Demonstration of Heat Treatment as a Viable Methyl Bromide Alternative for Disinfesting Grain-Processing Facilities

Final Report

Submitted by

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Introduction

Heat treatment of grain-processing facilities involves raising the ambient air temperatures of a food-processing facility or a storage structure to 50 to 60°C and holding these lethal temperatures for 24 to 36 h to manage stored-product insects. This final report describes the objectives, methods, and results from two heat treatments of commercial grain-processing facilities conducted during September 25-26, 2009 in St. Louis, MO, and September 25-26, 2010 in Columbia, MO.

The main project objectives, as proposed in the funded project, were to evaluate the effectiveness of heat treatments by monitoring temperatures, documenting efficacy against red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), life stages, and determining the degree and duration of insect suppression verified through trapping resident insect populations before and after the heat treatment. The goal was to document with empirical evidence that heat treatments are effective in eliminating insect infestations in food-processing facilities subjected to heat treatments. All progress reports pertinent to this project have been submitted to the EPA Region VII Office.

The grain-processing facility in St. Louis (Facility A) manufactures roasted sunflower seeds. Two rooms of this facility were subjected to heat treatment lasting 27.7 h during 25-26 September 2009. One of the rooms is small and stores sunflower seeds while the other large room is used for roasting the sunflower seeds. Both these rooms were in separate buildings and not within the same structure, and separate heaters and heating ducts were used for each facility. The grain-processing facility in Columbia, MO, (Facility B) manufactures rice cakes, and this facility was heat-treated for 24 h during 25-26 September 2010. In this facility, only one room was subjected to a heat treatment (tempering/processing room).

Heat treatments at both the locations were performed by Temp-Air (Burnsville, MN) using forced-air gas heaters, and propane was used as the fuel. Temperatures were monitored at various locations within each facility using temperature sensors. The effectiveness of the heat treatment was evaluated with insect bioassays placed adjacent to temperature sensors to measure insect mortality. The 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, KS.

The indirect efficacy of structural heat treatments was assessed by monitoring the resident insect populations before and after the heat treatment by monitoring insects in the facility using commercial food-baited traps. The traps, trap monitoring, and results were provided by Drs. James Campbell and Paul Flinn, USDA, Agricultural Research Service, 1515 College Avenue, Center for Grain and Animal Health Research, Manhattan, KS 66502.

Heat Treatment in Facility A

Facility A and heating equipment

The dry roast (DRR) room (45.4 x 37.8 x 5.2 m) and the bulk storage room (BBU) (52.7 x 70.4 x 9.2 m) 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 and 61.0 cm and 50.8 cm diameter ducts were placed in the DRR room. Uniform distribution of the heat was ensured with the help of 10 fans, each with a 91.4 cm fan diameter. Fans were placed in each room in such a fashion to assure uniform circulation of hot air, and

during hourly temperature monitoring by the service provider, using a hand-held infrared thermometer, fan locations were changed to allow cool spots (where temperatures were below 50°C) to reach 50°C and above during the heat treatment period.

Insect bioassays

Young larvae and adults of the red flour beetle, *Tribolium castaneum (*Coleoptera: Tenebrionidae)—an economically-important insect pest associated with food-processing facilities (Hagstrum and Subramanyam 2009), were reared on wheat flour plus 5% (by wt) brewer's yeast at 28°C and 65% relative humidity (RH) in laboratory. To obtain young larvae, 50 unsexed *T. castaneum* adults of mixed ages were introduced into 150-ml plastic containers holding 30 g of flour that was sifted through a sieve with 250-µm openings. These containers were incubated at 28°C and 65% RH, and were sifted after 6 d using a sieve with 250-µm openings to separate adults from young larvae. Plastic vials (2.6 cm inner diameter and 4.9 cm height) were cleaned and 5 g of white (bleached) wheat flour sifted using a 250-µm opening sieve was added to each vial. In each plastic vial, 20 young larvae (first instars—larvae that hatched from eggs) or adults were introduced. These vials were covered with plastic lids covered with wire mesh screens to allow air flow, but prevent insect escape. A total of 28 locations were selected in the DRR room. At each location four vials infested with young larvae and four vials infested with adults were placed. In the BBU room, at each of the 20 locations, four vials with young larvae and four vials with adults were placed. One vial each infested with young larvae or adults was placed outside the DRR and one outside the BBU room to determine insect mortality of these stages that were not exposed to heat (positive controls). Another set of vials (one for each life stage) held in the laboratory growth chamber at 28°C and 65% RH served as the negative controls. There were four sets of controls for the four sampling times. The layout of

DRR and BBU rooms with heating equipment and insect bioassay locations is shown in Figures 1 and 2, respectively.

