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EFFECTIVENESS OF HEAT TREATMENTS AGAINST *TRIBOLIUM CASTANEUM* LIFE STAGES IN TWO COMMERCIAL FOOD-PROCESSING FACILITIES

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ABSTRACT

Two facilities, A and B, were subjected to heat treatments using forced-air gas heaters that were fueled by propane. At facility A, two separate rooms were heated for ~28 h. Temperature sensors and insect bioassay vials with 20 young larvae (first instars) or 20 adults of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) were placed in 20 or 28 locations. Temperatures reached 50°C from the ambient temperature of 23 to 27°C in 5 to 6 h. Temperatures above 50°C were held for 22 h, and the maximum temperatures did not exceed 58°C. Mortality of young larvae and adults was 90 to 96% about 12 h into the heat treatment and reached 100% at 20 h. Little or no progeny was produced 42 d later in vials exposed to the heat treatment for 12 to 28 h. In facility B heated for 24 h, time to reach 50°C from the ambient temperature of 34°C took 1.5 h, and temperatures above 50°C were held for 23 h, and the maximum temperature observed was about 60°C. Mortality of *T. castaneum* eggs was 100% in vials exposed to heat for 3 h whereas that of adults was 57% with 100% mortality occurring at 24 h. A thermal death kinetic model predicted time to 99% mortality (LT_{99}) of *T. castaneum* young larvae, which is the most heat tolerant stage, as a function of time-dependent temperature data at each location. The LT_{99} values were positively related to time to 50°C, but inversely related to time above 50°C and the maximum temperature. Observed temperatures, insect responses in bioassays, and thermal death kinetic model predictions confirmed that successful commercial heat treatments can be conducted in 24 to 28 h. No adverse effects to the electrical or structural components of the facilities occurred during the two heat treatments.

Keywords: Food processing facilities, heat treatment, demonstration trials, *Tribolium castaneum*, life stages, efficacy assessment.

INTRODUCTION

Heat treatment involves raising the ambient air temperature of the whole or a portion of food-processing facility between 50 and 60°C and holding these lethal temperatures for 24 to 36 h to manage stored product insects (Dosland et al., 2006). Brijwani et al. (2012) reported that a successful heat treatment of a pilot food processing facility (flour mill) at Kansas State University, based on lethal temperatures attained and mortality of eggs, young larvae, pupae, and adults of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), can be

conducted in 24 h. In this paper, the effectiveness of heat treatment was demonstrated in two commercial facilities based on lethal temperatures attained and evaluating mortality of exposed *T. castaneum* eggs, young, larvae, and adults.

MATERIALS AND METHODS

Heat treatment effectiveness was demonstrated at two commercial facilities, A and B. Facility A manufactures roasted sunflower seeds. Two separate rooms of this facility were subjected to heat treatment lasting 27.7 h during September 25 to 26, 2009. One of the rooms is used for roasting the seeds (DRR; 52.7 m x 70.4 m x 9.2 m) while the other is used for storing sunflower seeds (BBU; 45.4 m x 37.8 m x 5.2 m). Facility B manufactures rice cakes, and in this facility a processing room (30.5 m x 12.2m x 12.2 m) was heat treated for 24 h during September 25 to 26, 2010. Heat treatments at both locations were performed by Temp-Air (Burnsville, Minnesota, USA) using forced air gas heaters, and propane was used as the fuel.

The DRR and BBU were subjected to heat treatment. A total of three heaters were used. Two heaters with a maximum heat energy output of 410.3 kW/h (1.4 million BTU/h) and 161.2 kW/h (0.55 million BTU/h) were used for heating the DRR, and one heater with a capacity of 1318.8 kW/h (4.5 million BTU/h) was used for heating the BBU. The hot air was transferred from the gas heaters into the processing rooms with fabric ductworks. A 91.4 cm diameter ductwork was placed in BBU room, and ducts of 61.0 and 50.8 cm diameter were placed in DRR. Uniform distribution of the heat was ensured with the help of 10 fans, each with a 91.4 cm fan diameter, that were placed in each room to circulate hot air.

