

Dynamic Model for Predicting Survival of Mature Larvae of *Tribolium confusum* During Facility Heat Treatments

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ABSTRACT Structural heat treatment, a viable alternative to methyl bromide fumigation, involves raising the ambient temperature of food-processing facilities between 50 and 60°C by using gas, electric, or steam heaters, and holding these elevated temperatures for 24 h or longer to kill stored-product insects. A dynamic model was developed to predict survival of mature larvae, which is the most heat-tolerant stage of the confused flour beetle, *Tribolium confusum* (Jacquelin du Val), at elevated temperatures between 46 and 60°C. The model is based on two nonlinear relationships: 1) logarithmic survival of *T. confusum* mature larvae as a function of time, and 2) logarithmic reduction in larval survival as a function of temperature. The dynamic model was validated with nine independent data sets collected during actual facility heat treatments conducted on two separate occasions at the Kansas State University pilot flour and feed mills. The rate of increase of temperature over time varied among the nine locations where mature larvae of *T. confusum* were exposed, and the approximate heating rates during the entire heat treatment ranged from 1.1 to 13.2°C/h. The absolute deviation in the predicted number of larvae surviving the heat treatment was within 3–7% of the actual observed data. Comparison of the absolute deviation in the time taken for equivalent larval survival showed that the model predictions were within 2–6% of the observed data. The dynamic model can be used to predict survival of mature larvae of *T. confusum* during heat treatments of food-processing facilities based on time-dependent temperature profiles obtained at any given location.

KEY WORDS *Tribolium confusum*, survival, heat treatments, modeling

Heat treatment of food-processing facilities is becoming popular as an alternative to methyl bromide fumigation. During structural heat treatments, the ambient temperature of the entire facility, or a portion of it, is raised to 50–60°C by using gas, electric, or steam heaters, and these elevated temperatures are held for 24 h or longer to kill stored-product insects (Dosland et al. 2006). Predicting mortality or survival of insects at elevated temperatures is necessary for optimizing heat treatments and for ensuring effective disinfestation of food-processing facilities. Without such models, facilities subjected to heat treatments could be over- (>60°C) or underheated (<50°C). Over heating wastes energy, and it may damage heat-sensitive equipment. Underheating may result in insects surviving the heat treatment. Optimizing heat treatments involves reducing energy costs while achieving 100% mortality of insects and monitoring temperatures and

redirecting heat to areas that are being underheated to achieve complete insect mortality.

Heat accumulation models that use the number of degree-minutes accumulated above a threshold temperature have been used to predict mortality of stored-product insect pests exposed to elevated temperatures (Banks and Fields 1995, Wright et al. 2002, Subramanyam et al. 2003). Wright et al. (2002) developed a degree-minute model for predicting the mortality of large larvae of the warehouse beetle, *Trogoderma variabile* Ballion, by obtaining time–mortality data at four constant temperatures between 50 and 56°C. The base temperature for accumulating degree-minutes, and the intercept and slope of the linear regression of mortality (expressed as the inverse of the standard normal deviate), and degree-minutes accumulated were different at each of the four temperatures. Despite these differences, Wright et al. (2002) pooled the data across 52, 54, and 56°C to describe the relationship between degree-minutes and mortality of large larvae of *T. variabile*. No statistical or biological basis was given for pooling the data across the three temperatures. Subramanyam et al. (2003) developed a simple heat-accumulation model for predicting the mortality of first instars of the red flour beetle, *Tribolium castaneum* (Herbst). The model was based on time–mortality data of young larvae of *T. castaneum* collected at the six constant temperatures between 42

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and 60°C (Mahroof et al. 2003a). Independent data on first instars, also collected at the same constant temperatures, were used to validate the model. The base temperature for accumulating degree-minutes was iteratively estimated to be 49.1°C. The degree-minute model underestimated mortality by 25%, but it explained $\approx 70\%$ of the variation in observed mortality of insects as a function of both temperature and time. The degree-minute models mentioned above have not been validated under field conditions, and as indicated by Subramanyam et al. (2003) have performed poorly in predicting mortality of insects. They are especially unsuitable for predicting incremental mortality of insects at dynamically changing temperature and time that occurs during heat treatment of food-processing facilities.

The use of thermal death kinetic models for predicting mortality or survival of insects subjected to heat treatments seems more appropriate than degree-minute models, because these models are dynamic (Tang et al. 2000), and they can be used to predict mortality of organisms (bacteria or insects) for any incremental temperature and time combination. Thermal death kinetic models were originally developed for thermal inactivation of bacteria (Van Impe et al. 1992, Baranyi et al. 1996), and these models have a rational and biological basis for describing temperature-time-mortality relationships in environments subjected to elevated temperatures.

