Relative Susceptibility of *Tribolium confusum* Life Stages Exposed to Elevated Temperatures

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ABSTRACT Methyl bromide, a space fumigant used in food-processing facilities, may be phased out in the United States by 2005. The use of elevated temperatures or heat treatment is gaining popularity as a methyl bromide alternative. During heat treatment, the temperature of the whole food-processing facility, or a portion of it, is raised and held between 50 and 60°C for 24–36 h to kill stored-product insects. We determined time–mortality responses of the confused flour beetle, *Tribolium confusum* (Jacquelin du Val), eggs, young larvae, old larvae, pupae, and adults exposed to six constant temperatures between 46 and 60°C. Responses of all five insect stages also were measured using exposure times of 160, 40, and 12 min at 46, 50, and 60°C, respectively. Time–mortality responses of all *T. confusum* life stages increased with an increase in exposure time and temperature. Both time–mortality and fixed time responses showed eggs and young larvae to be most susceptible at elevated temperatures and old larvae to be least susceptible. Our results suggest that old larvae should be used as test insects to gauge heat treatment effectiveness, because heat treatment aimed at controlling old larvae should be able to control all other *T. confusum* life stages. Besides providing baseline data for successful use of heat treatments, time–mortality data collected at the six temperatures can be used for developing thermal death kinetic models for this species to predict mortality during actual facility heat treatments.

KEY WORDS *Tribolium confusum*, elevated temperatures, methyl bromide alternative, heat tolerance

THE USE OF ELEVATED temperatures, or heat treatments, is becoming popular as a methyl bromide alternative for disinfesting food-processing facilities (Mahroof et al. 2003a, Roesli et al. 2003), because of the impending phase out of methyl bromide (Makhijani and Gurney 1995). Heat treatment consists of raising the ambient temperature of the entire facility, or a portion of it, to 50–60°C, and holding these temperatures for 24–36 h to help penetrate heat throughout the facility for effective disinfestation. Limited quantitative data are available on time–mortality relationships for economically important stored-product insects exposed to elevated temperatures used during facility heat treatments. Mahroof et al. (2003b) reported the time–mortality relationships for eggs, young larvae, old larvae, pupae, and adults of the red flour beetle, *Tribolium castaneum* (Herbst), exposed to six constant temperatures between 42 and 60°C. They found young larvae to be the most heat-tolerant stage. Wright et al. (2002) reported the large larvae or old larvae of the warehouse beetle, *Trogoderma variabile* Ballion, to be the most heat-tolerant stage at 56°C. Understanding relative susceptibility of insect life stages to elevated temperatures is important for identifying the most and least heat-tolerant stage (Mahroof et al. 2003b), because heat treatments aimed at the heat-tolerant life stage should be able to control all other stages. Furthermore, time–mortality data at constant temperatures can be used to develop dynamic thermal death kinetic models (Wang et al. 2002) for predicting mortality of insects during actual facility heat treatments in which temperatures are dynamically changing over time.

In the current study, we characterized time–mortality relationships at six constant temperatures between 46 and 60°C for eggs, young larvae, old larvae, pupae, and adults of the confused flour beetle, *Tribolium confusum* (Jacquelin du Val) (Coleoptera: Tenebrionidae), an important pest found in food-processing facilities (Sinha and Watters 1985, Mills and Pedersen 1990). The elevated temperatures tested (≥46°C) were well above the optimum range (27–32°C) for development and survival of *T. confusum* (Fields 1992). Although 50°C is the minimum temperature required for effective disinfestation (Wright

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et al. 2002, Roesli et al. 2003), vertical and horizontal stratification of temperatures during heat treatment results in temperatures below or above 50°C in some portions of the facility (Dowdy 1999, Dowdy and Fields 2002, Mahroof et al. 2003a). Therefore, temperatures between 46 and 60°C were used in this study. Our objectives were to determine the relative susceptibility of *T. confusum* life stages at elevated temperatures and to compare responses of *T. confusum* with responses of *T. castaneum* reported by Mahroof et al. (2003b).

