults of *L. serricorne* to elevated temperatures to identify a heat tolerant stage. Additional tests were conducted on the heat tolerant stage to determine time-mortality responses at elevated temperatures. The work reported in this thesis forms a valid basis for use of elevated temperatures for management of *L. serricorne* life stages in food-processing facilities.

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CHAPTER 2 - BIOLOGY OF THE CIGARETTE BEETLE, LASIODERMA SERRICORNE (F.), ON GROUND, PELLETED FEED

Introduction

The use of elevated temperatures for disinfesting food-processing facilities, also known as heat treatment, involves raising the ambient temperature of the whole or a portion of the facility to 50-60^{o}C for 24 h. Heat treatment is becoming an effective and viable alternative to fumigation with methyl bromide (Dosland et al. 2006), which is phased out in the United States as of 2005, except for certain critical uses. Since 1999, the research group in the Department of Grain Science and Industry, Kansas State University, Manhattan, KS, has been generating data on utilizing heat treatments as a non-chemical alternative to methyl bromide fumigation for management of stored-product insects associated with food and feed processing facilities (Mahroof et al. 2003a,b; 2004, 2005a,b; Roesli et al. 2003, Boina and Subramanyam 2004, Mahroof and Subramanyam 2007, Boina et al. 2008). Previous research at Kansas State University focused on evaluating susceptibility of various life stages of the red flour beetle, *Tribolium castaneum* (Herbst) (Mahroof et al. 2003a,b), confused flour beetle, *Tribolium confusum* (Jacquelin du Val) (Boina and Subramanyam 2004), and Indianmeal moth, *Plodia interpunctella* (Hübner) (Mahroof and Subramanyam 2006) to elevated temperatures. Very limited information is available on susceptibility of various life stages of the cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae), exposed to elevated temperatures used for structural disinfestations (Adler 2003, Collins and Conyers 2006).

In our laboratory, we have been successfully rearing *L. serricorne* on pelleted feed produced by the Department of Grain Science and Industry's pilot feed mill since 2006. In order to extract large numbers of insects of a specific life stage for exposure to elevated temperatures, it was important to first conduct a detailed biological study of *L. serricorne* on pelleted feed. The development and reproduction of *L. serricorne* is influenced by the food substrate (Howe 1957, Mahroof and Phillips 2008), and information on the development and reproduction of this species on pelleted feed is lacking. Therefore, laboratory tests were conducted at 28^{o}C and 65% RH to determine certain life history parameters, such as duration and survival of immature stages, number of instars, adult longevity, and female oviposition. The pelleted feed was finely ground for easy separation of life stages from the food substrate.

Materials and Methods

Insect Diet. The pelleted feed used for rearing *L. serricorne* at controlled environmental conditions and for all experiments consisted of 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 for a specific client. The poultry diet is made up of major and minor ingredients and these include: ground corn (69.8% by wt), 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%), dl-methionine (0.1%), L-lysine (0.06%). All ingredients were mixed for six minutes in a Forberg paddle mixer (Forberg International AS, Larvik, Norway) of 454-kg capacity. The mixture was thermally processed (85^{o}C) and pelleted using a pellet mill (model HD, series 1000, California Pellet Mill Company, Crawfordsville, IN) using a die to achieve a pellet diameter of 4 mm. The pelleted feed obtained from the feed mill was frozen for one week at -13^{o}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 one minute, after which they were sifted separately through a U.S. Standard Sieve No. 60 sieve (Fisher Scientific, Pittsburg, PA), with 250 μm openings. The *L. serricorne* diet consisted of ground feed (95% by weight) combined with ground yeast (5% by weight). About 50 g of the diet was placed in a 0.45-liter glass jar, with wire-mesh and filter paper lid, and seeded with 100 adults to start the cultures. All cultures were reared at 28°C and 65% RH in growth chambers (Model I-36 VL, Percival Scientific, Perry, IA).

Collection and Rearing of Eggs to Adulthood. To collect eggs, 100 male and female adults of *L. serricorne* were placed in 0.45-liter jars each with 50 g of the diet. After 24 h adults were separated from the diet using sieves of two different mesh sizes and a bottom pan. The top sieve had 425 μm openings (U.S. Standard Sieve No. 40) and the bottom sieve had 250 μm openings. Adults in the diet were collected on the top sieve and the eggs were retained on the 250 μm sieve, while the diet passed through the second sieve into the bottom pan. Eggs were gently removed from the sieve using a camel's hair brush into 9-cm glass Petri dishes.

A total of 288 eggs were individually placed in three 96-well assay microplates (Fisher Scientific, Pittsburg, PA), with 100 mg of diet in each well. Microplates were then placed in a growth chamber set at 28°C and 65% RH. Each well was examined daily until emergence of adults to record the duration and survival of immature life stages.