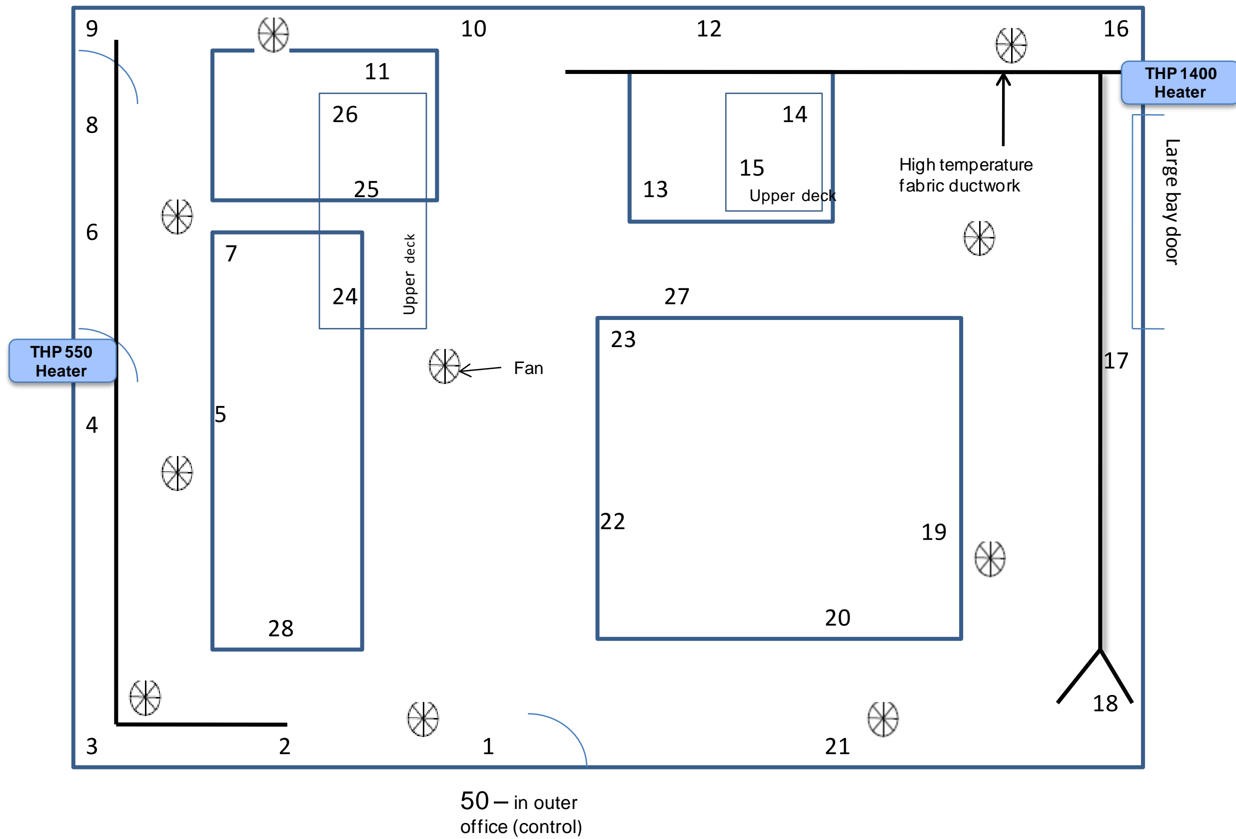


Figure 1. Heater and fan locations and locations of bioassay vials labeled 1 through 28 and 50 in the DRR room of facility A.