Materials and Methods

Insects. Cultures of *T. confusum* were reared on 95% whole wheat flour and 5% brewer's yeast (by weight) at 28 ± 0.5°C, 65 ± 5% RH, and a photoperiod of 14:10 (L:D) h. Approximately 50 g of the rearing medium, held in 0.94-liter glass jars with wire mesh screen and filter paper lids, were infested with 250–300 adults of *T. confusum* collected from culture jars. The adults were moved to new rearing medium every 2 d. This procedure allowed us to obtain *T. confusum* life stages of specific age for use in tests. Eggs (2–3 d after oviposition), young larvae (2–3 d from the time of eclosion from eggs), old larvae (22–23 d old), unsexed pupae (2–3 d after pupation), and unsexed adults (2 wk after eclosion from pupae) were used in tests. Eggs and young larvae were separated from the rearing media using a sieve with 0.25-mm openings, whereas old larvae, pupae, and adults were separated from the media using a sieve with 0.83-mm openings. The mean ± SE ($n = 10$) weight of an egg, young larva, old larva, pupa, and adult was 0.006 ± 0.002, 0.009 ± 0.005, 3.677 ± 0.035, 3.343 ± 0.022, and 2.738 ± 0.019 mg, respectively. Fifty individuals of a life stage were transferred to separate plastic test boxes (4.5 by 4.5 by 1.5 cm), each holding 1.5 g of the rearing medium, for exposure at various constant temperatures. Test boxes had perforated lids (3-cm-diameter perforation) for ventilation. Lid perforations were covered with wire mesh screens of 0.6-mm openings to prevent insect escape.

Growth Chambers. Growth chambers (model I-36 VL, Percival Scientific, Perry, IA) were used for exposing insects to constant temperatures of 46, 48, 50, 54, 58, or 60°C. A growth chamber set at 28°C served as the control treatment. The internal volume of each growth chamber was 0.84 m^3 (29.5 $feet^3$). Air velocity, measured with an electronic wind speed indicator (Davis Instruments, San Leandro, CA), inside the growth chambers at 46–60°C ranged from ≈0.6 to 1.2 m/s.

Temperature and Relative Humidity Measurements. The air temperature and relative humidity inside growth chambers and that of the rearing medium in test boxes with insects were measured by using HOBO data loggers (Onset Computer Corporation, Bourne, MA). At each temperature, an HOBO data-logging unit was placed in each of the four corners and the center of the top shelf of the growth chamber. The thermocouple wire of the HOBO unit was inserted through the wire mesh covering the lid of each test box with 1.5 g of medium such that the thermocouple wire was in contact with the rearing medium. These boxes also were placed in the four corners and center of the top shelf. The accuracy of each HOBO unit was verified with a mercury thermometer before use and was within 0.01°C of the reading from the mercury thermometer.

Determining Flour Equilibration Time. The rearing medium used in test boxes was at room temperature (25–26°C). Therefore, the time required (time lag) for the medium to reach the set chamber temperature differed at different chamber temperatures. Two test boxes with 1.5 g of flour were placed in growth chambers set at 46, 48, 50, 54, 58, or 60°C and 20–22% RH. Thermocouples of HOBO data-logging units inserted into the flour in test boxes recorded the time taken for the flour to reach the set chamber temperature. This experiment was replicated 15 times. The relationship between average flour equilibration time and temperature was determined by a linear regression (SAS Institute 1999).

