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Author(s): Rizana Mahroof , Bhadriraju Subramanyam , James E. Throne , and Anil Menon

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## Time-Mortality Relationships for *Tribolium castaneum* (Coleoptera: Tenebrionidae) Life Stages Exposed to Elevated Temperatures

RIZANA MAHROOF,<sup>1</sup> BHADRIRAJU SUBRAMANYAM,<sup>2</sup> JAMES E. THRONE,<sup>3</sup> AND ANIL MENON<sup>4</sup>

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**ABSTRACT** The use of elevated temperatures ( $\geq 40$ – $60^{\circ}\text{C}$ ) or heat treatments for managing insects in food-processing facilities is a viable alternative to space fumigation with methyl bromide. Quantitative data are lacking on the responses of life stages of the red flour beetle, *Tribolium castaneum* (Herbst), an important pest of food-processing facilities worldwide, to elevated temperatures used during heat treatments. We determined time-mortality relationships for eggs, young (neonate) larvae, old larvae, pupae, and adults of *T. castaneum*, exposed to constant temperatures of 42, 46, 50, 54, 58, and  $60^{\circ}\text{C}$ . Generally, mortality of each stage increased with an increase in temperature and exposure time. Young larvae were the most heat-tolerant stage, especially at temperatures  $\geq 50^{\circ}\text{C}$ . Exposure for a minimum of 7.2 h at  $\geq 50^{\circ}\text{C}$  was required to kill 99% of young larvae, whereas the other stages required  $\leq 1.8$  h. Heat treatments that control young larvae should control all other stages of *T. castaneum*, and young larvae should be used as test insects to evaluate efficacy against *T. castaneum* during an actual facility heat treatment. These results provide the basis for successful use of elevated temperatures for management of *T. castaneum* life stages associated with food-processing facilities.

**KEY WORDS** methyl bromide alternative, heat treatment, heat tolerance, *Tribolium castaneum*

THE USE OF ELEVATED temperatures or heat treatments has long been recognized as an effective strategy for managing stored-product insects associated with food-processing facilities, such as flour mills (Dean 1911, 1913). Heat treatment involves raising the ambient temperature of the whole or a portion of the facility to  $50$ – $60^{\circ}\text{C}$  and holding these elevated temperatures for 24–36 h (Dowdy and Fields 2002, Wright et al. 2002). The rate of heating of different floors of a food-processing facility during heat treatment can vary between 3 and  $14^{\circ}\text{C}/\text{h}$  (Roesli et al. 2003), and on some floors heating rates can be as low as  $0.3$ – $0.9^{\circ}\text{C}/\text{h}$  (Mahroof et al. 2003). This method of pest control was not readily adopted by the milling industry because anecdotal reports by users indicated damage to wooden structures, stretching of line belts, and degreasing of unsealed bearings (Imholte and Imholte-Tauscher 1999). Data have not been scientifically collected on the adverse effects of high temperatures on the structural integrity of the building, building ma-

terials, and equipment. Data are also lacking on the rate of heating during heat treatments on susceptibility of stored-product insects.

Facility heat treatments are labor intensive, because grain and grain products within the facility should be cleaned or removed, as they are poor conductors of heat. Insects may hide in these materials and escape the heat treatment. Therefore, heat treatments were abandoned soon after the commercialization and use of the fumigant methyl bromide in the 1930s, as the latter was inexpensive and more efficient in killing insects. Recent concern over the ozone-depleting ability of methyl bromide (Makhijani and Gurney 1995) has resulted in efforts to phase out production and use of this fumigant in the United States by the year 2005 (Fields and White 2001). The uncertain future of methyl bromide has renewed interest in exploring heat treatment as a potential methyl bromide alternative, not only for controlling insects in food-processing facilities, but also for disinfecting fresh horticultural products, dried fruits/nuts, and other perishables to provide quarantine security (Waddell et al. 2000, Wang et al. 2002a, b).

In facility heat treatments, gas, electric, or steam heaters are used to slowly heat the ambient air, and the long heat treatment period (24–36 h) is necessary for the heat to penetrate wall voids and equipment to kill insects harboring in them. In heat treatments of fresh fruits, nuts, or grains, high temperatures are used for short time periods to disinfest the products without

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<sup>1</sup> Department of Entomology, Kansas State University, Manhattan, KS 66506.

<sup>2</sup> Department of Grain Science and Industry, Kansas State University, Manhattan, KS 66506 (e-mail: bhs@wheat.ksu.edu).

<sup>3</sup> United States Department of Agriculture–Agricultural Research Service, Grain Marketing and Production Research Center, 1515 College Avenue, Manhattan, KS 66502.