The temperature sensor (HOBO® data loggers, Onset Computer Corporation, Bourne, MA) loggers were launched to record temperature every 2 min. A total of 28 loggers were placed at the floor level in DRR and 20 in BBU room. The effectiveness of the heat treatment was evaluated with insect bioassays placed adjacent to the temperature sensors in order to correlate temperature and insect mortality rates. Insect bioassays were prepared and mortality assessment was done in the Stored-Products Insects Research and Education Laboratory, Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas, following procedures described by Mahroof et al. (2003). Young larvae and adults of *T. castaneum* were reared on wheat flour plus 5% (by wt) brewer's yeast diet at 28°C and 65% r.h. Plastic vials (2.6 cm inner diameter and 4.9 cm height) were filled with 5 g of bleached wheat flour sifted using a 250-µm opening sieve. Into each vial 20 young larvae or adults were introduced. These vials were closed with plastic lids covered with meshes to allow air flow but prevent insect escape. Four vials infested with young larvae and four vials infested with adults were placed in each of 28 locations throughout DRR. Similarly, eight vials per location were placed in each of 20 different locations in the BBU room. Two vials each infested with adults or young larvae were placed outside the DRR and BBU rooms that were unheated to determine natural insect mortality of these stages. Another set of vials (one for each life stage) remained in the laboratory growth chamber set at 28°C and 65% r.h.

During the heat treatment one vial with larvae and one with adults was sampled at 4.8, 12.2, 20.2, and 27.7 h into the heat treatment from 28 locations in DRR and from 20 locations in BBU room. After the heat treatment, all vials were brought back to the laboratory on September 27, and mortality of adults determined 24 h after incubation at 28°C and 65% r.h. Adult mortality was based on number of dead adults out of the total exposed (20). Young larvae in vials were placed in a growth chamber at 28°C and 65% r.h. for 45 d. Mortality was determined based on number of adults that failed to emerge from each vial out of the total larvae exposed. Larval

mortality was corrected for mortality of larvae in vials not exposed to the heat treatment (control vials) (Abbott, 1925).

In facility B, one heater with a maximum heat energy output of 1318.8 kW/h (4.5 million BTU/h) was used. The hot air was transferred from the gas heater into the room with 91.4 cm diameter fabric ductwork. Uniform distribution of the heat was ensured with the help of 12 fans.

In each plastic vial 5 g of bleached wheat flour sifted using a 250-µm opening sieve was added. In each vial, 20 eggs or 20 adults of *T. castaneum* were introduced. These vials were closed with plastic lids covered with mesh. Four vials infested with eggs and four vials infested with adults were placed in 24 locations throughout the room. Two vials each infested with eggs or adults were placed outside the heated room to determine natural insect mortality, and another set of vials (four for each life stage) remained in the laboratory growth chamber set at 28°C and 65% r.h. Temperature profiles in each of the 24 locations were measured using SmartButton sensors (ACR Systems, Inc., Surrey, Canada) every 2 min. These sensors were placed in 5 g of flour in an additional vial without insects. This additional vial was placed at all 24 locations along with the set of vials holding eggs and adults. During the heat treatment one set of vials (eggs and adults) were sampled at 1.5, 3, 5 and 24 h into the heat treatment from all 24 locations in the tempering room. After heat treatment, all vials were brought back to the laboratory on September 27, 2010 and mortality of adults determined 24 h after incubation at 28°C and 65% r.h. Adult mortality was based on number of dead adults out of the total exposed (20). Eggs in vials were placed in a growth chamber at 28°C and 65% r.h. for 45 d. Egg-to-adult mortality was determined based on number of adults that failed to emerge from each vial out of the total eggs exposed. Eggs mortality was corrected for mortality of eggs in vials not exposed to the heat treatment (control vials).

The mean temperature profiles across all 28 and 20 data loggers within DRR and BBU rooms, respectively, and in the heated room of facility B were plotted as a function of time using SigmaPlot 11. The mean starting temperature (°C), time to 50°C (h), time above 50°C (h), and the maximum temperature (°C) attained within each heated room was determined from the mean time-dependent temperature data. At each of the four sampling periods, the mean ± SE of temperature and mortality of adults and young larvae in DRR and BBU rooms and mortality of eggs and adults in facility B were determined.