Insect Exposure. In the first test, test boxes each with 50 eggs, young larvae, old larvae, pupae, or adults were exposed in growth chambers set at 46, 48, 50, 54, 58, and 60°C. The relative humidity at these temperatures was maintained at 20–22%, typical of humidity levels observed during facility heat treatments (Mahroof et al. 2003a). At each temperature, two boxes were removed from the growth chamber at different time intervals after accounting for the flour equilibration time. The exposure time among stages at 46, 48, 50, 54, 58, and 60°C ranged from 40 to 290, 25 to 195, 10 to 76, 8 to 52, 5 to 33, and 3 to 13 min, respectively. HOBO data loggers showed that there was a slight drop in flour temperature when growth chamber doors were opened to remove the test boxes. This drop in temperature ranged from 0.0 to 1.0°C. However, the flour temperature returned to the set chamber temperature within 0–50 s. Natural mortality of each *T. confusum* life stage was determined by exposing insects in two test boxes with 1.5 g flour in a growth chamber set at 28°C and 65% RH for the maximum duration used for the elevated temperature treatments. Each temperature–time combination was replicated three times.

After removal from growth chambers, insects and flour in test boxes in the control and elevated temperature treatments were transferred to 150-ml plastic containers holding 10 g of flour. These plastic containers were placed in a growth chamber at 28°C and 65% RH to assess insect mortality. Containers were sifted after 72 h to count live and dead adults, and mortality was based on the number of adults that died out of the total exposed insects (50 per box). Containers with immature stages were held until emergence of adults. Mortality of immature stages was based on the number of insects that failed to emerge as adults out of the total exposed.

The time–mortality responses of *T. confusum* life stages at the temperatures tested were heterogeneous, and differences in heat tolerance among certain stages at temperatures <60°C were not obvious. Therefore,

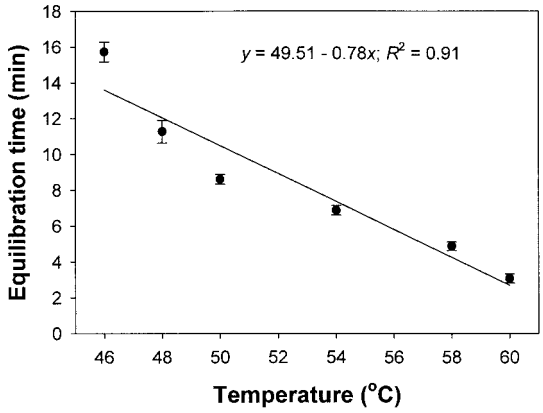

Fig. 1. The time required for the flour in test boxes to attain the set chamber temperature (flour equilibration time) at 46–60°C.

a second test was conducted with all five life stages by using fixed exposure times at 46, 50, and 60°C. The experimental methods used at these temperatures were similar to that described in the first test with a few exceptions. At each temperature, instead of two test boxes, one test box was used, with 100 individuals of a *T. confusum* life stage. The exposure time was 160 min at 46°C, 40 min at 50°C, and 12 min at 60°C. Insects exposed to 28°C and 65% RH for 160, 40, or 12 min served as the control treatment to determine natural mortality. This experiment was replicated three times. Mortality assessments of the various life stages were made according to procedures explained for the first test.

Data Analysis. Time–mortality data of each *T. confusum* life stage at 46, 48, 50, 54, 58, or 60°C from the first test were not corrected for control mortality, because it was <5%. The time–mortality data of each stage at an elevated temperature were fit to the complementary log-log (CLL) regression model (Robertson and Preisler 1992) for estimating the time required to obtain 99% mortality (LT_{99}) of the exposed insects using the PROBIT procedure (SAS Institute 1999). The χ^2 statistic was used to determine the goodness-of-fit of the CLL model to the data (SAS Institute 1999). LT_{99} values between any two life stages of *T. confusum* at a given temperature were compared by using lethal time ratios (Robertson and Preisler 1992). The two life stages being compared were significantly different ($P < 0.05$) from one another in their LT_{99} values if the 95% CL for the ratio did not include 1 (Robertson and Preisler 1992).