Determining Number of Instars. In a related experiment, newly hatched larvae (0-1-d old) were removed from Petri dishes and placed in 30-ml plastic condiment cups similar to that used by Subramanyam et al. (1985) with 100 mg of the diet per cup. The number of larvae per cup ranged from 5 to 10, depending on availability. The cups were incubated at 28°C and 65% RH, and the larvae sifted from the diet were preserved in 95% ethanol for measurement of head capsule widths. The measurements were made under ethanol by pinning abdomens of larvae such that the dorsal region of the head capsule was clearly visible. The widest portion of the head capsule was measured using a stereomicroscope (Model SMZ 1000, Nikon Corporation, Japan), fitted with an ocular micrometer, at 80X magnification.

Determining Adult Longevity and Female Oviposition. Pupae in 96-well microplates were sexed using differences in the genital papillae shown in Fig. 1 (Halstead 1963). When adults emerged from pupae, one male and one female were paired and placed in a plastic box (4.5 by 4.5 by 1.5 cm) with 100 mg of the diet. There were five mated pairs. Additionally, 13 unmated male adults and 17 unmated female adults were placed individually in the plastic boxes with 100 mg of the diet. Boxes were checked daily to record whether adults were alive or dead and for counting number of eggs laid by the females. To count number of eggs laid, the diet in each box (except boxes with males) was sifted through a sieve with 250 μm openings to separate eggs from the diet as explained above. Fresh diet (100 mg) was placed in boxes and checked daily until all the adults were dead.

Data Analysis. Means and standard errors (SE) for the duration of each immature stage and for the egg-to-adult development were calculated using the Statistical Analysis System (SAS Institute 2003). The number of individuals surviving at each immature stage was expressed as a percentage. The frequency distribution of head capsule widths were plotted by instar using SigmaPlot® 8.0 (Systat Software, Inc., San Jose, CA). Differences in head capsule widths among instars were determined by subjecting data to

one-way analysis of variance (ANOVA) and Fisher's protected least significant difference (lsd) test at $\alpha = 0.05$ using the GLM procedure of SAS (SAS Institute 2003). Data on the longevity of unmated and mated male and female adults were transformed to logarithmic scale to normalize variances, and subjected to one-way ANOVA using the GLM procedure, and means were separated using Fisher's protected lsd test. Data on the number of eggs laid by mated females over time were presented in tables as means and SE. Although data required transformation for certain analyses, in the tables untransformed means and standard errors are presented.

Results

Development and Survival of Immature Stages. Out of the 288 eggs, 92% hatched, and the mean ± SE duration of this stage at 28°C and 65% RH was 8.1±0.05 d (Table 1). Measurement of head capsule widths indicated four discrete instars (Fig. 2). There were significant differences in head capsule widths among the instars (F = 9216.74; df = 3, 119; $P < 0.0001$), and the widths among instars were significantly different from one another (Table 2). The first three instars took approximately 5 d each, whereas the fourth instar took 12 d (Table 1). Survivorship of the first through third instars was about 99% and survivorship of the fourth instar was 85%. About 96% of pupae became adults in 5 days, and the remaining 4% of the pupae died in this stage. The total egg-to-adult development on ground, pelleted feed took 38 days, and 73% of the 288 eggs survived to adulthood.

Adult Longevity. In general, the longevity of both male and female adults was lower than unmated males and females (Table 3). There were significant differences in the longevity of unmated and mated adults (F = 6.93; df = 3, 36; P = 0.0008). The longevity of unmated and mated males was essentially similar ($P > 0.05$). However, unmated females lived two times longer (35 d) than mated females (17 d), and this difference was significant ($P < 0.05$).

Female Oviposition. Among the five mated pairs, females started laying eggs on the third day after pairing with males and continued to lay eggs for an additional six to eight days (Table 4). The maximum number of eggs was laid on the fourth day after pairing. Females, on average, laid 105 eggs with a mean daily egg production of 12 eggs.