Thermal death kinetic models that have been developed for insect pests associated with fresh fruits (quarantine pests), dried fruits, and tree nuts are based on high temperature short time concept (Landolt et al. 1984; Neven 1994; Waddell et al. 2000; Wang et al. 2002a,b; Johnson et al. 2001, 2003), because preserving the quality of the commodity being treated is most important besides controlling insect pests (Neven 1998a,b). The typical heating rates vary anywhere from 1 to 18°C/min, and the final temperatures may exceed 50°C depending on the commodity being heated. In contrast, during facility heat treatments, gas, electric, or steam heaters are used to slowly heat the ambient air, and the duration of heat treatment typically lasts for 24–36 h (Imholte and Imholte-Tauscher 1999, Dowdy and Fields 2002, Wright et al. 2002). As a result, the temperature increase varies from location to location, and from floor to floor within a facility, due to stratification of heat both horizontally and vertically (Dowdy 2000). Heating rates (ambient to 50°C) range anywhere from 0.3 to 13.7°C/h (Mahroof et al. 2003a, Roesli et al. 2003), but typical heating rates should be around 3–5°C/h to prevent thermal shock to structural components of the facility (Imholte and Imholte-Tauscher 1999, Fields and White 2002). The rate of heating influences the susceptibility of insect species and life stages within a species (Mahroof et al. 2003b; Neven 1998a; Wang et al. 2002a,b) due to heat acclimation. However, such susceptibility differences tend to diminish at temperatures over 55°C (Fields and White 2002). Therefore, the thermal death kinetic models used for insect pests of fresh fruits, dried fruits, and tree nuts are not suitable for describ-

ing the survival of stored-product insects during facility heat treatments, and initial attempts to use these models to predict survival of insects exposed to facility heat treatments resulted in poor fit of data to the models (Bh.S., unpublished data). Therefore, development of new thermal death kinetic models was deemed necessary for predicting survival of insects subjected to higher temperatures for longer periods that occur during facility heat treatments.

To model survival of insects at dynamically changing temperatures over time, it is essential to obtain time-mortality data at constant temperatures (Van Impe et al. 1992, Baranyi et al. 1996). Fields (1992) suggested using the most heat tolerant developmental stage of an insect at a range of elevated temperatures when developing models to predict insect survival or mortality during facility heat treatments. In this article, we describe a novel dynamic model for predicting the survival of mature larvae of *Tribolium confusum* (Jacquelin du Val) (Coleoptera: Tenebrionidae), an economically important insect pest associated with feed and flour mills in the United States and throughout the world (Sinha and Watters 1985, Mills and Pedersen 1990). Mature larvae of *T. confusum* were used for model development and validation, because it is more heat tolerant than eggs, young larvae, pupae, and adults at elevated temperatures between 50 and 60°C (Boina and Subramanyam 2004). Our objectives were to develop a dynamic model using time-mortality data obtained at elevated constant temperatures and to validate it under field conditions with independent data collected during facility heat treatments in the pilot flour and feed mills at Kansas State University (Manhattan, KS).

Materials and Methods

Time-Mortality Relationships of *T. confusum* Mature Larvae. *T. confusum* cultures were reared on 95% whole wheat flour and 5% brewer's yeast (by weight) at $28 \pm 0.5^\circ\text{C}$, $65 \pm 5\%$ RH, and a photoperiod of 14:10 (L:D) h. Mature larvae that were 22–23 d old from the time of hatching from eggs, with a mean \pm SE weight of 3.68 ± 0.03 mg ($n = 10$), were separated from the rearing media by using a sieve with 0.83-mm openings. Fifty individuals were placed in separate plastic test boxes (4.5 by 4.5 by 1.5 cm), each holding 1.5 g of the rearing medium. The lids of the boxes had 3-cm diameter perforations that were covered with 600 μm wire mesh screens for ventilation. Test boxes with insects were exposed to constant temperatures of 46, 48, 50, 54, 58, and 60°C and 20–22% RH in growth chambers (model I-36 VL, Percival Scientific, Perry, IA). The low relative humidity used at 46–60°C is typical of humidity levels observed during facility heat treatments (Mahroof et al. 2003b, Roesli et al. 2003).