The change in LT_{99} values of each *T. confusum* life stage as a function of treatment temperature was described by a nonlinear equation (Jandel Scientific 1994):

$$lny = a + bx$$

where y is the LT_{99} in min, x is the temperature from 46 to 60°C, and a and b are the parameters estimated from the LT_{99} temperature data.

Mortality data of *T. confusum* life stages after 160 min at 46, 40 min at 50, and 12 min at 60°C were corrected for control mortality (>5%) (Abbott 1925). Corrected mortality data at each temperature were transformed to angular values and subjected to one-way analysis of variance to determine significant differences among stages using the GLM procedure (SAS Institute 1999). Means were separated by least squares means test at the $\alpha = 0.05$ level.

Results

Flour Equilibration Time. The time required for the flour in test boxes to attain the growth chamber temperature decreased with an increase in temperature. The linear regression showed that the equilibration time decreased by 47 s for every 1°C increase in temperature between 46 and 60°C (Fig. 1).

Temperature and Humidity Measurements. The temperatures recorded by HOBO data-logging units on the top shelf of growth chambers and inside test boxes with flour, before initiation of experiments, were similar to the set chamber temperatures (Table 1). This indicated that the insects were exposed to the same temperatures as reflected by the growth chamber digital display.

Table 1. Temperature and relative humidity (mean ± SE) of flour inside plastic test boxes, and air temperature and humidity near the top shelf of growth chambers at set chamber temperatures of 46 to 60°C

Set chamber temp (°C)	Top shelf of growth chamber[a]		Flour inside test boxes[a]	
	Temp (°C)	RH (%)	Temp (°C)	RH (%)
46	45.91 ± 0.03 (930)[b]	21.58 ± 0.01 (930)	45.97 ± 0.11 (314)[b]	21.75 ± 0.10 (314)
48	47.78 ± 0.05 (1,114)	21.46 ± 0.03 (1,114)	47.91 ± 0.17 (167)	21.76 ± 0.12 (167)
50	49.99 ± 0.01 (3,548)	21.18 ± 0.01 (3,548)	49.84 ± 0.10 (301)	20.93 ± 0.07 (301)
54	53.67 ± 0.07 (1,548)	21.52 ± 0.06 (1,548)	53.75 ± 0.15 (288)	20.94 ± 0.08 (288)
58	57.97 ± 0.15 (293)	21.53 ± 0.17 (293)	58.06 ± 0.13 (345)	20.73 ± 0.12 (345)
60	59.75 ± 0.15 (333)	20.96 ± 0.09 (333)	59.68 ± 0.18 (282)	20.45 ± 0.06 (282)
28 (Control)[c]	27.85 ± 0.00 (1,026)	65.54 ± 0.03 (1,026)	27.89 ± 0.00 (355)	64.82 ± 0.01 (355)

[a] Temperature and humidity measurements in each growth chamber were collected by five HOBO data-logging units placed at the four corners and center of the top shelf. Each mean represents pooled HOBO data from the five units collected continuously at 1-min intervals over time.

[b] Numbers in parenthesis represent the number of data observations collected over time used for computing means and associated standard errors.

[c] Control growth chamber.

Table 2. Time–mortality regression estimates (mean ± SE) and lethal time (LT_{99}) values for *T. confusum* life stages exposed to 46 to 60°C