Discussion

Howe (1957) reared *L. serricorne* on wheat feed (wheat bran) at 27.5°C and 70% RH, and observed the egg, larval, and pupal periods to be about 8.9, 22.4, and 4.8 d, respectively. On ground, pelleted feed, the egg, larval, and pupal periods were 8, 25.6, and 4.6 d, respectively, at 28°C and 65% RH. Given that the rearing conditions used by Howe (1957) and in this study were very similar, the similarity in immature developmental times for *L. serricorne* on wheat feed and ground, pelleted feed suggests that the latter is also an optimal diet for rearing this species in the laboratory. Development of *L. serricorne*, especially the larval stage, is influenced by the food substrate. Mahroof and Phillips (2008) reared *L. serricorne* on various food substrates at 28°C and 60% RH and found the duration of larval stage to be most influenced by the type of food. For example, the mean duration of egg, larval, and pupal stages on wheat flour took 4.8, 38.0, 4.6 d, respectively. Corresponding times on leaf tobacco were 5.2, 53.0, 8.2 d, and on paprika were 6.6, 63.0, and 7.6 d. On cayenne pepper the egg, larval, and pupal periods were 6.6, 75.3, 5.7 d, respectively, whereas on chili it was 5.0, 73.0, 18.3 d, respectively (Mahroof and Phillips 2008). The development on ground, pelleted feed was considerably shorter compared with those reported by Mahroof and Phillips (2008) (Table 5). Therefore, pelleted feed appears to be an optimal diet for mass rearing *L. serricorne* when compared with the diets reported by Mahroof and Phillips (2008).

The number of instars of *L. serricorne* reported in literature ranged from 3 to 10 on different food substrates (Jones 1913, Farage and Ismail 1986). On ground, pelleted feed four discrete instars were identified based on head capsule widths. Based on this study two instars—the first instars (young larvae) and fourth instars (old larvae), were selected for exposure to elevated temperatures (see Chapter 3). Young larvae and old larvae that were 3-4 d and 20-21 d old after eclosing from the eggs were used in experiments with elevated temperatures.

In our study, unmated male and female adults lived for 29-35 d and mated adults lived for 17 to 23 d. Powell (1931) found that unmated females lived 2-3 months. Shinoda and Fujisaki (2001) reported longevity of female and male adults to be 19.7 and 18.1 d, respectively, at 30°C and 70% RH. Allotey and Unanaowo (1993) found adult longevity of *L. serricorne* depended on the diet used for rearing the insects. For example,

at 28-32°C and 72.5- 80.5% RH, the longevity of male and female adults reared on rice, cowpea, groundnut, maize, and wheat ranged from 26.6-33.2, 22.6-26.5, 24.0-31.7, 26.0-30.8, and 24.6-27.7 d, respectively.

On ground, pelleted feed, female *L. serricorne* laid 105 eggs during its life time, and this value comes close to the 103 eggs reported by Runner (1919), who reared the beetles on tobacco leaves at 30^{o}C and 80% RH. Powell (1931) reported that female *L. serricorne* laid about 44 eggs. Allotey and Unanaowo (1993) found the number of eggs laid varied with the food substrate on which the insects were reared. For example, female *L. serricorne* laid 14, 19, 25, 37, and 40 eggs when reared on rice, cowpea, groundnut, maize, and wheat, respectively.

In summary, our results suggest that ground, pelleted feed to be an optimal laboratory diet for mass rearing *L. serricorne* at 28^{o}C and 65% RH based on the speed of development of immature stages and number of eggs laid by mated females. Characterizing stage-specific development and adult longevity of *L. serricorne* on ground, pelleted feed in the laboratory enabled us to harvest eggs, young larvae, old larvae, pupae, and adults of specific age in large numbers for detailed studies at elevated temperatures.

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Table 1. Development and survival of *L. serricorne* immature stages on ground, pelleted feed.

Life stage	No. insects[a]	Mean ± SE duration (d)	Survival (%)
Eggs	265	8.1 ± 0.05	92.0
First instars	262	4.7 ± 0.04	98.9
Second instars	260	4.5 ± 0.04	99.2
Third instars	259	4.7 ± 0.04	99.6
Fourth instars	219	11.8 ± 0.20	84.6
Pupae	210	4.6 ± 0.05	95.9
Egg-to-adult	209	38.3 ± 0.24	72.6

[a]The insects were individually reared on ground, pelleted feed diet using 288 eggs.

Table 2. Head capsule width measurements of *L. serricorne* instars.

Instar	No. insects	Head capsule width (mm)[a] Mean ± SE	Range within an instar (mm)
First	26	0.14 ± 0.002d	0.13 – 0.16
Second	23	0.26 ± 0.002c	0.25 – 0.29
Third	34	0.40 ± 0.002b	0.38 – 0.41
Fourth	40	0.64 ± 0.002a	0.63 – 0.66

[a]Means followed by different letters are significantly different ($P < 0.05$, by Fisher's protected lsd test).

Table 3. Longevity of unmated and mated *L. serricorne* adults.

Treatment	Number of individuals or mating pairs	Mean ± SE longevity (d)[a]
Unmated female	17	34.6 ± 2.8a
Unmated male	13	29.2 ± 2.2a
Mated male	5	23.4 ± 1.9a,b
Mated female	5	17.0 ± 0.5b

[a]Means followed by different letters are significantly different ($P < 0.05$, by Fisher's protected lsd test).

