HEAT TREATMENT OF GRAIN-PROCESSING FACILITIES: GAUGING EFFECTIVENESS AGAINST SELECT LIFE STAGES OF *TRIBOLIUM CASTANEUM* (HERBST) USING BIOASSAYS AND A THERMAL DEATH KINETIC MODEL

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Abstract

During heat treatment, the ambient temperature of grain-processing facilities is raised to 50-60°C for at least 24hours to manage stored-product insects. Young larvae (first instars) of the red flour beetle, *Tribolium castaneum* (Herbst), are the most heat tolerant stage at 50-60°C. A thermal death kinetic (TDK) model predicted survival of *T. castaneum* young larvae exposed to six constant elevated temperatures between 42 and 60ºC. The model is based on logarithmic survival of *T. castaneum* as a function of time and logarithmic reduction in larval survival as a function of temperature. The model was validated with 12 independent temperature datasets collected during heat treatments of pilot-scale and commercial grain-processing facilities. Young larval survival in plastic boxes/vials with flour was used to validate model predictions. The heating rate to 50°C from the ambient among the 12 datasets ranged from 0.9-7.8°C/h. Mean absolute deviations between observed and predicted larval survival for 10 of the 12 datasets ranged from 2.1-11.4%; it was 16.2 and 18.3% for two other datasets. The TDK model can be used to predict survival of young larvae of *T. castaneum* based on time-dependent temperature profile obtained at any given location during heat treatment of grain-processing facilities.

In three commercial grain-processing facilities heat treatments were conducted for 24-27.7 hours using forced-air gas heaters. Temperatures attained and survival of 20 eggs, 20 young larvae, and 20 adults of *T. castaneum* in bioassay vials at various locations were determined. Across all three facilities, 5 out of 2720 adults in 136 vials, 1 out of 960 young larvae in 48 vials, and 0 out of 1760 eggs in 88 vials were alive at the end of the heat treatment. In each facility, the time in hours for 1% predicted survival of *T. castaneum* young larvae was positively related to how quickly temperatures reached 50°C, and negatively related to rate of heating to 50°C from the ambient, time above 50°C in hours, and the maximum temperature. Bioassays with *T.*

castaneum life stages and the TDK model can be used to gauge effectiveness of facility heat treatments.

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Chapter 1 - A model for predicting survival of young larvae of *Tribolium castaneum* (Herbst) exposed to elevated temperatures during heat treatment of grain-processing facilities

Abstract

Heat treatment involves raising the ambient temperature of grain-processing facilities to 50-60°C for at least 24hours to manage stored-product insects. The young larvae (first instars) of the red flour beetle, *Tribolium castaneum* (Herbst), are the most heat tolerant stage at temperatures of 50-60°C when compared to eggs, old larvae, pupae, and adults. A thermal death kinetic (TDK) model was developed to predict survival of *T. castaneum* young larvae exposed to six constant elevated temperatures between 42 and 60ºC. The model was based on two non-linear relationships: 1) logarithmic decrease in survival of *T. castaneum* as a function of time, and 2) logarithmic reduction in young larval survival as a function of temperature. The model was validated with 12 temperature datasets collected during actual heat treatments of pilot-scale flour and feed mills and a commercial grain-processing facility. The observed survival of young larvae in plastic boxes/vials with flour was used to validate model predictions. The heating rate to 50°C from the ambient among the 12 datasets ranged from 0.9 to 7.8°C/h. The mean absolute deviation between observed and predicted larval survival for 10 of the 12 datasets ranged from 2.1-11.4%; for the other two data sets it was 16.2 and 18.3%. These results show that the TDK model can be used to predict survival of young larvae of *T. castaneum* based on time-dependent temperature profile obtained at any given location during heat treatment of grain-processing facilities.

Keywords: Heat treatment, Red flour beetle, Thermal death kinetic model, Model validation

Introduction

Heat treatment of grain-processing facilities involves raising the ambient temperature of facilities between 50 and 60°C using gas, electric, or steam heaters, and holding temperatures within this temperature range for 24 to 36 hours ((Imholte and Imholte-Tauscher, 1999; Dowdy and Fields, 2002; Wright et al., 2002; Dosland et al., 2006). Recent research proved that effective heat treatments can be conducted in 24 hours or less (Subramanyam et al., 2011; Brijwani et al., 2012a). During heat treatment, time-dependent temperatures vary from location to location, and from floor to floor within a facility, due to horizontal and vertical stratification of heat (Dowdy and Subramanyam, 1999; Dowdy 2000; Akdoğan et al. 2004; Brijwani et al., 2012b). Therefore, fans are placed in strategic locations to promote uniform distribution of heat within a facility (Dosland et al., 2006).

Predicting survival or mortality of insects during heat treatments of grain-processing facilities using statistical models is necessary for optimizing heat treatments and for ensuring effective disinfestation of facilities. Without such models, facilities subjected to heat treatments could be over or under-heated. Over-heating wastes energy, capital, and may damage heat-sensitive equipment. Under-heating may result in insects surviving the heat treatment or populations rebounding soon after a heat treatment (Roesli et al., 2003). Optimizing heat treatments involves reducing energy costs while achieving complete control of insects. This can be accomplished by monitoring temperatures and insect survival, and redirecting heat to areas that are being under-heated (<50°C) (Subramanyam et al., 2011).

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-morality data at constant temperatures of 50, 52, 54, and 56ºC. 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 were different at each of the four temperatures. Despite these differences, Wright et al. (2002) pooled data across 52, 54, and 56^{o}C to describe the linear 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, even though the linear regressions fit to data had different intercepts and slopes among the three temperatures. Subramanyam et al. (2003) developed a simple heat-accumulation model for predicting the mortality of young larvae (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 and 60^{o}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 49.1^{o}C and the model underestimated mortality by 25%, but explained about 70% of the variation in observed mortality of insects as a function of both temperature and time. The degree-minute models have not been validated under field conditions, and as indicated by Subramanyam et al. (2003) did not accurately predict mortality of insects. They are unsuitable for predicting incremental mortality of insects at dynamically changing temperature over time that occurs during facility heat treatments.

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, as these models are dynamic (Tang et al., 2000), and 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 bacterial inactivation (Van Impe et al., 1992; Baranyi et al., 1996), and they have a rational and biological basis for describing temperature-time-mortality relationships in environments subjected to heat treatments.

To model survival of insects at dynamically changing temperatures over time, it is important 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 species and a range of elevated temperatures when developing models to predict insect survival or mortality during facility heat treatments. Old larvae of *Tribolium confusum* (Jacquelin du Val), were found to be more heat tolerant than eggs, young larvae, old larvae, pupae, and adults at elevated temperatures between 50 and 60ºC (Boina and Subramanyam, 2004). A novel thermal death kinetic (TDK) model was developed for predicting the survival of *T. confusum* old larvae (Boina et al., 2008), and this model gave larval survival predictions during actual facility heat treatments that were within 3 to 7% of the observed survival. The only input needed to generate model predictions was the time-dependent temperature data during heat treatment of facilities. In this paper, the same TDK model was used to predict survival of young larvae of *T. castaneum*, which is the most heat tolerant stage at temperatures between 50 and 60°C (Mahroof et al., 2003a). The model predictions were validated by using 12 independent datasets collected during heat treatments of pilot-scale flour and feed mills and a commercial grain-processing facility.

Materials and methods

Exposure of young larvae to elevated temperatures

Cultures of *T. castaneum* were reared in 0.94-L glass jars with filter paper and wire-mesh lids were maintained in the Department of Grain Science and Industry's Stored-Product Entomology Research and Laboratory since 1999. Cultures were reared on a diet (250 g) consisting of 95% bleached wheat flour and 5% brewer's yeast (by wt) at 28^{o}C, 65% r.h., and 14:10 (L: D)hour photoperiod (Mahroof et al., 2003a). Eggs were collected every 2 d from cultures and reared on bleached flour plus yeast diet at the same rearing conditions. Newly hatched larvae (first instars; 6-d-old) were separated from the diet using a 250-µm sieve, and counted under a stereomicroscope. The mean $\pm$ SE (n = 15) weight of young larvae used in the experiment was 0.12 ± 0.01 mg.

Young larvae of *T. castaneum* were transferred to separate square plastic boxes (4.5 x 4.5 x 1.5 cm) with perforated lids covered with 600-µm wire-mesh screens for air diffusion. Each box held a mean $\pm$ SE (n = 20 replicates) of 305 ± 3 mg of bleached wheat flour and 20 young larvae. Boxes with larvae were exposed in growth chambers (Model I-36 VL, Percival Scientific, Perry, IA, USA) set at 42, 46, 50, 54, 58, and 60^{o}C for collecting time-mortality data. The humidity at each temperature was 20-22% (Mahroof et al., 2003a), because humidity during heat treatment generally drops to this level (Mahroof et al., 2003b; Roesli et al., 2003). At each temperature, five boxes (replicates) were removed at various time intervals, after accounting for the time required for the flour temperature to equilibrate with the set chamber temperature (Mahroof et al. 2003a). At 42°C, about 15 minutes were necessary for the flour temperature to reach the set chamber temperature, and for every 1°C rise in temperature between 42 and 60°C, the time required decreased by about 41 seconds. There were 11-32 exposure times across the six

temperatures studied. The control treatment consisted of boxes with 20 *T. castaneum* young larvae in boxes that were kept in a chamber at 28°C, 65% r.h., and 14:10 (L:D)hour photoperiod. There was a separate control treatment for each temperature treatment.

Boxes with young larvae collected over time from the six temperature treatments were transferred to 150-ml plastic containers, each containing 40 g of bleached wheat flour plus yeast (5% by wt), and held in the chamber at 28°C, 65% r.h. and 14:10 (L: D)hour photoperiod until emergence of adults. The control boxes also were handled similarly. The number of adults that emerged out of 20 young larvae was counted for each temperature, time, and replicate combination. At each collection time, data were pooled across the five replicates and the number of young larvae that survived to adulthood out of 100 exposed larvae was calculated. The mean ± SE (n = 30) mortality of young larvae (based on survival to adulthood) in the control treatment was only 1.7 ± 1.1%, and therefore, uncorrected survivorship values in temperature treatments were used for model development.

Model development

The number of young larvae surviving out of 100 was described as a function of time at each of the six constant temperatures by non-linear regressions after transforming survival data to logarithmic scale. Data at temperatures between 42 and 60°C were fit to non-linear equations using Table Curve 2D software (Jandel Scientific, 1994). Data at 42 and 60°C were fit to equation (1), data at 46°C were fit to equation (2), data at 50°C were fit to equation (3), data at 54°C were fit to equation (4), and data at 58°C were fit to equation (5):

$$y = \frac{(a+cx)}{(1+bx+dx^2)} \quad (1)$$

$$y = \frac{a+b}{(a+e^{(-\frac{x-c}{d})})} \quad (2)$$

$$y = a + bx^2(\ln x) \quad (3)$$

$$y = a + bx^3 + ce^x + de^{-x} \quad (4)$$

$$y = a + bx^3 \quad (5)$$

where, *y* is the logarithm of survival, *x* is the exposure time in minutes, and *a*, *b*, *c*, and *d*, are parameters estimated by fitting equations to logarithm of survival and exposure time data.

The slope of the fitted line describing logarithm of survival over time at each of the six temperatures was non-constant. Therefore, at each temperature an average instantaneous slope was calculated by determining slope every three minutes using differential equations derived from the six non-linear equations. The average instantaneous slope at each constant temperature was calculated by programming the differential equations in Microsoft Excel®. The inverse of the average instantaneous slope value yielded a mean instantaneous *D*-value, which is the time in minutes required for one log reduction in survival of young larvae of *T. castaneum* at each of the temperatures. Next, the *D*-values were plotted as a function of temperature, and equation 6 was fitted to the data:

$$D(T_t) = a + b\ln(T_t) + c^{-Tt} \quad (6)$$

where, $D(T_t)$ is the log reduction in survival of young larvae of *T. castaneum* (*D*-value) as a function of time-dependent temperature, $T_{t,,}$ and *a*, *b*, and *c* are parameters estimated from the equation fit to the mean instantaneous *D*-value and temperature data.

The TDK model (equation 7) described previously by Boina et al. (2008) for predicting survival of old larvae of *T. confusum* was used to predict survival of *T. castaneum* young larvae.

$$N_t = \frac{N_0}{10^{(\Sigma_0^t \frac{\Delta t}{D(T_t)})}} \quad (7)$$

where, N_t is the number of insects at time t during heat treatment, and Δt is the incremental exposure time at which temperatures were recorded during heat treatment. The survival of *T. castaneum* young larvae as a function of time-dependent temperatures measured during heat treatment of Kansas State University pilot flour and feed mills and a commercial grain-processing facility was predicted using equation 7. The observed survival of young larvae of *T. castaneum* in bioassays was verified during the same heat treatments. Model predictions were compared with observed larval survival to validate the model.

Model validation

The model predictions were validated using 12 different temperature datasets collected during heat treatments of the Kanas State University pilot flour and feed mills and a commercial grain-processing facility. The Kansas State University pilot flour mill is vertically separated into four floors. Each floor is horizontally separated into a cleaning house that has equipment for cleaning and a milling house that has equipment for milling wheat. Each cleaning house floor is 12.1 x 8.5 x 3.7 m, and each milling house floor is 11.6 x 9.9 x 3.7 m. Concrete walls separate both houses and vertical floors of the flour mill. The feed mill is located on the west side of the flour mill, and is separated by a concrete wall. The flour mill was heated on two separate occasions, once during May 25-26, 2002 and once during May 27-28, 2005. The milling house floors were heated using a built-in steam generator that vents hot air into each floor. The steam pressure was 6.3 kg/cm^2, and the outlet steam temperature was 68°C. The cleaning house floors were heated using two portable steam heaters (Armstong-Hunt, Cat. No. 3507, Milton, FL, USA), one each on the first and third floors. Portable heaters had a measured steam pressure of 17.6 kg/cm^2 and were equipped with a built-in fan that operated at 1725 rpm (Baldor Electric

Corporation, Fort Smith, AR, USA). No additional heating or air circulation systems were used in the pilot flour mill.

The Kansas State University pilot feed mill was heated using natural gas heaters from Temp-Air (Rupp Industries, Inc., Burnsville, MN, USA) on August 5-6, 2003. To heat the mill, three THP-550 and one THP-1400 commercial heaters were used with a maximum heat energy output of 161.19 kW/h and 410.30 kW/h, respectively. The heaters were placed outside the mill and heated air was forced into the building using 50.8-cm diameter nylon ducts with circular openings. Two Bayley fans (RUPP Industries, Inc., Burnsville, MN, USA) with a 1.5 horse power motor, a fan blade diameter of 0.78 m, and an airflow rate of 391 cm^3/min were placed in the first, second, and third floors to facilitate heat distribution.

Young larvae of *T. castaneum* were transferred to 4.5 x 4.5 x 1.5 cm square plastic boxes covered with lids that had 600 μm aperture mesh screens. Each box held a mean ± SE (n = 20) of 305 ± 3 mg of bleached wheat flour and 20 individual young larvae of *T. castaneum*. During May 24-26, 2002, heat treatment 21 boxes with larvae were kept on the first floor of the milling house. All boxes were kept at the floor level. A HOBO® data logger (Onset Computer Corporation, Bourne, MA, USA) was placed next to the bioassay boxes to record temperature every 15 minutes. Three boxes were sampled on seven different occasions. During May 27-28, 2005, heat treatment three locations on the third floor of the cleaning house close to the steam heater were selected for insect exposure. Distance between locations was >60 cm. At each location, 42 square plastic boxes each with 305 mg of bleached wheat flour and 20 *T. castaneum* young larvae were placed on the floor. HOBO® data loggers placed next to bioassay boxes at each location recorded temperatures every minute. At 21 different occasions during heat treatment, two boxes were removed from the heat treatment area. Young larvae of *T. castaneum*

in boxes sampled at times coinciding with heat treatment sampling times served as the control treatment.