Two boxes were removed from the growth chamber at each temperature treatment at different time intervals, after accounting for the time it takes for the flour in test boxes to attain the set chamber temperature, reported earlier by Boina and Subramanyam (2004). The larvae and rearing medium in each box

Table 1. Parameters of the nonlinear equations describing the relationship between logarithm of survival of *T. confusum* mature larvae and exposure time at six constant temperatures

Temp (°C)	n ^a	Parameter				Adj R ²
		a	b	c	d	
46 ^b	18	1.94 ± 0.10	-3.15 ± 2.15	272.05 ± 63.45	28.95 ± 6.09	0.97
48 ^c	18	2.04 ± 0.13	-0.005 ± 0.00	-0.01 ± 0.00		0.97
50 ^c	22	1.97 ± 0.07	-0.01 ± 0.00	-0.03 ± 0.00		0.95
54 ^c	18	2.03 ± 0.02	-0.02 ± 0.00	-0.05 ± 0.00		0.99
58 ^c	12	1.98 ± 0.06	-0.03 ± 0.00	-0.07 ± 0.00		0.98
60 ^c	11	1.97 ± 0.07	-0.05 ± 0.00	-0.10 ± 0.00		0.95

^a n, number of observations.

^b The four parameter model was $y = a + b/(1 + \exp(-(x - c)/d))$.

^c The three parameter model was $y = (a + cx)/(1 + bx)$.

were transferred to separate 150-ml plastic containers holding 10 g of flour. These plastic containers were placed in a growth chamber at 28°C, 65% RH, and 14:10 (L:D) h photoperiod until adult emergence to assess mortality. Mortality of mature larvae, expressed as a percentage, was based on the number of insects that failed to emerge as adults out of the total exposed. Natural mortality of *T. confusum* mature larvae was determined by exposing them in two test boxes with 1.5 g of flour in a growth chamber set at 28°C, 65% RH, and 14:10 (L:D) h for the maximum duration used at each elevated temperature treatment. Each temperature-time combination was replicated three times by conducting tests at three different times.

Model Development. Larval survival (100 - mortality) as a function of time at each of six constant temperatures was described by nonlinear regressions, after transforming the survival data to logarithmic scale. Data at temperatures between 46 and 60°C were fit to two nonlinear equations by using Table Curve 2D software (Jandel Scientific 1994). Time-survival data at 48 - 60°C were well described by a three-parameter nonlinear equation 1. However, this equation did not describe well the time-survival data at 46°C. Therefore, another four-parameter nonlinear equation 2 was fit to the data at 46°C.

$$y = (a + cx)/(1 + bx) \quad [1]$$

$$y = a + b/(1 + \exp(-(x - c)/d)) \quad [2]$$

where *y* is the logarithm of survival, *x* is the exposure time in minutes, and *a*, *b*, *c*, and *d* are parameters estimated from the logarithm of survival and exposure time data (Table 1).

The slope of the logarithm of survival as a function of time was nonlinear. Therefore, an average instantaneous slope at each temperature was calculated by determining slope every 3 min by using differential equations derived from the two nonlinear equations 1 and 2 (Table 2). The slope every 3 min and the average instantaneous slope at each constant temperature were calculated by programming the differential equations in Microsoft Excel (Microsoft 2000). Inverse of the average instantaneous slope value yielded a mean instantaneous *D*-value, which gave the time required for one log reduction in survival of mature larvae of *T. confusum* at each of the temperatures.

Table 2. Differential equations used for calculating mean instantaneous *D*-values from the nonlinear relationship between logarithm of survival of *T. confusum* mature larvae and exposure time at six constant temperatures

Temp (°C)	Equation (dy/dx) ^a	Mean ± SE instantaneous <i>D</i> -value (min)
46	$-b/(1 + \exp(-(x - c)/d))^2 - 1/d$	912.86 ± 172.54 (64) ^b
48	$((1 + bx)^* c - (a^* cx)^* b)/(1 + bx)^2$	207.22 ± 25.50 (40)
50	$((1 + bx)^* c - (a^* cx)^* b)/(1 + bx)^2$	94.75 ± 15.68 (18)
54	$((1 + bx)^* c - (a^* cx)^* b)/(1 + bx)^2$	67.46 ± 14.67 (14)
58	$((1 + bx)^* c - (a^* cx)^* b)/(1 + bx)^2$	55.97 ± 15.07 (10)
60	$((1 + bx)^* c - (a^* cx)^* b)/(1 + bx)^2$	24.84 ± 9.47 (6)

^a The nonlinear equation for 46°C was $y = a + b/(1 + \exp(-(x - c)/d))$, and for 48 to 60°C was $y = (a + cx)/(1 + bx)$.

^b Number of observations for computing mean ± SE.

The change in mean instantaneous *D*-value as a function of temperature was described by equation 3:

$$D(T_t) = a + b \exp(c * T_t) \quad [3]$$

where *D*(*T*_{*t*}) is mean instantaneous *D*-value, *T*_{*t*} is temperature, and *a*, *b*, and *c* are parameters estimated from the equation fit to the mean instantaneous *D*-value and temperature data.