<sup>4</sup> Wil-Kil Pest Control, Sun Prairie, Madison, WI 53590.

adversely affecting product quality (Evans 1986, Tang et al. 2000, Wang et al. 2002a). Furthermore, quantitative data are lacking on the temperature-time-mortality relationships for economically important stored-product insects at temperatures typically used during heat treatments (Oosthuizen 1935, Wright et al. 2002). In contrast, there is a wealth of information on optimizing heat treatments for disinfesting horticultural products (Landolt et al. 1984, Armstrong 1994, Neven 1994, Waddell et al. 2000, Wang et al. 2002a, b), dried fruits/nuts (Johnson et al. 2001, Wang et al. 2002a), and grains (Dermott and Evans 1978, Evans 1986, Beckett et al. 1998, Mourier and Poulsen 2000, Beckett and Morton 2003). Quantifying responses of stored-product insects at elevated temperatures is the first step toward determining the minimum temperature and time combinations needed for effective control of pests during heat treatment of a food-processing facility.

The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is a common insect pest associated with food-processing facilities worldwide (Sinha and Watters 1985, Mills and Pedersen 1990). The susceptibility of life stages of *T. castaneum* to elevated temperatures used for heat treatments is unknown. Dowdy (1999a) reported  $\leq 29\%$  mortality of *T. castaneum* adults when they were exposed for 15–30 min at 50°C and then allowed to recover without food for 1 d. Increasing the recovery period to 7 d resulted in 51–65% mortality. However, adult survival was greatly enhanced, or the lethal effects of temperature were reversed, if *T. castaneum* was allowed to recover on food (Dowdy 1999a). Dowdy and Fields (2002) reported mortality of *T. castaneum* adults during steam heat treatment of a pilot flour mill at Kansas State University (Manhattan, Kansas). Mortality of adults varied among mill floors, and in some mill areas where temperatures reached 47°C, 100% mortality of adults occurred after 25–43 h.

The present laboratory study was designed to determine time-mortality relationships for various life stages of *T. castaneum* at six constant temperatures between 42 and 60°C. Temperatures below and above 50°C were chosen, because during heat treatment temperatures stratify both vertically and horizontally, resulting in nonuniform heating of a food-processing facility (Dowdy 1999b, Dowdy and Fields 2002, Mahroof et al. 2003). Some areas may be underheated ( $<50^\circ\text{C}$ ), while others may be overheated ( $>50^\circ\text{C}$ ). Heat treating a facility with a temperature range below 42°C may typically take several days to achieve required killing of insects. At slow heating rates, most insects may have adequate time to adapt to the heat and increase thermal tolerance (Waddell et al. 2000). Furthermore, such a lengthy heat treatment may not be economically feasible. Temperatures  $>60^\circ\text{C}$  generally are not recommended for heat treatments because of possible damage to heat-sensitive equipment in the food-processing facility. Understanding minimum temperatures and time periods necessary to kill all stages of *T. castaneum* is important for optimizing facility heat treatments. The objectives of the work

reported in this study were to determine stage-specific susceptibility of *T. castaneum* to elevated temperatures experienced by insects during a heat treatment (Dowdy and Fields 2002, Roesli et al. 2003), and to identify the most heat-tolerant life stage.

### Materials and Methods

**Insects.** *T. castaneum* was reared on 95% whole-wheat flour and 5% (by weight) brewer's yeast at 28°C and 65% RH. Eggs and newly hatched (neonate) larvae were collected every 2 d from cultures and reared on bleached flour and powdered brewer's yeast in the same ratio as above. Eggs (2 d old), 6-d-old larvae (young larvae), 22-d-old larvae (old larvae), unsexed pupae (26 d old), and unsexed adults (2 wk old after emergence) were used in tests. The mean  $\pm$  SE ( $n = 15$ ) weight of young larvae was  $0.12 \pm 0.01$  mg, and that of old larvae was  $3.59 \pm 0.11$  mg. Old larvae, pupae, and adults were separated from the diet using a 0.83-mm sieve. Eggs and young larvae were separated from the diet using a 250- $\mu\text{m}$  sieve, and counted under a stereomicroscope.

**Insect Exposure.** Life stages of *T. castaneum* were transferred to separate square plastic boxes ( $4.5 \times 4.5 \times 1.5$  cm) with perforated lids covered with 600- $\mu\text{m}$  wire mesh screens. The diameter of the lid perforation was 3 cm. Each box held a mean  $\pm$  SE ( $n = 20$  replicates) of  $305 \pm 3$  mg of bleached wheat flour. Twenty individuals of each *T. castaneum* life stage were introduced into each box. Boxes with insects were exposed in growth chambers (model I-36 VL; Percival Scientific, Perry, IA) to 42, 46, 50, 54, 58, and 60°C for establishing time-mortality relationships for each life stage. The interior volume of the growth chamber was  $0.84 \text{ m}^3$  (29.6  $\text{feet}^3$ ). The plastic test boxes were placed on the top shelf of the growth chamber. Air speed, measured with an electronic wind speed indicator (Davis Instruments, San Leandro, CA), near the top shelf ranged from 0.6 m/s at 42°C to 1.2 m/s at 60°C. The relative humidity at each temperature was 20–22%, typical of humidity levels during commercial heat treatments (Dowdy and Fields 2002, Mahroof et al. 2003).