In the pilot feed mill, during the August 5-6, 2003 heat treatment, 42 boxes each with 305 mg bleached flour and 20 *T. castaneum* young larvae were placed on the first floor of the mill. HOBO® data logger placed next to the bioassay boxes recorded temperature every five minutes. Two boxes were removed from the heat treatment area on 21 different sampling occasions. All boxes were brought to the laboratory. The flour and insects from each box were transferred to 150 ml round plastic containers containing 40 g of bleached flour and held at 28°C and 65% r.h. until emergence of adults. Observed survival of larvae was calculated on number of larvae that failed to emerge as adults out of the total exposed. Data for the two or three boxes sampled at different occasions was pooled (40 or 60 larvae/sampling occasion), and the survival was expressed as a percentage out of the total exposed.

Except for the 2002 steam heat treatment in the pilot flour mill which lasted 46.5 h, all other bioassay experiments were completed in less than eight hours. The sampling occasions were based on checking temperatures near bioassay boxes during heat treatment using an infrared thermometer (Raytek MX4 model 4TP78, Raytek, Santa Cruz, CA, USA) to ensure that the bioassays were removed at regular intervals based on the temperature. In cases where the temperatures reached 50°C quickly and were above 50°C, bioassay boxes were removed at a greater frequency to obtain a range of survival values to compare against model predictions.

The commercial grain-processing facility in the Midwestern United States has been using steam heaters for more than 50 years at monthly intervals to control stored-product insects. The heaters used were antiquated, and it was difficult to obtain information on the make and model of the steam heaters from the company representatives. Natural gas from the city is used to fuel a

boiler to generate the steam heat. Each heater was rated to deliver maximum heat energy of 278.43 kW/h. Aerovent portable fans (Aerovent, Piqua, OH, USA) were used during heat treatment to facilitate even heat distribution.

Bioassays with *T. castaneum* young larvae were conducted during heat treatment of specific rooms of the facility during June 1-3, 2007 and August 31-September 2, 2007. Round plastic vials (2.6 cm inner diameter and 4.9 cm high) were filled with 5 g of bleached wheat flour that was sifted using a 250 μm sieve. Into each vial 30 young larvae of *T. castaneum* were added. The vials were closed with plastic lids covered with 600 μm wire mesh screens to allow air flow but prevent insect escape. Bioassay vials were transported to the facility by car. Bioassay vials were placed on the floor at three different locations in June (corn mill bin annex area) and at four different locations in September (8^{th} floor of the corn mill), and a HOBO® data logger was placed next to the vials in each location to record temperature every minute. During June and September, the total heat treatment lasted for 35.2 and 33.5 h. The bioassay vials were removed on five different occasions during the June heat treatment (Commercial 43, 48, 49). One vial from each location was removed one start of the heat treatment and the other four at 4, 12.5, 21, and 27.5hours into the heat treatment. During the September heat treatment, one vial from each location was removed at the start of the heat treatment, and at 2, 3, 4, 5, 6, 7, 8.2, 9, 12, 17, and 24hours into the heat treatment (Commercial 24, 27, 30, and 31). Vials handled similarly but left outside the heat treated area served as the control treatment. Vials were brought to the laboratory and incubated at 28°C and 65% r.h. until emergence of adults. Larval survival, expressed as a percentage, was based on number of adults that failed to emerge from the total exposed.

The total time-dependent temperature profile from HOBO® data loggers was used to determine the starting ambient temperature, time in hours required to reach 50°C from the starting temperature, heating rate to 50°C (°C/h), number of hours temperatures were maintained above 50°C, and the maximum temperature for each of the 12 datasets.

Equation 7 programmed in Microsoft Excel® was used to predict survival of *T. castaneum* young larvae based on the time-dependent temperature profile for each of the 12 datasets. The survival of young larvae observed in bioassays was compared with the predicted survival using equation 7 over the temperature range. Model predictions from the grain-processing facility for the June and September 2007 heat treatments were truncated at 27.5 and 24 h, respectively as the survival reached 0% prior to these times.

The predicted survival of young larvae (y) for each dataset was regressed on the observed larval survival (x) using a liner regression. The slope of the regression was tested for deviation from 1 ($y = x$) using a t-test at $n - 2$ df (Zar, 1984). A non-significant t-test ($P > 0.05$) indicated that the model predictions were not significantly different from the observed survival. Furthermore, the observed and predicted larval survival at specific sampling occasions for each dataset were used to determine the overall mean ± SE percent deviation in survival. The percentage of observations of each dataset out of the total, where the difference between observed and predicted larval survival was within 1%, greater than 1%, and less than -1%, was determined. A difference in observed and predicted larval survival within 1% indicated that the model accurately predicted larval survival (predicted=observed), where as a difference in observed and predicted larval survival greater than 1% indicated that the model under-predicted larval survival (predicted < observed), and a difference in observed and predicted larval survival

less than -1% indicated that the model over-predicted survival (predicted > observed). A model is considered suitable if the predictions are within 15% of the observed values.

Results

Survival of insects as a function of time and constant temperatures

The logarithmic survival of *T. castaneum* over time was nonlinear at each of the six constant temperatures. The relationship of logarithm of survival and time at each of the six constant temperatures was adequately described by equations 1-5 (adjusted r^2 = 0.813 - 0.993) (Fig. 1), and the parameter estimates are given in Table 1. The decrease in survival was inversely related to exposure time at all temperatures. The decrease in logarithmic survival as a function of time tended to be faster as the temperature increased from 42 to 60°C.

The differential form for equations 1-5 are shown in Table 2. The mean instantaneous *D*-value calculated every three minutes from the fitted line was 5766.03 minutes at 42°C, and these values decreased in a non-linear fashion as a function of temperature to 112.73 minutes at 60°C (Fig. 2). Equation 6 best described the non-linear relationship between *D*-values and temperature (n = 6; adjusted r^2 = 0.999), and the values for parameter a, b, and c were 5742.32, -1379.89, and 9.01 x 10^{21}, respectively.

Model validation datasets from pilot mills and a commercial grain-processing facility

The starting ambient temperature, time to 50°C, heating rate to 50°C, time above 50°C, and the maximum temperature for each of the 12 independent datasets collected in 2002, 2003, 2005, and 2007 are presented in Table 3. The starting temperature among the datasets ranged from 24.8-38.3°C. The time to 50°C was quite rapid (3-8 h) in all heat treatments, except the 2002 steam heat treatment of the pilot flour mill where it took 28.3 h. The rate of heating to 50°C was very slow in the 2002 steam heat treatment of the pilot flour mill (0.9°C/h), whereas it

was greatest in the 2003 pilot feed mill heat treatment (7.8°C/h), perhaps a result of more heat energy being used relative to the size of the mill. The heating rates in the 2005 pilot flour mill and all commercial heat treatments ranged from 1.8-3.2°C/h. Time above 50°C was shortest (1.4-3.6 h) in the 2003 pilot feed mill and the 2005 pilot flour mill heat treatments. This is due to truncating temperature data when all of the bioassay samples were removed. In the 2002 pilot flour mill and the commercial facility, temperatures were held above 50°C for 18.5-30.6 h. The maximum temperatures rarely exceeded 60°C, except at three locations in the commercial grain-processing facility (Commercial 24, 43, and 49).

The predicted survival of *T. castaneum* young larvae as a function of time-dependent temperature profile using the TDK model (Equation 7) for the pilot flour and feed mills is shown in Fig 3, and for the commercial grain-processing facility in Fig. 4. The observed survival of young larvae was plotted as a function of time in these figures. Larval survival data were not corrected because control mortality of larvae was 5% or less in all bioassays. The linear regressions of predicted and observed *T. castaneum* young larval survival for each of the 12 independent datasets are shown in Table 4. There was a good fit between predicted and observed larval survival (r^2 = 0.805-0.995), and the slope values, except for the 2003 pilot feed mill data and 2005 location 2 pilot flour mill data, ranged from 0.79-1.03. All of these slopes were not significantly different from 1 ($P > 0.082$). The slopes for the 2003 pilot feed mill data and the 2005 location 2 data were significantly lower than 1, indicating the model generally under-predicted larval survival.

The mean absolute deviation in predicted and observed larval survival, except for the 2002 pilot flour mill data and for 2005 location 2 pilot flour mill data, ranged from 2.1-11.4% (Table 5). In the 2002 and 2005 location 2 pilot flour mill data, overall model deviations across

the temperature range were16.2 and 18.3%, respectively. In general, except for 2005 location 2 pilot flour mill data, the TDK model predictions were accurately predicted or under-predicted. This is especially true for data from the commercial grain-processing facility. In 2005 location 2 data from the pilot flour mill, the TDK model over-predicted larval survival (61.9% of the observations), whereas 38.1% of the predictions were either accurately predicted or were under-predicted. These results suggest that the TDK model can be used for predicting survival of young larvae of *T. castaneum* as a function of time-dependent temperature.

Discussion

The decrease in logarithm of survival of *T. castaneum* young larvae was slower at temperatures below 50°C than at temperatures at or above 50°C. The greatest decrease in the mean instantaneous *D*-value (10-fold) was between 42 and 46°C. In *T. confusum*, a closely related species, the old larvae are heat tolerant (Boina and Subramanyam, 2004), and the decrease in logarithm of survival of larvae was slower at 46 and 48°C than at 50, 54, 58, and 60°C (Boina et al., 2008). Wang et al. (2002a,b) reported a similar relationship in tests with fifth instars of the navel organeworm, *Amyelois trasitella* (Walker), exposed to temperatures of between 46 and 54°C at a constant heating rate of 18°C/minute. Several researchers have hypothesized that a minimum temperature of 50°C was necessary for effective disinfestation of grain-processing facilities subjected to heat treatments (Dowdy and Fields, 2002; Wright et al., 2002; Mahroof et al., 2003b; Roesli et al., 2003; Boina and Subramanyam, 2004). The logarithm of survival as a function of time at temperatures >50°C rapidly reached 0% in less than 60 minutes. Similarly, the observed *T. castaneum* young survival data in the pilot flour and feed mills and the commercial grain-processing facility heat treatments showed 0% survival at temperatures above 50°C. Boina et al. (2008) reported 0% survival of *T. confusum* old larvae at

temperatures of 52-54°C. Subramanyam et al. (2011) reported that the time to 1% survival of *T. castaneum* young larvae (lethal time for 99% mortality or LT_{99}) using the TDK model was positively related to how quickly temperatures reached 50°C from the ambient, and negatively related to how long temperatures were held above 50°C and the maximum temperature. In location 1 and 3, during the 2005 steam heat treatment of the flour mill, the survival of *T. castaneum* young larvae at the end of the treatment was 35 and 51.5%, respectively. In location 1, temperatures were above 50°C, but only for about 1.4 h, and in location 3 temperatures never reached 50°C. On the contrary, data from the 2003 pilot feed mill and the 2005 location 2 showed 0% survival of *T. castaneum* larvae because temperatures reached 50°C in 7.7 and 3.2 h, respectively, and temperatures were held above 50°C for 1.4-3.6 h, and the maximum temperatures were 54.7-56.6°C. In these two cases, the TDK model under-predicted larval survival. Under-prediction by the model is not as risky as over-prediction because in cases where larval survival is under-predicted, the heat treatment will be allowed to continue until the predicted survival approaches 0%.

The 2002 pilot flour mill heat treatment is not typical of heat treatments of commercial grain-processing facilities. Effective heat treatments can be conducted with 24hours (Subramanyam et al., 2011; Brijwani et al., 2012a, 2012b). However, the pilot flour mill does not have enough heat energy, and as a result the heat treatment lasted 46.5 h. This is also evident from the heating rate which was 0.9°C/h. Typical heating rates for effective disinfestations, without adversely affecting the structural components of a facility, should be between 3 and 5°C/h (Boina et al., 2008; Subramanyam et al., 2011). Temperatures reached 50°C in 28.3hours and were held above 50°C for 18.5 h, and the maximum temperature recorded was 59.9°C. Despite the slow heating rate, 0% survival of *T. castaneum* young larvae was observed 31hours

into the heat treatment where the temperature was 50.6°C (data not shown in Fig. 3). These data support Subramanyam et al.'s (2011) conclusions that larval survival is related to time to 50°C, time above 50°C, and the maximum temperature attained.

Except in two cases out of the 12 independent model validations (2003 pilot feed mill data and 2005 location 2 flour mill data), the predicted survival of *T. castaneum* young larvae was not significantly different from the observed larval survival. In the two exceptional cases, the model over-predicted larval survival by 47.6 and 61.9% of the time. However, in the 10 other cases, the model predictions were accurate or were under-predicted. As stated earlier, under-prediction will lead to extending the heat treatment to ensure 0% survival of larvae, and hence, may improve heat treatment effectiveness.

The TDK model assumes 100% survival of *T. castaneum* young larvae at temperature below 42°C, and it does not account for development of thermo tolerance in larvae. Young larvae of *T. castaneum* are capable of expressing heat shock proteins (HSP) at elevated temperatures (Mahroof et al., 2004, 2005). HSPs are a cellular response to environmental stressors including elevated temperatures, desiccation, and ultraviolet radiation exposure (Currie and Tufts, 1997; Mahroof et. al., 2005). Thermotolerance in *T. castaneum* young larvae at 50-60C (Mahroof et al., 2003a) could be due to increased expression of HSPs. Jian et al. (2013) recently developed a model that can account for thermotolerance when predicting survival of *T. castaneum* young larvae during facility heat treatments. Thermo tolerance may be an issue if larvae are acclimated for longer time periods at temperatures below 42°C prior to exposure to lethal temperatures. Gonen (1977a,b) reported that the granary weevil, *Sitophilus granarius* (L.), adults exposed to 32°C for 14 d were able to withstand a 40°C exposure, but a 2 d exposure to 32°C did not confer any thermo tolerance. At the recommended heating rates of 3-5°C/h and at

temperatures above 50°C during facility heat treatments, lethal temperatures for larvae will be reached quickly to prevent development of thermo tolerance.

In summary, the new TDK model can be used to predict survival of *T. castaneum* young larvae during facility heat treatments. The mean absolute deviation in predicted larval survival from that observed ranged from 2.1 to 11.4% in 10 out of the 12 independent validations. The only input needed is time-dependent temperature data collected from different locations of the facility subjected to a heat treatment. A software program in C++ was developed to provide heat treatment summary data such as starting temperature, time to 50°C, time above 50°C, and the maximum temperature from time and multiple temperature data stored in Microsoft Excel®) spread sheet in the .xls format. The program also provides time in hours for 10, 5, and 1% survival of both *T. castaneum* young larvae and *T. confusum* old larvae, plus output in Excel® showing time, temperature, and predicted percent survival of *T. castaneum* young larvae (this study) and *T. confusum* old larvae (Boina et al., 2008) for graphing purposes. In locations where the temperatures does not reach 50°C, the program shows expected percent mortality based on the TDK models for the *Tribolium* spp. The program analyzes all of this information in less than five seconds. The program may be of use to pest management service providers, as it can give a summary that can be shared with clients. The use of this program should help grain-processing facilities improve their heat treatment effectiveness. The use of bioassays is not needed, as some facility managers may be reluctant to have live insects brought in to gauge effectiveness of a heat treatment. In addition, the methods presented here can be used to develop and validate TDK models for predicting survival of many other stages and species of stored-product insects associated with grain-processing facilities, during facility heat treatments.

Acknowledgements

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Figure 1.1 Relationship describing survival of *T. castaneum* young larvae as a function of exposure time. The *x*-axis scale is different among graphs.