The dynamic model for predicting survival of mature larvae of *T. confusum* was derived from the following relationship based on first-order kinetics (Stumbo 1973):

$$\log\left(\frac{N}{N_o}\right) = \frac{-t}{D_T} \quad [4]$$

where, *N*_o is the initial number of insects, *N* is the number of insects surviving at time *t*, and *D*_{*T*} is *D*(*T*_{*t*}) as shown in equation 3. Equation 4 can be written in a differential form as follows:

$$\log\left(\frac{N_{dt}}{N_o}\right) = \frac{-dt}{D(T_t)} \quad [5]$$

where, *N*_{dt} is the insect survival at time *dt*. Upon integration, equation 5 becomes:

$$\int_{N_o}^{N_t} \log\left(\frac{N_{dt}}{N_o}\right) = \int_0^t \frac{-dt}{D(T_t)} \quad [6]$$

$$\log\left(\frac{N_t}{N_o}\right) = \int_0^t \frac{-dt}{D(T_t)} \quad [7]$$

$$\log\left(\frac{N_o}{N_t}\right) = \int_0^t \frac{dt}{D(T_t)} \quad [8]$$

where, *N*_{*t*} is the number of insects at time *t*. By changing the mathematical equation 8 to a numerical equa-

tion, and taking the antilog on both sides, yields equations 9 and 10.

$$\log \frac{N_t}{N_o} = - \sum_0^t \frac{\Delta t}{D(T_i)} \quad [9]$$

$$N_t = \frac{N_o}{\left(\sum_0^t \frac{\Delta t}{D(T_i)} \right)} \quad [10]$$

where Δt is the incremental exposure time.

Model Validation. The model was validated with nine different data sets (a set includes insect survival data from a given location in a mill over time from independent samples) collected during heat treatments of the Kansas State University pilot feed and flour mills. The pilot feed mill was heated using natural gas heaters from Temp-Air (Rupp Industries, Inc., Burnsville, MN) during 5–7 August 2003. Three THP-550 heaters and one THP-1400 heater with a production capacity of 550,000 BTU/h (138,545 kcal/h) and 1,400,000 BTU/h (352,660 kcal/h), respectively, were used for heating the mill. The heaters were placed outside the mill and the heat generated was discharged into the mill using 50.8-cm-diameter nylon ducts with circular openings (≈ 10 cm in diameter). Bayley fans (Rupp Industries, Inc., Burnsville, MN), each with a 1.5-horsepower motor, with a fan blade diameter of 0.78 m, and an airflow rate of 391 cm³/min, were placed in the first, second, and third floors (two fans per floor) to recirculate the hot air. In the first floor of the feed mill, two locations at ≈ 2 and 3 m from a heat-discharging duct, were selected for exposing the mature larvae of *T. confusum*. Different distances from the heating duct were used to obtain different heating rates. At each location, 15 test boxes (4.5 by 4.5 by 1.5 cm) were placed. Each test box held 1.5 g of medium and 50 *T. confusum* mature larvae. Temperature data at each location were collected at 1-min intervals (Δt in equation 10) by placing thermocouples of two HOBO data-logging units in the flour of two of the test boxes with rearing media and insects.

Seven additional data sets were collected from 27 February to 1 March 2004 heat treatment of the Kansas State University (KSU) pilot flour mill. Details about the pilot mill and its built-in and portable heater steam heaters were described in a previous article (Mahroof et al. 2003b). Tests were conducted on the fourth floor of the cleaning house, as this floor had a portable steam heater (no. 3507, Armstrong-Hunt, Milton, FL). The heater had a built-in fan (Baldor Electric Corporation, Fort Smith, AR) that operated at 1,725 rpm. Seven locations, at 3–10 m from the steam heater, were selected for collecting the validation data. At each location, 22 test boxes, each holding 1.5 g of medium and 50 individuals of *T. confusum* mature larvae, were placed on the floor. Temperature data at each location were collected at 1-min intervals (Δt in equation 10) by placing two HOBO data-logging units next to the

boxes with thermocouple wires from the units inserted into two of the boxes as mentioned above.

During the August 2003 heat treatment, one box from each location was removed at different time intervals. During the February to March 2004 heat treatment, two boxes from each location were removed at different time intervals. Test boxes with insects, collected at the beginning of the heat treatment, were placed in a growth chamber at 28°C and 65% RH and served as controls for determining natural mortality. The time intervals for removing boxes from the heat-treated area were decided based on the surface temperatures measured inside the test boxes using an infrared thermometer (model 4TP78, Raytek, Santa Cruz, CA). The accuracy of measurement with this instrument is $\pm 0.25\%$ for temperatures $\leq 600^\circ\text{C}$. Test boxes were sampled after the infrared thermometer indicated temperatures inside boxes were above 40°C. Insects and wheat flour in test boxes, collected at different time intervals during heat treatment, were transferred to 150 ml plastic containers with 10 g of wheat flour. The plastic containers were placed in growth chambers set at 28°C and 65% RH until emergence of adults. The number of larvae that survived heat treatment temperatures was based on the number of adults that emerged out of the 50 or 100 larvae originally exposed to heat (N_o in equation 10). At each location, T_i was determined by averaging the readings from the two HOBO data-logging units. $D(T_i)$ for any given temperature-time combination was estimated by using T_t in equation 3. The survival of mature larvae (N_t) as a function of dynamically changing T_t was predicted using equation 10 by writing a program in Microsoft Excel. The approximate heating rate observed at each location was determined from the time-dependent temperature profile (T_i) as follows: (final temperature – starting temperature in °C)/total heat treatment time in hours.