The flour introduced into boxes was at room temperature before exposure to elevated temperatures. Therefore, to determine the time required for the flour to reach the chamber temperatures of 42–60°C, a preliminary trial was conducted. At each temperature, thermocouples from three HOBO data-logging units (Onset Computer Corporation, Bourne, MA) were placed in the flour inside three separate test boxes. At temperatures of 42, 46, 50, 56, and 60°C, it took 16 (range, 15.5–16.5), 13 (12.7–13.5), 8 (7.9–9.1), 5 (4.7–5.5), and 4 (3.7–4.1) min ( $n = 3$ ), respectively, for the flour to reach the set chamber temperature. A linear regression model was fit to these data (SAS Institute 1998) to determine the time needed for the flour to reach the set chamber temperature of 42–60°C.

At each of the six constant temperatures, boxes with a particular *T. castaneum* life stage were removed at

**Table 1.** Temperature and humidity (mean  $\pm$  SE) of flour inside the plastic test boxes and air temperature and humidity near the top shelf of the growth chamber relative to the set chamber parameters

| Set chamber parameters |        | Top shelf of growth chamber         |                          | Flour inside test boxes            |                          |
|------------------------|--------|-------------------------------------|--------------------------|------------------------------------|--------------------------|
| Temp. ( $^{\circ}$ C)  | RH (%) | Temp. ( $^{\circ}$ C) <sup>a</sup>  | RH (%) <sup>a</sup>      | Temp. ( $^{\circ}$ C) <sup>a</sup> | RH (%) <sup>a</sup>      |
| 42                     | 22     | 42.01 $\pm$ 0.03 (369) <sup>b</sup> | 21.57 $\pm$ 0.02 (369)   | 42.62 $\pm$ 0.71 (47)              | 22.54 $\pm$ 1.27 (47)    |
| 46                     | 22     | 46.01 $\pm$ 0.04 (245)              | 21.80 $\pm$ 0.00 (245)   | 46.34 $\pm$ 0.17 (35)              | 21.80 $\pm$ 0.01 (35)    |
| 50                     | 21     | 50.01 $\pm$ 0.13 (315)              | 21.50 $\pm$ 0.01 (315)   | 50.17 $\pm$ 0.12 (15)              | 21.51 $\pm$ 0.01 (15)    |
| 54                     | 22     | 54.02 $\pm$ 0.04 (311)              | 21.60 $\pm$ 0.30 (311)   | 54.19 $\pm$ 0.09 (11)              | 21.68 $\pm$ 1.65 (11)    |
| 58                     | 21     | 58.02 $\pm$ 0.09 (260)              | 21.40 $\pm$ 0.50 (260)   | 58.27 $\pm$ 0.15 (24)              | 20.90 $\pm$ 0.01 (24)    |
| 60                     | 21     | 60.09 $\pm$ 0.01 (315)              | 20.70 $\pm$ 0.01 (315)   | 60.03 $\pm$ 0.08 (15)              | 20.74 $\pm$ 0.37 (15)    |
| 28 <sup>c</sup>        | 25     | 28.26 $\pm$ 0.11 (2,496)            | 44.52 $\pm$ 0.28 (2,496) | 28.26 $\pm$ 0.11 (2,496)           | 44.52 $\pm$ 0.28 (2,496) |

<sup>a</sup>Temperature and humidity data were collected by two HOBO® data-logging units. Each mean represents pooled HOBO® data from the two units collected at every 3-min interval over time. Data at 28°C were collected at 5-min intervals.

<sup>b</sup>Numbers in parentheses represent the number of data observations collected over time used for computing the means and associated standard errors.

<sup>c</sup>Control growth chamber.

different exposure periods, after accounting for the time required for the flour to reach the set chamber temperature. Five boxes were removed at each exposure period. The control treatment consisted of five boxes with a *T. castaneum* life stage held in a chamber at 28.2  $\pm$  0.2°C and 44.5  $\pm$  0.3% RH. There was a separate control treatment for each temperature treatment.

