Models to Predict Mortality of *Tribolium castaneum* (Coleoptera: Tenebrionidae) Exposed to Elevated Temperatures During Structural Heat Treatments

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J. Econ. Entomol. 106(5): 2247–2258 (2013); DOI: http://dx.doi.org/10.1603/EC12278

ABSTRACT Novel thermal death models were developed with certain assumptions, and these models were validated by using actual heat treatment data collected under laboratory conditions at constant temperatures over time and in commercial food-processing facilities where temperatures were dynamically changing over time. The predicted mortalities of both young larvae and adults of the red flour beetle, *Tribolium castaneum* (Herbst), were within 92–99% of actual measured insect mortalities. There was good concordance between predicted and observed mortalities of young larvae and adults of *T. castaneum* exposed to constant temperatures in laboratory growth chambers and at variable temperatures during structural heat treatments of commercial food-processing facilities. The models developed in this study can be used to determine effectiveness of structural heat treatments in killing young larvae and adults of *T. castaneum* and for characterizing insect thermotolerance.

KEY WORDS *Tribolium castaneum*, mortality, heat treatment, food-processing facility, model

Heat treatment is an effective method to control pest insects in grain-processing facilities (Fields 1992, Beckett and Morton 2003, Subramanyam et al. 2011) and a viable alternative to fumigation with methyl bromide or sulfuryl fluoride (Fields and White 2001). During heat treatment, the entire building or portions of it are heated to 50–60°C for 24–36 h (Imholte and Imholte–Tauscher 1999, Fields and White 2001), although commercial heat treatments can be accomplished within 15–24 h (Subramanyam et al. 2011). Mortality of insects is a function of how quickly temperatures reach 50°C, how long temperatures are maintained between 50 and 60°C, and the maximum temperature (Subramanyam et al. 2011).

Thermal death of insects has been modeled using fundamental kinetic to semi- or purely empirical models (Hansen et al. 2004, Wang et al. 2007, Boina et al. 2008). Some developed models, such as fundamental kinetic model (Gazit et al. 2004), complementary log-log transformation, degree-day model (Subramanyam et al. 2002), and logarithmic model (Stumbo 1973) cannot be used to calculate insect mortality where temperatures are dynamically changing over time, which happens during a heat treatment.

Selection of an appropriate model depends on researcher's preference, intended use, target insects, and temperature range (Wang et al. 2007). The reason for selecting different models is that the published models can only be used under certain conditions or for certain insect species (Throne et al. 1995, Wang et al. 2002). Subramanyam et al. (2002) found that heat accumulation model accounted for only 75% of the mortality of first instars of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Mathematical models and concepts, which can be used for different insect species and under different treatment conditions, are required. In this study, we propose a novel concept of "heat treatment zones," which we define as a combination of treatment time and temperature. The combination of treatment time and temperature is nonlinearly additive and cumulative. Inside different zones, insects experience different mortalities. Given this novel concept, we developed and validated a thermal death model for predicting mortality of first instars (the heat tolerant stage [Mahroof et al. 2003a]) and adults of the red flour beetle, *T. castaneum*, an economically important insect pest of food-processing facilities worldwide (Sinha and Waters 1985, Mills and Pedersen 1990), using independent data collected in the laboratory and commercial food-processing facilities subjected to heat treatments.

Model Hypotheses and Premise

The mathematical model that describes insect mortality under elevated temperatures was based on the following two assumptions: 1) during heat treatment, insect mortality is primarily caused by elevated temperatures and there is zero mortality because of other natural causes, especially those related to insect age,

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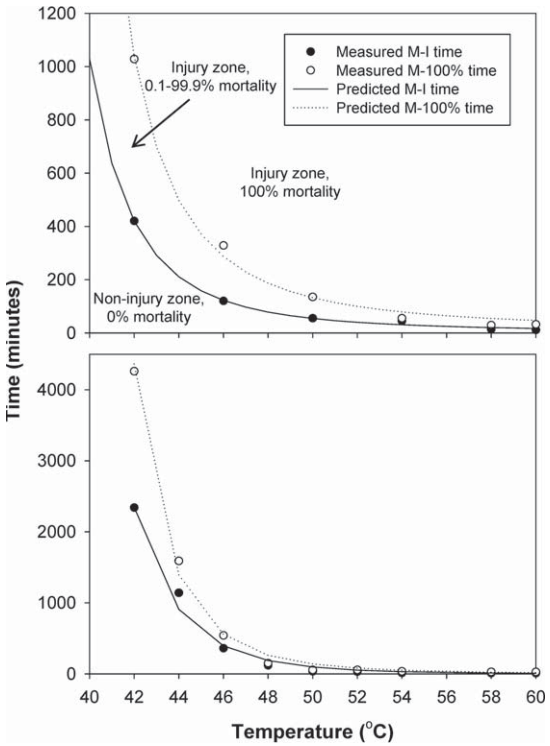

Fig. 1. Measured and predicted M-I and M-100% times of young larvae (top graph) and adults (bottom graph) of *Tribolium castaneum*. The predicted M-I time and predicted M-100% time were based on equation 1 and parameter values given in Table 2.

and 2) zones that cause heat injury or noninjury can be categorized. Inside the heat-injury zone, the survival rate of insects is adversely affected by elevated temperatures and treatment times. Heat injury, which results in the death of insects, could not be repaired at any temperature and treatment times within this zone. In the noninjury zone insect mortality is zero. This zone was based on the assumption that minor heat injuries could be repaired during or after the heat treatment. The outside boundary of the heat injury zone involves temperatures where death occurs instantaneously. For example, the mortalities of *T. castaneum* were tested at various temperatures under laboratory at constant temperatures over time. After the heat treatment, insect mortalities were determined under room temperatures (24–28°C). We assumed the room temperature and exposure time to be in the noninjury zone. At an elevated temperature (42–60°C), the minimum treatment time where insect mortality is significantly larger than unexposed insects, is referred to as M-I-time. The line connecting the M-I-times at different elevated temperatures separates the injury and noninjury zones (Fig. 1). The time equal to or greater than the M-I-time is located in the injury zone and the time less than the M-I-time is located in the noninjury zone. In the injury zone the minimum time for 100% mortality of insects is referred to as the M-100% time. The line connecting the M-100% times separates the injury zone into two zones: injury zone with 0.1–99.9% mortality and injury zone with 100% mortality. The theories supporting the above hypothesis were that heat transfer into insect bodies requires time; the basic variables for heat treatments are temperature and time (Hallman and Armstrong 1994, Sharp 1994, Neven 2000). The mortality of insects is additive and cumulative because continuous exposure to elevated temperature increases mortality in a linear or nonlinear fashion (Subramanyam et al. 2002, Hansen et al. 2004, Yu et al. 2011).