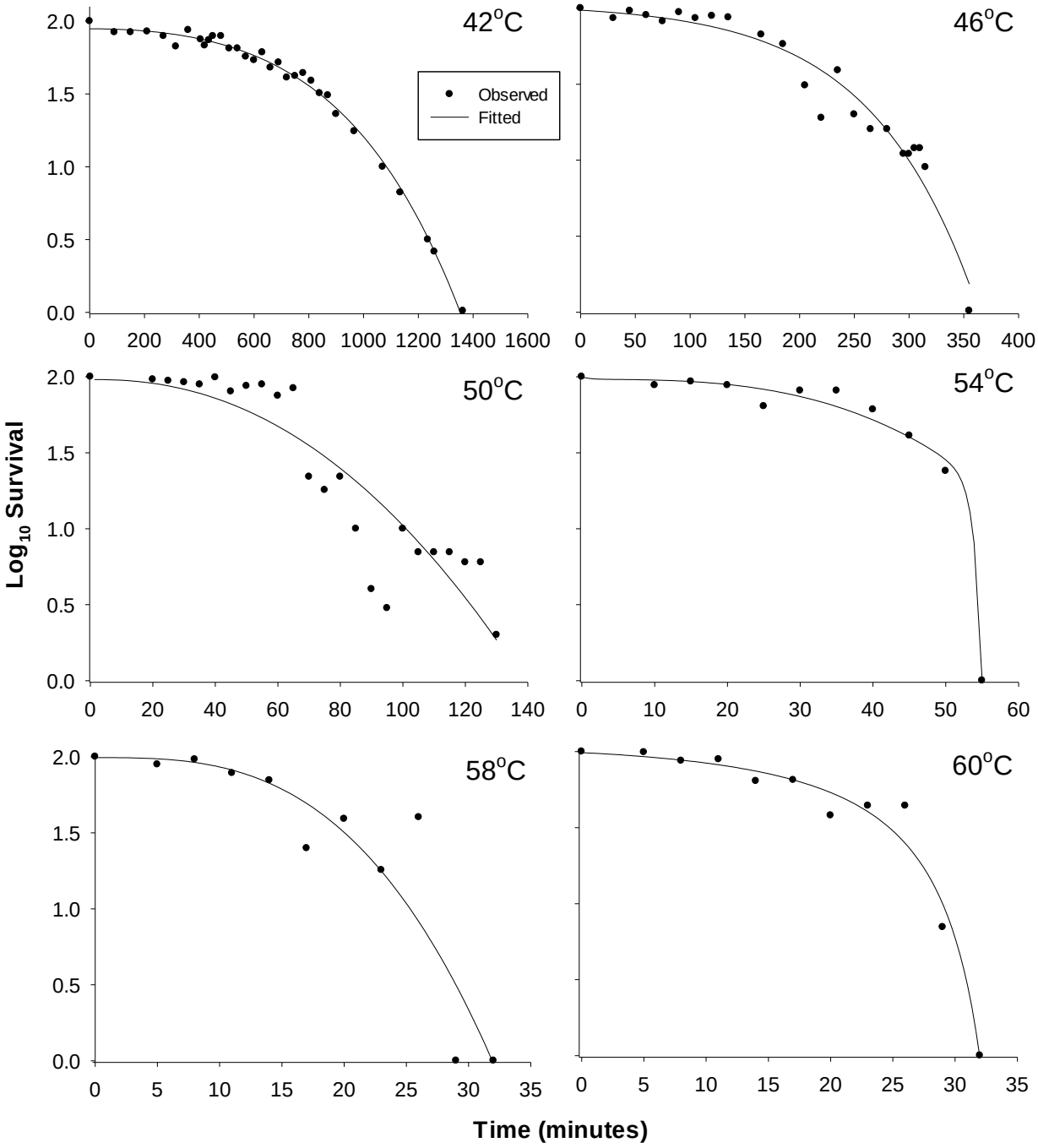


Figure 1.2 Relationship describing mean instantaneous *D*-value as a function of temperature.

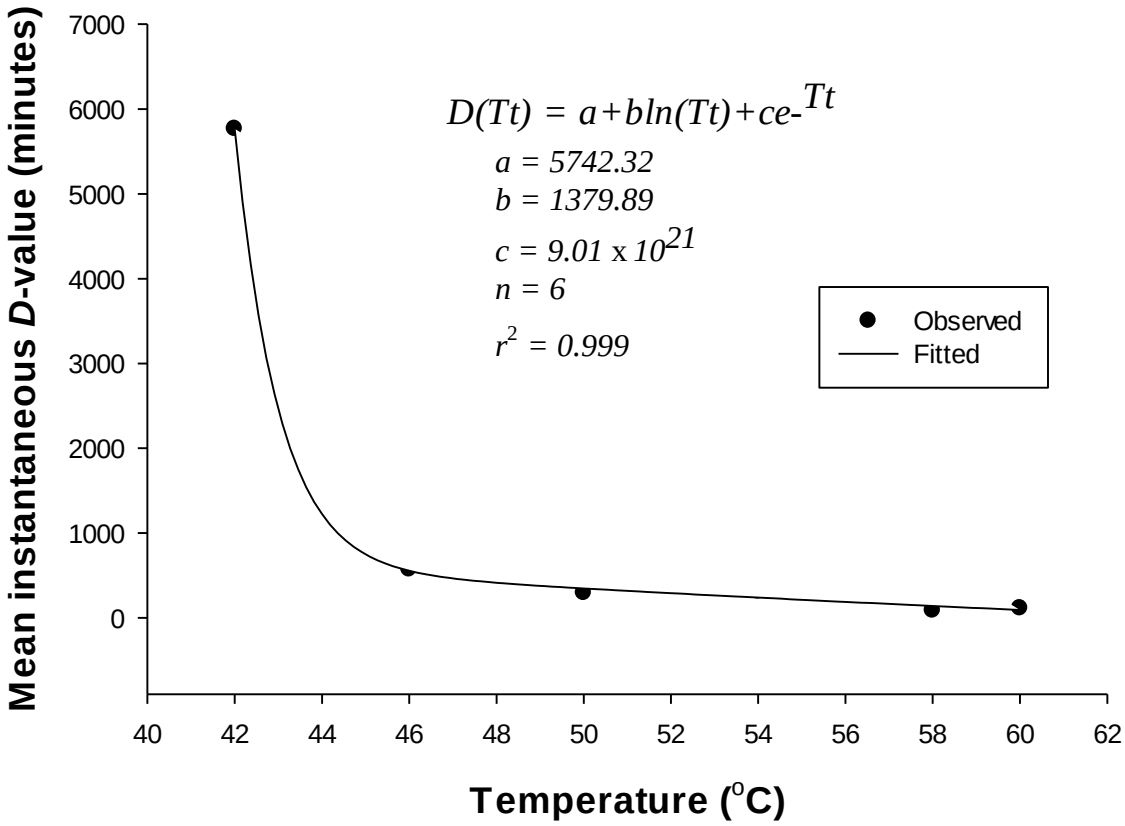


Figure 1.3 Observed and predicted survival of *T. castaneum* young larvae during heat treatment of Kansas State University pilot flour and feed mills. Note that the *x*-axis scale is different among graphs.

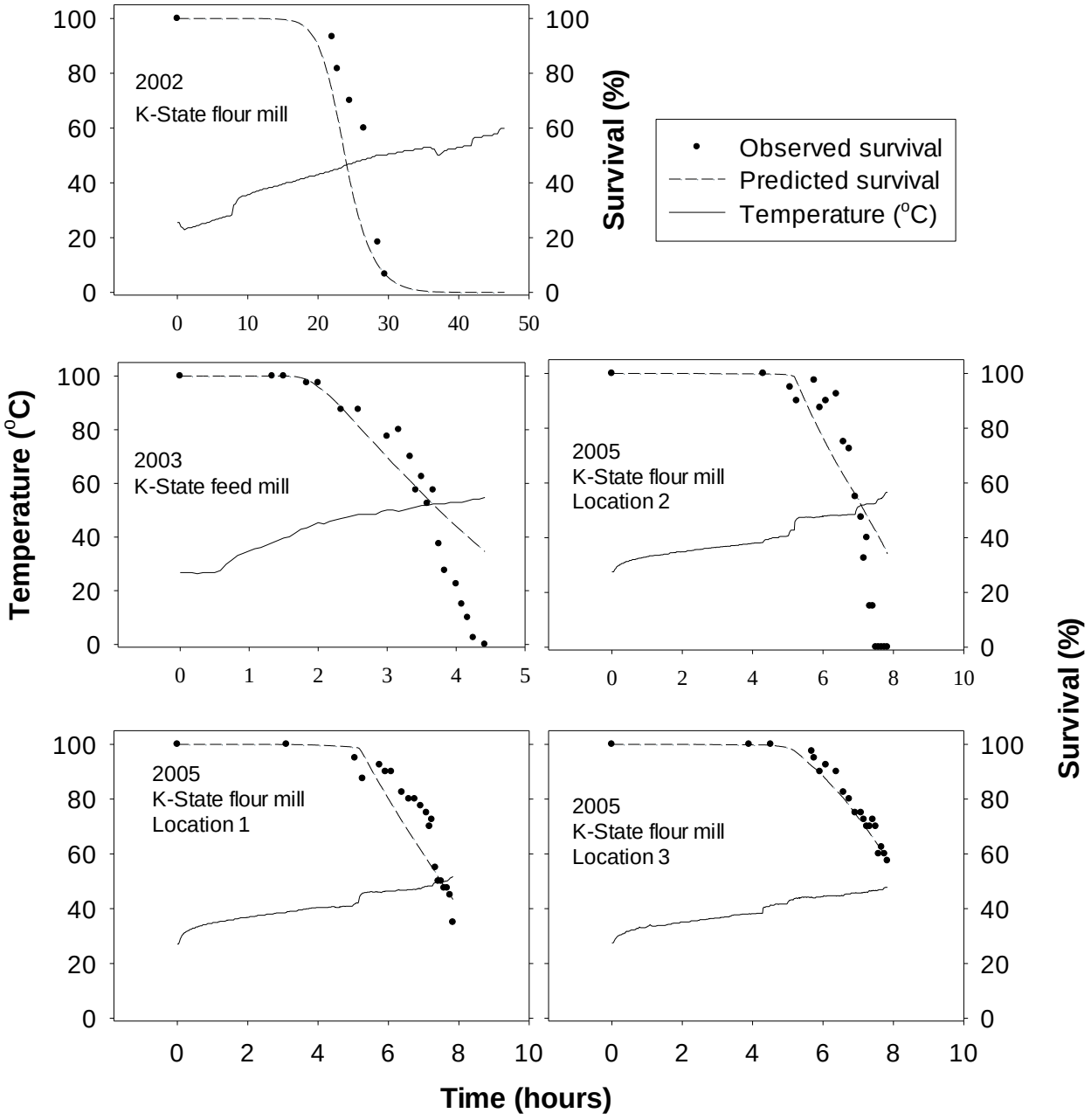


Figure 1.4 Observed and predicted survival of *T. castaneum* young larvae during June (Commercial 43, 48, and 49) and September (Commercial 24, 27, 30, and 31), 2007 heat treatment of a grain-processing facility.

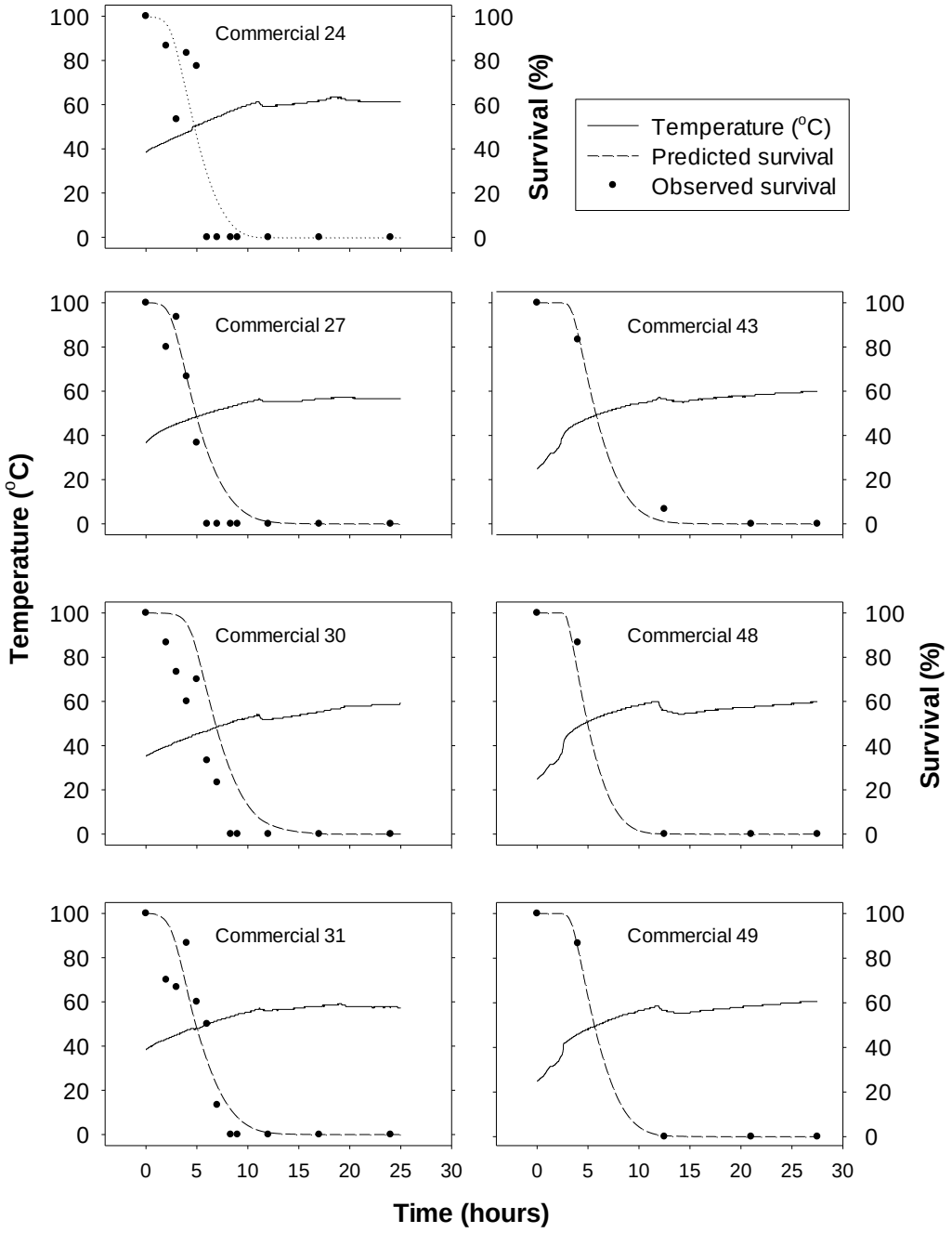


Table 1.1 Parameters of equations describing the relationship between logarithm of survival of *T. castaneum* young larvae and exposure time at six constant temperatures.

Temp. (°C)	n[a]	r^2[b]	Equation	Parameter			
				a	*b*	*c*	*d*
42	32	0.993	$a+cx/(1+bx+dx^2)$	1.95	-0.00074	-0.0014	$1.60*10^{-7}$
46	23	0.937	$a+b/(1+e^{(-(x-c)/d)})$	-50.59	52.62	660.575	-92.12
50	24	0.801	$a+bx^2(lnx)$	1.98	$-2.08*10^{-5}$		
54	11	0.982	$a+bx^3+ce^x+de^{-x}$	1.98	$-4.16*10^{-6}$	$-1.7*10^{-24}$	0.018
58	11	0.813	$a+bx^3$	1.99	$-6.14*10^{-5}$		
60	11	0.949	$a+cx/(1+bx+dx^2)$	1.99	-0.029	-0.062	$3.84*10^{-5}$

[a]*n* = Number of observations.

[b]Adjusted r^2.

Table 1.2 Mean instantaneous *D*-values and the differential equations used to compute them from the relationship between survival of young larvae of *T. castaneum* and exposure time at six constant temperatures.

Temp. (°C)	Equation (*dy/dx)*	Number of 3-minute observations[a]	Mean instantaneous *D*-value (minutes)
42	$(c(1+bx+dx^2)-(a+cx)(b+2dx))/((1+bx+dx^2)^2)$	455	5766.03
46	$((b/d)e^{-((x-c)/d)})/((1+e^{-((x-c)/d)})^2)$	119	569.64
50	$2bx(lnx)+bx$	44	291.96
54	$3bx^2+ce^x-de^{-x}$	19	310.51
58	$3bx^2$	11	85.43
60	$(c(1+bx+dx^2)-(a+cx)(b+2dx))/((1+bx+dx^2)^2)$	11	112.73

[a]Number of 3-minute observations used to calculate the mean instantaneous *D*-value.

Table 1.3 Data obtained from temperatures measured during heat treatment of pilot mills and a commercial grain-processing facility.