**Temperature and Humidity Measurements.** Temperatures measured by the HOBO units were compared with those measured by mercury thermometers, and found to be accurate ( $\pm$ 0.01°C). Two HOBO data-logging units were used to measure temperature and relative humidity inside 2 of the 50 plastic boxes placed on the top shelf of the growth chamber at each of the six temperatures. Temperature and relative humidity near the top shelf of the chamber (next to the test boxes) were measured with two HOBO units. All HOBO units were launched by a computer to record temperature and RH data at 3- to 5-min intervals from the beginning until the end of the experiments.

**Determining Insect Mortality.** Boxes with adults in wheat flour were removed at different time periods from growth chambers and held for an additional 24 h at 28.2  $\pm$  0.1°C and 44.5  $\pm$  0.3% RH (control chamber) before assessing mortality. Mortality of adults was based on number dead out of the total exposed. Pupae were held in the same boxes in the control chamber until emergence of adults. Wheat flour from boxes containing eggs, young larvae, and old larvae was transferred to 150-ml plastic containers, each holding 40 g of whole-wheat flour plus 5% (by weight) brewer's yeast. Containers were placed in a control chamber until emergence of adults. Mortality of all immature stages was based on those that failed to emerge into adults.

**Data Analyses.** The mean  $\pm$  SE ( $n$  = 30) mortality of eggs, young larvae, old larvae, pupae, and adults in the control chamber was 2.6  $\pm$  1.3, 1.7  $\pm$  1.1, 1.2  $\pm$  0.8, 1.0  $\pm$  0.7, and 1.6  $\pm$  0.8%, respectively. Therefore, time-mortality data for *T. castaneum* life stages exposed to elevated temperatures were not corrected for control mortality. Time-mortality data for each *T. castaneum* life stage at 42–60°C were fit to the comple-

mentary log-log (CLL) regression model (Robertson and Preisler 1992), to estimate the time required to kill 50% (LT<sub>50</sub>) and 99% (LT<sub>99</sub>) of the exposed insects (SAS Institute 1998). In the CLL model, percentage mortality ( $y$ ) is transformed to  $\log_e(-\log_e[1 - y/100])$  scale, and exposure time ( $x$ ) is transformed to  $\log_{10}$  scale. The goodness-of-fit of the CLL model to the data was compared using a  $\chi^2$  statistic (SAS Institute 1998, Robertson and Preisler 1992).

The LT<sub>99</sub> values of any two red flour beetle life stages at each temperature were compared using lethal time ratios, as described by Robertson and Preisler (1992). Lethal time ratios make no assumptions about the parallelism of the responses being compared. At each temperature, there were 10 pairwise comparisons among the five stages tested. The LT<sub>99</sub> values are not significantly different from one another ( $P$  > 0.05) if the 95% confidence limit (CL) for the ratio includes one (Robertson and Preisler 1992).

The change in LT<sub>99</sub> with temperature for each stage was described using equation 1 (Jandel Scientific 1994):  $\ln y = a + bx$  (1), where  $y$  is the LT<sub>99</sub> in min,  $x$  is the temperature between 50 and 60°C, and  $a$  and  $b$  are constants estimated from the LT<sub>99</sub>-temperature data. Temperatures  $\geq$ 50°C were used because, during heat treatment, the target temperature for effective disinfestation should be at least 50°C (Imholte and Imholte-Tauscher 1999, Wright et al. 2002).

## Results

**Temperature and Humidity Measurements.** The time required for the flour temperatures inside boxes to reach the set chamber temperature decreased with an increase in temperature. The linear regression,  $y = 44.24 (\pm 4.77) - 0.69 (\pm 0.09) x$  (where  $y$  = time in min and  $x$  = temperature in  $^{\circ}$ C;  $n$  = 5;  $R^2$  = 0.948), indicated that at 42°C  $\approx$ 15 min were necessary for the flour temperature to reach the set chamber temperature, and for every 1°C rise in temperature from 42 to 60°C the time required decreased by  $\approx$ 41 s. Both the temperature and relative humidity measured outside and inside the test boxes were similar (Table 1) at the set chamber temperatures of 42–60°C. The measured

Table 2. Time-mortality regression estimates for *T. castaneum* life stages exposed to six constant temperatures