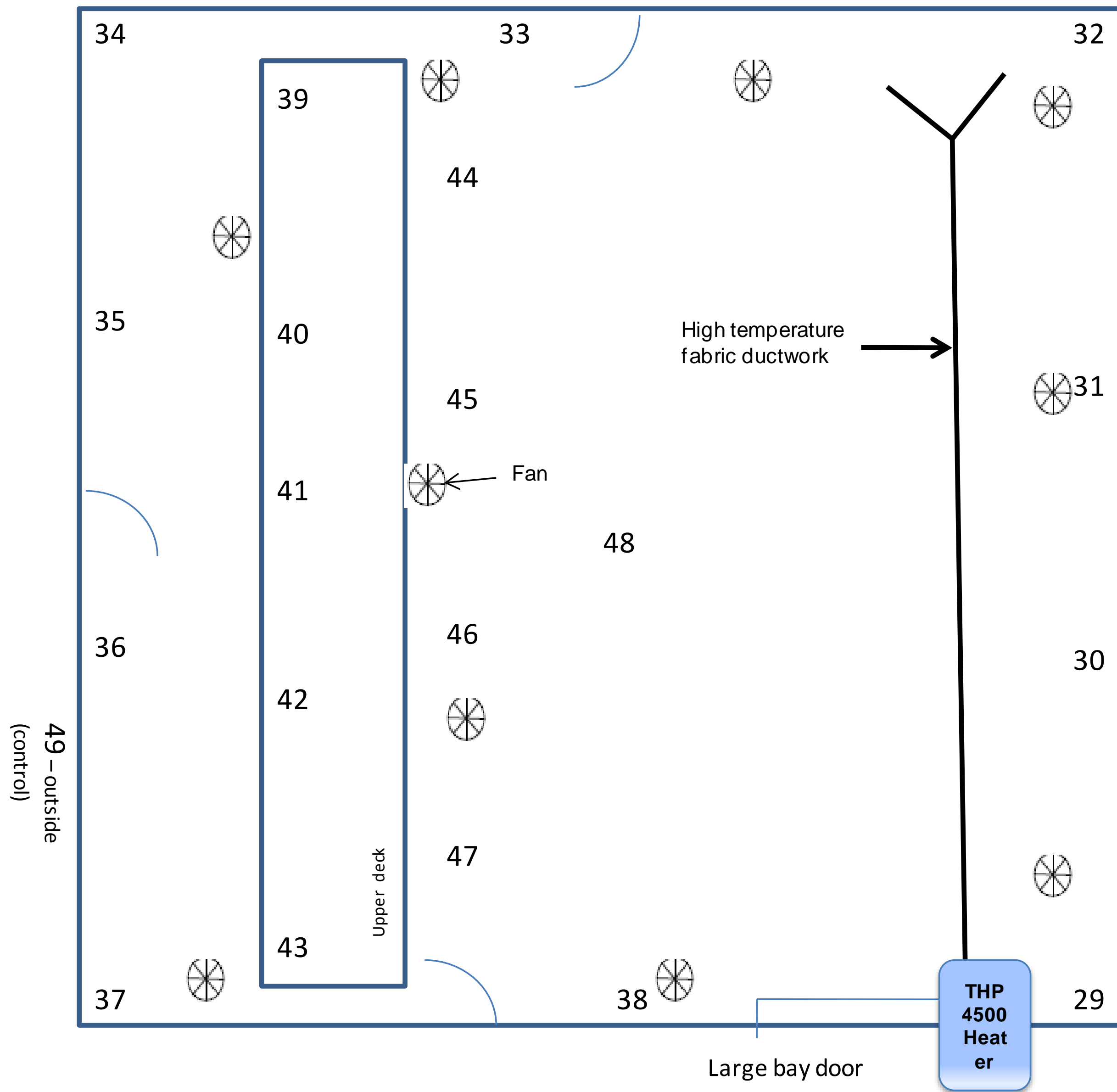


Figure 1. Heater and fan locations and locations of bioassay vials labeled 29 through 49 in the BBU room of facility A. For simplicity, the vials were numbered consecutively.