A novel thermal death kinetic model was developed and validated to predict survival of old larvae of the confused flour beetle, *Tribolium confusum* (Jacquelin du Val), based on time-dependent temperature measured during facility heat treatments (Boina et al., 2008). The same modelling approach was used to validate survival of young larvae of *T. castaneum* (Subramanyam Bh, Mahroof R, unpublished data; Subramanyam et al., 2011) during facility heat treatment. Young larvae of *T. castaneum* are the most heat resistant life stage (Mahroof et al., 2003) among important stored-product insects and stages we tested (Boina and Subramanyam, 2004; Mahroof and Subramanyam, 2006; Yu et al., 2011; Subramanyam et al., 2011). Therefore, this stage was used in model predictions, because controlling this stage would control other stages of *T. castaneum*. Temperature data at each location in the DRR and BBU room, and in the heated room in facility B, were used to predict time in hours for 99% mortality (LT_{99}) of *T. castaneum* young larvae. The temperature data from each data logger were also used to determine the time to 50°C (h), time above 50°C (h), and maximum temperature (°C) obtained at each of the locations sampled in each heated room. The relationship between LT_{99} and time to 50°C (h), time above 50°C (h), or maximum temperature (°C) was described using regression models (SAS Institute, 2002).

RESULTS AND DISCUSSION

The mean temperature in the BBU room was higher than the DRR room up to 6.7 h, after which the mean temperature was higher in the DRR than the BBU room (Fig. 1). Mean temperature reached 50°C in 6.3 h in the DRR and in 5.1 h in the BBU room. During the heat treatment across all 48 locations in both rooms, 37.5% of the locations were above 50°C in 4.8 h, 87.5% of locations were above 50°C in 12.2 h and 93.7% locations were above 50°C in 20.2 h. There was a sudden drop in the percent locations above 50°C (60.4%) at 27.7 h (Table 1) because the amount of heat input was reduced a few hours before the end of heat treatment.

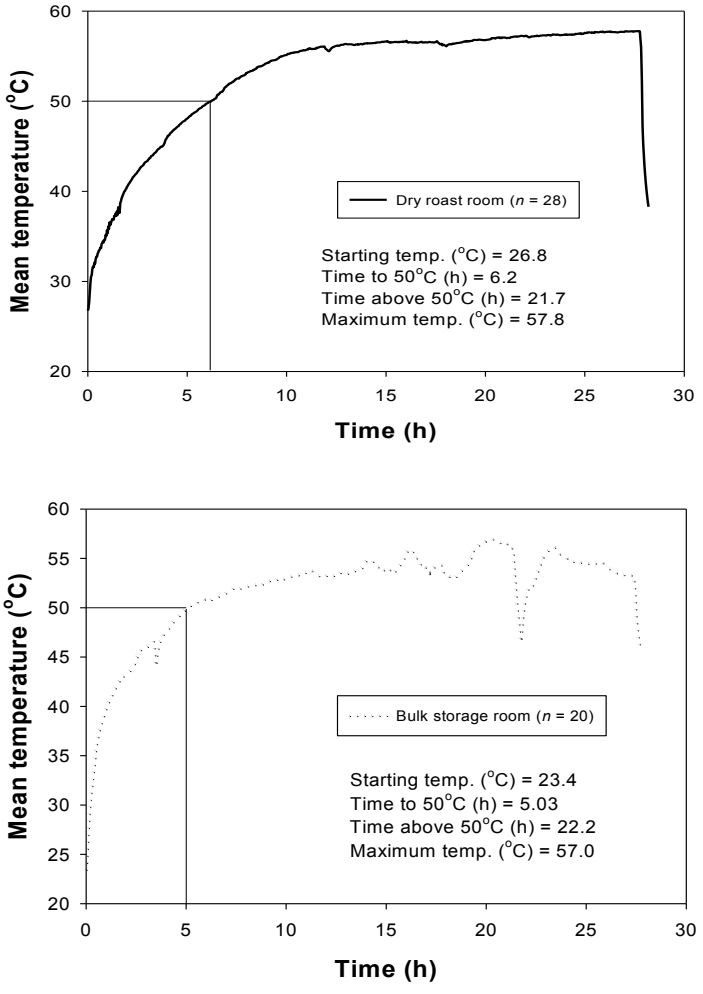

Fig. 1- Mean temperatures observed in the dry roast room (DRR) and bulk storage room (BBU) in facility A. The solid black line shows time taken to reach 50°C.