Validation dataset[a]	Starting temp. (^oC)	Time to 50^oC (h)	Heating rate to 50°C (^oC/h)	Time above 50^oC (h)	Max temp. (^oC)
2002	25. 6	28.3	0.9	18.5	59.90
2003	26.7	3.0	7.8	1.42	54.74
2005 location 1	27.1	7.6	3.08	1.42	51.79
2005 location 2	27.5	7.0	3.28	3.62	56.60
2005 location3	27.5	----[b]	----	----	47.79
Commercial 24	38.3	4.6	2.6	28.93	63.48
Commercial 27	36.6	5.9	2.3	56.53	57.24
Commercial 30	35.3	8.0	1.8	82.03	59.90
Commercial 31	38.3	6.0	2.0	109.43	59.22
Commercial 43	24.8	6.2	4.1	30.65	61.29
Commercial 48	24.8	4.6	5.5	62.97	59.90
Commercial 49	24.8	5.8	4.3	94.03	61.29

[a]2002 and 2005 data are from the pilot flour mill; 2003 data are from the pilot feed mill, and Commercial 24-49 are from a grain-processing facility.

[b]Temperature did not reach 50°C.

Table 1.4 Parameter estimates from the linear regressions of predicted survival of *T. castaneum* young larvae using the TDK model and observed survival during heat treatment of pilot mills and a commercial grain-processing facility.

Validation dataset[a]	r^2	Mean ± SE parameter estimate		*t*-value (df)[b,c]	*P*-value
		Intercept	Slope		
2002	0.841	-9.76±12.17	0.89±0.17	0.895 (5)	0.4120
2003	0.881	28.90±3.58	0.62±0.05	7.214 (19)	<0.0001*
2005 location 1	0.835	3.41±6.77	0.88±0.09	1.310 (19)	0.2059
2005 location 2	0.834	35.15±3.47	0.52±0.05	9.112 (19)	<0.0001*
2005 location 3	0.968	1.67±3.23	0.95±0.04	1.200 (19)	0.2448
Commercial 24	0.805	2.06±6.49	0.79±0.12	1.685 (10)	0.1229
Commercial 27	0.949	3.31±3.26	0.88±0.06	1.933 (10)	0.0820
Commercial 30	0.961	5.53±3.27	1.03±0.06	0.403 (10)	0.6952
Commercial 31	0.831	-1.92±6.52	0.87±0.12	1.041 (10)	0.3224
Commercial 43	0.995	-2.28±2.31	1.00±0.04	0.131 (3)	0.9041
Commercial 48	0.963	-0.60±5.97	0.89±.010	1.103 (3)	0.3505
Commercial 49	0.994	-0.26±2.59	0.95±0.04	1.102 (3)	0.3509

[a]See footnote to Table 3.

[b]*t*-test for H_0: slope=1.

[c]Number of observations (*n*) used=df +2.

*The slope is significantly lower than 1 ($P < 0.05$).

Table 1.5 Mean absolute deviation in observed and predicted percent survival of *T. castaneum* young larvae based on data collected during heat treatment of pilot mills and a commercial grain-processing facility, and accuracy of model predictions.

Validation dataset[a]	*n*	Mean±SE absolute deviation[b]	% of observations out of *n* that were:		
			Within 1% survival[c]	Greater than 1% survival[d]	Less than -1% survival[e]
2002	7	16.2±5.5	28.6	71.4	0.0
2003	21	11.4±2.7	14.3	38.1	47.6
2005 location 1	21	7.4±6.3	19.1	57.1	23.8
2005 location 2	21	18.3±3.1	9.5	28.6	61.9
2005 location 3	21	2.5±0.5	28.6	66.7	4.7
Commercial 24	12	9.6±4.8	50	50	0.0
Commercial 27	12	6.1±2.2	33.3	66.7	0.0
Commercial 30	12	10.2±4.0	33.3	66.7	0.0
Commercial 31	12	7.4±2.0	33.3	66.7	0.0
Commercial 43	5	2.5±1.5	60.0	40.0	0.0
Commercial 48	5	4.8±4.8	80.0	20.0	0.0
Commercial 49	5	2.1±2.1	80.0	20.0	0.0

[a]See footnote to Table 3.

[b]Σ (% observed survival - % predicted survival)/*n*.

[c]Accurate prediction (prediction=observed)

[d]Under-prediction (prediction<observed)

[e]Over-prediction (prediction>observed)

Chapter 2 - Gauging effectiveness of three commercial heat treatments against *Tribolium castaneum* (Herbst) eggs, young larvae, and adults using bioassays and a thermal death kinetic model

Abstract

Heat treatments were conducted in three commercial grain-processing facilities for 24-27.7hours using forced-air gas heaters fueled by propane. Temperatures attained and survival of 20 eggs, 20 young larvae, and 20 adults of *T. castaneum* in bioassay vials at various locations were determined. In facility A, mean starting temperature was 34.1°C, the mean time to 50°C was 1.5 hours, the mean time above 50°C was 22.5 hours, and the mean maximum temperature was 59.6°C. In facility B, two separate rooms underwent heat treatment, the dry roast room (DRR) and bulk bin storage unit (BBU). In the DRR, the mean starting temperature was 26.8°C, the mean time to 50°C was 6.2 hours, the mean time above 50°C was 21.7 hours, and the mean maximum temperature was 57.8°C. In the BBU, the mean starting temp was 23.4°C, the mean time to 50°C was 5.0 hours, the mean time above 50°C was 22.2 hours, and the mean maximum temperature was 57°C. The BBU was the only facility where any survival was observed and had the only location that did not reach 50°C during the heat treatment. In facility C, the mean starting temp was 32.8°C, the time to 50°C was 4.51 hours, the mean time above 50°C was 23.52 hours, and the mean maximum temperature was 60.5°C. Eggs were more susceptible to heat treatment when compared with young larvae and adults. Across all three facilities, 5 out of 2720 adults in 136 vials, 1 out of 960 young larvae in 48 vials, and 0 out of 1760 eggs in 88 vials were alive at the end of the heat treatment. In each facility, the time in hours for 1% predicted survival of *T. castaneum* young larvae was positively related to how quickly temperatures reached 50°C, and negatively related to time above 50°C and the maximum temperature. Bioassays with *T. castaneum* life stages and the TDK model can be used to gauge effectiveness of facility heat treatments.

Keywords: Commercial heat treatments, Red flour beetle, *Tribolium castaneum*, Efficacy assessment

Introduction

Heat treatments have been used for over 100 years to manage stored-product insects associated with grain-processing facilities (Dean, 1911, 1913; Goodwin, 1922). During heat treatment, the ambient temperature of the facility is slowly raised to 50-60°C, and these elevated temperatures are held for 24-36 hours to kill stored-product insect pests (Imholte and Imholte-Tauscher, 1999; Dosland et al. 2006). Failing to achieve a minimum temperature of 50^{o}C during heat treatment can lead to incomplete mortality of target species and accelerated population rebound of stored-product insects (Boina et al., 2008; Subramanyam et al., 2011).

In the last 20 years, there has been renewed interest in using heat treatment as a means of controlling stored product pests in modern commercial grain-processing facilities because of the phase out of methyl bromide as a structural fumigant, due to its adverse effects on stratospheric ozone (Subramanyam et al., 2011). There are several published papers on the efficacy of heat treatments against life stages of the red flour beetle, *Tribolium castaneum* (Herbst); confused flour beetle, *Tribolium confusum* Jacquelin duVal; Indian meal moth, *Plodia interpunctella* (Hübner); cigarette beetle, *Lasioderma serricorne* (L.) based on laboratory trials (Mahroof et al., 2003a; Boina and Subramanyam, 2004; Mahroof and Subramanyam, 2006; Abdelghany et al., 2010; Yu et al., 2011) at constant elevated temperatures and during heat treatments of pilot flour and feed mills at Kansas State University (Dowdy and Fields, 2002; Mahroof et al., 2003b; Roesli et al., 2003; Brijwani et al., 2012a,b). Yu et al. (2011) reported on the stage-specific susceptibility of *L. serricorne* during a single heat treatment of a grain-processing facility. However, these results were inconclusive and did not show eggs to be the most heat tolerant stage in this species as confirmed by laboratory studies. Except for a few reports (Norstein, 1996; Fields, 2012; Campolo, et. al., 2013), very limited published information is available on the effectiveness of heat treatments of grain-processing facilities on the efficacy against life stages of

stored-product insects. Norstein (1996) reported on a heat treatments conducted at three commercial facilities in Sweden. Two of the mills underwent facility-wide treatment, while the third facility only underwent spot treatment of the roll stands. Majority of the report deals with temperatures attained. Limited data are presented on the death of different insect species; however, six or fewer insects of a species were used in bioassays. Fields (2012) reported on commercial heat treatments in three Canadian flour mills. Two of the heat treatments were done using forced-air gas heaters fueled by propane and one mill was heated using steam heaters. Pheromone trapping was done using commercial food and pheromone-baited traps to capture *Tribolium* spp. 3-9 weeks before a heat treatment and 20 weeks after heat treatments. Bioassay vials with 16 g of flour and 20 adults of *T. castaneum* were used. The adults were introduced 4-8 days before heat treatments, so eggs and/or young larvae would be present in vials. No live stages of *T. castaneum* were found in vials 6-21hours into the heat treatment and temperatures at this time were between 53 and 57°C. *Tribolium* spp. in pheromone traps did not return to pretreatment levels 20 weeks after the heat treatments. Campolo, et al. (2013) reported on a heat treatment of a single flour mill in Greece. The facility was heated using portable electric heaters with attached fans. Bioassays filled with 10 g of flour with either 20 eggs, 20 larvae, or 20 adults of four different species (*T.. confusum*, the Broad-horned flour beetle, *Gnatocerus cornutus* (F.); rice weevil, *Sitophilus orzyae* (L.); lesser grain borer, *Rhyzopertha dominica* (F.), were used to evaluate the efficacy of the heat treatment. Temperatures were measured at 15-minute increments and bioassays were sampled at 12, 24, and 36 hours into the heat treatment. Time to 50°C ranged from 9.0- 24.4 h and maximum temperatures ranged from 52.8 to 59.1°C. Mortality ranged from 53.9- 100% across all species and life stages after 24 h into the treatment.

At 36hours mortality, of all species and life stages was 100%. Campolo et al. (2013) reported a positive linear relationship between time above 50°C and insect mortality.

In this paper, we report on the temperatures measured at multiple locations in three grain-processing facilities in the United States subjected to forced-air gas treatment, and on the efficacy of the heat treatments using two approaches. The first approach involved using specific life stages (eggs, young larvae, or adults) of *T. castaneum* used in bioassay vials to determine survival at specific intervals during the heat treatment. The second approach involved predicting survival of *T. castaneum* young larvae (first instars), which is the most heat tolerant stage compared with eggs, old larvae, pupae, and adults, using the thermal death kinetic model developed in chapter 1(Bingham, 2014. Heat treatment effectiveness was verified using *T. castaneum* as it is the most common stored-product insect pest insect associated with food-processing facilities in the United States (Good, 1937; Hagstrum and Subramanyam, 2009; Buckman et al., 2013), and a majority of pest management interventions are targeted against this pest insect.

Materials and methods

Insect bioassays

Eggs, young larvae, and adults of *T. castaneum* were reared on wheat flour plus 5% (by wt) brewer's yeast at 28^{o}C and 65% r.h. in the Stored Products Insects Research and Education Laboratory, Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas. Plastic vials (2.6 cm inner diameter and 4.9 cm high) were filled with 5 g of bleached wheat flour that was sifted using a 250 µm opening sieve. In each vial, 20 eggs, 20 young larvae, or 20 adults of mixed ages and sex of *T. castaneum* were introduced. These vials were closed with plastic lids covered with a fine mesh to allow air flow but prevent insect escape.

Heat treatments

Facility A

Facility A was a rice cake production facility, and it was subjected to heat treatment during September 25-26, 2010. The area of the facility subjected to heat treatment measured 30.48 m x 12.20 m x 12.20 m. Plastic bioassay vials (2.6 cm diameter and 4.9 cm height), each with 5 g of bleached flour, were placed in 24 locations in the room being heated. At each location there were 4 vials, each with 20 eggs, and four vials, each with 20 adults of *T. castaneum*. In addition, six vials, each with 20 eggs of T. castaneum, and six vials, each with 20 adults of *T. castaneum,* served as the control treatment. The control treatment vials were placed outside the heat-treated area. SmartButton temperature sensors (ACR Systems, Surrey, Canada) were launched to read temperature at two minute intervals. One sensor was placed next to the vials at each location. The facility was heated using a 1318.82 kw/h forced-air gas heaters fueled by propane heater (THP-4500; Rupp Industries Inc., Burnsville, MN). The hot air was transferred from the gas heaters into the rooms with high temperature nylon ductwork with holes. Duct diameters ranged from 50-91 cm. Fifteen 91.44 cm fans (Schaffer Ventilation Equipment, Sauk Rapids, MI) were placed throughout the treatment area to ensure proper heat distribution. A set of vials with eggs and adults from each of the 24 locations was collected at 1.5, 3, 5, and 24hours into the heat treatment. Six vials with eggs and 6 vials with adults unexposed to heat were collected at the same time periods. After the heat treatment, all vials were brought back to the laboratory, and mortality of adults determined 24hours after incubation at 28^{o}C and 65% r.h. After 45 days, vials with eggs were checked to count the number of adults that survived to adulthood. Adult survival was calculated based on number of adults that survived based on the total exposed. The number of adults that survived to adulthood out of the total eggs exposed

gave survival rate of eggs exposed to heat treatments. Egg and adult survival in control vials were determined similarly. Temperature data collected from the start of the heat treatment until the end of the heat treatment was used to determine starting temperature, time to 50°C, time above 50°C, and the maximum temperature.

Facility B

Facility B was a commercial sunflower seed processing and packaging facility, and it was subjected to heat treatment during September 25-26, 2009. Two separate rooms, the dry roasting room (DRR) and a bulk bin storage unit (BBU), in two separate buildings were subjected to heat treatment at this facility. Bioassay vials, each with 5 g of bleached flour, were placed throughout the dry roasting room (DDR) and the bulk bin storage unit (BBU). The DRR measured 45.42 m x 37.80 m x 5.18 m and the BBU measured 52.73m x 70.41 m x 9.14 m. There were 28 locations selected in DRR and 20 in BBU for vial placement. At each location in the DRR and BBU rooms, there were four vials with *T. castaneum* young larvae and four with adults. Three vials, each with 20 young larvae, or 20 adults of *T. castaneum* served as the control treatment. A SmartButton sensor was placed next to the vials at each location lauched to record temperature every two minutes, to record temperatures during the heat treatment.

Two forced-air gas heaters, fueled by propane, with a maximum heat energy output of 410.30 (THP-1400) and 161.0 kw/h (THP-550) from Temp-Air were used for heating the DRR, and one heater with a capacity of 1318.82 kw/h (THP-4500) was used for heating the BBU room. High temperature nylon ductwork transferred heat from the heaters into the processing rooms. Uniform distribution of the heat was ensured with the help of 10 Schaefer fans (91.44 cm diameter) placed in each room to circulate hot air and ensure uniform heating. One vials with young larvae and one with adults was sampled from each location at 4.7, 12.2, 20.2, and

27.7hours into the heat treatment. Control vials (3 vials with young larvae and 3 with adults) were sampled at each of these time periods. After the heat treatment, all vials were brought back to the laboratory and mortality of adults determined 24 hours after incubation at 28^{o}C and 65% RH. Larvae were reared to adulthood to determine survival. Temperature data collected from the start of the heat treatment until the end of the heat treatment was used to determine starting temperature, time to 50°C, time above 50°C, and the maximum temperature.

Facility C

Facility C is a commercial brewing facility with a volume of 280,416 m3, which was heat-treated during August 24-25, 2010. A total of 5 forced air propane heaters were used to heat the entire 280,416 cubic meters of the facility. There were four 1318.82 kw/h THP4500 propane heaters (THP, Rupp Industries Inc., Burnsville, MN), and one 410.30 kw/h THP 1500 propane heater (THP, Rupp Industries Inc., Burnsville, MN). Heat distribution was accomplished with 19 Schaffer fans (91.44cm), 8 Bailey fans (71.12 cm), and 18 box fans (121.92 cm). Bioassay vials (528 with 5 g of bleached flour in each) were placed throughout the first and second floors. In each of 264 the vials, 20 unsexed adult *T. castaneum* were introduced. The remaining 264 vials were infested with *T. castaneum* eggs. Vials were arranged in sets of eight: four adult and four egg vials to be set at each location in the facility. Eight vials with eggs and eight vials with *T. castaneum* adults were outside the heat-treated area served as the control vials. SmartButton sensors were launched to read temperature at two-minute intervals. One sensor was placed next to a group of vials at each of the 64 locations to record temperature from the start until the end of the heat treatment. After 2.5, 4.5, 8.5, and 27.5hours (end) into the heat treatment two vials (one vial each with eggs and adults) were removed from each of the 64 locations. Two control vials with eggs and two with adults were removed at each of the four

collection times. After the heat treatment, all vials were brought back to the laboratory and mortality of adults determined 48 hours after incubation at 28^{o}C and 65% RH. After 45 days, vials with eggs were observed to count the number of live adults that emerged in control and heat-exposed vials. Adult survival was based on number of adults that survived out of the total exposed. Egg survival was based on number of adult that emerged out of the total exposed.