Vial sampling and mortality assessment

During the heat treatment one vial with young larvae and one with adults was collected at 4.8, 12.2, 20.2, or 27.7 h (end of heat treatment) into the heat treatment from each of the 28 locations in the DRR room and from each of the 20 locations in the BBU room. After the heat treatment, all vials were brought back to the laboratory on September 27, 2009, and adult mortality was determined 24 h after incubation in a growth chamber at 28°C and 65% RH. Adult mortality was based on number of dead adults out of the total exposed (20). After the mortality counts were taken, adults from the vials were discarded and the flour was added back into the vials and held for 45 days to determine adult progeny production (F_1 progeny) from eggs laid by adults before they succumbed to the heat treatment. This assessment indirectly also documents whether adults exposed to heat were reproductively impaired. The vials with young larvae were placed in a growth chamber at 28°C and 65% RH 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 and adult mortality counts were corrected for mortality of larvae in vials not exposed to the heat treatment (negative and positive control vials) using Abbott's (1925) formula.

Temperature monitoring

Temperature data-logging units (HOBO® data loggers, Onset Computer Corporation, Bourne, MA) were launched to record temperature every 2 minutes. A data logger was placed at the floor level, one with each set of vials in the DRR and BBU rooms.

Data Analysis

The mean temperature profiles across all 28 data loggers in the DRR room and 20 loggers in the BBU rooms were plotted as a function of time using the SigmaPlot®11 software. The mean starting temperature (^{o}C), time to 50°C (h), time above 50°C (h), and the maximum

temperature (°C) attained within the DRR room or the BBU room was determined (Mahroof et al. 2003).

The thermal death kinetic (TDK) model, first developed and validated for old larvae of the confused flour beetle (Boina et al. 2008), was developed and validated for the young larvae of the red flour beetle (Subramanyam and Mahroof, unpublished data), to predict insect survival as a function of the time-dependent temperature. These models were developed for the most heat resistant life stages of these insects, because controlling such stages should control all other stages.

For temperature data at each location in the DRR and BBU rooms, the predicted time in hours for 99% mortality (LT_{99}) was obtained based on the TDK model. The temperature data from each data logger was also used to determine the time to 50°C (h), time above 50°C (h), and maximum temperature (°C). The relationship between time to 50°C (h), time above 50°C (h), or maximum temperature (°C) and predicted LT_{99} for 28 locations within the DRR room and 20 locations within BBU room were described by fitting appropriate linear or nonlinear regression models (SAS Institute 2002).

Results and Discussion for Facility A

Temperature profiles

The mean temperature over time for DRR and BBU rooms is shown in the Figures 3 and 4, respectively. The mean temperature in the BBU room was higher than the DRR room during the first 6.7 h, after which the mean temperature was higher in the DRR room than the BBU room. The mean time to 50°C was 5.1 h in the BBU room and 6.3 h in the DRR. During heat treatment in all 48 locations of both rooms, 37.5% of the locations were above 50°C in 4.8 h, 87.5% of locations in 12.2 h and 93.7% locations in 20.2 h. Close to the end of the heat treatment

(27.7 h) there was a temperature drop, and only in 60.4% of the locations temperatures were above 50°C (Table 1), because the amount of heat input was lowered prior to terminating the heat treatment.

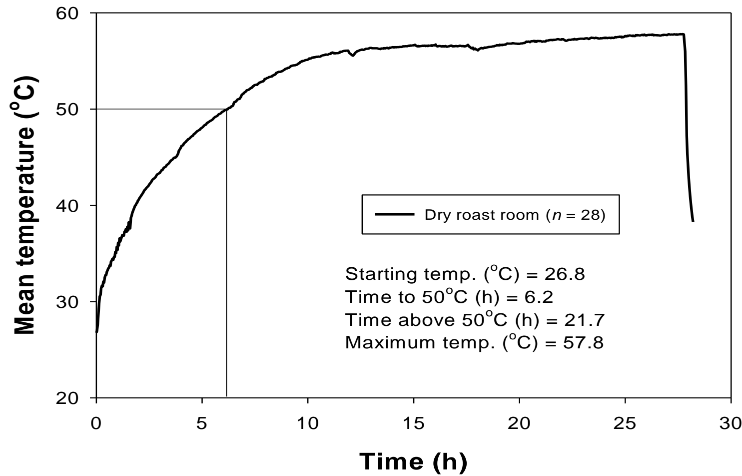


Figure 3. Mean temperature observed in the dry roast room (DRR) in facility A. The black line shows time taken to reach 50°C. Other temperature data are shown in the inset box.