Materials and Methods

Data Used for Model Development. The mortality data for eggs, young larvae, old larvae, pupae, and adults of *T. castaneum* at different temperatures and treatment times at constant temperatures in the laboratory were taken from published literature (Mahroof et al. 2003a). We verified and validated the developed models using all *T. castaneum* stages and found the data associated with all of the stages had the same degree of accuracy. Therefore, in this article we present validation results using laboratory data for young larvae (first instars) and adults, because first instars are more heat tolerant than other insect stages under laboratory conditions (Mahroof et al. 2003a, b), and the decision to use pest management interventions is usually based on observing the presence, distribution, and numbers of the adult stage.

Data on *T. castaneum* at Elevated Temperatures. Cultures of *T. castaneum* were reared on whole wheat flour plus 5% (by weight) brewer's yeast at 28°C and 65% relative humidity (RH) in the laboratory. Young larvae (6-d old from the time of egg hatching) and adults (mixed 2–4-wk old) were sifted from the rearing media using a sieve with 250-μm opening. The same materials and methods used by Mahroof et al. (2003a, b) were used to determine insect mortality at different temperatures. The humidity used in tests at constant temperatures was 22–25% (Mahroof et al. 2003a), which is typical of humidity levels observed during heat treatment of food-processing facilities (Mahroof et al. 2003b, Roesli et al. 2003). The treatment time was based on insect responses that varied from 0 to 100% mortality (Table 1). The temperatures used for young larvae were 42, 46, 50, 54, 58, and 60°C, and for the adults they were 42, 44, 46, 48, 50, 52, 54, 58, and 60°C. The control treatment for both insect stages was 28°C and 65% RH. There were five replications at each temperature-treatment time combination for each insect stage. Twenty insects were exposed in each replicate. All of the data (insect mortality expressed as a percentage) collected at different temperatures except 46°C for young larvae and 52°C for adults were used to find the time for M-I and M-100%. The first three replications at 46°C for young larvae and 52°C for adults were used to find the M-I and M-100% times. The last two replications at 46°C for young larvae and 52°C for adults were used for the model development.

Table 1. Treatment times for *Tribolium castaneum* young larvae and adults at different temperatures

Temperature (°C)	Treatment time (minutes)		
	Minimum time	Increment	Maximum time
Young larvae			
42	90	Variable[a]	900
46	30	Variable[b]	315
50	20	5	130
54	10	5	55
58	5	3	32
60	5	3	32
Adults			
42	1,440	Variable[c]	4,260
44	660	Variable[d]	1,620
46	240	30	570
48	60	15	165
50	25	5	60
52	15	5	60
54	5	Variable[e]	35
58	5	3	26
60	3	3	27

[a] The time interval between 90 and 270 min was every 60 min; between 270 and 405 min it was every 45 min; and between 405 and 450 min it was every 15 min. For other time periods it was every 30 min.

[b] The time interval between 135 and 165 min was every 30 min; between 165 and 205 min it was every 20 min; between 305 and 315 min it was every 5 min. For all other time periods it was every 15 min.

[c] The times used at this temp were 1,440, 1,560, 1,800, 1,980, 2,340, 2,550, 2,700, 2,820, 3,000, 3,240, 3,420, 3,780, 4,140, and 4,260 min.

[d] The time interval between 750 and 810 min was every 60 min; between 810 and 930 min it was every 120 min; between 930 and 1,140 min it was every 210 min; between 1,140 and 1,200 min it was every 60 min; between 1,470 and 1,590 min it was every 60 min; between 1,590 and 1,620 min it was every 30 min. For other time periods it was every 90 min.

[e] The time interval between 5 and 10 min was every 5 min, but it was every 3 min for other time periods.

Model Development. The M-I times at each temperature were found as follows: a Student's *t*-test with Bonferroni correction was conducted between percentage mortality in the control treatment and mortality observed at a given temperature at each treatment time (SAS Institute 2008). The minimum time that showed a significant difference in insect mortality ($\alpha = 0.05/n$, where n = number of treatment times at a given temperature) with the control, was selected as the M-I time at a given temperature. An exponential decay model (equation 1, Table 2) satisfactorily described the relationship between the M-I times and temperatures ($R^2 = 0.99$) for both young larvae and adults (Fig. 1).

$$y = ae^{\frac{b}{T+c}} \quad [1]$$

Table 2. Mean ± SE values for equation 1 parameters used to calculate the M-I and M-100% times for young larvae and adults of *Tribolium castaneum*

Insect stages	Parameter	M-I time	M-100% time
Young larvae	*a*	1.11 ± 1.84	5.78 ± 3.01
	b	90.56 ± 61.65	63.07 ± 11.39
	c	−26.74 ± 6.17	−29.85 ± 0.96
Adults	*a*	$(0.07 \pm 0.05) \times 10^{-2}$	0.11 ± 0.29
	b	446.09 ± 418.52	175.30 ± 87.12
	c	−12.31 ± 13.73	−25.44 ± 4.17

where, y = M-I-times; a, b, and c are estimated model parameters; and T = temperature (°C) (Table 2).

The M-100% times were found by checking the insect mortality at each temperature with different treatment times. The minimum time at which the insect mortality in all of the replicates reached 100%, was selected as the M-100% time. The relationship between the M-100% times and temperatures also followed the exponential decay equation with an R^2 of 0.99 for both young larvae and adults (Fig. 1; Table 2).

Base Temperature. The base temperature is the temperature at which there is no insect mortality during the entire heat treatment period. It was calculated using equation 1 and the parameters associated with the M-I times and at $y = 36$ h. The reason for choosing 36 h was that 1) heat treatments are usually conducted for 36 h or less (Imholte and Imholte–Tauscher 1999, Mahroof et al. 2003b), 2) zero mortality during the entire heat treatment period would indicate that heat treatment temperature with the associated treatment time had no adverse effects on insects, and 3) the base temperature should be at or near the M-I time line if our basic assumption related to the noninjury and injury zones is correct. The calculated base temperatures were 38.7°C for young larvae and 42.0°C for adults.

Scaling Factors. If mortalities of insects under elevated temperatures were additive, two scaling factors could be determined between two different temperatures.

$$f_{M\text{-}I} = \frac{\theta_{M\text{-}I,1}}{\theta_{M\text{-}I,2}} \quad [2]$$

$$f_{M\text{-}100\%} = \frac{\theta_{M\text{-}100\%,1}}{\theta_{M\text{-}100\%,2}} \quad [3]$$

where, $f_{M\text{-}I}$ is the scaling factor associated with the M-I time and $f_{M\text{-}100\%}$ is the scaling factor associated with the M-100% time; $\theta_{M\text{-}I,1}$ and $\theta_{M\text{-}I,2}$ are the M-I times in minutes at temperature 1 and 2 and $\theta_{M\text{-}100\%,1}$ and $\theta_{M\text{-}100\%,2}$ are the M-100% times in minutes at temperature 1 and 2, respectively. The assumptions supporting equation 2 and 3 were that 1) insect mortalities at different temperatures inside the injury zone with 0.1–99.9% mortality could be mapped based on the scaling factors; 2) the heat stress inside insect bodies reached injury zone in $\theta_{M\text{-}I,1}$ or $f_{M\text{-}I} * \theta_{M\text{-}I,2}$ time period at temperature T_1 and T_2, respectively; and 3) the mortality of insects reached 100% in $\theta_{M\text{-}100\%,1}$ or $f_{M\text{-}100\%} * \theta_{M\text{-}100\%,2}$ time period at temperature T_1 and T_2, respectively. When an arbitrary temperature was chosen as a reference temperature (T_{ref}), values of the scaling factors related to the T_{ref} can be found using equation 4.

$$f_T = \frac{b}{e^{T_{ref}+c}} - \frac{b}{T+c} \quad [4]$$

where, f_T is the value of scaling factor at the temperature T (°C) and the chosen reference temperature (T_{ref}).