Table 4. Daily egg production of mated *L. serricorne* females.

Days after pairing	Mean ± SE no. eggs	Percentage of total[a]	Range among pairs
3	7.8 ± 4.9	7.4	0 – 23
4	24.0 ± 6.2	22.8	8 – 42
5	18.6 ± 2.1	17.7	12 – 24
6	14.8 ± 1.6	14.1	11 – 20
7	14.8 ± 2.2	14.1	10 – 22
8	9.0 ± 2.0	8.6	4 – 16
9	7.2 ± 2.1	6.8	0 – 12
10	5.8 ± 2.0	5.5	0 – 12
11	3.2 ± 1.1	3.0	0 – 6

Females were observed over a 13-d period. Eggs were not laid on the first two days and on the 12th and 13th days after pairing.

[a]Percentages are based on the mean total number of 105.2 eggs laid during a female's life time.

Table 5. Development of immature stages of *L. serricorne* on ground, pelleted feed compared with various food substrates.

Temp (°C)	RH	Food substrate	Development time for:				Reference
			Egg	Larva	Pupa	Egg-to-adult	
28	65	Pelleted feed	8.1	25.6	4.6	38.3	This study
27	70	Wheat/corn flour	6.8	28.7	7.4-9.8	42.9-45.3	Farage and Ismail (1986)
27.5	70	Wheat feed	8.9	22.4	4.8	36.1	Howe (1957)
28	60	Wheat flour	4.8	38.0	4.6	47.4	Mahroof and Phillips (2008)
28	60	Leaf tobacco	5.2	53.0	8.2	66.4	Mahroof and Phillips (2008)
28	60	Paprika	6.6	63.0	7.6	77.2	Mahroof and Phillips (2008)
28	60	Cayenne pepper	6.6	75.3	5.7	87.6	Mahroof and Phillips (2008)
28	60	Chili	5.0	73.0	18.3	96.3	Mahroof and Phillips (2008)
28	60	Cigar tobacco	4.8	92.2	10.4	107.4	Mahroof and Phillips (2008)
28	60	Navel orangeworm bait	4.6	92.0	12.6	109.2	Mahroof and Phillips (2008)
30	70	Wheat feed	6.0	19.9	4.0	29.9	Howe (1957)
30	60	English broad wheat bran	6-8	17-27	7-12	30-47	Lefkovitch and Currie (1963)

Temp (°C)	RH	Food substrate	Development time for:				Reference
			Egg	Larva	Pupa	Egg-to-adult	
30	80	Tobacco seed	6-8	29-30			Runner (1913)
30	80	Pressed yeast cake		27-30			Runner (1913)
30	80	Plug chewing tobacco		29			Runner (1913)
30	80	Loose granulated tobacco		35-38			Runner (1913)
30	80	Cigars		34- 36			Runner (1913)
30	80	Cigarettes		42			Runner (1913)
32	75	Magic yeast	4	16	9	29	Powell (1931)

Figure 1. Characteristics of the genitial papillae (arrows) used for sexing male (left) and female (right) *L. serricorne* in the pupal stage (Halstead 1963).