Table 1. Number of locations in dry roast room (DRR) and bulk storage room (BBU) with temperatures below and above 50°C at each of the four vial sampling periods in facility A

Time (h)	Dry roast room (DRR)		Bulk storage room (BBU)	
	No. locations	Mean ± SE Temp (°C)	No. locations	Mean ± SE Temp (°C)
4.8	22	45.9 ± 0.6	8	42.0 ± 2.2
	6	54.4 ± 1.6	12	54.5 ± 1.7
12.2	2	48.2 ± 0.8	5	44.6 ± 3.2
	26	56.2 ± 0.6	15	56.1 ± 1.4
20.2	1	43.4 ± 0.0	2	48.3 ± 0.5
	27	57.3 ± 0.6	18	58.0 ± 1.1
27.7	1	45.4 ± 0.0	18	45.3 ± 0.5
	27	58.3 ± 0.6	2	51.0 ± 0.1

The mean mortality of adults and young larvae in DRR and BBU rooms is shown in Tables 2 and 3, respectively. Mortality of adults and young larvae in vials in the DRR sampled at 27.7 h, close to the end of the heat treatment, was 100% and the mean temperature at this time was 57.8°C. In the BBU room, at 27.7 h, 19 out of 20 locations with mean temperature of 46°C achieved 100% mortality while 1 out of 20 locations with mean temperature 44°C achieved 75% mortality for adults and 92% mortality for young larvae. In general, commercial kill of adults and young larvae of red flour beetles were observed during this heat treatment at 20.2 and 27.7 h. In both DRR (Fig. 2) and BBU rooms (Fig. 3), the time required to 50°C was positively related to LT_{99}, whereas the time above 50°C and the maximum temperature were inversely related to LT_{99}.

The mean time to reach 50°C was 1.5 h after the start of the heat treatment in the room being heated in facility B (Fig. 4). During the heat treatment, across all 24 locations, 50.0% of the locations were above 50°C in 1.5 h while 91.7% of locations were above 50°C at 3 and 6 h. There was a sudden drop in the percent locations above 50°C (79.2%) at 24 h (Table 4), because the heat was turned down a few hours before terminating the heat treatment. All eggs in vials were killed 3 h into the heat treatment. Adult mortality increased with time and was 100% at 24 h (Table 5).

Table 2. Mean temperature and mean mortality of young larvae and adults of *T. castaneum* in dry roast room (DRR) in facility A

Time (h)	Mean ± SE		
	Temperature (°C)	Mortality (%) of:	
		Young larvae[a]	Adults[b]
4.8	47.7 ± 0.9	35.6 ± 10.7	15.9 ± 8.2
12.2	55.6 ± 0.7	96.2 ± 4.3	90.4 ± 6.6
20.2	56.8 ± 0.8	99.5 ± 1.6	100.0 ± 0.0
27.7	57.8 ± 0.7	100.0 ± 0.0	100.0 ± 0.0

Each mean is based on $n = 28$ samples or vials.
[a]Mean control mortality at 4.8. 12.2, 20.2, and 27.7 h was 23.4, 35.0, 33.4, and 36.4%, respectively. Mortality of young larvae was corrected for control mortality.
[b]Mean control mortality of adults was 0%.