We found equation 4 had a low prediction accuracy of insect mortality (MRE values, box plots of residuals,

and the slopes of regression, refer to the model validation section), because equation 4 was based on equation 1 and the calculated M-I or M-100% times using equation 1 had about ±20 min difference from the determined M-I or M-100% times. This difference generated a wrong prediction at 58–60°C, because the mortality of insects at 58–60°C was 100% in <30 min. Therefore, the following changes were made to find the f_T: we arbitrarily chose 46°C as the T_{ref} for the young larvae and 52°C for the adults. During model validation, we tested six temperatures (42, 46, 50, 54, 58, and 60°C) and found the reference temperature could be any of these temperatures. However, temperatures of 46°C for the young larvae and 52°C for the adults produced higher prediction accuracies than the other temperatures.

For each constant temperature we tested under laboratory conditions, we calculated the $f_{M\text{-}I}$ and $f_{M\text{-}100\%}$ using the determined M-I and M-100% times at the chosen T_{ref} and equation 2 and 3. In addition, for each temperature, we found the maximum value of the $f_{M\text{-}I}$ and $f_{M\text{-}100\%}$. This maximum value was the f_T at the tested temperature. The interpolation method was used to calculate the f_T at the temperatures that were not tested under constant temperatures. For example, mortalities of young larvae were tested at 46 and 50°C. The f_T between 46 and 50°C was calculated using equation 5.

$$f_T = \frac{(f_{50} - f_{46})(T - 46)}{50 - 46} + f_{46} \quad [5]$$

where, f_{46} and f_{50} are the f_T at 46 and 50°C, respectively. We assumed that $f_T = 0$ at the calculated base temperature (38.7°C for young larvae and 42.0°C for adults).

Prediction of Insect Mortality. The time at which a mortality effect occurred (θ_{efc}) at each temperature T (°C) was calculated as:

$$\theta_{efc} = f_T \theta \quad [6]$$

where, θ is the total heat treatment time in minutes. Mortality of insects at each heat treatment temperature was calculated as:

$$M = M_0 + \frac{a}{1 + e^{-\frac{\Sigma\theta_{efc} - \theta_0}{b}}} \quad [7]$$

$$\Sigma\theta_{efc} = \int_0^{\theta} \theta_{efc} d\theta - \theta_{M\text{-}I,ref} \quad [8]$$

where, $\theta_{M\text{-}I,ref}$ is the M-I time at T_{ref}, M is the percent mortality of insects at θ_{efc}. M_0, a, θ_0, and b are estimated parameters (Table 3). Equation 7 shows the relationship between insect mortality and treatment time.

Model Validation. Models were validated using data collected on insect responses during heat treatments of two commercial food-processing facilities. In facility A, heat treatment was conducted in a room during 25–26 September 2010 (total 28.2 h heat treatment time). The room dimensions were 45.4 by 37.8 by 5.2 m, and deck above the ground floor was made of metal. The room was equipped with roasting machines and conveyors. It was heated by two heaters (Temp-Air, Burnsville, MN) with a maximum heat output of 410.3 kW/h (1.4 million BTU/h) and 161.2 kW/h (0.55 million BTU/h). To create uniform distribution of temperatures during heat treatment, 10 fans (91.4 cm blade diameter and 311.5 m^3/min volume flow rate [Schaeffer Fan, Temp-Air, Burnsville, MN]) were placed within the room. The locations of fans were adjusted if some cold spots (<50°C) were found during heat treatment. Twenty young larvae and 20 unsexed adults of mixed ages of *T. castaneum* were introduced into separate plastic vials (2.6 cm inner diameter and 4.9 cm high), each holding 5 g of bleached wheat flour. The vials were covered with lids of 600 μm wire mesh screen. The vials were placed inside the room at 28 locations (Fig. 2). The vials were placed on floors of the room at locations 1–13, 16–18, 21, 22, and 27; at locations 14, 15, 24, 25, and 26 the vials were placed at floor level on decks; and at locations 19, 20, and 28 vials were placed on top of conveyors or machines; and at location 23 inside a closed conveyor. At each location there were eight vials (four with young larvae and four with adults). Four vials with young larvae and four vials with adults were placed inside an unheated room served as the control treatment. Temperatures were recorded at each location using HOBO data loggers (Onset Computer Corporation, Bourne, MA) at 2-min intervals. Temperature sensors were located next to the vials at each location. At each location, one vial with young larvae and one with adults were collected at 4.8, 12.2, 20.2, or 27.7 h into the heat treatment. All collected vials were incubated in a chamber at 28°C and 65% RH, a day after the heat treatment. Adult mortality was determined 24 h later (Mahroof et al. 2003a, Boina and Subramanyam 2004, Boina et al. 2008). Mortality of young larvae was determined after 45 d and was based on the number of adults that emerged from exposed larvae (Mahroof et al. 2003a, Boina and Subramanyam 2008). Mortality observed in vials placed in the heated room was corrected for control mortality using Abbott's (1925) formula.