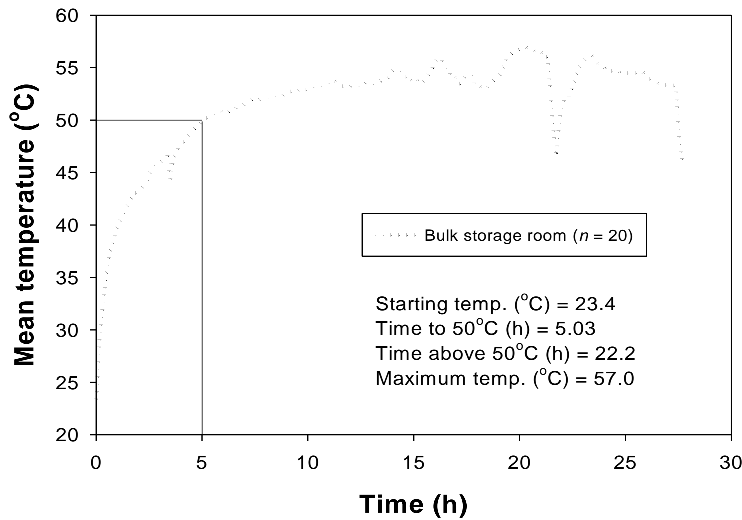


Figure 4. Mean temperature observed in the bulk storage room (BBU) in facility A. The black line shows time taken to reach 50°C. Other temperature data are shown in the inset box.

Table 1. Number of locations with temperatures below and above 50°C at the four pick-up times in two rooms of facility A subjected to heat treatment during September 25-26, 2009.

	Dry roast room (DRR)		Bulk storage room (BBU)	
Time (h)	No. locations	Temp. (°C) (Mean)[a]	No. locations	Temp. (°C) (Mean)[a]
4.8	22	45.9	8	42.0
	6	54.4	12	54.5
12.2	2	48.2	5	44.6
	26	56.2	15	56.1
20.2	1	43.4	2	48.3
	27	57.3	18	58.0
27.7	1	45.4	18	45.3
	27	58.3	2	51.0

[a]The mean temperature data does not have a SE because the treatment was applied only once in each room and therefore, errors for temperature data from multiple locations are not independent.

Insect mortality

Mortality of adults that were not exposed to heat was 0%, whereas mortality of young larvae ranged from 20 to 55% for vials placed outside the heated sites, which was close to what

was observed with vials in the growth chamber (15 to 45%) at ideal conditions (28°C and 65% RH).

The mean mortality of adults and young larvae in DRR and BBU rooms is shown in Tables 2 and 3. Mortality of adults and young larvae in vials in the DRR room sampled at the 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.

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

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

Each mean is based on mortality in *n* = 28 vials, and each vial contained 20 young larvae or 20 adults. Separate vials were used for each life stage.

[a]Mean and standard error for mortality were determined using the binomial distribution.

[b]The mortality of young larvae in control vials at 4.8, 12.2, 20.2, and 27.7 h into the heat treatment were 23.4% (14/60), 35% (21/60), 33.4% (20/60) and 36.4% (22/60), respectively.

[c]Mortality of adults (n = 20/vial) in control vials at each pick-up time was 0%.

Table 3. Mean temperature and mean corrected mortality of young larvae and adults of the red flour beetle in bulk storage room (BBU) in facility A.

Time (h)	Mean temp (°C)	Mean mortality (%)[a] of:	
		Young larvae[b]	Adults[c]
4.8	49.5	68.9 ± 10.3	58.8 ± 11.0
12.2	53.2	84.6 ± 8.1	85.0 ± 8.0
20.2	57.0	96.3 ± 4.2	95.3 ± 4.8
27.7[d]	45.8	99.6 ± 1.4	98.8 ± 2.5

Each mean is based on mortality in n = 28 vials and each vial contained 20 young larvae or 20 adults. Separate vials were used for each life stage.

[a]Mean mortality and standard error using the binomial approximation.