Data analysis

The mean temperature across all data loggers was plotted as a function of time using SigmaPlot 12.5 (Systat Software Inc., San Jose, California, USA). The mean temperature and survival of individual life stages exposed to heat and those unexposed to heat at each of the four collection times were reported by facility. The thermal death kinetic (TDK) model developed in chapter 1(Bingham et al., 2014) predicted 1% survival of *T. castaneum* young larvae based only on time-dependent temperature data collected by SmartButton sensors at each of locations sampled in the three facilities. In each facility, the time for 1% predicted survival of young larvae at each of the locations temperatures measured was related to time to 50°C, time above 50°C, or the maximum temperature. Regressions models were fit to these data using TableCurve 2D (Jandel Scientific, San Rafael, CA, USA) to characterize this relationship, and the best fit was reported.

Results

Facility A

In Facility A, all 24 locations that were monitored reached well above 50^{o}C. The maximum temperatures ranged from 56.0^{o}C to 66.0^{o}C, averaging 59.8^{o}C. Heating rates ranged from 5.55^{o}C/hr to 48.75^{o}C/hr. In the facility, it took an average of 2.08 hours to reach 50^{o}C and the average time spent above 50^{o}C was 22.36 hours. Starting temperatures fell in a range

between 22°C and 24°C. Using a previously developed model (Bingham, 2014), *T. castaneum* 1% survival was estimated to take between 2.63 hours and 12.43 hours.

After 1.5 hours of treatment, 7 out of the 24 locations showed 0% adult survival. After 3 hours, 11 of the 24 locations showed 0% adult survival. After 5 hours, 17 of the 24 locations showed 0% adult survival. By the conclusion of the heat treatment, all locations showed 0% adult survival. After 1.5 hours of treatment, 11 of the 24 location showed 0% egg survival. After 3, 5, and 24 hours of treatment, 100% of the locations showed 0% egg survival.

At 1.5 hours into treatment, 12 of the 24 locations had reached or exceed 50°C, and by 3 hours, 22 of the 24 location had reached or exceeded 50°C. By 5 hours into the treatment, all location had reached 50°C; the last to do so was location 6, which reached 50°C after 4.5 hours.

Figure 2.4 shows average temperature across all locations along with total observed survival. Figure 2.1 shows the relationship between predicted time to 1% survival and maximum temperature, time above 50°C, and time required to achieve 50°C. Predicted time to 1% survival was calculated using the model generated from Chapter 1 (Bingham, 2014). In these figures, heating rate was calculated from the temperature profile as follows:
(50°C – ambient temperature/time in hours to reach 50°C). Time above 50°C was directly calculated from the temperature data as was maximum temperature.

Maximum temperature, time required to attain 50°C, and duration of time for which temperatures were held at or above 50°C all had an influence on predicted time to 99% mortality (n=24). The maximum temperature and duration of time temperatures were held at or above 50°C were both negatively related time to predicted 1% survival (r^2 = 0.948, r^2 = 0.948, respectively). Time that temperatures were maintained at or above 50°C was positively related with time to predicted 99% mortality (r^2 = 0.928).

Facility B

The mean temperature in the BBU room was higher than the DRR room up to 400 minutes, after which the mean temperature was higher in the DRR when compared with the BBU room until the termination of the heat treatment. Mean time to reach 50^0C was 5.1 hours in the BBU room and 6.23 hours in the DRR. During the heat treatment across all 48 locations in both rooms, 97.92% of the locations reached 50^0C. 37.5% of the locations were above 50^0C in 4.77 hours, 87.5% of locations were above 50^0C in 12.17 hours, and 93.7% locations were above 50^0C in 20.17 hours. There was a sudden drop in the percent locations above 50^0C (60.4%) at 27.7 hours because the heat was down regulated a few hours before terminating the heat treatment. In the DRR, 21.1% of the locations had reached 50°C after 4.77 hours, 92.9% of the locations were at or above 50°C at 12.17 hours. All locations in the DRR had reached 50°C by 12.5 hours of heat treatment, however location 3 was not able to sustain 50°C throughout the rest of the heat treatment. At 20.17 hours and 27.7 hours, 96.4% of the locations were at or above 50°C. In the BBU, 55% of the locations had reached 50°C after 4.77 hours of heat treatment. After 12.17 hours of heat treatment, 75% of the locations were at or above 50°C, and 90% of the locations were at or above 50°C after 20.17 hours of heat treatment. After 25 hours, all but one location (location 6) had reached 50°C. Due to the down regulation of heaters, approximately 2 hours before the end of the heat treatment, only 15% of the locations in the BBU were at or above 50°C at 27.7 hours.

Survival of adults that were not exposed to heat was 100%; whereas for young larvae, natural survival ranged from 80 to 45% for vials at the heated site. For vials in the growth chamber in the laboratory, larval survival ranged from 85 to 55%.

The survival of adults and young larvae is shown in Table 2. Survival of adults and young larvae in vials in the DRR sampled at 27.7 hours, close to the end of the heat treatment, was 0% and the mean temperature at this time was 57.8°C. At 4.77 hours into the heat treatment, 4 of the 28 sampled locations showed 0% adult survival, at 12.17 hours into the heat treatment, 23 of the 28 locations showed 0% adult survival, at both 20.17 and 27.7 hours into the heat treatment, 0% adult survival was observed at all locations in the DRR. Similar results were observed with survival of young larvae in the DRR; 4 of 28 locations showed 0% survival after 4.77 hours, 25 of 28 locations showed 0% survival after 12.17 hours, and at 20.17 and 27.7 hours all 28 sampled locations showed 0% observed young larvae survival.

In the BBU room, both adult and young larvae survival were initially lower than in the DRR with 11of 20 and 12 of 20 locations showing 0% adult and young larvae survival respectively at 4.77 hours. After 12.17 hours, both adults and young larvae had 15 out of 20 locations showing 0% observed survival. After 20.17, only one location (location 35, see figure 2.10) showed any observed survival in both adults and young larvae. After 27.7 hours, 19 out of 20 locations with mean temperature of 50.28^0C achieved 0% survival, while 1 out of 20 locations (location 35, see figure 2.10) with mean temperature 44.3^0C achieved 20% survival for adults and 5% survival for young larvae

Figure 2.4 shows average temperature across all locations in both the BBU and the DRR, and tables 2.3 and 2.4 show control and observed survival for both young larvae and adults at each sample time. Figure 2.2 shows the relationship between predicted time to 1% survival and maximum temperature, time above 50°C, and time required to achieve 50°C.

All these variables have been shown to influence insect survival. In the DRR, time to predicted 1% survival (n = 28 observations) was negatively related with the duration for which

the temperatures were held above 50°C (r^2 = 0.837) and the maximum temperatures attained (r^2 = 0.841). Time to predicted 1% survival was positively related with time required to attain 50°C (r^2 = 0.894). Similar results were found in the BBU: time to predicted 1% survival was negatively related to time above 50°C (n=19, r^2 = 0.924) and maximum temperature (n=19, r^2 = 0.862); time to predicted 1% survival was positively related with time to 50°C (n=19, r^2 = 0.599).

Facility C

In Facility C, all 64 monitored locations reached or exceeded the target temperature of 50°C. The maximum temperatures ranged from 51.5°C to 70°C with an average maximum temperature of 61.8°C. Heating rates ranged from 0.54°C/hr to 14.61°C/hr, averaging 5.8°C/hr. The mean time to reach 50°C was 4.51 and the mean time above 50°C was 23.52 hours. Using the previously developed and validated model (Bingham, 2014), 1% survival of *T.castaneum* was estimated to take between 2.93 and 19.97 hours.

After 2.5 hours, 35.94% of locations had reached 50^{o}C; after 4.5 hours, 65.63% of locations had reached 50°C; after 8 hours, 90.63% of locations had reached 50°C. One of the locations was missing bioassay vials for sample times 3 and 4 (8.5 and 27.7 h) and were thus excluded from the results. As such, adult survival data is calculated as n=63 samples. After 2.5 hours, 12 of the 63 sampled locations showed 0% adult survival. By the second sampling at 4.5 hours, 54 of the 63 locations showed 0% adult survival. After 8 hours, only 3 sampling location showed any adult survival; there was no adult survival at the conclusion of the heat treatment (27.5 hours). At sample time 3 (8 hours), locations 12, 36, and 40 (figure 2.11) had 100%, 15.80%, and 100% adult survival respectively. All three locations had lower than recommended heating rates and had not yet reached 50°C. *T. castaneum* egg survival data showed that after 2.5

hours of heat treatment, 28 of the 64 locations had reached 0% survival. At 4.5 hours, only one location (location 38, figure 2.11) showed any egg survival. All locations showed 0% egg survival by the 8 hour sampling time. At all sample times where insect survival was seen, temperatures had not yet reached 50°C.

Figure 2.4 shows average temperature across all locations along with total observed survival. Figure 2.3 shows the relationship between predicted time to 1% survival and heating rate, maximum temperature, time above 50°C, and time required to achieve 50°C.

Maximum temperature (r^2 = 0.707), and time above 50°C (r^2 = 0.904) were both negatively related with time to predicted 1% survival. Time to reach 50°C was positively related with time to predicted 1% survival (r^2 = 0.901).

Discussion

Across all three facilities, 5 of 2720 adults survived the heat treatment. The adults that did survive were from the single sample location that did not reach 50°C during the heat treatment. This agrees with prior work showing that 50°C is required to achieve complete disinfestation of a facility within a 24-36 hour timetable that is feasible for commercial facility heat treatment (Immohote and Immoholte-Tauscher, 1999). Additional analysis of the data shows that once temperatures of 50°C are reached, all sampled life stages of *T.castaneum* perish rapidly. Results here agree with general trends reported by Fields (2012). Fields reported ranges of 0-6% adult *T. castaneum* survival and 0-0.4% immature *T. castaneum* survival occurring between 5.5 and 21 hours. The average time above 50°C reported by Fields (2012) were 24.4 hours, 26.4 hours, and 24.9 hours were slightly higher than those reported here (22.5, 21.7, 22.2, 23.52), however, no data was given on duration of the heat treatments, so we are unable to

compare time to 50°C. While it is difficult to directly compare results across different species, general trends observed by Campolo (2013) were also observed here. While Campolo (2013) initially had much higher survival, he reported rapid decreases in survival as temperatures reached 50°C. The difference in initial survival reports likely comes from the large difference in reported time to 50°C: 9-25 hours in Campolo's case compared to 1.5-5 hours reported here. These findings also agree with prior work showing that insect death occurs within minutes to hours at temperatures of 50°C or greater (Fields, 1992). The data presented here shows that following the heating profile recommendations from pilot scale studies can be suitably scaled up for commercial facility disinfestation (Boina, et. al.2008; Mahroof et.al., 2003b; Dosland, 2006).

Across all facilities, time to predicted 1% survival was negatively related to time above 50°C and maximum temperature. Time to predicted 1% survival was positively related to time required to attain 50°C. These findings were in agreement with prior research on *T. confusum, G. cornutus, S. orzyae, and R. dominica* (Campolo et. al., 2013). At all three locations, maximum temperature was also shown to be negatively related to time to predicted 1% survival. All three factors were shown to adversely affect the survival of *T. castaneum* eggs, young larvae, and adults. While the data may seem to contradict laboratory findings that first instars are the most heat tolerant stage of *T. castaneum* (Mahroof, et. al., 2003b), this is likely a function of increased mobility and ability of adult life stages to seek refuge from the elevated temperatures within the 5 g of flour placed within the bioassay vials.

Carefully monitoring temperatures and using a modeling approach, industry professionals should be able to optimize their heat treatment operation to occur in 24 hours or less (Subramanyam, 2011). For this to occur, it is critical that air circulation is sufficient to facilitate even heating throughout the facility and that all locations reach a minimum 50^{o}C (Dowdy, 1999).

A heating rate between 2 and 5^{o}C/hr is sufficient in most normal conditions to reach and hold 50^{o}C for the required amount of time.

As methyl bromide and other fumigants are relied upon less and less, alternative stored product insect management techniques must be researched and refined. Heat treatment as a means of facility disinfestation has been used commercially for over 100 years (Dean, 1911; 1913). However, only recently has research in the area been focused on making heat treatment a viable alternative to pesticide fumigation (Madsen, 1994; Heaps and Black, 1994; Heaps, 1994; Mueller, 1994; Heaps, 1996; Dowdy, 1998; Subramanyam, 2011). While multiple pilot scale studies have shown the effectiveness of heat treatment pests (Boina, et. al.2008; Mahroof et.al., 2003b; Dosland, 2006), in-depth studies of commercial facilities have been sparse (Norstein, 1996; Dowdy, 1999; Fields, 2012; Campolo, 2013).

In conclusion, this data shows that heat treatment, when done properly, can achieve less than 1% survival across all *T.castaneum* life stages. This data also shows the practically significant relationships between time to 50°C, time above 50°C, and maximum temperature and *T. castaneum* survival.

Table 2.1 Observed and control *T. castaneum* adult survival at Facility A

Sample Time (h)	Mean temperature[a] (°C)	Observed Adult Survival[a] Survival /Total (%)	Control Adult Survival[b] Survival/ Total (%)
1.5	49.8	340/480 (70.83)	120/120 (100)
3	55.3	208/480 (43.33)	120/120 (100)
5	57.5	104/480 (21.67)	120/120 (100)
24	53.8	0/480 (0.00)	120/120 (100)

[a]*n*=24 sampled locations

[b]*n*=6 control vials

Table 2.2 Observed and control *T.castaneum* egg survival at Facility A

Sample Time (h)	Mean temperature[a] (°C)	Observed Egg Survival[a] Survival /Total (%)	Control Egg Survival Survival/ Total (%)
1.5	49.8	84/480 (17.5)	81/120 (67.5)
3	55.3	0/480 (0.00)	79/120 (65.83)
5	57.5	0/480 (0.00)	84/120 (70.0)
24	53.8	0/480 (0.00)	80/120 (66.67)

[a]*n*=24 sampled locations

[b]*n*=6 control vials

Table 2.3 Observed and control *T. castaneum* adult survival at Facility B

Sample Time (h)	Mean temperature (°C) in DRR[a]	Observed Adult Survival[a] DRR Survival /Total (%)	Mean temperature (°C) in BBU[b]	Observed Adult Survival[b] BBU Survival /Total (%)	Control Adult Survival[c] Survival/ Total (%)
4.7	47.7	471/560 (84.11)	49.1	165/400 (41.25)	60/60 (100)
12.2	55.7	54/560 (9.64)	53.3	59/399 (14.49)	60/60 (100)
20.2	56.8	0/560 (0.00)	56.9	19/400 (4.75)	60/60 (100)
27.7	57.2	0/560 (0.00)	46.4	5/400 (1.25)	60/60 (100)

[a] *n*=28 sampled locations

[b] *n*=20 sampled locations

[c] *n*=3 control vials

Table 2.4 Observed and control *T. castaneum* young larvae survival at Facility B

Sample Time (h)	Mean temperature (°C) in DRR[a]	Observed Young Larvae Survival[a] DRR Survival /Total (%)	Mean temperature (°C) in BBU[b]	Observed Young Larvae Survival[b] BBU Survival /Total (%)	Control Egg Survival Survival/ Total (%)
4.7	47.7	258/560 (46.07)	49.1	93/400 (23.25)	46/60 (80)
12.2	55.7	13/560 (2.32)	53.3	39/400 (9.75)	39/60 (70)
20.2	56.8	2/560 (0.36)	56.9	10/400 (2.50)	38/70 (63)
27.7	57.2	0/560 (0.00)	46.4	1/400 (0.25)	35/70 (60)