In facility B, heat treatment was conducted during 25–26 September 2011 (total 24 h heat treatment time). One heater with a maximum heat output of 1318.8 kw/h (4.5 million BTU/h) was used to heat a room with dimensions of 30.5 by12.2 by 12.2 m. The same two *T. castaneum* life stages, confined in plastic vials with flour, were used for insect bioassays. Tem-

Table 3. Mean ± SE parameter values for equation 7 parameters used to calculate the M-I and M-100% times for young larvae and adults of *Tribolium castaneum*

Parameter	Young larvae	Adults
M_0	−20.95 ± 35.08	−3.49 ± 40.50
a	121.12 ± 45.17	103.11 ± 41.97
b	44.42 ± 20.60	5.06 ± 1.76
θ_0	54.91 ± 23.35	3.99 ± 4.85
R^2	0.87	0.98

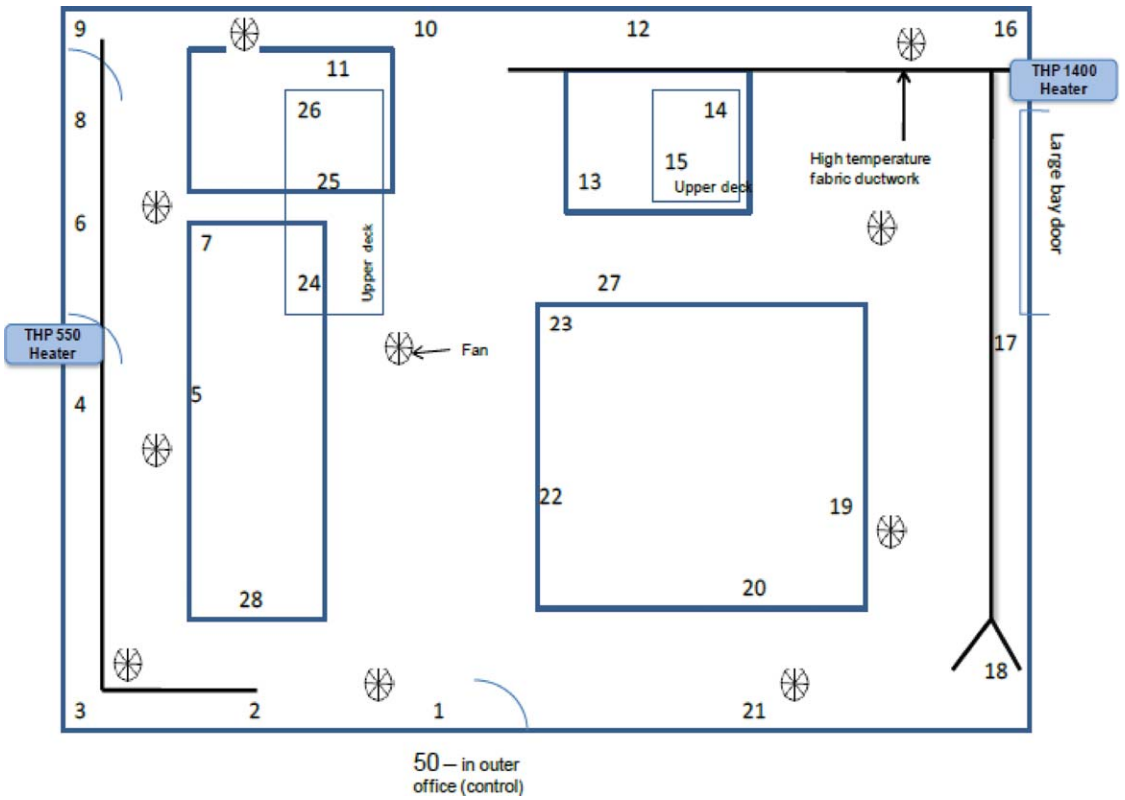

Fig. 2. Locations of vials labeled 1–28 in a room at facility A subjected to heat treatment. (Online figure in color.)

peratures at each vial location were monitored using data loggers as described above. The experimental exceptions were as follows: 1) there were 12 fans inside the room; 2) the vials were collected at 1.5, 3.0, 5.0, and 24 h from each of the 24 locations in the room during heat treatment; and 3) temperature at each location was measured inside one additional vial (containing 5 g of flour and without insects) and outside next to the vial. Heat treatment data at both laboratory and field conditions were used to validate developed models. The developed models were coded in a C^{++} program. Input data for the program included measured temperatures over time. Measured temperature at floor level next to the vials in facility A and on the outside next to the vial and inside the vial at facility B were used to predict insect mortalities. The magnitude of relative error (MRE) was used to measure the prediction accuracy (Kemerer 1987),

$$MRE = \frac{|M_{measured} - M_{predicted}|}{M_{measured}} \quad [9]$$

where, $M_{measured}$ is the measured insect mortality (%) and $M_{predicted}$ is the predicted percent insect mortality. If $M_{measured} = 0$, the measured and predicted mortalities were converted to measured ($S_{measured}$) and predicted ($S_{predicted}$) percent survival and the MRE was calculated as:

$$MRE = \frac{|S_{measured} - S_{predicted}|}{S_{measured}} \quad [10]$$

Although MRE is a standard evaluation criterion to assess the accuracy of prediction of models (Stensrud et al. 2002), Kitchenham et al. (2001) showed that MRE are measures of the spread and kurtosis of z ($z = M_{predicted}/M_{measured}$). They suggested box plots of residuals ($M_{measured} - M_{predicted}$) (Pickard et al. 1999) as one useful alternative to summarize measures because they can give a good indication of the distribution of residuals (Kitchenham et al. 2001). Therefore, we compared the accuracy of predictions using box plots of residuals.

To evaluate the relationship between the observed or measured (y) and predicted (x) mortalities under laboratory and field conditions, linear regressions with a zero intercept were used ($y = bx$). The slope of the regression (b) was tested for departure from one using a Student's t-test (SAS Institute 2008). A slope that was not significantly different ($P \geq 0.05$) from one indicated good correspondence between predicted and measured mortalities. A slope significantly less ($P \leq 0.05$) than one indicated that the predicted mortality overestimated measured mortality, whereas a slope significantly >1 ($P \leq 0.05$) indicated that the predicted mortality underestimated measured mortality.

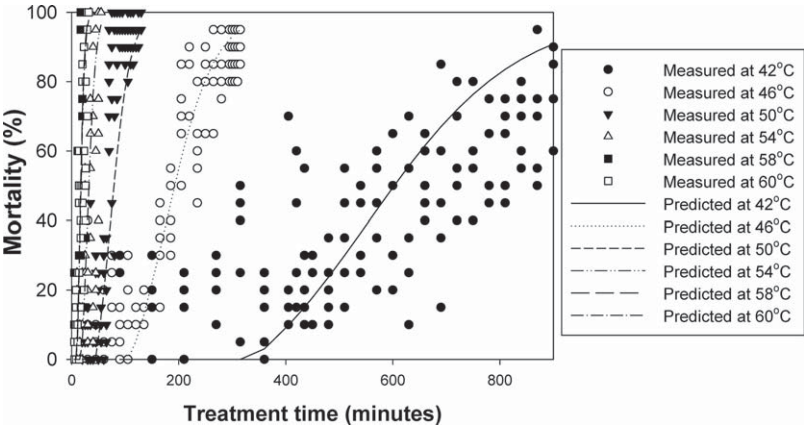

Fig. 3. Observed and predicted insect mortality of young larvae of *Tribolium castaneum* at different constant temperatures in the laboratory. The predicted mortalities were based on equation 7 and parameter values given in Table 3.