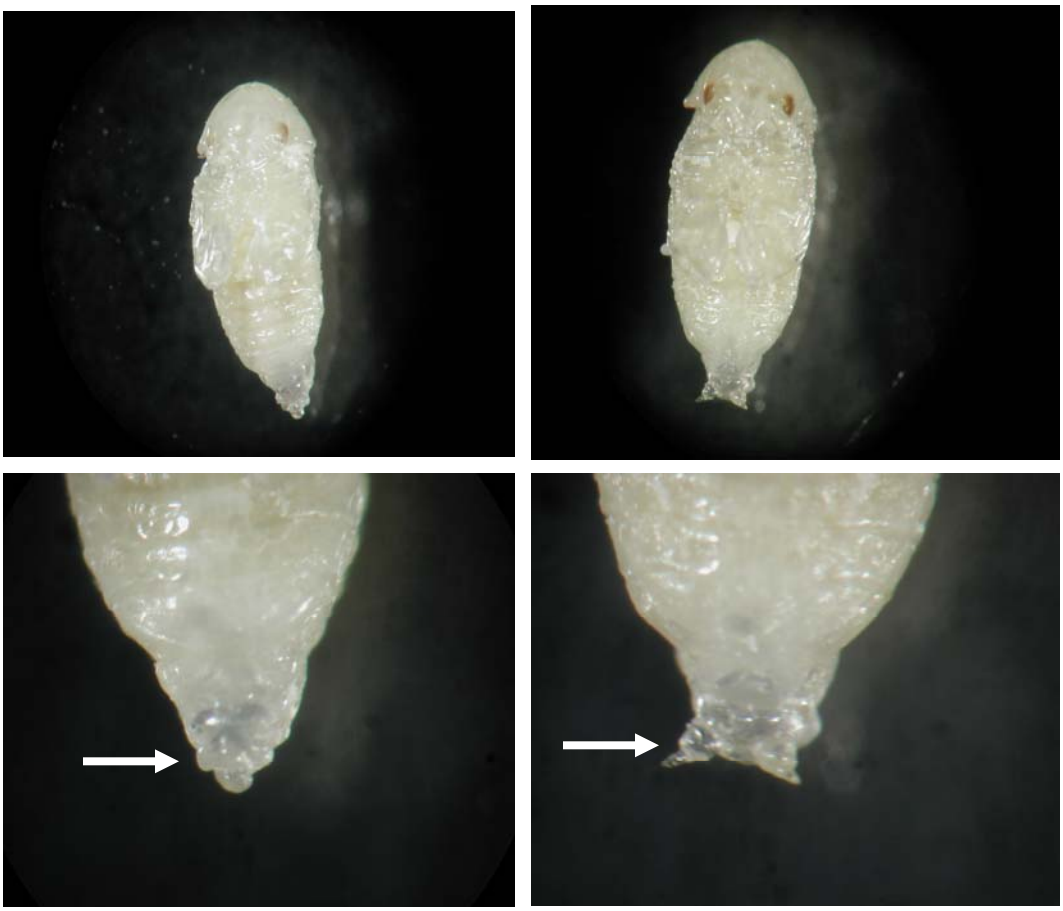
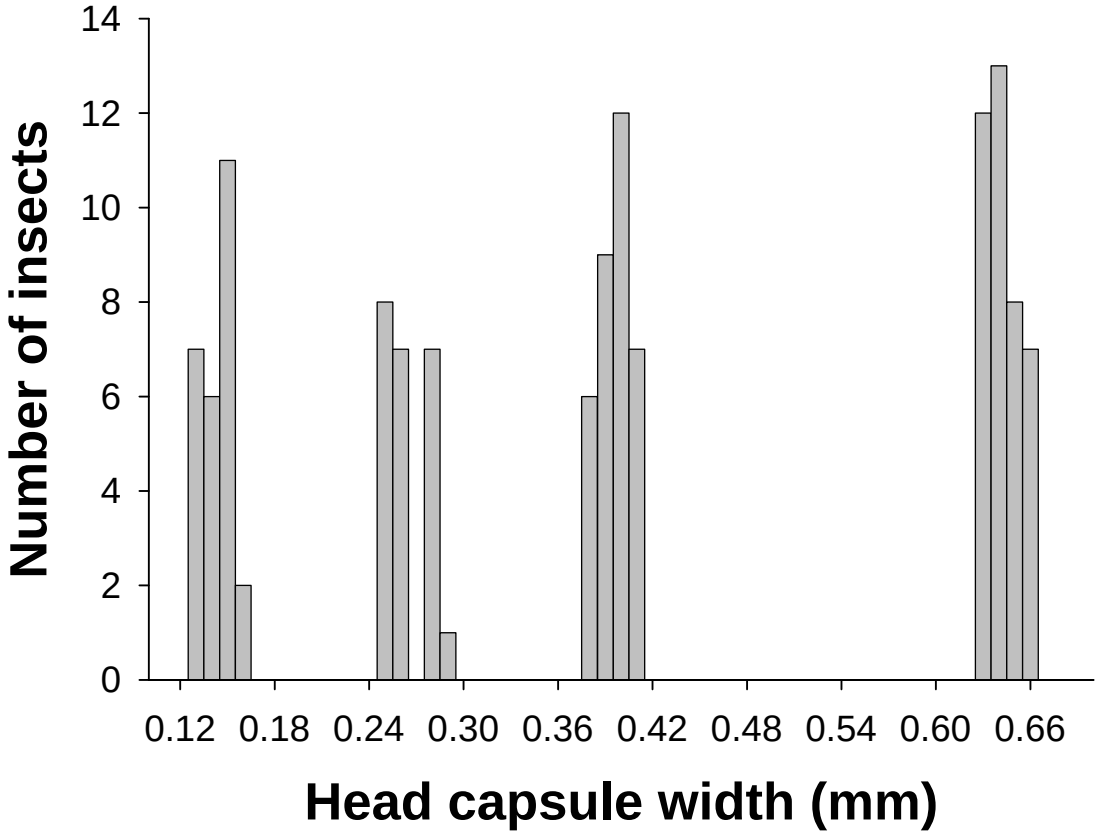


Figure 2. Frequency distribution of head capsule widths of four instars of *L. serricorne* reared on ground, pelleted feed.