| Temp. (°C) | Stage <sup>a</sup> | Total no. insects | Intercept ± SE | Slope ± SE | LT <sub>50</sub> (95% CL) (min) | LT <sub>99</sub> (95% CL) (min) | χ <sup>2</sup> (df) <sup>b</sup> |
|------------|--------------------|-------------------|----------------|------------|---------------------------------|---------------------------------|----------------------------------|
| 42         | E                  | 1,100             | -6.3 ± 1.3     | 2.5 ± 0.5  | 225.5 (175.8–276.2)             | 1,273.0 (734.5–5,574.0)         | 40.2 (9)*                        |
|            | YL                 | 2,520             | -8.9 ± 1.1     | 3.0 ± 0.4  | 667.9 (604.8–752.2)             | 2,850.0 (2,023.0–5,096.0)       | 85.8 (23)*                       |
|            | OL                 | 1,100             | -22.7 ± 8.2    | 6.6 ± 2.4  | 2,371.0 (947.8–2,690.0)         | 4,579.0 (3,705.0–28,377.0)      | 99.7 (9)*                        |
|            | P                  | 980               | -17.9 ± 1.7    | 5.2 ± 0.5  | 2,186.0 (1,959.0–2,383.0)       | 5,022.0 (4,536.0–5,747.0)       | 7.6 (8)                          |
|            | A                  | 1,400             | -32.1 ± 1.6    | 9.3 ± 0.4  | 2,645.0 (2,579.0–2,706)         | 4,235.0 (4,079.0–4,424.0)       | 16.6 (12)                        |
| 46         | E                  | 1,800             | -3.4 ± 0.4     | 1.8 ± 0.2  | 53.5 (35.4–69.3)                | 635.9 (448.4–1,135.0)           | 56.1 (15)*                       |
|            | YL                 | 2,100             | -11.7 ± 1.6    | 5.0 ± 0.7  | 181.3 (156.6–202.1)             | 430.7 (364.3–573.6)             | 190.6 (19)*                      |
|            | OL                 | 1,180             | -11.4 ± 1.8    | 4.7 ± 0.7  | 219.0 (187.6–249.2)             | 551.5 (432.2–889.7)             | 61.8 (10)*                       |
|            | P                  | 1,400             | -34.0 ± 2.4    | 12.3 ± 0.9 | 547.5 (531.3–562.3)             | 780.4 (745.9–828.1)             | 25.4 (12)*                       |
|            | A                  | 1,200             | -42.4 ± 3.9    | 16.2 ± 1.5 | 380.3 (365.9–392.9)             | 497.4 (476.0–528.7)             | 30.9 (10)*                       |
| 50         | E                  | 1,000             | -5.3 ± 1.1     | 3.4 ± 0.7  | 28.9 (19.7–34.6)                | 105.1 (77.0–230.8)              | 42.3 (8)*                        |
|            | YL                 | 2,100             | -11.7 ± 1.4    | 5.0 ± 0.7  | 181.3 (156.6–202.1)             | 432.8 (365.3–572.6)             | 191 (19)*                        |
|            | OL                 | 1,000             | -30.5 ± 6.9    | 17.6 ± 3.9 | 51.6 (45.2–55.9)                | 66.1 (60.1–83.2)                | 135.9 (8)*                       |
|            | P                  | 700               | -22.4 ± 2.8    | 12.3 ± 1.5 | 61.2 (56.4–64.9)                | 87.2 (80.5–99.9)                | 13.3 (5)*                        |
|            | A                  | 800               | -25.8 ± 3.2    | 15.9 ± 1.9 | 39.9 (37.4–41.9)                | 52.5 (49.3–58.3)                | 22.6 (6)*                        |
| 54         | E                  | 1,000             | -7.7 ± 1.7     | 5.9 ± 1.2  | 17.6 (12.0–21.3)                | 36.9 (29.9–58.6)                | 87.1 (8)*                        |
|            | YL                 | 1,000             | -8.5 ± 1.9     | 5.3 ± 1.2  | 35.9 (28.2–43.4)                | 81.9 (60.4–207.7)               | 93.3 (8)*                        |
|            | OL                 | 1,000             | -6.9 ± 1.1     | 4.7 ± 0.8  | 25.4 (19.4–29.9)                | 64.4 (51.9–98.4)                | 66.5 (8)*                        |
|            | P                  | 1,000             | -12.6 ± 1.4    | 9.2 ± 1.0  | 21.1 (19.4–22.5)                | 33.8 (30.8–39.2)                | 30.6 (8)*                        |
|            | A                  | 1,000             | -7.3 ± 1.4     | 5.9 ± 1.0  | 15.4 (11.7–17.9)                | 32.3 (27.2–45.1)                | 72.8 (8)*                        |
| 58         | E                  | 1,000             | -8.8 ± 1.1     | 8.1 ± 1.0  | 11.0 (9.8–12.1)                 | 18.9 (16.9–22.8)                | 26.5 (8)*                        |
|            | YL                 | 1,000             | -6.7 ± 1.5     | 5.2 ± 1.1  | 16.6 (11.8–19.9)                | 38.0 (29.4–75.8)                | 109.3 (8)*                       |
|            | OL                 | 1,000             | -3.4 ± 0.7     | 3.3 ± 0.7  | 7.9 (5.2–9.9)                   | 29.1 (20.5–69.1)                | 69.2 (8)*                        |
|            | P                  | 1,000             | -11.4 ± 2.6    | 10.7 ± 2.4 | 10.9 (8.8–12.4)                 | 16.4 (14.1–24.0)                | 74.5 (8)*                        |
|            | A                  | 800               | -11.0 ± 1.8    | 9.2 ± 0.9  | 14.4 (13.7–15.4)                | 23.3 (21.1–26.4)                | 15.8 (6)*                        |
| 60         | E                  | 980               | -5.7 ± 1.0     | 6.0 ± 1.0  | 7.7 (5.9–8.9)                   | 15.9 (13.5–21.8)                | 34.9 (8)*                        |
|            | YL                 | 1,020             | -7.5 ± 1.2     | 5.5 ± 0.9  | 19.1 (16.0–21.7)                | 41.9 (33.8–65.7)                | 57.2 (8)*                        |
|            | OL                 | 1,000             | -10.8 ± 1.7    | 9.8 ± 1.5  | 11.3 (9.9–12.5)                 | 17.6 (15.8–21.5)                | 39.3 (8)*                        |
|            | P                  | 1,000             | -7.5 ± 1.3     | 7.1 ± 1.2  | 10.2 (8.3–11.6)                 | 18.8 (16.1–25.4)                | 46.1 (8)*                        |
|            | A                  | 900               | -5.9 ± 1.5     | 5.7 ± 1.4  | 9.1 (5.4–11.5)                  | 19.4 (15.1–37.2)                | 99.5 (7)*                        |