Results and Discussion

Validation Using Laboratory Data at Constant Temperatures. The model (equation 7) and parameter values given in Table 3 fit the mortality data of young larvae of *T. castaneum* at different constant temperatures (Fig. 3). The slope of the predicted and observed mortality regression for young larvae of *T. castaneum* (b (SE) = 0.96 (0.01); $R^2 = 0.84$) was significantly <1 ($t = 3.68$; df = 596; $P = 0.0001$) indicating that the predicted mortality overestimated the measured mortality. The model (equation 7) and parameter values given in Table 3 also best described insect mortality data of adults of *T. castaneum* at temperatures of 48°C or less and at temperatures of 50–60°C (Fig. 4). The

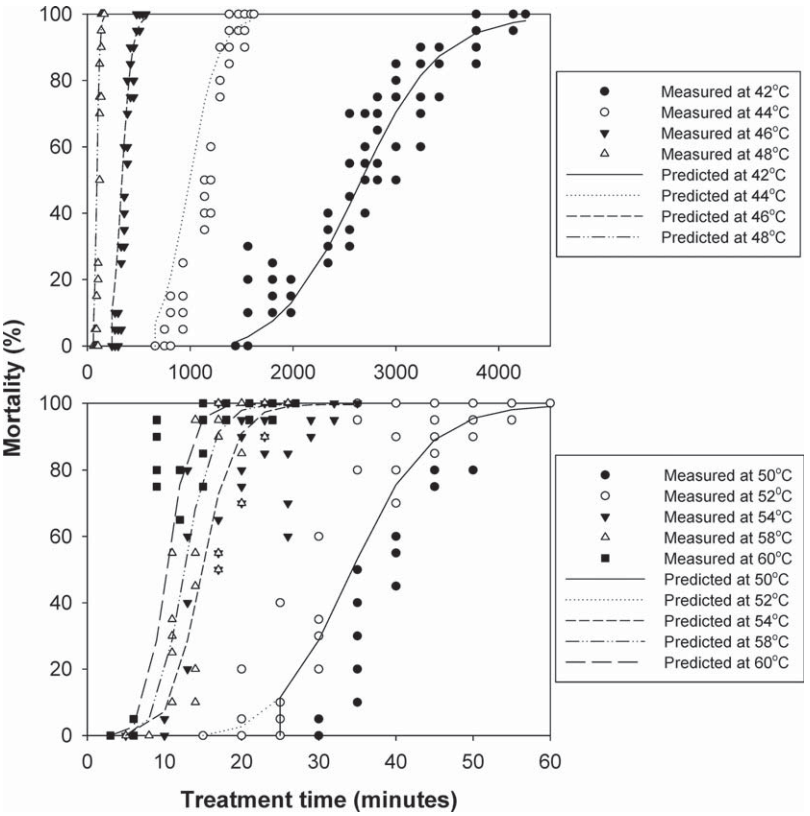

Fig. 4. Observed and predicted insect mortality of adults of *Tribolium castaneum* when temperatures were ≤48°C (top graph) or ≥50°C (bottom graph) in the laboratory. The predicted mortalities were based on equation 7 and parameter values given in Table 3.

Table 4. Mean ± SE magnitude of relative error (MRE, equation10) values associated with mortality of young larvae and adults of *Tribolium castaneum* exposed to different constant elevated temperatures in the laboratory

Temperature (°C)	Mean ± SE MRE values for	
	Young larvae	Adults
42	0.57 ± 0.05	0.20 ± 0.02
44		0.58 ± 0.16
46	0.37 ± 0.04	0.63 ± 0.20
48		1.38 ± 0.43
50	0.47 ± 0.06	0.62 ± 0.34
52		0.20 ± 0.04
54	1.21 ± 0.35	0.17 ± 0.03
58	0.55 ± 0.12	0.48 ± 0.21
60	0.48 ± 0.09	0.14 ± 0.04
Over all	0.55 ± 0.04	0.45 ± 0.06

slope of the predicted and measured mortality regression for adults of *T. castaneum* (b = 0.99 (SE) (0.01); R^2 = 0.90) was not significantly different from one (t = 0.84; df = 432; P = 0.20); indicating that the model predicted measured mortality well (Fig. 4). The highest mean of MRE was 1.38 ± 0.43 (Table 4). This MRE value was associated with adult data at 48°C (Table 4), and was caused by over-prediction when treatment time was <120 min. The overall MRE associated with the prediction of larval mortality was larger than that of adult mortality. This was caused by the large standard errors associated with larval mortality than with adult mortality under the same temperature regimes. The box plots show that: 1) the medians of the residuals were zero or close to zero (<10% of the measured insect mortality) in all of the predictions (Fig. 5), 2) the maximum and minimum residuals were 60 and −55% for the adults, and 3) the maximum and minimum residuals were 55 and −65% for the larvae. These results indicated that the differences between the mortalities measured under the same laboratory conditions were greater than the differences between the predicted and measured mortalities (Figs. 3 and 4). Residuals were skewed at 60% of the tested temperatures (Fig. 5). However, when residuals at all tested temperatures were pooled there was no skewness.

Validation Using Field Data. When data collected in both facilities A and B were used, the slope of the linear regression of predicted and measured mortality for young larvae of *T. castaneum* (b (SE) = 1.08 (0.02); df = 115; R^2 = 0.99) was not significantly different from one (t = −4.00; P = 0.9999). The slope of the regression of predicted and measured mortality for adults (b (SE) = 1.01 (0.01); df = 115; R^2 = 0.99) also was not significantly different from one (t = −1.00; P = 0.8403).

The overall MRE values associated with both the larval and adult mortalities in facility A were smaller than those measured under laboratory conditions (Tables 4 and 5). The overall MRE value associated with larval mortality was greater than that of adult mortality (Table 5). This might have been caused by higher control mortality of the larvae than that of adults (the control mortality of the adults was <1% while the larval mortality was >10%). The trend of the predicted

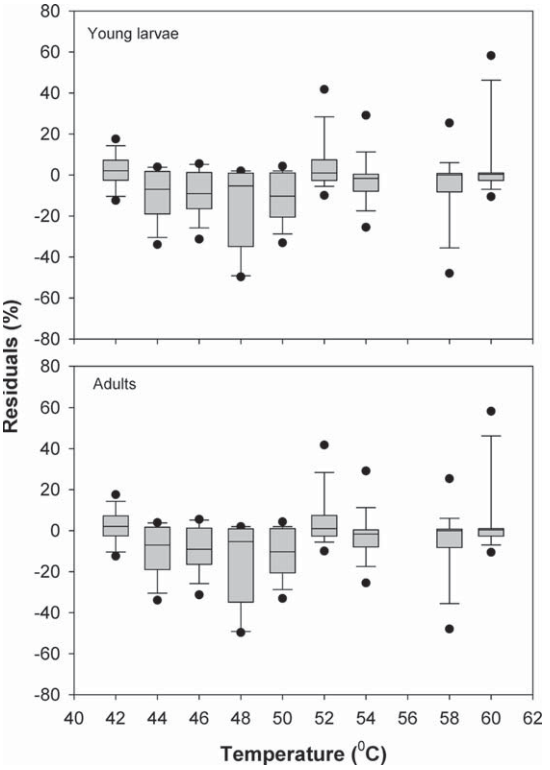

Fig. 5. Box plots of residuals between observed and predicted mortalities for young larvae (top graph) and adults (bottom graph) of *Tribolium castaneum* at constant temperatures in the laboratory. A line within the box marks the median of the residuals, whiskers (error bars) above and below the box indicate maximum and minimum nonoutliers. Dots show the 5th/95th percentile outliers.

insect mortality agreed with the measured insect mortality (Fig. 6). The skewness associated with the mortality of the larvae and adults at each location was not in the same direction (Fig. 7) and there was no skewness in the overall predictions (Fig. 8).