Temp (°C)	Stage	Total no. insects	Intercept ± SE	Slope ± SE	LT_{99} (95% CL) (min)	χ^2 (df)[a]
46	Eggs	4,200	−10.18 ± 1.49	5.04 ± 0.72	208.71 (175.16–287.37)	138.33 (12)
	Young larvae	4,500	−11.31 ± 1.34	5.64 ± 0.65	188.27 (164.83–232.59)	141.77 (13)
	Old larvae	5,400	−22.61 ± 1.77	9.74 ± 0.76	299.46 (281.81–324.88)	147.40 (16)
	Pupae	3,900	−13.90 ± 1.16	6.43 ± 0.52	251.28 (230.80–282.30)	75.60 (11)
	Adults	3,900	−21.53 ± 1.20	9.90 ± 0.54	213.53 (205.91–223.27)	35.47 (11)
48	Eggs	3,600	−10.72 ± 2.45	5.92 ± 1.33	116.93 (95.63–200.31)	515.72 (10)
	Young larvae	3,600	−11.52 ± 1.36	6.12 ± 0.70	135.11 (120.62–161.74)	112.34 (10)
	Old larvae	6,300	−18.14 ± 0.99	8.75 ± 0.47	176.53 (168.41–187.05)	85.53 (18)
	Pupae	3,600	−15.56 ± 1.95	7.83 ± 0.97	152.23 (137.78–179.33)	135.94 (10)
	Adults	4,500	−20.75 ± 2.15	10.18 ± 1.04	153.69 (144.26–168.88)	145.26 (13)
50	Eggs	5,100	−3.57 ± 0.41	3.15 ± 0.33	41.24 (34.08–55.98)	173.26 (15)
	Young larvae	5,100	−2.94 ± 0.26	2.71 ± 0.21	44.45 (37.62–55.56)	101.92 (15)
	Old larvae	6,600	−7.96 ± 0.59	4.85 ± 0.35	90.05 (81.80–102.26)	143.28 (20)
	Pupae	4,500	−10.59 ± 0.82	7.18 ± 0.55	48.70 (45.21–53.83)	87.28 (13)
	Adults	6,600	−14.86 ± 1.35	8.82 ± 0.79	72.17 (67.15–79.80)	291.27 (20)
54	Eggs	3,300	−2.50 ± 0.48	3.34 ± 0.56	16.10 (12.22–28.42)	168.90 (9)
	Young larvae	2,700	−3.70 ± 0.67	4.39 ± 0.72	15.46 (12.42–24.31)	111.52 (7)
	Old larvae	5,400	−5.47 ± 0.52	4.01 ± 0.36	55.71 (48.75–67.25)	138.26 (16)
	Pupae	4,800	−4.15 ± 0.65	4.01 ± 0.59	26.02 (21.27–37.35)	294.59 (14)
	Adults	5,400	−8.63 ± 0.80	5.77 ± 0.53	57.50 (51.45–67.47)	156.23 (16)
58	Eggs	2,400	−3.19 ± 0.59	4.40 ± 0.76	11.76 (9.21–20.44)	93.84 (6)
	Young larvae	3,000	−2.82 ± 0.61	4.01 ± 0.81	12.10 (8.88–25.86)	233.38 (8)
	Old larvae	3,600	−7.40 ± 1.62	5.64 ± 1.23	38.15 (30.19–71.00)	332.72 (10)
	Pupae	2,700	−3.04 ± 0.45	4.20 ± 0.55	12.19 (10.09–16.96)	75.88 (7)
	Adults	5,100	−8.77 ± 1.52	6.71 ± 1.15	34.15 (29.15–46.57)	539.16 (15)
60	Eggs	2,100	−2.53 ± 0.37	4.89 ± 0.62	6.77 (5.61–9.38)	48.64 (5)
	Young larvae	2,100	−0.82 ± 0.07	3.09 ± 0.15	5.73 (5.30–6.29)	3.21 (5)
	Old larvae	3,600	−5.04 ± 0.76	4.77 ± 0.67	23.91 (20.07–32.63)	164.95 (10)
	Pupae	2,400	−3.99 ± 0.91	5.44 ± 1.18	10.32 (7.99–21.30)	181.13 (6)
	Adults	2,700	−7.93 ± 2.04	8.10 ± 2.03	14.67 (12.26–26.61)	273.75 (7)

[a] The χ^2 values for goodness-of-fit of the CLL regression model to the observed mortality data were significant ($P < 0.05$), except for young larvae at 60°C.