CHAPTER 3 - RELATIVE SUSCEPTIBILITY OF LASIODERMA SERRICORNE (F.) LIFE STAGES EXPOSED TO ELEVATED TEMPERATURES

Introduction

The use of elevated temperatures, also termed heat treatments, has long been documented as an effective approach for managing stored-product insects infesting food-processing facilities (Mahroof et al. 2003a). It is becoming popular as a methyl bromide alternative because of the phase out of methyl bromide by 2005 in the United States (Boina and Subramanyam 2004, 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^{o}C, and hold these elevated temperatures for 24-36 h to help heat penetrate throughout the entire space of the facility for effective disinfestation. Limited quantitative data are available on relative susceptibility of life stages and time-mortality relationships for the cigarette beetle, *Lasioderma serricorne* (F.), an important pest associated with food-processing facilities (Sinha and Watters 1985), exposed to elevated temperatures. Adler (2003) carried out tests using mixed life stages of *L. serricorne* to determine their tolerance to elevated temperatures in a heated water bath; however, he was unable to find the effects of elevated temperatures on specific life stages of *L. serricorne.* Conyers and Collins (2006) assessed the effects of elevated temperatures on the mortality of *L. serricorne* life stages; nevertheless, not all of the life stages were tested, and insects were exposed to only two temperatures, 45 and 50^{o}C. Neither of them could definitively identify the most heat tolerant life stage of *L. serricorne* at elevated temperatures. Therefore, the studies described in this paper were conducted using all life stages of *L. serricorne* exposed to three constant elevated temperatures. Our objectives were to determine the relative susceptibility of *L. serricorne* life stages, develop time-mortality relationships, and compare lethal time 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 (Fields 1992); this should ensure control of all other stages. Furthermore, time-mortality responses 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 most heat-tolerant insect life stage during real facility heat treatments in which temperatures are dynamically 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 optimum range (28-32°C) 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 wt) plus brewer's yeast (5% by wt) diet. About 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 in 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. 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. 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. All life stages were separated from the rearing media with a U.S. Standard Sieve No. 60 (Fisher Scientific, Pittsburg, PA) with 250 µm openings. For field experiments, 20 individuals of a life stage were transferred to separate plastic test boxes (4.5×4.5×1.5 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 the food-processing facility subjected to heat treatment. This

particular facility conducts heat treatments in several rooms on a monthly basis 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 3-5 m). At each of the five locations, a HOBO® data-logging unit (Onset Computer Corp, Pocasset, MA) was placed to record temperature at one minute intervals during the entire heat treatment period (33 h). Test boxes were collected at 11, 24, and 33 h into the heat treatment. The number of test boxes collected at the specific time intervals varied from 1 to 12. 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* diet. The plastic containers were placed in a growth chamber set at 28°C and 65% RH. After 72 h, the diet was sifted using a 425 μm sieve to separate adults from the diet. Adult mortality, expressed as a percentage, was determined based on number of dead adults out of the total exposed. Immature stages were reared to adulthood 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.

Growth Chambers. Three growth chambers (Model I-36 VL, Percival Scientific, Perry, IA) were used for exposing life stages of *L. serricorne* to elevated constant temperatures of 46, 50, and 54°C and 22% RH. A humidity of 22% was used because during heat treatment, the humidity inside the facility is around 22-25% (Roesli et al. 2003, Mahroof et al. 2003a). A fourth growth chamber, was set at 28°C and 65% RH, served as the control treatment. The internal volume of growth chambers was 0.84 m^3 (29.5 ft^3). Air velocity, measured with an electronic wind speed indicator (Davis Instruments, San Leandro, CA), inside the growth chambers at 46-54°C ranged from ≈0.6-1.2 m/s.

In order to verify that the insects in test boxes are exposed to the set chamber temperature (46, 50, or 54°C) and humidity (22%), the air temperature and relative humidity inside growth chambers and inside test boxes with that of *L. serricorne* diet were measured using HOBO® data-logging units. At each temperature, a HOBO® unit was placed in each of the four corners and the center of the top shelf inside growth chambers. The thermocouple wire of the HOBO® unit was inserted through the wire mesh covering the lid of each test box containing 100 mg of *L. serricorne* diet such that

the thermocouple wire was in contact with the diet. These boxes also were placed in the four corners and center of the top shelf. This experiment was replicated three times. The accuracy of each HOBO® unit was verified with a mercury thermometer before use and was within 0.1°C of the reading from 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 20-22% RH. Thermocouples of HOBO® units were stuck to the bottom of test boxes below the diet and the time taken for the diet to reach the set chamber temperature was recorded. Each experiment was replicated three times.