At each location in facility A, the predicted mortality was skewed toward underestimation (19 out of 28 locations for larvae, 11 out of 28 for adults) or overestimation (8 out of 28 locations for larvae, 3 out of 28 for adults) (Fig. 7). The medians of the residuals were zero in 27 of 28 locations for the larvae and 26 of 28 locations for the adults. These results could be explained by the following facts: 1) at each location, there were a total of four residuals for larvae and four for adults; 2) three out of the four residuals were zero in >78.6% cases for larvae and 39.3% for adults (Table 5); and 3) if three out of four residuals were zero, then only one of the four residuals showed a skewed distribution.

In facility A, the MRE value was ≤0.15 in 109 out of 112 cases (97%) for the larvae and 108 out of 112 cases (96%) for the adults (Table 5). These results indicated that there were wrong predictions in 3% of cases for the larvae and 4% for the adults. These wrong predictions were at location 4, 8, 17, 20, and 21 (Figs. 6 and

Table 5. Mean ± SE magnitude of relative error (MRE, equation10) values associated with mortality of young larvae and adults of *Tribolium castaneum* exposed to elevated temperatures during heat treatment of a room at facility A

Location	Young larvae				Adults			
	Mean ± SE	=0[a]	≤0.15[b]	>0.5[c]	Mean ± SE	=0[a]	≤0.15[b]	>0.5[c]
1	0.023 ± 0.022	3	1	0	0.024 ± 0.022	3	1	0
2	0.002 ± 0.000	4	0	0	0.021 ± 0.021	3	1	0
3	0.024 ± 0.022	3	1	0	0.001 ± 0.000	4	0	0
4	0.246 ± 0.245	3	0	1	0.000 ± 0.000	4	0	0
5	0.024 ± 0.022	3	1	0	0.023 ± 0.009	3	1	0
6	0.059 ± 0.035	2	2	0	0.039 ± 0.023	2	2	0
7	0.024 ± 0.022	3	1	0	0.000 ± 0.000	4	0	0
8	2.653 ± 2.651	3	0	1	0.167 ± 2.651	2	1	1
9	0.002 ± 0.000	4	0	0	0.000 ± 0.000	4	0	0
10	0.024 ± 0.011	3	1	0	0.000 ± 0.000	4	0	0
11	0.024 ± 0.022	3	1	0	0.000 ± 0.000	4	0	0
12	0.024 ± 0.020	3	1	0	0.019 ± 0.012	3	1	0
13	0.024 ± 0.022	3	1	0	0.000 ± 0.000	4	0	0
14	0.024 ± 0.022	3	1	0	0.000 ± 0.000	4	0	0
15	0.024 ± 0.012	3	1	0	0.000 ± 0.000	4	0	0
16	0.023 ± 0.017	3	1	0	0.019 ± 0.021	3	1	0
17	0.423 ± 0.394	2	1	1	0.168 ± 0.166	3	0	1
18	0.025 ± 0.012	3	1	0	0.000 ± 0.000	4	0	0
19	0.029 ± 0.027	3	1	0	0.039 ± 0.037	3	1	0
20	0.016 ± 0.014	3	1	0	0.251 ± 0.249	3	0	1
21	0.142 ± 0.064	1	1	0	2.150 ± 2.149	3	0	1
22	0.000 ± 0.000	4	0	0	0.025 ± 0.020	3	1	0
23	0.024 ± 0.023	3	1	0	0.000 ± 0.000	3	1	0
24	0.021 ± 0.025	3	1	0	0.037 ± 0.022	2	2	0
25	0.021 ± 0.009	3	1	0	0.023 ± 0.024	2	2	0
26	0.021 ± 0.012	3	1	0	0.000 ± 0.000	4	0	0
27	0.020 ± 0.008	3	1	0	0.000 ± 0.000	4	0	0
28	0.023 ± 0.009	3	1	0	0.000 ± 0.000	4	0	0
Overall	0.143 ± 0.096	83	24	3	0.108 ± 0.075	93	15	4

[a] Number of locations where the MRE value = 0.0.
[b] Number of locations where the MRE value ≤0.15 but >0.
[c] Number of locations where the MRE value >0.50.

7). There was larger than ±20% residuals at locations 4, 8, and 17 for larvae and at locations 8, 17, 20, and 21 for adults. These locations were close to heaters (location 4 and 17), door (location 8), fan (location 21), and on top of a machine (location 21) (Fig. 2). At these locations, the measured temperatures next to the vial (that were the temperatures used to predict insect mortalities) might not be the actual temperatures experienced by the insects. This assumption was supported by the fact that the flour temperature at the bottom of vials is most influenced by floor temperatures because of thermal conduction (Jian et al. 2012a). Additionally, when vials were located at places close to the door, the floor temperature would be lower than the air temperature because of heat lost through conduction of the floor; a similar phenomenon may occur where vials were placed on metal surfaces (Jian et al. 2012b). In case of vials placed close to heaters or near fans blowing hot air, higher temperatures may have forced insects to burrow deep into the flour (Jian et al. 2005). Adding (at location 4 and 17) and deducting (at locations 8, 20, and 21) 5°C to the measured temperature can make the MRE value at these locations smaller than 0.01 and the residuals smaller than ±10%. These plausible explanations were further supported by the results associated with the data collected in facility B, where temperatures were measured both inside and outside vials. Depending on the location either temperature measured inside the vial or outside the vial gave a better prediction of observed insect mortality. Generally, predictions based on temperatures measured inside vials gave better insect mortality prediction. If better predictions were used, all of the MRE and residuals values were smaller than 0.9 (Table 6) and within 20% of the measured mortality (Fig. 9), respectively. In facility A, MRE values and residuals were zero in 83 out of 112 cases (74%) for larvae and 93 out of 112 cases (83%) for adults (Table 5). This trend was also found in facility B (Table 6). The zero MRE or residuals indicated that the predicted insect mortality was equal to the measured insect mortality. These equal mortalities mostly occurred when the mortality was zero or 100% (Fig. 6). When the measured mortality at the facility A was zero or 100%, the developed model could predict the measured insect mortality in 84 out of 85 cases (99%) for larvae and 93 out of 97 cases (96%) for adults. In facility B, when temperatures inside the vials were used, the corrected prediction rate was 96%. Therefore, the developed model predicted the M-I and M-100% times with a high degree of accuracy.