Model Performance. The predicted survival of larvae at each location and observation time was compared with the observed survival. The deviation (difference) in the observed and predicted number of larvae surviving at each observation time during heat treatment, and deviation in time for equivalent larval survival at each observation time, were determined. These deviations were averaged across all observation times to determine the absolute deviation, which provided information on the overall performance of the model. Differences in the observed and predicted larval survival and time for equivalent larval survival at each heating rate were compared using the chi-square goodness-of-fit test statistic at the $\alpha = 0.05$ level (Zar 1984). The absolute deviation in the number of larvae surviving the heat treatment relative to the total exposed was expressed as a percentage by multiplying it with 100. Similarly, the absolute deviation in time for equivalent larval survival was divided by the total heat treatment time and multiplied by 100 to express the absolute deviation as a percentage.

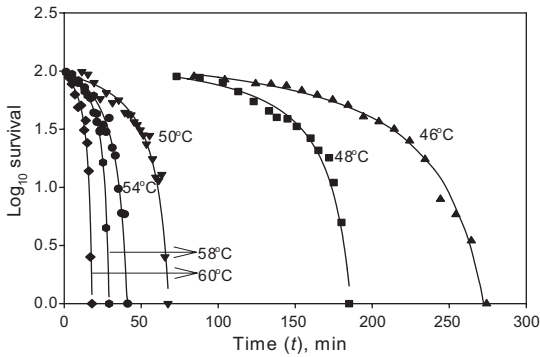


Fig. 1. Nonlinear relationship describing survival of mature larvae of *T. confusum* as a function of exposure time.

Results

Survival of Mature Larvae as a Function of Time.

The mean ± SE ($n = 18$, pooled across the controls for the six constant temperatures tested) natural mortality of mature larvae was 3.7 ± 1.8 . Therefore, insect survival data at the six temperatures were not corrected for natural mortality. The logarithm of survival of *T. confusum* mature larvae over time was nonlinear at each of the six constant temperatures (Fig. 1). The relationship between logarithm of survival and exposure time was satisfactorily described by equations 1 and 2 at 46–60°C (adjusted [Adj] $R^2 = 0.95$ –0.98). The decrease in logarithm of survival was inversely related to temperature. At 46, 48, or 50°C, an initial lag period with near 100% survival was observed. Thereafter, larval survival decreased slowly, followed by a linear decrease. At temperatures of 54, 58, or 60°C, the initial lag period was short, and the logarithm of survival decreased in an almost linear fashion over time.

Mean Instantaneous *D*-Values. In general, the mean instantaneous *D*-values decreased nonlinearly with an increase in temperature (Fig. 2), and the relationship between *D*-values and temperature was adequately described by equation 3 (Adj $R^2 = 0.90$).

Temperature Measurements and Approximate Heating Rates. The starting temperature at different locations during the August 2003 and February to March 2004 heat treatments ranged from 24 to 31°C (Table 3). The temperature increase during the heat treatment was not uniform among locations. Locations (where test boxes with insects and HOBO data-logging units were placed) that are closer to steam heaters in the flour mill or hot-air duct in the feed mill showed a faster temperature increase, whereas locations that were away from heaters or hot-air ducts, especially those located at floor-wall junctions, showed a slower increase in temperature. The final temperature at different locations during validation, when the last test box with larvae was removed, ranged from 52 to 54°C (Table 3). The calculated approximate heating rates at different locations ranged from 1.1 to 13.2°C/h (Table 3).