[a]*n*= 28 sampled locations

[b]*n*=20 sampled locations

Table 2.5 Observed and control *T. castaneum* adult survival at Facility C

Sample Time (h)	Mean temperature[a] (°C)	Observed Adult Survival Survival /Total (%)	Control Adult Survival[c] Survival/ Total (%)
2.5	47.0	1015/1280 (79.30)[a]	40/40 (100)
4.5	53.4	135/1280 (10.55)[a]	40/40 (100)
8	57.5	43/1260 (3.36)[b]	40/40 (100)
27.7	59.6	0/1260 (0.00)[b]	40/40 (100)

[a]*n*=64 sampled locations

[b]*n*=63 sampled locations

[c]*n*=2 control vials

Table 2.6 Observed and control *T. castanuem* egg survival at Facility C

Sample Time (h)	Mean temperature[a] (°C)	Observed Egg Survival[a] Survival /Total (%)	Control Egg Survival[b] Survival/ Total (%)
2.5	47.0	434/1280 (33.90)	31/40 (77.5)
4.5	53.4	2/1280 (1.56)	28/40 (70.0)
8	57.5	0/1280 (0.00)	28/40 (70.0)
27.7	59.6	0/1280 (0.00)	28/40 (70.0)

[a]*n*=64 sampled locations

[b]*n*=2 control vials

Figure 2.1 Relationship between time to 50°C, time above 50°C, maximum temperature, and predicted time to 1% survival at Facility A

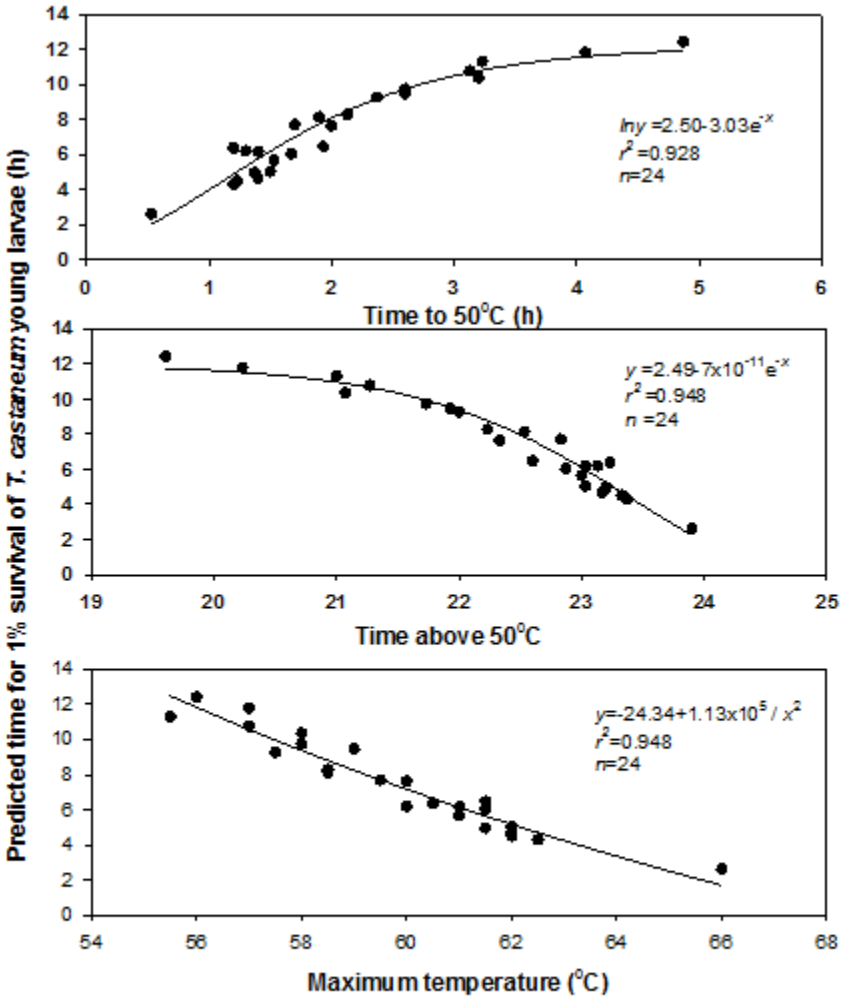


Figure 2.2 Relationship between time to 50°C, time above 50°C, maximum temperature, and predicted time to 1% survival in the dry roast room (DDR) and the bulk bin storage unit (BBU) at Facility B

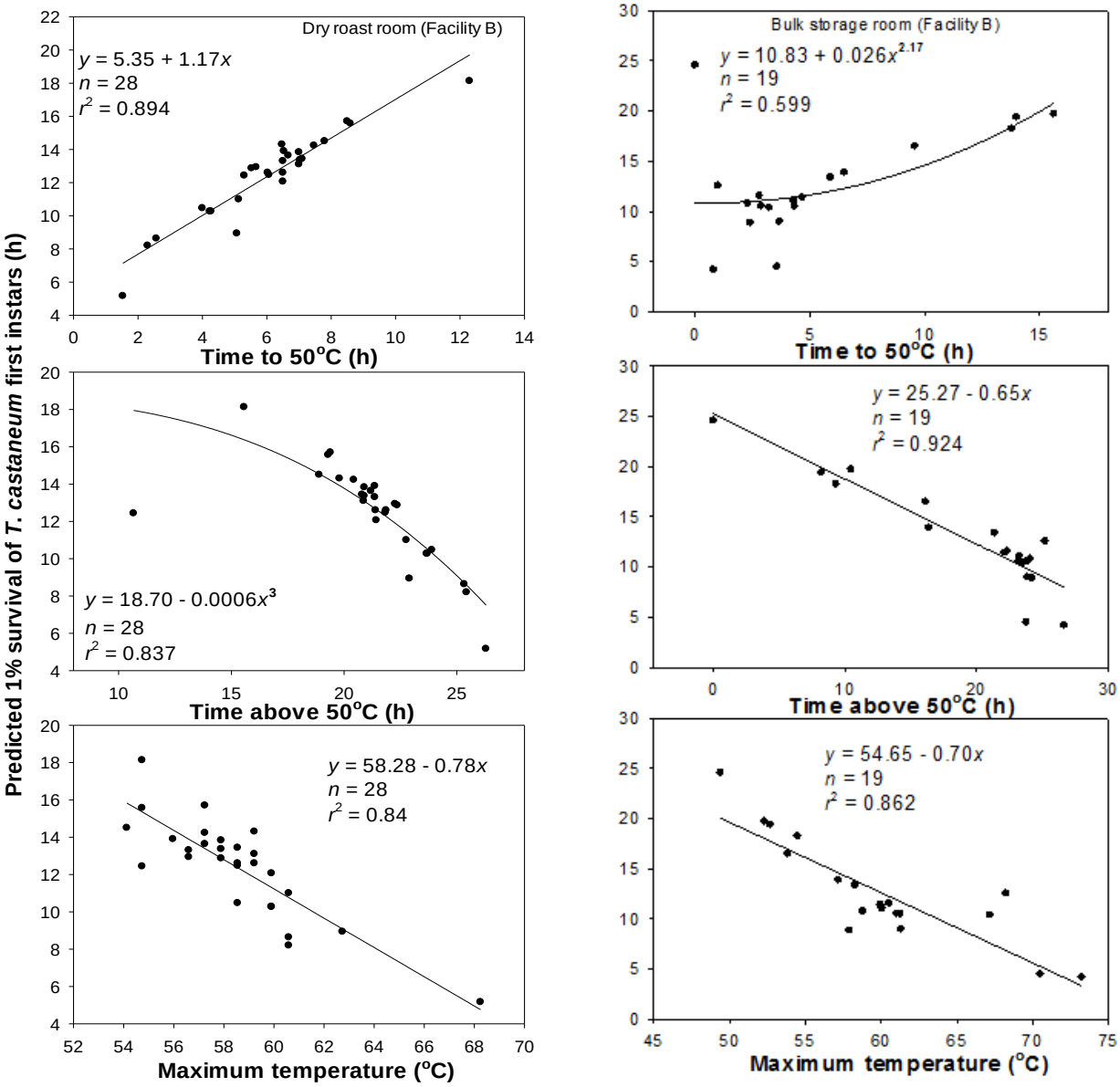


Figure 2.3 Relationship between time to 50°C, time above 50°C, maximum temperature, and predicted time to 1% survival at Facility C

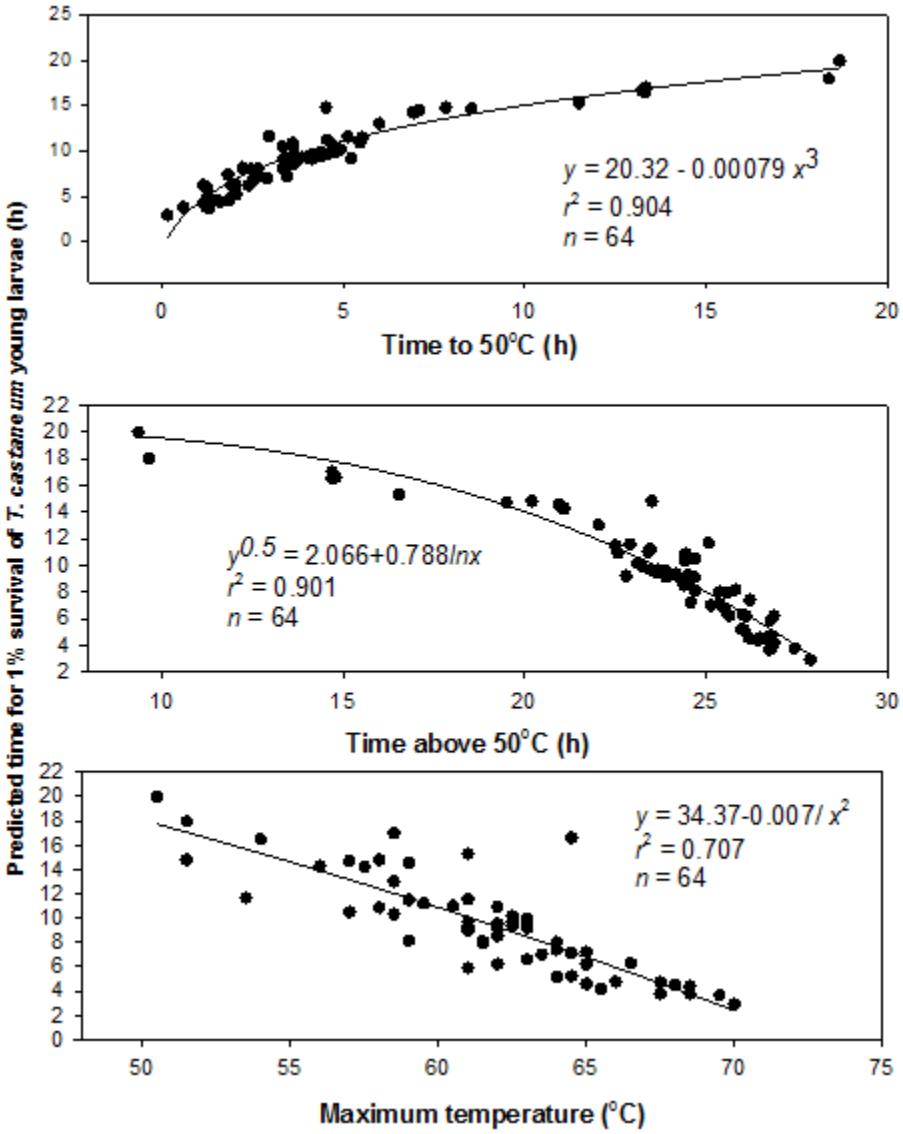


Figure 2.4 Average temperature, starting temperature, time to 50°C, time above 50°C, and maximum temperature across 24 sample locations at facility A

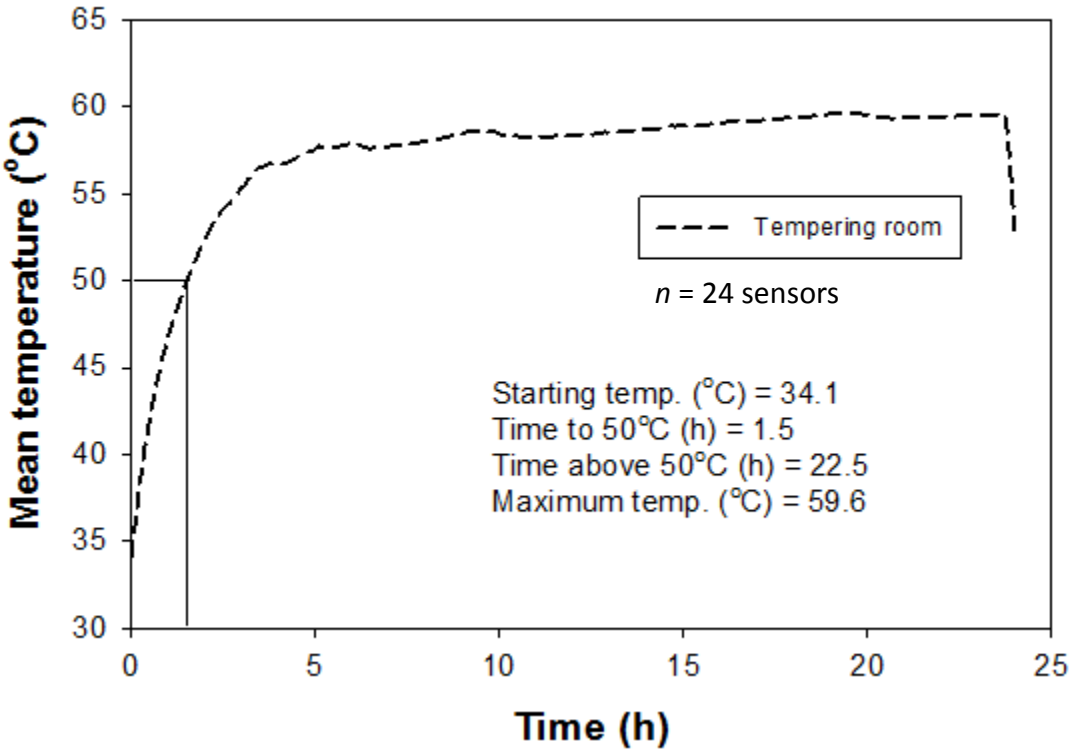


Figure 2.5 Average temperature, starting temperature, time to 50°C, time above 50°C, and maximum temperature across 28 sample locations at facility B dry roast room (DRR)

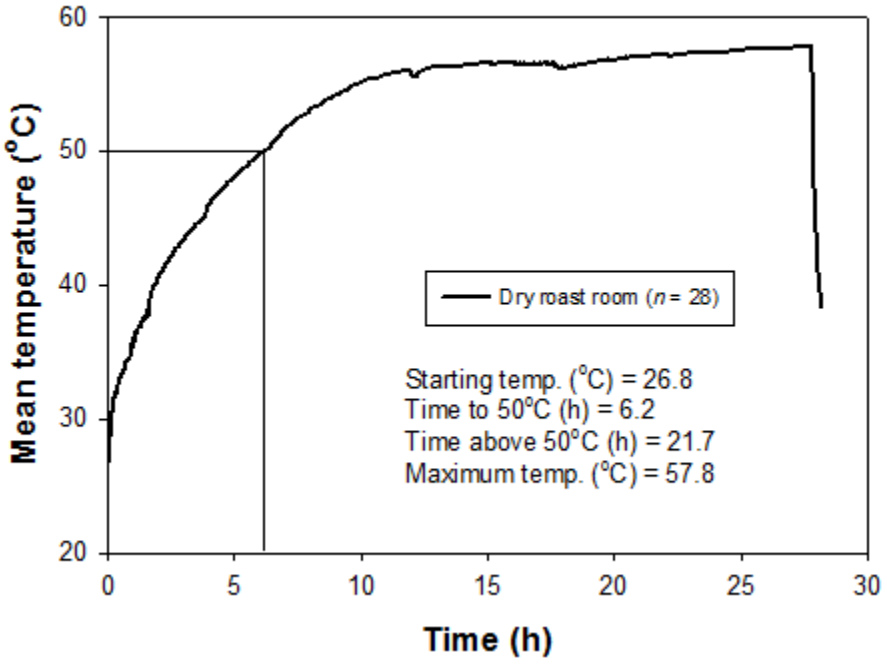


Figure 2.6 Average temperature, starting temperature, time to 50°C, time above 50°C, and maximum temperature across 20 sample locations at facility B bulk bin storage unit (BBU)