The temperatures that were lower than the base temperature at the beginning of the heat treatment contributed nothing to insect lethality until the temperature was higher than the base temperature. Temperatures that were higher than the base temperature but lower than 42°C contributed very little to the total insect mortality. The developed models accounted for

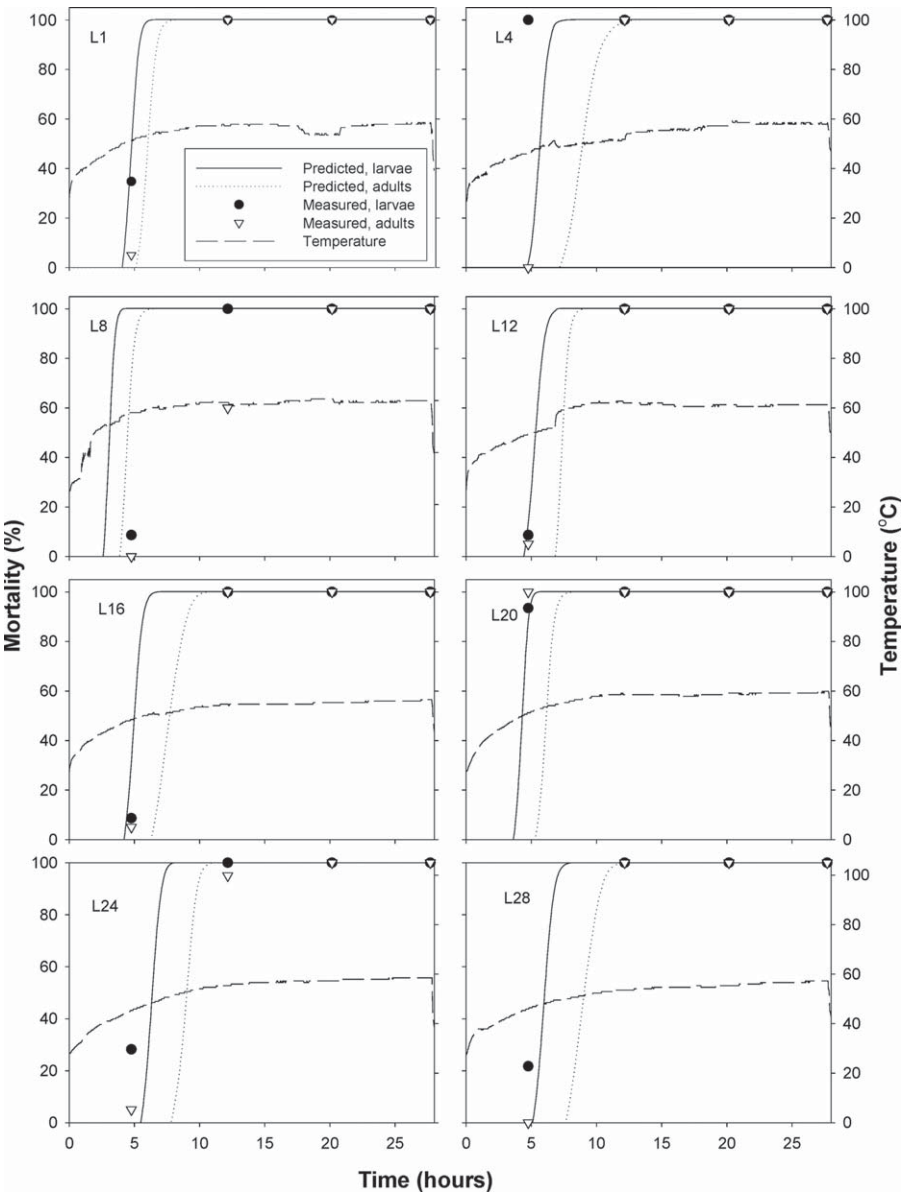

Fig. 6. Observed and predicted insect mortality of *Tribolium castaneum* adults exposed to elevated temperatures during heat treatment at facility A. In the graph, "L" and the number after the L is the location. The location was selected at location 1 and from 4 to 28 with four increments. The predicted mortalities were based on equation 7 and parameter values given in Table 3.

the low temperatures and the ramp-up time. The effective times could be easily calculated from the measured temperature-time history regardless of how treatments were set up. Therefore, the developed models will be useful to predict mortality under new treatment conditions.

Inside the injury zone insect mortality could be predicted by using equation 7, a double-logarithmic model (Jones et al. 1995, Thomas and Mangan 1997, Waddell et al. 1997), which has been used successfully to predict insect mortality at constant temperatures. This model can be used to predict insect mortality under dynamic temperature conditions after mathematical conversion (Jian et al. 2010). However, the model (after conversion) should only be used inside the injury zone where mortality is 0.1–99.9%, because insect mortality would be zero in less than M-I time and insect mortality would be 100% in higher than M-100% time.

The models developed in this study did not account for insect acclimation to elevated temperatures. Boina et al. (2008) did not observe the effect of acclimation in their thermal death validations, and did not have a factor to adjust the model for thermal acclimation.

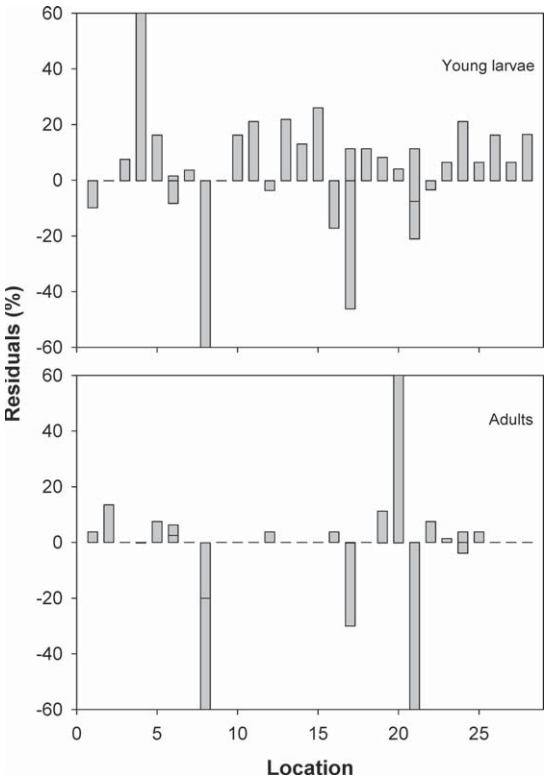

Fig. 7. Box plots of residuals between observed and predicted mortalities of young larvae (top graph) and adults (bottom graph) of *Tribolium castaneum* at each location exposed to elevated temperatures during heat treatment at facility A. If there is no line within the box, the median of the residual is zero.