Fixed-Times Responses of Life Stages at Three Elevated Temperatures. In order to determine the most heat-tolerant stage, test boxes each with 50 specific life stages of *L. serricorne* were exposed to 46, 50 and 54°C and 20-22% RH. Two experiments were conducted, because in the first experiment, 100% of young larvae, old larvae, pupae and adults died at 50°C and 100% of all life stages died at 54°C. 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. In the second experiment, the exposure times at 46, 50 and 54°C were 240, 60, and 30 min, respectively. 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, and sampled at the same fixed times corresponding to each elevated temperature.

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 20-22% 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 mortality of eggs was determined by placing three boxes, each with 50 eggs and 100 mg of *L. serricorne* diet at 28°C and 65% RH (control treatment) for the maximum duration corresponding to each elevated temperature treatment. The control treatment was replicated three times. The experiment

at each elevated temperature was replicated three times, and for each temperature, replication and exposure time combination, three boxes were removed to assess egg mortality using two different approaches. In the first approach, 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 for a week before examining each box for egg hatchability. In the second approach, time-mortality experiments were performed as explained above, except that test boxes with eggs removed from the elevated temperature treatments and the control treatment were immediately transferred to 150-ml plastic container with 10 g of *L. serricorne* diet. These containers were placed at 28^{o}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.

Data Analysis. The temperature data from HOBO® units at each of the five locations was used to determine the starting temperature, time required to reach 50^{o}C, time above 50^{o}C, and the maximum temperature. The mortality of *L. serricorne* life stages were summarized by location, 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 elevated temperatures were corrected for natural mortality using Abbott's (1925) formula. Corrected mortality data at each temperature were transformed to angular values (Zar 1984) and subjected to one-way analysis of variance (ANOVA) and Fisher's protected least significant difference (lsd) test $\alpha = 0.05$ level to determine significant differences among stages using the GLM procedure of SAS (SAS Institute 2003).

Corrected time-mortality responses 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) 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 was compared using a χ^2 statistic (SAS Institute 2003).

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

test (Robertson and Preisler 1992). The two LT_{99} values being compared are 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 6). Despite slow heating rates (1.3-2.5°C/h), temperatures above 50°C were maintained for 21 to 27 h. Except for one location, the maximum temperature did not exceed 60°C.

The number of eggs, young larvae, old larvae, and adults in test boxes available at the time of the facility heat treatment were sufficient for exposure in locations 1, 2, and 3, and therefore, only the egg stage was available in adequate numbers for exposure in locations 4 and 5. 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 7). 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. Only in location 2, where the heating rate was 2.5°C/h, it appeared that the eggs were the most heat tolerant stage when compared with other stages based on 10% survival at 11 h. However, in locations 1 and 3, where the heating rate was 1.3 to 1.5°C/h, young larvae and adults appeared to be more heat tolerant than eggs. The lack of consistent trends in stage-specific susceptibility made it difficult to discern a heat-tolerant stage based on results at locations 1 through 3. Therefore, laboratory experiments were needed to determine relative susceptibility of *L. serricorne* life stages to elevated temperatures.

None of the eggs survived heat treatment 24 and 33 h into the heat treatment, but some survival was observed 11 h into the heat treatment. Egg survival at location 4 was comparable to survival at location 2 and survival at location 5 was comparable to that of location 1, and these survival rates could be related to the heating rates. The survival rates of life stages, at least at 11 h into the heat treatment, seems to be higher at low heating rates than high heating rates.

Temperature and Humidity Measurements in Growth Chambers. The temperatures 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 8). 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 took 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 periods.

Fixed Time-Mortality Responses of Life Stages. In the first experiment, the exposure time of 40 min at 54°C resulted in 100% mortality of all life stages (Table 9). Similarly, the 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 46 and 50°C showed eggs to be the most heat tolerant stage.

In the second experiment, exposure times that were 10 to 60 min shorter than the first experiment were used to ensure survival of the life stages, especially at 50 and 54°C. There were significant differences in the mortality of life stages at 46°C ($F = 323.04$, df = 4, 10, $P < 0.0001$), 50°C ($F = 51.56$, df = 4, 10, $P < 0.0001$), and 54°C ($F = 59.37$, df = 4, 10, $P < 0.0001$), respectively. Clear cut susceptibility differences among stages were observed at 46°C, but at 50 and 54°C differences among certain stages disappeared because of increased susceptibility (Table 10). However, eggs were always significantly ($P < 0.05$) less susceptible when compared with other stages at each of the temperatures. These experiments also confirmed eggs to be the most heat tolerant stage.