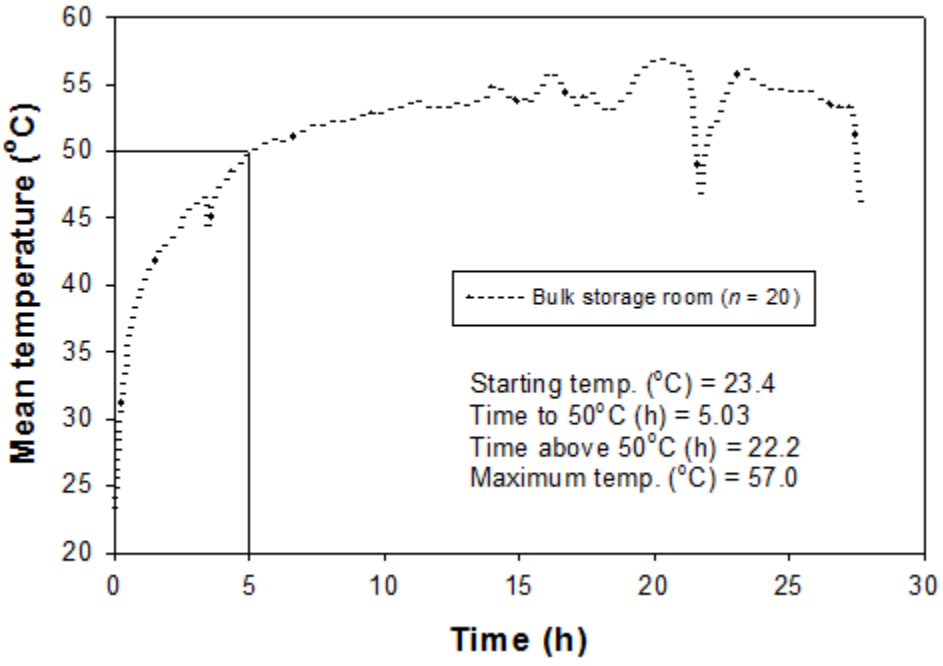


Figure 2.7 Average temperature, starting temperature, time to 50°C, time above 50°C, and maximum temperature across 64 sample locations at facility C

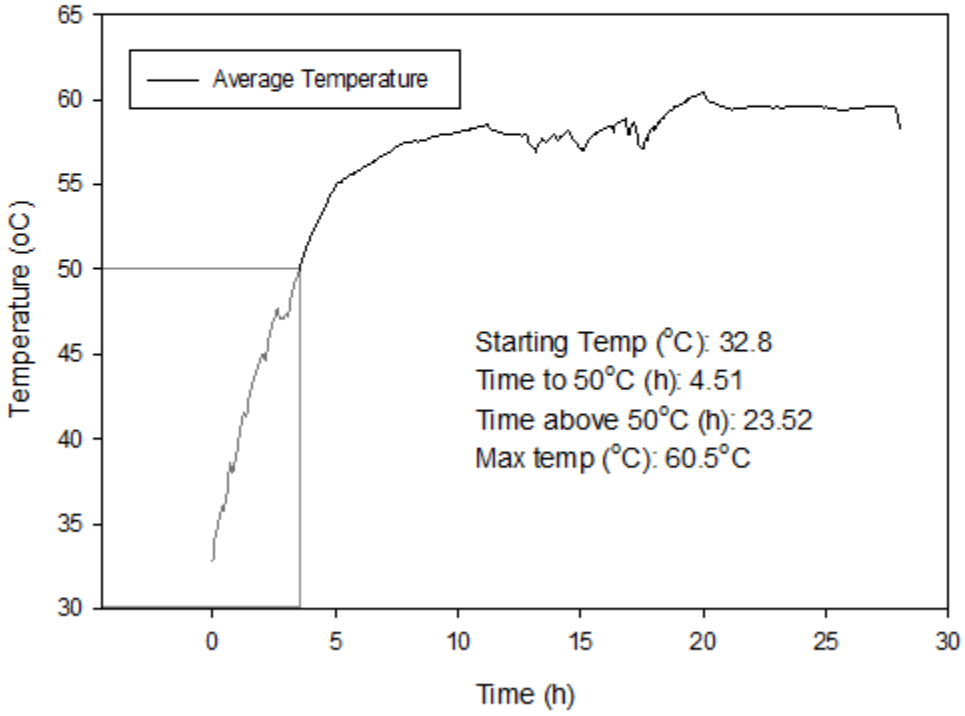


Figure 2.8 Floor diagram and bioassay sample locations at facility A

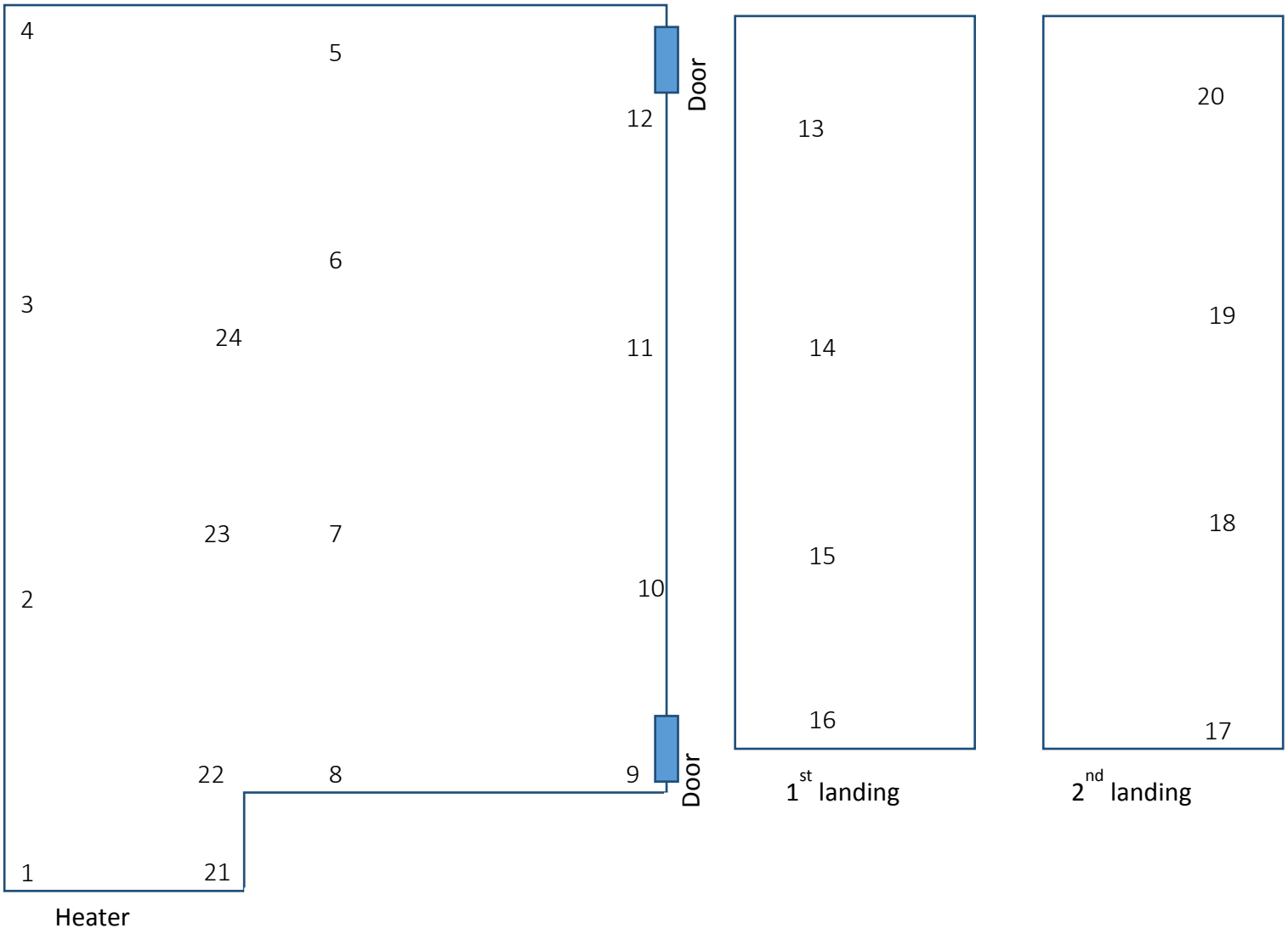


Figure 2.9 Facility B dry roast room (DRR) floor diagram and bioassay vial locations

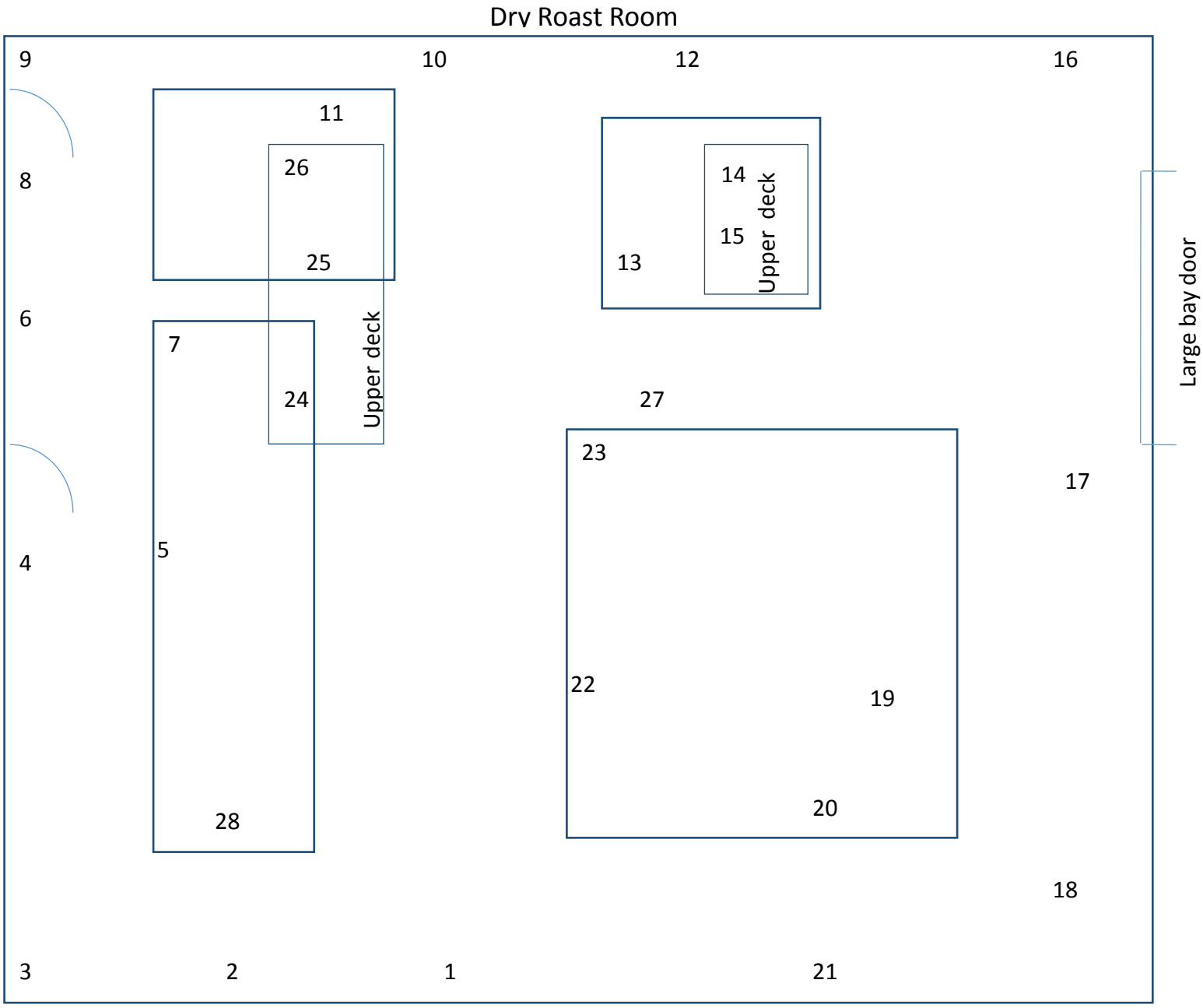


Figure 2.10 Facility B bulk bin storage unit (BBU) floor diagram and bioassay vial locations.