Table 6. Mean ± SE magnitude of relative error (MRE, equation10) values associated with mortality of adults of *Tribolium castaneum* exposed to elevated temperatures during heat treatment of a room at facility B

Location	Mean ± SE	=0[a]	≤0.15[b]	>0.5[c]
1	0.000 ± 0.000	3	1	0
2	0.015 ± 0.014	2	2	0
3	0.000 ± 0.000	3	1	0
4	0.022 ± 0.022	3	1	0
5	0.026 ± 0.026	3	1	0
6	0.000 ± 0.000	4	0	0
7	0.032 ± 0.032	3	1	0
8	0.026 ± 0.026	3	1	0
9	0.025 ± 0.025	2	2	0
10	0.027 ± 0.027	3	1	0
11	0.000 ± 0.000	4	0	0
12	0.045 ± 0.044	3	1	0
13	0.000 ± 0.000	3	1	0
14	0.000 ± 0.000	3	1	0
15	0.019 ± 0.019	3	1	0
16	0.027 ± 0.027	3	1	0
17	0.030 ± 0.030	3	1	0
18	0.015 ± 0.015	3	1	0
19	0.000 ± 0.000	3	1	0
20	0.000 ± 0.000	3	1	0
21	0.082 ± 0.082	3	0	0
22	0.083 ± 0.083	3	0	0
23	0.016 ± 0.016	3	1	0
24	0.012 ± 0.012	3	1	0
Overall	0.026 ± 0.062	72	22	0

[a] Number of locations where the MRE value = 0.0.
[b] Number of locations where the MRE value ≤0.15 but >0.
[c] Number of locations where the MRE value >0.50.

They suggested that: 1) irrespective of heating rate, the survival of insects decreased at temperature >40°C and was not influenced by the heating rate; and 2) minimal or no acclimation occurred at temperatures >40°C or when insects were heat-treated for short periods (i.e., 24 h). This hypothesis was supported by Gonen (1977a, b). The fact that developed models could predict insect mortality with a high degree of accuracy might indicate that there was no acclimation effect during structural heat treatments at facilities A and B.

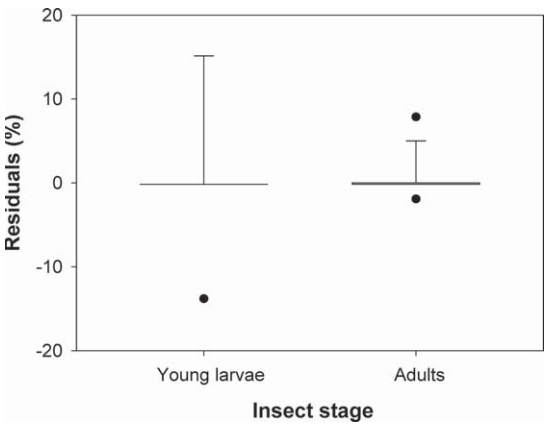

Fig. 8. Box plots of residuals between observed and predicted mortalities of young larvae and adults of *Tribolium castaneum* at all locations exposed to elevated temperatures during heat treatment at facility A. The median, 25th and 75th percentile are zero. Whiskers indicate the maximum and minimum nonoutliers. Dots show the 5th/95th percentile outliers.

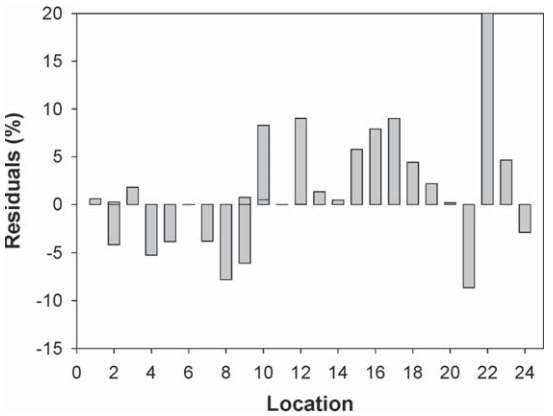

Fig. 9. Box plots of residuals between observed and predicted mortalities of adults of *Tribolium castaneum* exposed to elevated temperatures during heat treatment at facility B. The best predicted adult mortality at each location was used. If there is no line within the box, the median of the residual is zero.

Degree-minute models also use a base temperature (threshold temperature) to calculate insect mortality (Subramanyam et al. 2002). Threshold temperature varies with treatment insect species and stage (Hansen et al. 2004). Unlike our study, the selection of this value has been arbitrary (Subramanyam et al. 2002, Hansen et al. 2004, Boina et al. 2008). The procedure to calculate the base temperature in this study has a logical and valid quantitative basis.

The models developed here could be used to visualize insect mortality during the heat treatment process, because the injury and noninjury zones have a practical meaning. For example, to achieve a successful heat treatment, both the treatment time and temperature must be located inside the injury zone with 0.1–99.9% mortality. If the effective time is less than the M-I time, there will be no mortality of insects. If the effective time is less than M-100% time and larger than M-I time, insect mortality will be <100%. If both heat treatment temperature and its associated time were located inside the zone of injury with 100% mortality, 100% of the insects will be killed. However, heat energy may be wasted. Therefore, a successful heat treatment should use temperatures and times to reach exactly the M-100% time. From the view of energy efficiency and to control *T. castaneum* young larvae and adults, the heat treatment temperature should be around 50°C (Fig. 1). If the temperature is <46°C both the M-I and M-100% times would increase exponentially (Fig. 1). If the heat treatment temperature was higher than 50°C, both the M-I and M-100% times have an almost linear decrease with an increase in temperature (Fig. 1). Higher temperature differences between outside and inside of the heat treated building would also result in higher heat energy losses.

The developed concepts and calculated parameters in this study might be used as comparison factors. For example, the M-100% time might be used as a factor to compare thermotolerance of different stages of an insect species or among insect species. If M-100% times of two insect species or two life stages of a species at the same reference temperature were compared, the higher M-100% time would indicate a higher thermotolerance. The M-100% time of young larvae and adults of *T. castaneum* at 50°C was 135 and 55 min, respectively. This indicated that young larvae were more heat tolerant than adults; this was supported earlier by Mahroof et al. (2003a) based on time-mortality responses at elevated temperatures. Therefore, the zones, M-I and M-100% times, and base temperature developed in this study could be used as a basic guideline for controlling *T. castaneum* young larvae and adults during facility heat treatments.

Acknowledgments

We thank the facility managers for allowing us access to conduct bioassays and measure temperatures during heat treatments of their facilities. Research reported here was supported by grants from the Propane Education and Research Council, Washington, DC, Temp-Air, Burnsville, MN, conducted the heat treatment and provided information on fan placement. This article is published as contribution number 13-014-J of the Kansas State University Agricultural Experiment Station.

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Received 17 July 2012; accepted 19 March 2013.