Time-Mortality Responses of Eggs at Elevated Temperatures. The probit regression estimates based on egg hatchability and egg-to adult emergence are shown in Table 11. In general, the intercepts, slopes, and lethal time estimates at each of the three temperatures based on the two approaches for egg mortality assessment were very similar. In general, the lethal time estimates decreased with an increase in temperature. Irrespective of the mortality assessment method used, the lethal time estimates (LT_{50}

and/or LT_{99}) are 3 to 6-fold lower at 50°C when compared to 46°C and 4 to 5-fold lower at 54°C when compared to 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 12), but the LT_{99} values between any two elevated temperatures based on egg hatchability or adult emergence were significantly different from one another ($P < 0.05$). These results indicated that approximately 10 h are needed to kill 99% of *L. serricorne* eggs at 46°C, 3 h at 50°C and about 0.6 h at 54°C.

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). It is unclear why there is a discrepancy in definitively identifying a heat tolerant stage during facility heat treatments. It is plausible that the heating rate may have an impact on which stages develop influence heat tolerance during facility heat treatments, and this aspect warrants further study.

The fixed-time mortality experiments showed eggs to be the most heat-tolerant of all *L. serricorne* stages. A comparison of LT_{99} value of *L. serricorne* eggs at 50 and 54°C with that of other species showed that the *L. serricorne* eggs were relatively more heat tolerant than other stored-product insect species. For example, at 50°C the time required to kill 99% of eggs of *L. serricorne*, *T. castaneum* (Mahroof et al. 2003b), the confused flour beetle, *Tribolium confusum* (Jacquelin du Val) (Boina and Subramanyam 2004), and the Indianmeal moth, *Plodia interpuntella* (Hübner) (Mahroof and Subramanyam 2006), was 165, 105, 41 and 29 min, respectively. Similarly, at 54°C the time required to kill 99% of eggs of *L. serricorne*, *T. castaneum*, and *T. confusum* was 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 is 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 stages may vary based on heating rates or temperature. The limited data of Adler (2003) and Conyers and Collins (2006) also suggested 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 10 h observed by Conyers and Collins (2006) for eggs of *L. serricorne* at 50°C was 7 h higher 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.

The rapid drop in lethal time estimates at 50 and 54°C as compared to 46°C is due to rapid mortality of eggs at higher temperatures. A comparison of LT_{99} values based on egg hatchability and egg-to-adult emergence yielded identical results at the three elevated temperatures. Therefore, either method can be used for assessing egg mortality. 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 takes only 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 as test insects 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.

Developing and validating a dynamic thermal death kinetic model for predicting survival of eggs of *L. serricorne* during real facility heat treatments will be conducted in the ongoing term.

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Table 6. Temperature data at five locations where test boxes with *L. serricorne* life stages were placed during heat treatment of a food-processing facility, July 20-22, 2007.

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, P, pupae, and A, adults.

[b]Rate = (50°C – 34°C)/time to 50°C.

Table 7. Mortality of *L. serricorne* life stages in test boxes at five locations sampled at three different time intervals during a heat treatment.

Location	Stage[a]	Sample collection time (h)	Temp at sample collection	No. test boxes	No. dead/ 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	1	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	1	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

Location	Stage[a]	Sample collection time (h)	Temp at sample collection	No. test boxes	No. dead/ total	Mortality (%)
2	A	33.7	64.2	1	20/20	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, P, pupae, and A, adults.

Table 8. Comparison of temperature 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 (^{o}C)	RH (%)	Temp (^{o}C)	RH (%)	Temp (^{o}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 data observations collected over time used for computing means and associated standard errors.

[b]Control growth chamber.

Table 9. Corrected mortality (%mean ± SE) of *L. serricorne* life stages exposed for fixed time periods at three elevated temperatures (experiment I).

Stage	46°C (300 min)	50°C (90 min)	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

Mean (n = 3) mortality of eggs, young larvae, old larvae, pupae, adults in the control treatment over the three temperatures ranged from 15 to 20, 13 to 16, 12 to 16, 6 to 7, and 0% , respectively.

Table 10. Corrected mortality (%mean ± SE) of *L. serricorne* life stages exposed for fixed time periods at three elevated temperatures (experiment 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 9 for control mortality.

Table 11. 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

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

[b]N, total number of insects exposed.

[c]χ^2 values for goodness-of-fit of the CLL regression model to the observed mortality data.

*Significant ($P < 0.05$).

Table 12. Pair wise 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)*

[a]The 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).

*Significant ($P < 0.05$).