<sup>a</sup>E, eggs; YL, young larvae; OL, old larvae; P, pupae; and A, adults.<sup>b</sup>χ<sup>2</sup> values for goodness-of-fit of the CLL regression model to the observed mortality data.\* Significant ( $P < 0.05$ ).

humidity in the control chamber was 1.8 times higher than the set humidity. This increase in humidity was of little practical significance, because control mortality of all stages was negligible (<3%).

**Time-Mortality Responses.** The χ<sup>2</sup> values were not significant ( $P > 0.05$ ) for 2 of the 30 time-mortality regressions, indicating good fit of data to the CLL model (Table 2). Significant χ<sup>2</sup> values ( $P < 0.05$ ) for the remaining 28 time-mortality curves indicated that the responses of *T. castaneum* life stages were heterogeneous at each of the six temperatures. In general, the LT<sub>99</sub> values decreased with an increase in temperature.

Ratio tests indicated that differences among the stages in the LT<sub>99</sub> values were not consistent across the six constant temperatures (Table 3). For example, the LT<sub>99</sub> value for young larvae at 42°C was not significantly different ( $P > 0.05$ ) from similar values for eggs, old larvae, and adults. The LT<sub>99</sub> values at 42°C were similar ( $P > 0.05$ ) between old larvae and pupae or between old larvae and adults. The remaining five pairwise comparisons among stages showed that the LT<sub>99</sub> values were significantly different from one another (Table 3). Eggs and young larvae were more susceptible to heat than other stages at 42°C, and eggs were more susceptible than young larvae. Of the 10 possible pairwise comparisons at 46°C, 7 were not significant ( $P > 0.05$ ). The only significant LT<sub>99</sub> values

were between young larvae and pupae, old larvae and pupae, and old larvae and adults. The LT<sub>99</sub> values for eggs and young larvae and eggs and pupae at 50°C were the only two comparisons that were not significant. In general, young larvae were the most tolerant stage at 50°C, because it had the highest LT<sub>99</sub> value (432.8 min, Table 2). Young larvae also had the highest LT<sub>99</sub> value at 54°C (81.9 min). The LT<sub>99</sub> value for young larvae was significantly different from that for eggs, pupae, and adults, but it was not significantly different from that for old larvae at 54°C. Young larvae had significantly higher LT<sub>99</sub> value at 58°C when compared with that for eggs and adults. Similarly, young larvae had significantly higher LT<sub>99</sub> value at 60°C when compared with that for eggs, old larvae, pupae, and adults.

The change in LT<sub>99</sub> with temperature between 50 and 60°C for each stage (Fig. 1) was best described by equation 1 ( $R^2 = 0.74 - 0.99$ ). The estimated parameters of the nonlinear regression model for all stages were significantly greater than zero (parameter *a*: *t*-value range among stages, 8.52–13.54; df = 2;  $P < 0.01$ ; parameter *b*: *t*-value range among stages, -9.66 to -1.59; df = 2;  $P < 0.02$ ). In general, young larvae of *T. castaneum* were relatively more heat tolerant than other life stages. The LT<sub>99</sub> for young larvae decreased by 5-fold as the temperature increased from 50 to 54°C. Beyond 54°C, the decrease in LT<sub>99</sub> was <2-fold.