Responses of *T. confusum* Life Stages at Elevated Temperatures. The natural mortality (% mean ± SE; $n = 18$ [pooled across the controls for the six temperatures tested]) of eggs, young larvae, old larvae, pupae and adults at the end of the first test was 3.6 ± 0.4, 3.0 ± 0.4, 3.7 ± 1.8, 2.3 ± 0.5, and 0.9 ± 0.3. In general, mortality of *T. confusum* life stages at each elevated temperature increased with an increase in exposure time. Similarly, the mortality of each stage increased with an increase in temperature. The χ^2 value was not significant ($P > 0.05$) for only one of the 30 time–mortality regressions, indicating adequate fit of the CLL model to data (Table 2). However, significant χ^2 values for the remaining 29 time–mortality regressions indicated that the responses of *T. confusum* life stages were heterogeneous at each of the temperatures tested.

The CLL regression estimates (intercepts and slopes) and LT_{99} values were not consistent among stages at each temperature and among temperatures for a given stage (Table 2). LT_{99} values for each stage decreased with an increase in temperature. Generally, old larvae and adults had greater LT_{99} values than other stages at all the temperatures tested.

Ratio tests showed that the LT_{99} values among the five stages at 46 or 48°C were not significantly different ($P > 0.05$) from one another (data not shown). At 50, 54, 58, and 60°C, the LT_{99} value of old larvae was significantly greater ($P < 0.05$) than that of eggs, young larvae, and pupae (Table 3). LT_{99} values of old larvae and adults were not statistically different ($P > 0.05$) from one another at 50, 54, or 58°C, but they were statistically different ($P < 0.05$) at 60°C. At 54°C, the LT_{99} value of pupae was significantly greater than that of eggs. The LT_{99} value of adults was greater ($P < 0.05$) than that of eggs, young larvae, and pupae at both 54 and 58°C. At 60°C, the LT_{99} value of adults was significantly greater ($P < 0.05$) than that of eggs and young larvae.

The decrease in LT_{99} values for each *T. confusum* life stage as a function of temperature (Fig. 2) was best described by the equation above ($R^2 = 0.957–0.986$). The rate of decrease in LT_{99} for eggs, young larvae, old larvae, pupae, and adults was 2.2-, 2.3-, 1.0-, 1.7-, and 1.0-fold, respectively, for every 1°C increase in temperature between 46 and 60°C.

The mortality of *T. confusum* life stages exposed for 160 min at 46°C ranged from 47 to 88%, whereas a 40-min exposure at 50°C resulted in 39–100% mortality (Table 4). Similarly, exposure for 12 min at 60°C produced 72–100% mortality of the five life stages. Eggs were the most susceptible stage followed by young larvae and pupae. At the three temperatures, mortality of the old larvae was significantly lower ($P < 0.05$) than that of the other stages. The mortality of adults at each temperature was significantly lower ($P < 0.05$) compared with eggs, young larvae, and pupae, but was

Table 3. Significant ($P < 0.05$) pairwise comparisons of LT_{99} values among *T. confusum* life stages at 50 to 60°C

Temp (°C)	Stages compared[a]	LT_{99} ratio (95% CL)[b]
50	*Old larvae*-eggs	2.18 (1.14–4.18)
	Old larvae-young larvae	2.02 (1.12–3.66)
	Old larvae-pupae	1.22 (1.01–3.33)
54	*Old larvae*-eggs	3.45 (2.05–5.82)
	Old larvae-young larvae	3.60 (2.18–5.93)
	Old larvae-pupae	2.14 (1.16–3.93)
	Pupae-eggs	1.61 (1.01–2.56)
	Adults-eggs	3.57 (2.10–6.06)
	Adults-young larvae	3.71 (2.23–6.17)
	Adults-pupae	2.20 (1.19–4.09)
58	*Old larvae*-eggs	3.24 (1.24–8.41)
	Old larvae-young larvae	3.15 (1.25–7.94)
	Old larvae-pupae	3.12 (1.32–7.39)
	Adults-eggs	2.90 (1.33–6.29)
	Adults-young larvae	2.82 (1.34–5.90)
	Adults-pupae	2.80 (1.45–5.39)
60	*Old larvae*-eggs	3.53 (1.74–7.16)
	Old larvae-young larvae	4.16 (2.11–8.19)
	Old larvae-pupae	3.21 (1.37–3.88)
	Old larvae-adults	1.62 (1.06–2.49)
	Adults-eggs	2.16 (1.12–4.18)
	Adults-young larvae	2.55 (1.36–4.78)