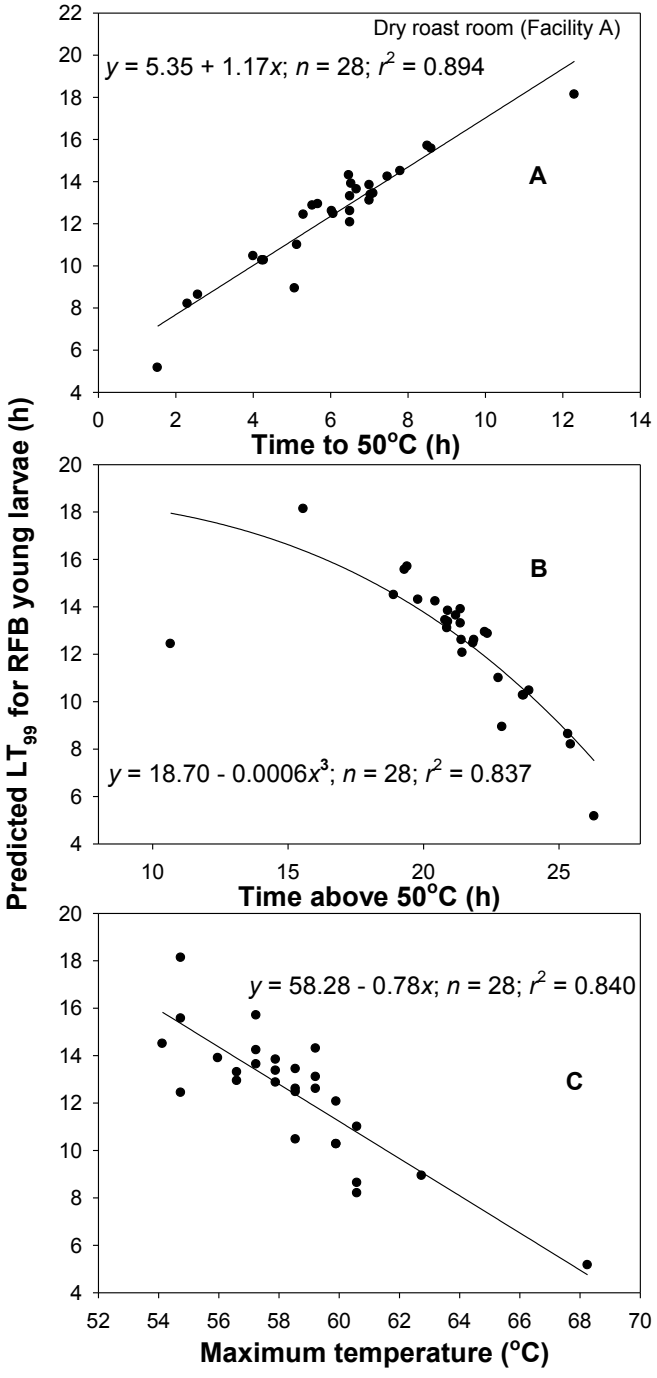

Fig. 2- Relationship between time to 50°C, time above 50°C, and the maximum temperature and time for predicted 99% mortality (LT_{99}) of *T. castaneum* young larvae in the dry roast room (DRR) of facility A.

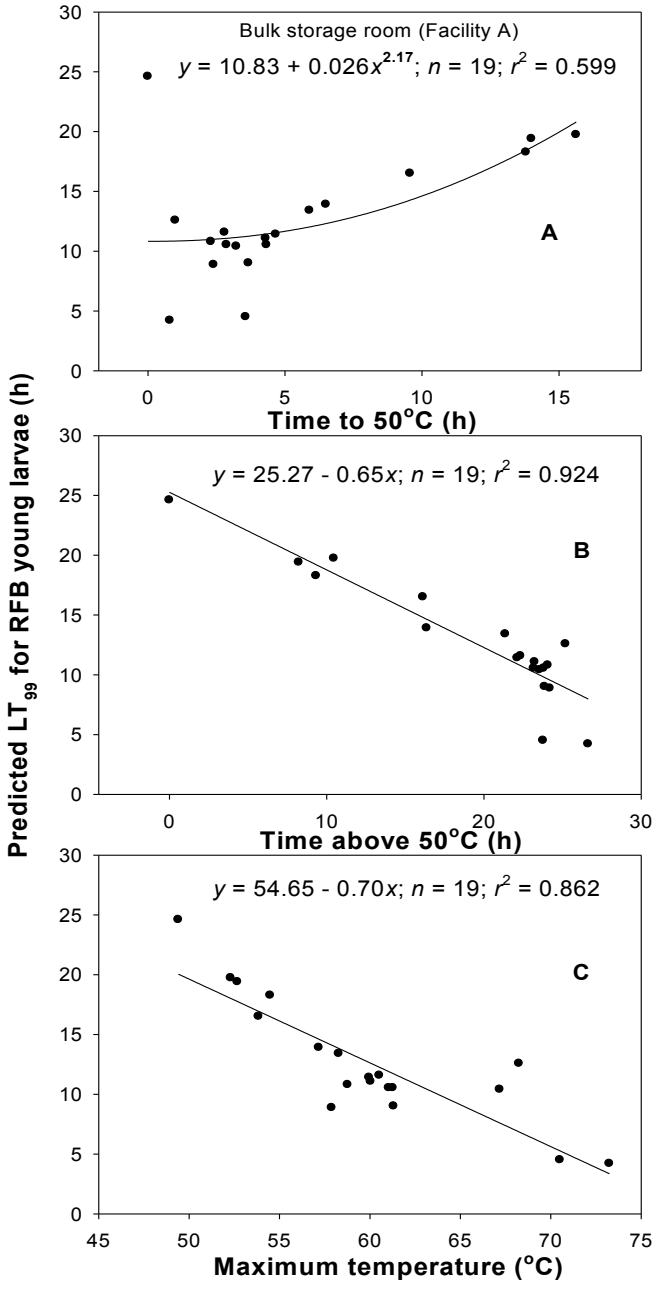

Fig. 3- Relationship between time to 50°C, time above 50°C, and the maximum temperature and time for predicted 99% mortality (LT_{99}) of *T. castaneum* young larvae in the bulk storage room (BBU) of facility A.