**Table 3.** Significant ( $P < 0.05$ ) pairwise comparisons of  $LT_{99}$  values among *T. castaneum* life stages at each of the six constant temperatures

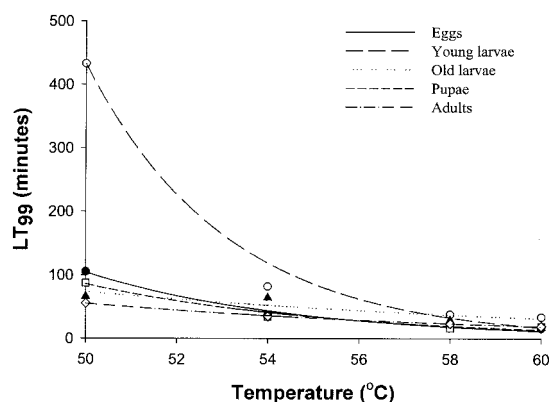
| Temp. (°C) | Stages compared <sup>a</sup> | $LT_{99}$ ratio (95% CL) <sup>b</sup> |
|------------|------------------------------|---------------------------------------|
| 42         | Eggs vs old larvae           | 3.6 (1.7–7.8)                         |
|            | Eggs vs pupae                | 3.9 (1.9–8.1)                         |
|            | Eggs vs adults               | 3.3 (1.6–6.7)                         |
|            | Young larvae vs pupae        | 1.8 (1.1–2.7)                         |
|            | Pupae vs adults              | 1.1 (1.04–1.34)                       |
| 46         | Young larvae vs pupae        | 1.8 (1.5–2.2)                         |
|            | Old larvae vs pupae          | 1.4 (1.1–1.9)                         |
|            | Old larvae vs adults         | 1.6 (1.5–1.7)                         |
| 50         | Eggs vs old larvae           | 1.6 (1.1–2.3)                         |
|            | Eggs vs adults               | 1.9 (1.4–2.9)                         |
|            | Young larvae vs old larvae   | 6.6 (5.2–8.3)                         |
|            | Young larvae vs pupae        | 5.0 (4.0–6.2)                         |
|            | Young larvae vs adults       | 8.3 (6.7–10.3)                        |
|            | Old larvae vs pupae          | 1.3 (1.2–1.5)                         |
|            | Old larvae vs adults         | 1.3 (1.1–1.4)                         |
| 54         | Pupae vs adults              | 1.5 (1.5–1.8)                         |
|            | Eggs vs young larvae         | 2.2 (1.4–3.5)                         |
|            | Eggs vs old larvae           | 1.7 (1.2–2.4)                         |
|            | Young larvae vs pupae        | 2.4 (1.6–3.6)                         |
|            | Young larvae vs adults       | 2.5 (1.6–3.9)                         |
| 58         | Old larvae vs pupae          | 1.9 (1.4–2.5)                         |
|            | Old larvae vs adults         | 2.0 (1.4–2.7)                         |
|            | Eggs vs young larvae         | 2.0 (1.4–2.8)                         |
|            | Young larvae vs adults       | 1.7 (1.2–2.2)                         |
| 60         | Old larvae vs pupae          | 1.8 (1.1–2.8)                         |
|            | Eggs vs young larvae         | 2.6 (1.9–3.6)                         |
|            | Young larvae vs old larvae   | 2.4 (1.8–3.1)                         |
|            | Young larvae vs pupae        | 2.2 (1.6–3.0)                         |
|            | Young larvae vs adults       | 1.8 (1.4–2.4)                         |

<sup>a</sup>Stage in bold letters has the higher  $LT_{99}$  in the pair being compared.

<sup>b</sup>Ratio = larger  $LT_{99}$ /smaller  $LT_{99}$ . The  $LT_{99}$  between the two stages are significantly different ( $P < 0.05$ ) if the ratio does not include 1 (Robertson and Preisler 1992).

## Discussion

The six constant temperatures used in the present tests were above the physiological upper limit for *T. castaneum* and are not conducive for development or



**Fig. 1.** Observed and fitted lines (equation 1), describing  $LT_{99}$  of *T. castaneum* life stages as a function of temperature. Regression equations for the stages are: eggs,  $\ln y = 15.560 - 0.218x$ ; young larvae,  $\ln y = 22.220 - 0.323x$ ; old larvae,  $\ln y = 8.578 - 0.085x$ ; pupae,  $\ln y = 13.719 - 0.185x$ ; and adults,  $\ln y = 9.374 - 0.107x$ .

survival (Howe 1956, Fields 1992). In all tests, unsexed individuals of *T. castaneum* used were within 2 d of the specified age. The inadequate fit of the CLL model to time-mortality data in 28 of the 30 regressions may be attributed to the heterogeneous responses of age (both within and among life stages) or sex-related differences in susceptibility to heat. Furthermore, thermal acclimation of certain individuals at the six temperatures tested, especially at longer exposure times, may have contributed to the heterogeneity observed. The  $LT_{50}$ ,  $LT_{99}$  values, and associated 95% CL, and time-mortality regression slopes indicated that the mean heat tolerances and distribution of tolerances varied among the stages and temperatures. Heat tolerance in insects has been reported to vary within a developmental stage (Davison 1969, Bursell 1973) and among stages (Oosthuizen 1935, Evans 1981).