[a] Stage in italics has the greater LT_{99} value in the pair being compared.

[b] Ratio = larger LT_{99}/smaller LT_{99}. The LT_{99} between the two stages being compared is significantly different ($P < 0.05$) if the 95% CL for the ratio does not include 1 (Robertson and Preisler 1992).

significantly higher ($P < 0.05$) compared with old larvae.

Discussion

The time required for the flour in test boxes to attain the growth chamber air temperatures decreased with an increase in temperature. Therefore, the flour equilibration time was taken into account when determining time-mortality relationships for various *T. confusum* life stages at the six elevated temperatures.

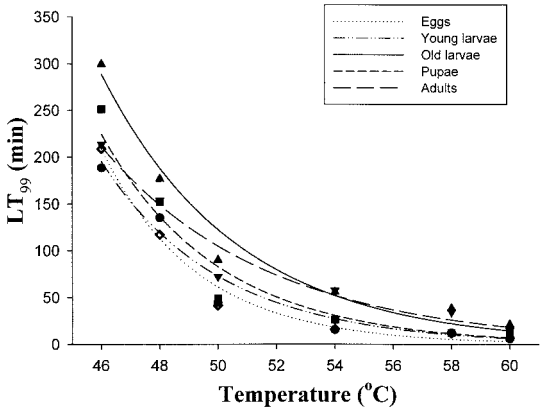

Fig. 2. Observed and fitted lines (see equation in text) describing LT_{99} of *T. confusum* life stages as a function of temperature. Regression equations for the stages are as follows: eggs, $\ln y = 19.464–0.307x$; young larvae, $\ln y = 16.640–0.247x$; old larvae, $\ln y = 15.550–0.214x$; pupae, $\ln y = 16.887–0.249x$; and adults, $\ln y = 13.433–0.175x$.

Table 4. Corrected mortality (% mean ± SE) of *T. confusum* life stages at 46, 50, and 60°C exposed for specific time periods

Stage	46°C[a] (160 min)	50°C[a] (40 min)	60°C[a] (12 min)
Eggs	87.67 ± 1.86a	100.0 ± 0.00a	100.0 ± 0.00a
Young larvae	74.65 ± 3.30b	96.40 ± 0.95b	100.0 ± 0.00a
Old larvae	46.50 ± 1.20d	38.82 ± 1.49e	72.10 ± 1.48c
Pupae	73.65 ± 2.10b	76.11 ± 1.79c	100.0 ± 0.00a
Adults	65.42 ± 1.86c	48.66 ± 2.72d	88.96 ± 2.65b

Mean ($n = 3$) mortality of eggs, young larvae, old larvae, pupae, and adults in the control treatment over the three temperatures ranged from 3.7 to 5.3, 3.0 to 7.3, 0.3 to 3.0, 1.3 to 3.7, and 0 to 0.7%, respectively.

[a] Each mean is based on $n = 3$ replications of 100 insects each. At each temperature, means among stages followed by different letters are significantly different ($P < 0.05$; by least squares means test).

However, the decrease in flour equilibration time as a function of temperature (47 s for 1°C rise in temperature) would vary with the amount of flour used in the test boxes and may indirectly have an impact on insect mortality. Mahroof et al. (2003b) used a similar protocol for determining time-mortality relationships for *T. castaneum.* They reported that the flour equilibration time decreased by 41 s for every 1°C rise in temperature between 42 and 60°C.