[b]The mortality of young larvae in control vials at 4.8, 12.2, 20.2, and 27.7 h into the heat treatment were 23.4% (14/60), 35% (21/60), 33.4% (20/60) and 36.4% (22/60), respectively.

[c]Mortality of adults (n = 20/vial) in control vials at each pick-up time was 0%.

[d]In one location, where the temperature was 44°C, the mortality of adults in a vial was 75% at this time while the mortality of young larvae was 92%.

Adult progeny

The adults in vials that were sampled at 4.8 h were able to produce greater number of adult progeny compared to those sampled at 12.2 and 20.2 h (Table 4), because a majority of these adults were alive. This is expected because at 4.8 h temperatures did not reach 50°C and the mean adult mortality ranged from 16 to 59%. No adult progeny emerged from vials subjected to 27.7 h of heat treatment. This indicated that the heat treatment was effective in killing either eggs or young larvae that hatched from eggs. A 1.5 to 2.4 times more adult progeny was observed in control vials when compared to the number of F_1 adults produced from vials in the heated rooms. This shows that the heat treatment killed most of the eggs/young larvae that were produced by adults initially used to infest the vials.

Relation between temperature variables and predicted time to 99% mortality (LT_{99})

In the dry roast room, the time required to 50°C was positively related to the predicted time for 99% mortality and this was described by a linear regression model ($y = a + bx$) (r^2 = 0.894) (Figure 5A). The mean ± SE intercept and slope values for this model were 5.35 ± 0.51 and 1.17 ± 0.08, respectively. The time above 50°C was inversely related to the predicted time for 99% mortality and this was described by a three parameter power equation ($y = a + bx^c$) (r^2 = 0.837) (Figure 5B). The mean ± SE for parameters *a*, *b*, and *c* were 18.70 ± 2.07, -0.0006 ± 0.002, and 3.00 ± 1.09, respectively. The maximum temperature (°C) was also inversely related to the predicted time for 99% mortality and this was described by a linear regression model ($y = a – bx$) (r^2 = 0.84) (Figure 5C). The mean ± SE intercept and slope values for this model were 58.28 ± 5.76 and -0.78 ± 0.10, respectively.

Table 4. Mean adult progeny[a] production in vials from DRR and BBU rooms subjected to heat treatments and in control vials.

Time (h)	Heat treated		Unheated (control) (no. vials)
	DRR (no. vials)	BBU (no. vials)	
4.8	13.3 (26)	12.4 (8)	14.5 (2)
12.2	1.0 (2)	3.6 (4)	12.3 (3)
20.2	0.0 (28)	3.0 (1)	11.6 (3)
27.7	0.0 (28)	0.0 (20)	21.6 (3)

[a]Adults after heat treatment from infested vials were removed and the vials were incubated at 28°C and 65% RH for 45 days to determine adult progeny (F_1) production. Progeny is based on the number of live and dead adults that emerged after 45 days.