Comparative susceptibility of *T. castaneum* life stages to heat at each of the six temperatures yielded results that were inconsistent. However, at temperatures  $\geq 50^{\circ}\text{C}$ , young larvae appeared to be relatively more heat tolerant than eggs, old larvae, pupae, and adults. The lack of significant differences between young larvae and other stages at  $\geq 50^{\circ}\text{C}$  could be attributed to the heterogeneous responses observed in the study, which resulted in the large 95% CL around the lethal test ratios (Table 3) or because of the increased susceptibility of all stages, especially at 58–60°C. Nevertheless, equation 1 fit to  $LT_{99}$  and temperature data (Fig. 1) showed that young larvae were generally more heat tolerant than the other stages. Wright et al. (2002) reported that large larvae of the warehouse beetle, *Trogoderma variabile* (Ballion), were the most heat-tolerant stage, based on experiments in a growth chamber at 56°C and 0% RH. A 4-min exposure at this temperature resulted in 79% mortality of large larvae, whereas mortality of eggs, diapausing larvae, pupae, and female adults was 93–100%. Our results cannot be directly compared with that of Wright et al. (2002), because they did not report the susceptibility of small larvae of *T. variabile* to elevated temperatures. Furthermore, Wright et al. (2002) did not determine the stage-specific susceptibility of *T. variabile* at different elevated temperatures.

The decreased susceptibility of young larvae at elevated temperatures could be caused by higher metabolism or production of heat shock proteins. Bijok (1996) and Emekci et al. (2002) reported that newly hatched *T. castaneum* larvae had greater oxygen consumption per unit weight compared with eggs, old larvae, and pupae. Emekci et al. (2002) reported that respiration rates, measured in terms of carbon dioxide ( $\text{CO}_2$ ) emission, for *T. castaneum* eggs, young larvae, old larvae, pupae, and adults at 30°C were 0.012, 9.25, 8.45, 1.45, and 4.67 ml of  $\text{CO}_2$  /insect/h, respectively. Respiration rates corrected for insect weight were 0.3 (eggs), 29.1 (young larvae), 3.3 (old larvae), 0.6 (pupae), and 2.4 (adults) ml of  $\text{CO}_2$  /insect/h. Bijok (1996) inferred that the higher respiration rates may be attributable to higher metabolic rates often connected with a reaction to stress, and may enhance survival under unfavorable environmental conditions.

Currie and Tufts (1997) and Lewis et al. (1999) concluded that tolerance to high temperatures could be caused by the synthesis of stress proteins and other key metabolites in many organisms. In response to a sudden increase in temperature, the normal pattern of protein synthesis is halted, and a new set of proteins, called the heat shock proteins, is expressed. Generally, these proteins are thought to provide the cell with protection by preventing aggregation or improper folding of proteins. In addition, they are involved in redissolving and stabilizing proteins by targeting denatured proteins for degradation and removal, thereby ensuring the survival of the organism under stressful conditions that promote cell damage and death (Currie and Tufts 1997).

The longer time periods (21–83 h) required to kill 99% of all exposed stages at 42°C suggest that it is preferable to use temperatures  $\geq 50^\circ\text{C}$  during heat treatment, because of the reduced time necessary to kill insects. At temperatures  $\geq 50^\circ\text{C}$ , eggs, old larvae, pupae, and adults become highly susceptible to heat. At 50°C, to kill 99% of the exposed young larvae required 7.2 h, as opposed to 1.8 h for eggs, 1.1 h for old larvae, 1.5 h for pupae, and 0.9 h for adults. Similarly, at 60°C, a 42-min exposure was necessary to kill 99% of the exposed young larvae, whereas the remaining stages required <20 min. Therefore, during heat treatments, temperatures should be monitored to ensure that  $\geq 50^\circ\text{C}$  is achieved throughout the area being heated for at least 7.2 h to kill the most heat-tolerant young larvae of *T. castaneum*. Our data suggest that heat treatments aimed at controlling young larvae should be able to control all other stages. In addition, young larvae should be used as test insects to gauge the effectiveness of heat treatment against *T. castaneum* in a food-processing facility. The information reported in this work provides baseline data for successful use of elevated temperatures for management of *T. castaneum* life stages associated with food-processing facilities.

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