The heterogeneous responses of *T. confusum* life stages at elevated temperatures may be attributed to differences in age, sex, exposure time on acclimation, and inherent ability to tolerate heat (Oosthuizen 1935, Davison 1969, Evans 1981). In all our tests, we used unsexed insects that were within 2 d of the specified age. The lack of significant differences in relative susceptibilities of certain *T. confusum* life stages, especially at temperatures ≥50°C, also can be attributed to the heterogeneous responses observed. Mahroof et al. (2003b) observed responses of eggs, young larvae, old larvae, pupae, and adults of a related species, *T. castaneum*, to be heterogeneous when exposed to elevated temperatures between 42 and 60°C. In their study, 28 of the 30 CLL time–mortality regressions were found to be significant. Nevertheless, the fixed time mortality data at 46, 50, and 60°C consistently showed the old larvae to be the most heat tolerant of all *T. confusum* life stages.

The lack of significant differences in LT_{99} values among *T. confusum* life stages at 46 and 48°C and the increased susceptibility and differences among life stages at temperatures ≥50°C suggest that the temperature during heat treatment should be at least 50°C. Both the time–mortality responses and the fixed time responses generally indicated old larvae to be the most heat-tolerant stage, followed by adults, and pupae. Eggs and young larvae were most susceptible to elevated temperatures. However, in *T. castaneum* the young larvae were the most heat tolerant compared with eggs, old larvae, pupae, and adults (Mahroof et al. 2003b). The differences observed in these two closely related species suggest that generalizations regarding heat tolerance of various life stages of stored-product insects cannot be made without generating data on each insect species separately. Shayesteh and Barthakur (1996) exposed, eggs (12–24 h old), mature

larvae, pupae (12–24 h old), and adults (1–7 d old) of *T. confusum* in 50 g of flour for 5, 10, 20, and 40 min to microwave radiation (2,450 MHz). The flour temperature during the 5- to 40-min interval increased from 40 to 87°C. Of all the stages, mature larvae were found to be the most heat tolerant because their survival over time decreased much more slowly than that of the other stages. Wright et al. (2002) also reported that large larvae of *T. variabile* were the most heat-tolerant stage compared with eggs, diapausing larvae, pupae, and adult females at 56°C and 0% RH.

The physiological or biochemical mechanisms involved in conferring heat tolerance in old larvae of *T. confusum* relative to other life stages requires further study. It is likely that heat-shock proteins may be involved in conferring heat tolerance. Heat-shock proteins play a vital role in the heat tolerance of insects by protecting ordinary cell proteins when the organism is exposed to stresses, including high temperatures (Lewis et al. 1999). Heat-shock proteins are involved in the removal of denatured proteins across cell membranes, interact with other cell proteins to keep them in a folded state, and minimize aggregation of proteins at high temperatures (Currie and Tufts 1997). Heat tolerance in young larvae of *T. castaneum* was attributed to increased expression of a 70 kDa heat-shock protein (Mahroof et al. 2005). It would be interesting to know the family of heat-shock proteins involved in conferring heat tolerance in old larvae of *T. confusum*, because of notable variation in the developmental regulation of heat shock protein expression for a given species.

In summary, old larvae of *T. confusum* were consistently the most heat tolerant of all the stages tested at 50–60°C. For example, at 50°C, 90 min was required to kill 99% of old larvae, whereas 41–72 min was required to kill 99% of eggs, young larvae, pupae, and adults. Therefore, old larvae should be used as test insects in bioassays for gauging heat-treatment effectiveness during facility heat treatments, because treatments targeted at controlling old larvae should be able to control all other stages. The information presented in this article provides a quantitative basis for successful use of elevated temperatures for management of *T. confusum* life stages associated with food-processing facilities.

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