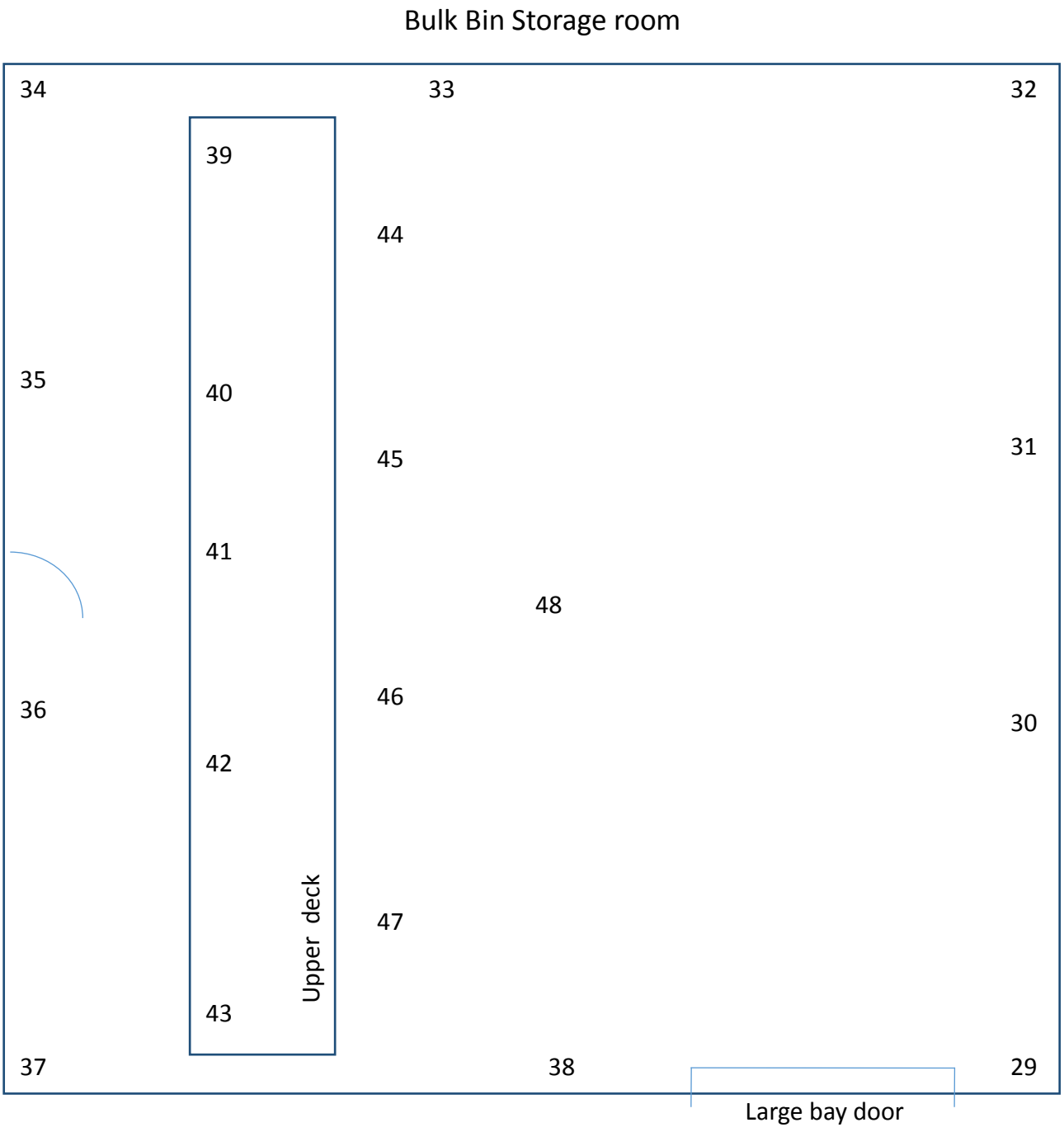
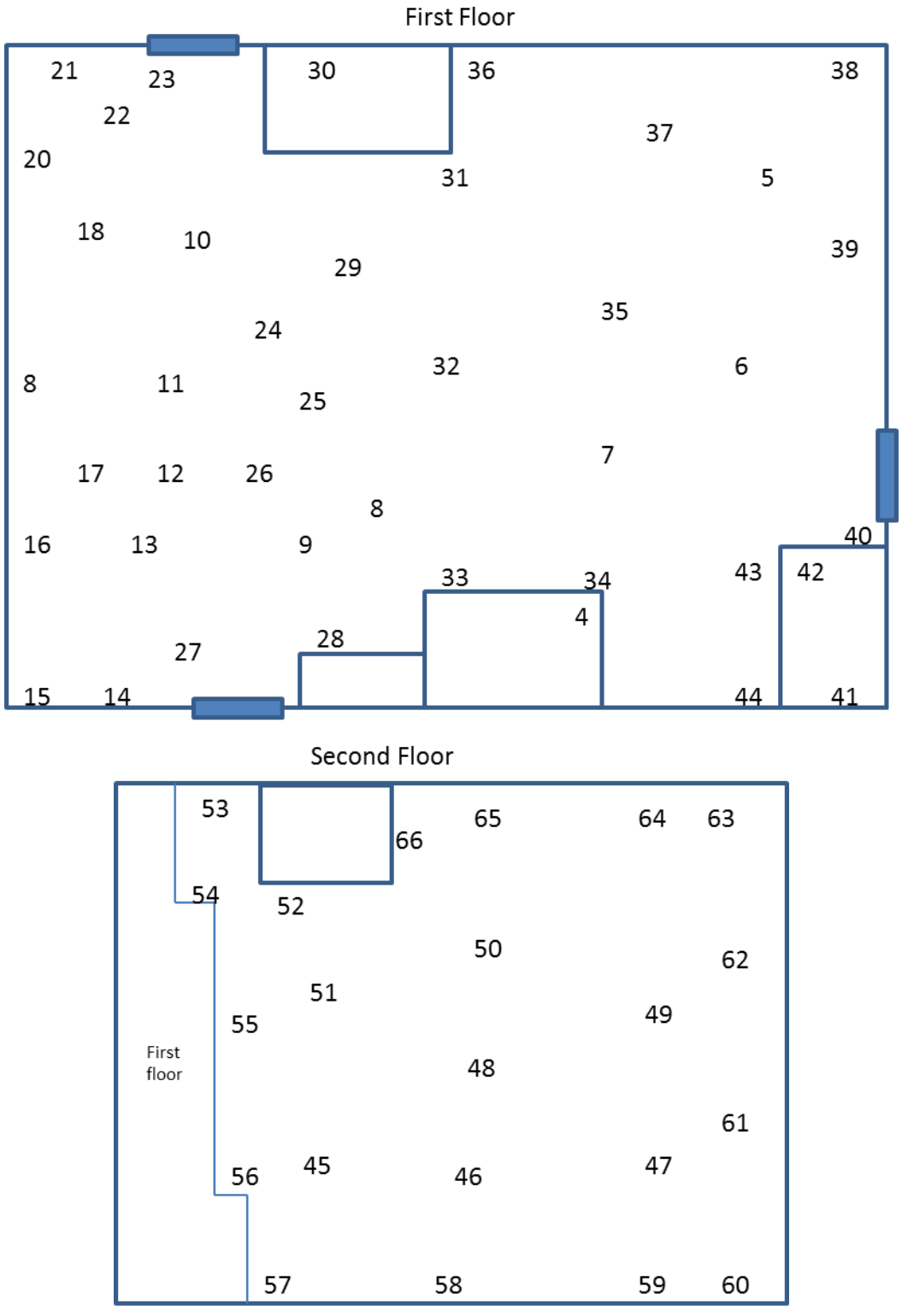


Figure 2.11 Facility C floor plan and bioassay vial locations.



References

Abdelghany, A.Y., Awadalla, S.S., Abdel-Baky, N.F., EL-Syrafi, H.A., and Fields, P.G. (2010). Effect of high and low temperatures on the drugstore beetle (Coleoptera: Anobiidae).", Journal of Economic Entomology. 103: 1909-1914.

Akdoğan, H., M. E. Casada, A. K. Dowdy, and Bh. Subramanyam. 2005. A novel method for analyzing grain facility heat treatment data. J. Stored Prod. Res. 41: 175-185.

Banks, J., and Fields, P., 1995 Physical methods for insect control in stored-grain ecosystems, pp 353-409. *In* D.S. Jayas, N.D.G. White, and E.E. Muir [eds.], Stored-grain ecosystems. Mercel Decker, New York.

Baranyi, J.., A. Jones, C. Walker, A. Kaloti, T.P. Robinson, and B.M. Mackey. 1996. A combined model for growth and subsequent thermal inactivation of *Brochothix thermosphacta.* Appl. Environ. Microbiol. 62: 1029-1035.

Bingham, A.C. 2014. Heat treatment of grain-processing facilities: gauging effectiveness against select life stages of *Tribolium castaneum* (herbst) using bioassays and a thermal death kinetic model. M. S. Thesis, Kansas State University, Manhattan, KS

Boina, D., and Bh. Subramanyam. 2004. Relative susceptibility of Tribolium confusum (Jacquelin duVal) life stages to elevated temperatures. J. Econ. Entomol. 97: 2168-2173.

Boina, D., Bh. Subramanyam, S. Alavi. 2008. Dynamic model for predicting survival of mature larvae of *Tribolium confusum* during facility heat treatment. J. Econ Entomol. 101: 989-997.

Buckman, K. A., Campbell, J. F., & Subramanyam, B. (2013). Tribolium castaneum (Coleoptera: Tenebrionidae) associated with rice mills: Fumigation efficacy and population rebound. Journal of economic entomology, 106(1), 499-512.

Brijwani, M., Bh. Subramanyam, P. W. Flinn, M. R. Langemeier, M. Hartzer, and R. Hulasare. 2012a. Susceptibility of Tribolium castaneum life stages exposed to elevated temperatures during heat treatments of a pilot flour mill: influence of sanitation, temperatures attained among mill floors, and costs. J. Econ. Entomol. 105: 709-717.

Brijwani, M., Bh. Subramanyam, and P. W. Flinn. 2012b. Impact of varying levels of sanitation on mortality of Tribolium castaneum eggs and adults during heat treatment of a pilot flour mill. J. Econ. Entomol. 105: 703-708.

Campolo, O., M. Verdone, F. Laudani, A. Malacrino, E. Chiera, V. Palmeri. 2013 Response of four stored products insects to a structural heat treatment in a flour mill. Journal of Stored Product Research. 54: 54-58

Currie, S. and B. Tufts. 1997. Synthesis of stress protein 70 (Hsp 70) in Rainbow Trout (Oncorhyncus mykiss) red blood cells. J Exp. Biol. 200:607-614.

Dean, G. A. 1911. Heat as a means of controlling mill insects. Journal of Economic Entomology, 4(2), 142-161.

Dean, G. A. 1913. Further data on heat as a means of controlling mill insects. Journal of Economic Entomology, 6(1), 40-55.

Dosland, O., Bh. Subramanyam, G. Sheppard, and R. M. Mahroof. 2006. Temperature modification for insect control, pp. 89-103. In, Heaps, J. (ed.), Insect Management for Food Storage and Processing. Second Edition, American Association of Cereal Chemists, St. Paul, MN.

Dowdy, A. K. 1998,. Heat sterilization as an alternative to methyl bromide fumigation in cereal processing plants. In Proceedings of the Seventh International Working Conference on Stored Product Protection (Vol. 2, pp. 14-19).

Dowdy, A. K. 1999. Mortality of red flour beetle,< i> Tribolium castaneum</i>(Coleoptera: Tenebrionidae) exposed to high temperature and diatomaceous earth combinations. Journal of Stored Products Research, 35: 175-182.

Dowdy, A. K., and Bh. Subramanyam, (1999). Comparison of temperature distribution during heat treatments using TempAir or Aggreko Systems. *https://www.ksre.ksu.edu/grsc_subi/New_CV_Folder/Publications/contents/HeatandIPM/1999_Dowdy_TempDist_Report.pdf.*

Dowdy, A.K., 2000. Heat sterilization as an alternative methyl bromide fumigation in cereal processing plants, pp. 1089-1095. *In* J. Zuxun, L. Quan, L. Yongshen, T. Xianchang, and G. Lianghua [eds.] Proceedings of the Seventh International Working Conference on Stored Product Protection., 14-19 October, Beijing, China. Sichuan Publishing House of Science and Technology, Chengdu, Sichuan Province, People's Republic of China.

Dowdy, A.K., and P.G. Fields. 2002. Heat combined with diatomaceous earth to control the confused flour beetle (Coleoptera: Tenebrionidae) in a flour mill. J. Stored Prod. Res. 38:413-425.

Fields, P.G. 1992. The control of stored-product insects and mites with extreme temperatures. J. Stored Prod. Res. 28:89-118.

Fields, P. G. 2012. Novel fumigants and heat treatment for flour mills. *In* Navarro S, Banks HJ, Jayas DS, Bell CH, Noyes RT, Ferizi AG, Emekci M, Isikber AA, Alagusundaram K [Eds.] Proc 9th Int. Conf. on Controlled Atmosphere and Fumigation in Stored Products,

Antalya, Turkey. 15-19 October 2012, ARBER Professional Congress Services, Turkey pp: 333-344

Gonen, M. 1977a. Susceptibility of *Sitophilus granaries* and *S. oryzae* (Coleoptera: Curculionidae) to high temperature after exposure to supra-optimal temperature. Entomol. Exp. Appl. 21:243-248.

Gonen, M. 1977b. Survival and reproduction of heat- acclimated sitophilus granarius (coleoptera, curculionidae) at a moderately high temperature. Entomol. Exp. Appl, 21: 249-253.

Good, N. E. 1937. Insects found in the milling streams of flour mills in the southwestern milling area. Journal of the Kansas Entomological Society, 10: 135-148.

Goodwin, W. H., 1914. Some factors affect the use of high temperatures in the control of insects injuring cereal products. Journal of Economic Entomology 7:313-322

Goodwin, W.H. 1922. Heat for control of cereal insects. (No. 354) Ohio Agricultural Experiment Station.

Hagstrum, D. W. and Bh. Subramanyam. 2009. Stored product insect resource. American Association of Cereal Chemists International, St. Paul, MN, 509 pages.

Heaps, J. W. 1994. Temperature control for insect elimination. In Methyl Bromide Conference (Vol. 56, pp. 1-3).

Heaps, J. 1996. Heat for stored product insects. IPM Practitioner, May/June, 18-19.

Heaps, J. W., & Black, T. 1994. Using portable rented electric heaters to generate heat and control stored product insects. Association of Operative Millers Bulletin,(July), 6408-6411.

Imholte, T.J., and T. Imholte-Tausher. 1999. Engineering for food safety and sanitation. Technical Institute of Food Safety. Woodinville, Washington DC.

Jian, F., Subramanyam, B., Jayas, D. S., & White, N. D. (2013). Models to Predict Mortality of Tribolium castaneum (Coleoptera: Tenebrionidae) Exposed to Elevated Temperatures During Structural Heat Treatments. Journal of economic entomology, 106(5), 2247-2258.

Madsen, B. 1994. Heat treatment as an alternative to methyl bromide fumigation in mills. Alternatives to methyl bromide, TermaNord, 1995 574:51-55

Mahroof, R., Bh. Subramanyam, J. Throne, and A. Menon. 2003a. Time-mortality relationships for *Tribolium castaneum* (Herbst) life stages exposed to elevated temperatures. Journal of Economic Entomology. 96: 1345-1351.

Mahroof, R., Bh. Subramanyam, and D. Eustace. 2003b. Temperature and relative humidity profiles during heat treatment of mills and its efficacy against *Tribolium castaneum* (Herbst) life stages. Journal of Stored Product Research. 39: 555-569.

Mahroof, M., K. Y. Zhu, and Bh. Subramanyam. 2004. Changes in expression of heat shock proteins in Tribolium castaneum (Coleoptera: Tenebrionidae) in relation to developmental stage, exposure time, and temperature. Annals of the Entomological Society of America 98: 100–107. [PDF]

Mahroof, R., K. Y. Zhu, L. Neven, Bh. Subramanyam, and J. Bai. 2005. Expression patterns of three heat shock protein 70 genes among the developmental stages of the red flour beetle, Tribolium castaneum. Comparative Biochemistry and Physiology, Part A 141: 247-256.

Mahroof, R. Bh. Subramanyam. 2006. Susceptibility of *Plodia interpunctella* (Lepidoptera: Pyralidae) developmental stages to high temperatures used during structural heat treatments. Bulletin of Entomological Research 96: 539-545

.Mueller, D. K. 1994. Phosphine, Heat and Carbon Dioxide Deliver Death Punch to Insects. Pest Control, 62: 42-48.

Neven, L.G. 1994. Effects of combined heat treatments and cold storage on fifth-instar codling moth (Lepidoptera: Tortricidae) mortality. Journal of Economic Entomology. 87:1262-1265.

Norstein, S. 1996. Heat treatment in the Scandinavian Milling Industry: Heat treatment as an alternative to methyl bromide. SFT Miljøteknologi, 96, 02E.

Roseli, R., Bh. Subramanyam, F.J. Fairchild, and K.C. Behnke. 2003. Trap catches of stored product insects before and after heat treatment in a pilot feed mill. Journal of Stored Product Research. 39:521-540.

Subramanyam, Bh. P.W. Flinn, and R. Mahroof. 2003. Development and validation of a simple heat accumulation model for predicting mortality of *Tribolium castaneum* (Herbst) first instarts exposed to elevated temperatures, p.p. 369-374. *In* P.F. Creedland, DM. Armitage, C.H. Bell, P.M. Cogan, and E. Highley [eds.] Proceedings of the Eighth International Working Conference on Stored Product Protection, 22-26 July 2002, York, United Kingdom. CAB International, Wallingford, Oxon, United Kingdom.

Subramanyam, Bh., R. Mahroof, and M. Brijwani. 2011. Heat treatment of food-processing facilities for insect management: a historical overview and recent advances, pp. 1-11. Stewart Postharvest Review 3: 1-11.

Tang, J., J.N. Idediala, S. Wang, J.D. Hansen, and R. Cavalieri. 2000. High-temperature-short-time quarantine methods. Postharvest Biol. Techno. 21: 129-145.

Van Impe, J.F., B.M. Nicolai, T. Martens, J. De Baerdemaeker, and J. Vandewalle. 1992. Dynamic Mathematical model to predict microbiological growth and inactivation during food processing. Appl. Envirion. Microbiol. 58:2901-2909

Wang, S., J.N. Ikediala, J. Tang, and J.D. Hansen. 2002a. Thermal-death kinetics and heating rate effects for fifth-instar codling moths (*Cydia pomonella* (L.)*)*. Journal of Stored Product Research. 38:441-453.

Wang, S., J.N. Ikediala, J. Tang, and J.D. Hansen. 2002b. Thermal-death kinetics of fifth-instar *Amyelois transitella* (Walker) (Lepidoptera: Pyralideae). Journal of Stored Product Research. 38:427-440.

Wright, E.J., E.A. Sinclair, and P.C. Annis. 2002. Laboratory determination of the requirements for control of *Trogoderma variabile* (Coleoptera: Dermestidae) by heat. Journal of Stored Product Research. 38:147-155.

Yu, C., Bh. Subramanyam, P. W. Flinn, and J. A. Gwirtz. 2011. Susceptibility of Lasioderma serricorne (Coleoptera: Anobiidae) life stages to elevated temperatures used during structural heat treatments. J. Econ. Entomol. 104: 317-324.

Zar, J.H., 1984. Biostatistical analysis, 2nd ed. Prentice Hall, Englewood Cliffs, New Jersey, United States of America