Model Predictions. Natural mortality of *T. confusum* mature larvae was <1% (mean ± SE, 0.8 ± 0.4 , $n = 9$,

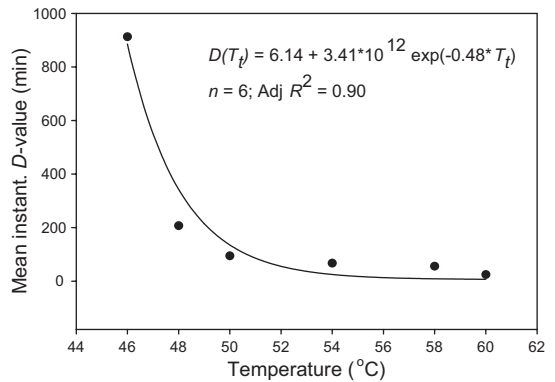


Fig. 2. Nonlinear relationship describing mean instantaneous *D*-value as a function of temperature. The SE for parameters *a*, *b*, and *c* were 56.14, 2.26×10^{13} , and 0.14, respectively.

pooled across the controls for the nine validation data sets). Therefore, survival data from test boxes at different heating rates were not corrected for natural mortality. At low heating rates of 1.1 and 1.8°C/h, the lag period (where survival is 100%) estimated by the model lasted 695 and 336 min, respectively (Fig. 3). The corresponding actual observed lag periods for the above-mentioned heating rates from our data were 676 and 310 min. Predicted lag periods decreased with an increase in the heating rate, and they were 51 and 61 min at high heating rates of 5.5 and 13.2°C/h, respectively. Corresponding observed lag periods for these heating rates were 64 and 61 min (Fig. 3). The slightly longer lag period predicted by the model in 13.2°C/h compared with 5.5°C/h was mainly due to a slow increase in temperature (up to 42°C) observed at the beginning of heat treatment at 13.2°C/h location as against a rapid increase observed at 5.5°C/h location (Fig. 3).

The model underpredicted and overpredicted the actual observed data at heating rates of 1.1–1.8°C/h. At heating rates of 2.1–13.2°C/h, the model consistently underpredicted the survival of mature larvae. The predicted times for 0% survival (complete mortality) of mature larvae were 146 and 1565 min at 13.2 and 1.1°C/h, respectively, whereas the corresponding actual observed times were 119 and 1550 min.

Model Performance. At approximate heating rates of 1.1–13.2°C/h, the absolute deviation in larval survival from observed values ranged from 2 to 7% (Table 3), and the absolute deviation in terms of time for equivalent larval survival ranged from 2 to 6%. The chi-square goodness-of-fit test showed that the larval survival predicted by the model was not statistically significant from the observed larval survival ($P > 0.05$) at all heating rates except at 2.4°C/h (Table 3). However, when goodness-of-fit test comparisons were based on time for equivalent larval survival, the model predictions were not significantly different ($P > 0.05$) from observed data for five of the nine heating rates.

Table 3. Performance of the model in predicting survival of *T. confusum* mature larvae during heat treatment of the Kansas State University pilot mills during validation experiments

Location ^a	Starting temp (°C)	Final temp (°C)	Time (min)	Approx. heating rate (°C/h) ^b	Mean ± SE absolute deviation in no. larvae surviving/100 larvae (%)	χ^2 (df)	P value	Mean ± SE absolute deviation in time for equivalent larval survival in min (%) ^c	χ^2 (df)	P value
1	24.4	53.5	1,550	1.1	4.1 ± 1.1 (4.1)	10.2 (9)	0.33	49.0 ± 14.3 (3.0)	44.3 (9)	0.00
2	24.8	52.4	1,424	1.2	3.9 ± 0.9 (3.9)	7.7 (9)	0.56	38.6 ± 13.5 (2.7)	35.9 (9)	0.00
3	26.7	51.8	1,260	1.2	3.5 ± 0.8 (3.5)	8.6 (9)	0.47	28.9 ± 4.6 (2.2)	10.6 (9)	0.30
4	27.5	51.8	1,193	1.2	2.5 ± 0.4 (2.5)	2.5 (9)	0.98	28.7 ± 7.2 (2.4)	12.4 (9)	0.19
5	25.6	52.7	914	1.8	3.0 ± 0.7 (3.0)	1.9 (9)	0.99	21.4 ± 5.4 (2.3)	9.4 (9)	0.40
6	25.9	52.4	748	2.1	3.7 ± 0.8 (3.7)	9.0 (9)	0.44	38.6 ± 12.7 (4.8)	90.8 (9)	0.00
7	27.5	52.4	609	2.4	4.4 ± 0.9 (4.4)	18.0 (9)	0.03	20.4 ± 4.1 (2.2)	10.3 (9)	0.33
8	31.1	54.1	251	5.5	3.5 ^d ± 0.6 (7.0)	18.1 (13)	0.15	15.1 ± 1.8 (5.8)	26.9 (13)	0.01
9	27.9	54.1	119	13.2	2.1 ^d ± 0.5 (4.0)	6.0 (13)	0.94	7.7 ± 2.6 (5.9)	16.6 (13)	0.22

^a Locations 1–7 were in the pilot flour mill, and locations 8 and 9 were in the feed mill.