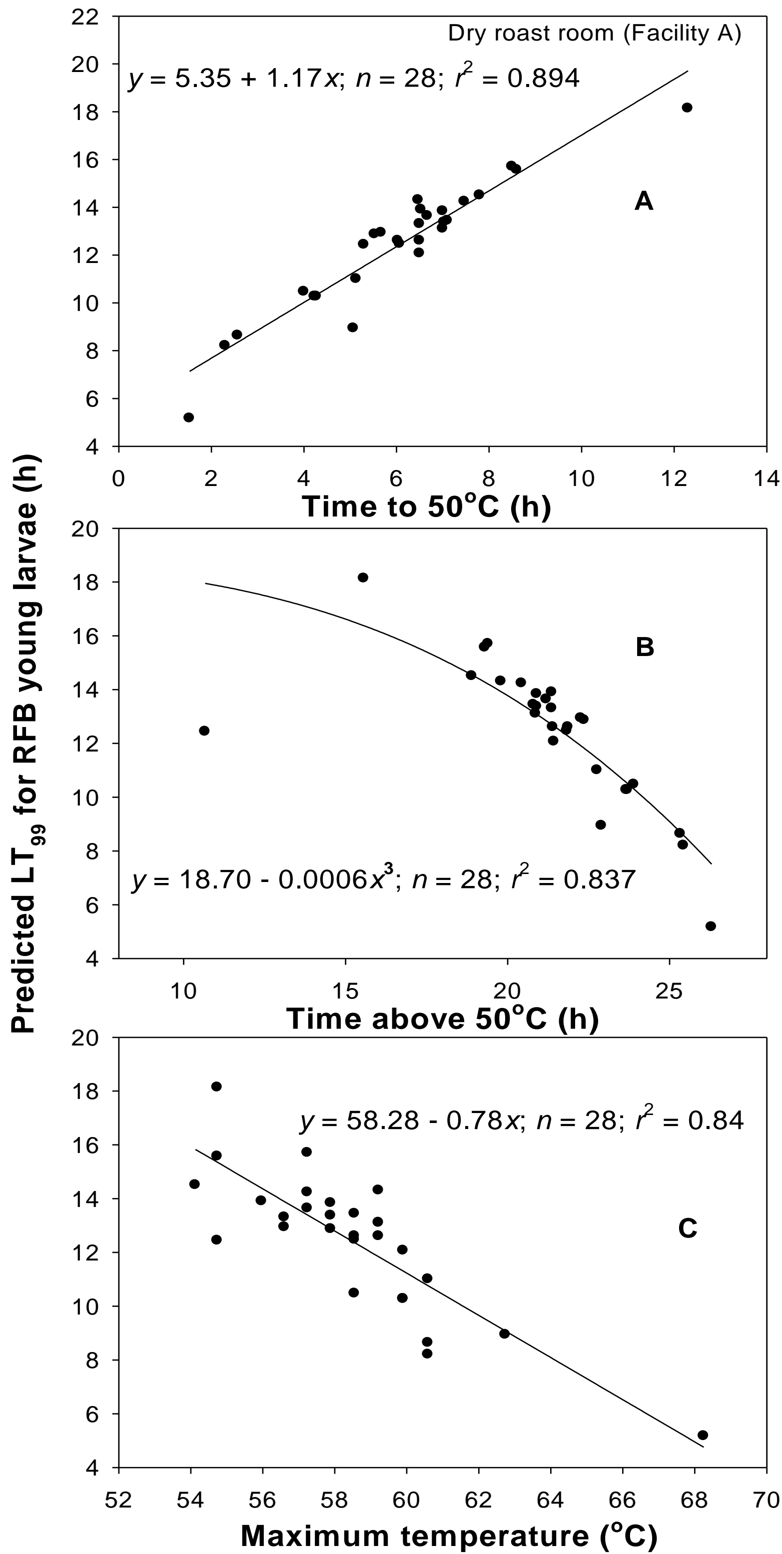


Figure 5. Regression models for temperature variables and predicted time to 99% mortality of red flour beetle in dry roast room in facility A.

In the bulk storage room, the time required to 50°C was positively related to the predicted time for 99% mortality and this was described by a three parameter power model ($y = a + bx^c$) (r^2 = 0.599) (Figure 6A). The mean ± SE for parameters a, b, and c were 10.83 ± 1.75, 0.026 ± 0.15 and 2.17 ± 2.24, respectively. The time above 50°C was inversely related to the predicted time for 99% mortality and this was described by a linear regression model ($y = a + bx$) (r^2 = 0.924) (Figure 6B). The mean ± SE intercept and slope values for this model were 25.27 ± 1.37 and -0.65 ± 0.07, respectively. The maximum temperature (^{o}C) was inversely related to the predicted time for 99% mortality and this was described by a linear regression model ($y = a – bx$) (r^2 = 0.862) (Figure 6C). The mean ± SE intercept and slope values for this model were 54.65 ± 6.01 and -0.70 ± 0.09, respectively.