Table 3. Mean temperature and mean mortality of young larvae and adults of *T. castaneum* in bulk storage room (BBU) in facility A

Time (h)	Mean ± SE		
	Temperature (°C)	Mortality (%) of:	
		Young larvae[a]	Adults
4.8	49.5 ± 1.9	68.9 ± 10.3	58.8 ± 11.0
12.2	53.2 ± 1.7	84.6 ± 8.1	85.0 ± 8.0
20.2	57.0 ± 1.2	96.3 ± 4.2	95.3 ± 4.8
27.7[b]	45.8 ± 0.6	99.6 ± 1.4	98.8 ± 2.5

Each mean is based on $n = 20$ samples or vials.
[a]Mortality of young larvae was corrected for control mortality (see footnote to Table 2).
[b]In one location, where the temperature was 44°C, the mortality of adults in a vial was 75% whereas that of young larvae was 92%.

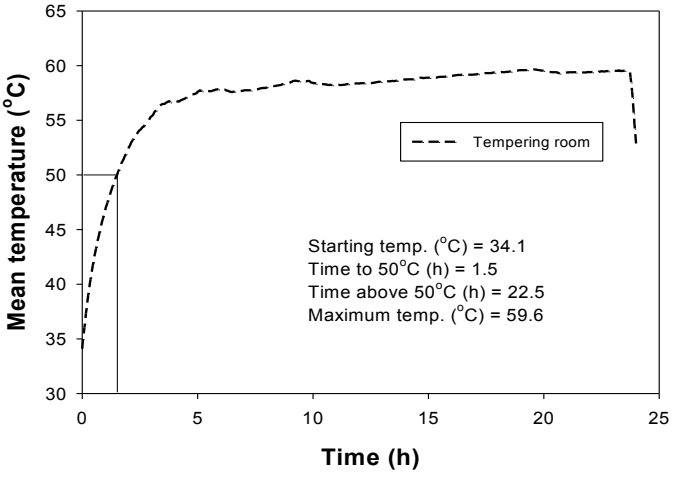

Fig. 4- Mean temperature observed in the heated room of facility B. The solid black line shows time taken to reach 50°C.

Table 4. Number of locations in the heated room with temperatures below and above 50°C at each of the four vial sampling periods in facility B

Time (h)	No. locations	Mean ± SE Temp (°C)
1.5	12	46.5 ± 0.7
	12	53.5 ± 0.7
3.0	2	47.8 ± 0.8
	22	56.0 ± 0.8
6.0	2	47.8 ± 0.8
	22	56.0 ± 0.9
27.7	5	47.7 ± 0.7
	19	54.2 ± 0.6

Our results show that an effective heat treatment to control eggs, young larvae, and adults of *T. castaneum* can be performed in 24 to 28 h. Brijwani et al. (2012) also showed that a successful heat treatment to control eggs, young larvae, old larvae, pupae, and adults of *T. castaneum* can be accomplished in 24 h. Despite careful management of temperature, in two locations in the DRR and four locations in BBU room, the maximum temperature exceeded 60°C. Except for these six locations, temperatures in all other locations in facilities A and B were maintained between 50 and 60°C. Both the facilities were heated at different heating rates and a faster heating rate (facility B) did not cause any adverse effects on the electrical or structural components. The thermal death kinetic model predictions showed that the mortality of *T. castaneum* young larvae is related to how quickly temperatures reach 50°C, and how long temperatures are held above 50°C, and the maximum temperature. In conclusion, heat treatment is an effective and environmentally benign pest management tactic to kill life stages of *T. castaneum* within food-processing facilities.

Table 5. Mean temperature and mean mortality of eggs and adults of *T. castaneum* in the heated room of facility B.

Time (h)	Mean ± SE		
	Temperature (°C)	Mortality (%) of:	
		Eggs[a]	Adults[b]
1.5	50.0 ± 0.9	74.5 ± 10.0	33.3 ± 9.7
3.0	55.4 ± 0.9	100.0 ± 0.0	56.7 ± 9.9
6.0	55.4 ± 0.9	100.0 ± 0.0	78.3 ± 8.0
24.0	52.9 ± 0.7	100.0 ± 0.0	100.0 ± 0.0

Each mean is based on $n = 24$ samples or vials.
[a]Mean control mortality at 1.5, 3.0, 6.0, and 24.0 h was 32.5, 34.2, 30.0, and 33.3%, respectively. Mortality of eggs was corrected for control mortality.
[b]Mean control mortality of adults was 0%.

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