^b Approximate heating rates were calculated as (final temp – starting temp in °C)/total heat treatment time in hours (time in minutes/60).

^c Percentages were calculated as: (time for equivalent larval survival/total heat treatment time) * 100.

^d Number of larvae out of 50 exposed larvae.

Discussion

The decrease in logarithm of survival was slower at constant temperatures below 50°C than at temperatures above 50°C. Wang et al. (2002b) also reported a

similar relationship in tests with fifth instars of the navel orangeworm, *Amyelois transitella* (Walker), exposed to temperatures between 46 and 54°C at a constant heating rate of 18°C/min. During model validation tests, our observed data for 0% survival of mature

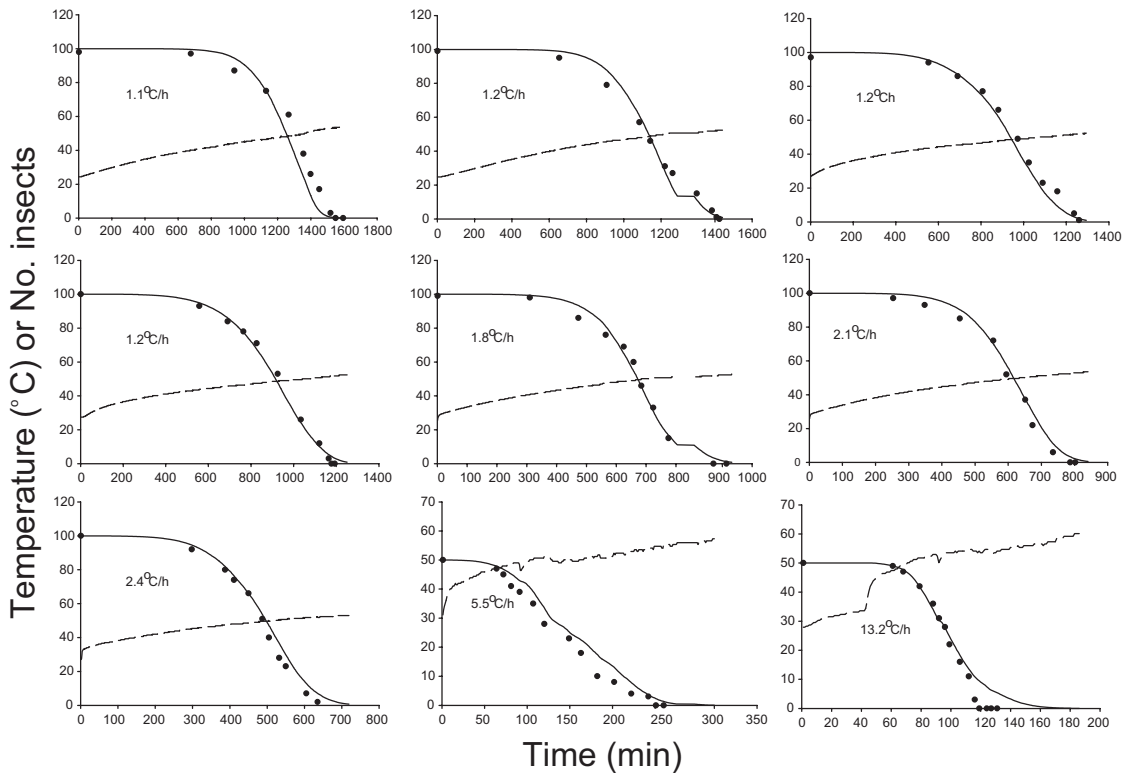


Fig. 3. Observed and predicted survival of *T. confusum* mature larvae at approximate heating rates of 1.1–13.2°C/h. The dashed line is the time-dependent temperature data (T_i) collected every 1 min (Δt), and the solid line is the predicted survival of insects over time (N_i). The solid circles represent actual number of insects surviving heat treatment temperatures at specific observations times. The initial number of insects (N_o) is either 100 or 50. NOTE: the x-axis scale is different among the nine graphs.

larvae (complete larval mortality) was achieved between 52 and 54°C. The temperature at 0% survival, based on the model predictions was 52–55°C. These findings suggest that temperatures $\geq 50^\circ\text{C}$ should be the minimum temperature to kill exposed mature larvae of *T. confusum* during facility heat treatments. Several researchers have hypothesized that 50°C may be the minimum temperature for effective disinfestation of facilities during heat treatments (Dowdy and Fields 2002, Wright et al. 2002, Mahroof et al. 2003b, Roesli et al. 2003, Boina and Subramanyam 2004).

Although the model does not have a factor to account for thermotolerance, it may be possible to characterize thermotolerance by examining the lag period (the time in minutes where larval survival is 100%) or the difference in lag periods between model predictions and observed data at the beginning of the heat treatment. The lag periods observed at different heating rates and those predicted by the model were essentially similar, which suggested that our model satisfactorily described the lag at the different heating rates tested. The longer lag period at low heating rates is an artifact of the model. Thus, at temperatures $< 46^\circ\text{C}$, the model assumes 100% survival of insects because the model was based on data at 46–60°C. The longer lag period at slow heating rates (1–2°C/h) may predispose larvae to become heat tolerant (physiological acclimation to heat) by altering their metabolic activities (respiration) or by producing heat shock proteins (Currie and Tufts 1997; Lewis et al. 1999; Neven 1998a, 2000). As a consequence, the insects' ability to survive exposures to high temperatures such as 40°C would be higher and may result in insects surviving longer exposures than that predicted by the model. However, we did not observe this effect in our validation tests, and irrespective of heating rate, the survival of mature larvae decreased at temperatures $> 40^\circ\text{C}$. This suggests minimal or no role for physiological acclimation to heat in insects when temperatures were $> 40^\circ\text{C}$ or when insects were acclimated to heat for shorter periods (i.e., 1 d) such as in heat treatments. Similarly, Gonen (1977a,b) showed that physiological acclimation to heat in the granary weevil, *Sitophilus granarius* (L.), adults exposed to 32°C for 14 d increased their ability to tolerate a 40°C exposure, whereas shorter acclimation period for 2 d under the same conditions did not. However, the heating rates close to 5°C/h, which are recommended for heat treating facilities, the heating rate is low enough not to cause any structural damage due to overheating and at the same time is adequate for providing quick mortality of *T. confusum* mature larvae (Mahroof et al. 2003b, Roesli et al. 2003).

The chi-square goodness-of-fit tests also indicated that the model predictions were close to the observed values, except at a few heating rates. The significant chi-square values at certain heating rates with respect to larval survival and time for equivalent larval survival was a result of large deviations between the observed and predicted values at the beginning and end of the heat treatment period. Despite significant chi-square values, assessment of model performance based on

absolute deviations showed that the model predictions were within 7% of the observed values, both with respect to larval survival or time for equivalent larval survival at all nine heating rates. Overall, the model was robust and flexible in predicting survival at a range of heating rates observed during facility heat treatments. Obtaining information on incremental mortality of mature larvae during facility heat treatments in "real time" should help in taking corrective actions to improve the speed of kill of insects, such as placing an additional heater or by redirecting heat to an area that is being under heated from an area that is being over heated. Taking corrective action in real time requires monitoring temperatures remotely and making predictions with the acquired temperature data. This is currently being explored using wireless temperature sensors and a user-friendly software program.

Besides incremental mortality, determining the time for 0% survival (complete larval mortality) will greatly help in optimizing heat treatments. In our study, the model consistently underpredicted the end point mortality or 0% survival. At heating rates of 1.1 and 5.5°C/h, the model underpredicted time to 0% survival by 79 and 15 min, respectively. Similarly, this time at the heating rate of 13.2°C/h was 27 min. Underprediction by the model is in fact beneficial, because the heat treatment would be extended for slightly longer than necessary, ensuring complete kill of insects.

Susceptibility of insects to heat (high temperatures) exposure varies within life stage (Mahroof et al. 2003a, Boina and Subramanyam 2004), among life stages of an insect (Wright et al. 2002, Mahroof et al. 2003a, Boina and Subramanyam 2004), and between same life stage of related insect species (Mahroof et al. 2003a, Boina and Subramanyam 2004). Similarly, physiological acclimation to heat also varies between same life stages of related insect species (Gonen 1977a,b). Therefore, it is essential to model the survival of the most heat-tolerant stages of insect pests associated with food-processing facilities. As a first step toward this, we chose to model the survival of mature larvae of *T. confusum*. Subsequently, we will be modeling the survival of *T. castaneum* first instars that we found to be more heat tolerant than the mature larvae of *T. confusum* at $\geq 50^\circ\text{C}$ based on time–mortality relationships at 50–60°C (Mahroof et al. 2003a, Boina and Subramanyam 2004).

In summary, the new dynamic model satisfactorily predicted survival of mature larvae of *T. confusum* during actual facility heat treatments. Time-dependent temperature data are the only input needed for making model predictions. Real-time temperature data collected during heat treatments can be used by the model to predict survival of mature larvae in various locations of the facility being heat-treated, and the heat treatment can be stopped when 0% survival is attained in the area being heat-treated. Further validations using mature larvae of *T. confusum* in commercial mills subjected to heat treatment should make this model more acceptable to the flour and feed milling industries. The methods outlined in this paper

can also be used to develop and validate models to predict survival of many other economically important stored-product insects, associated with food-processing facilities, during facility heat treatments.

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