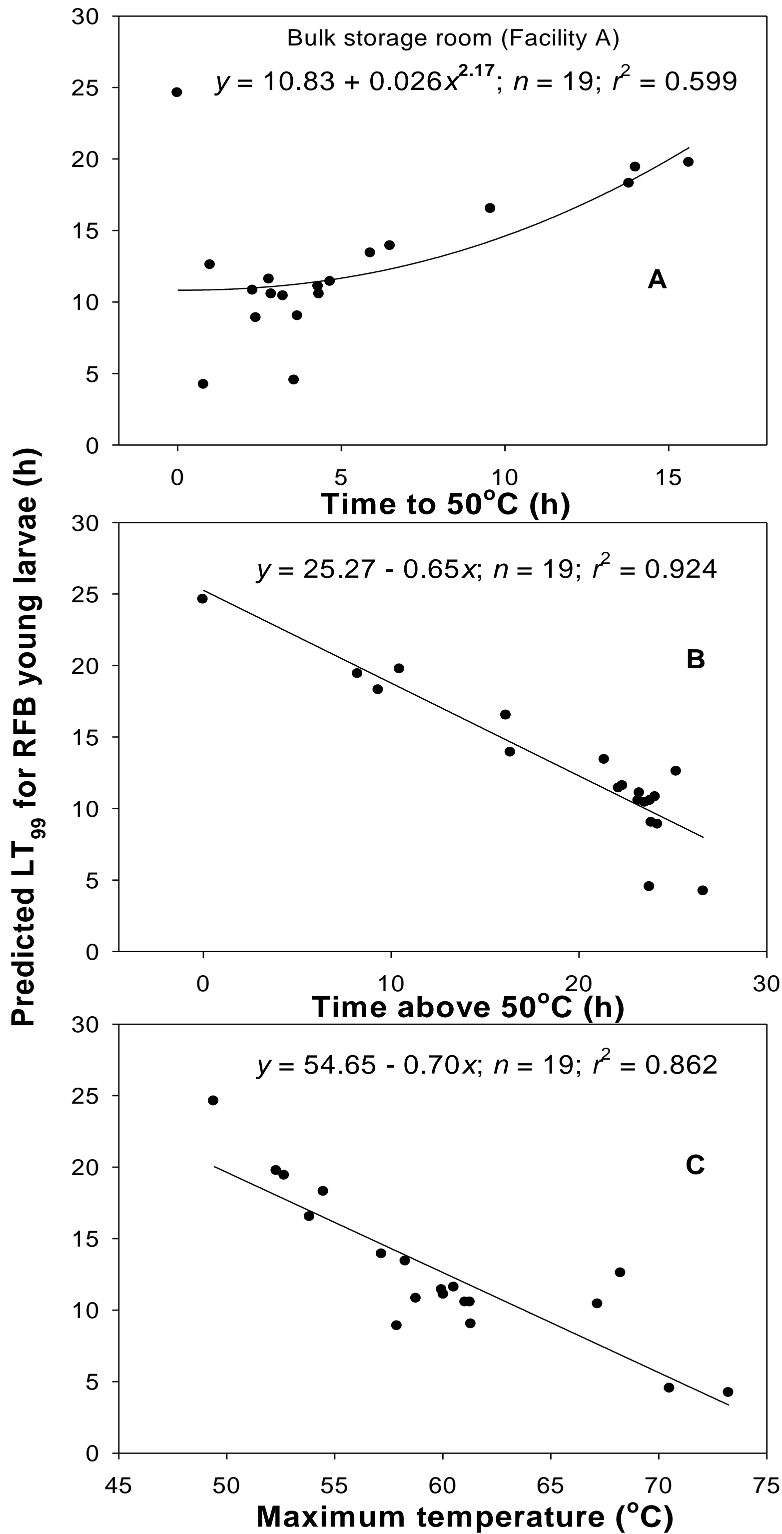


Figure 6. Regression models for temperature variables and predicted time to 99% mortality of red flour beetle in bulk storage room in facility A.

Insect Monitoring Data in Facility A (*Provided by Drs. James Campbell/Paul Flinn*)

Trap locations and set-up

Twenty six Pherocon II sticky traps and 24 Dome traps (Trece, Adair OK) were placed at various locations (Figure 7). Traps were placed in paired locations in the bulk storage room, warehouse, dry roast room, and outside facility. Only bulk storage room and dry roast rooms were the areas that were subjected to the heat treatment. Pherocon II traps were baited with pheromone lures for Indianmeal moth and warehouse beetle and the Dome traps were baited with pheromone lure for red flour beetle, but since food oil bait was used, it will also capture species for which no pheromone lure was used.

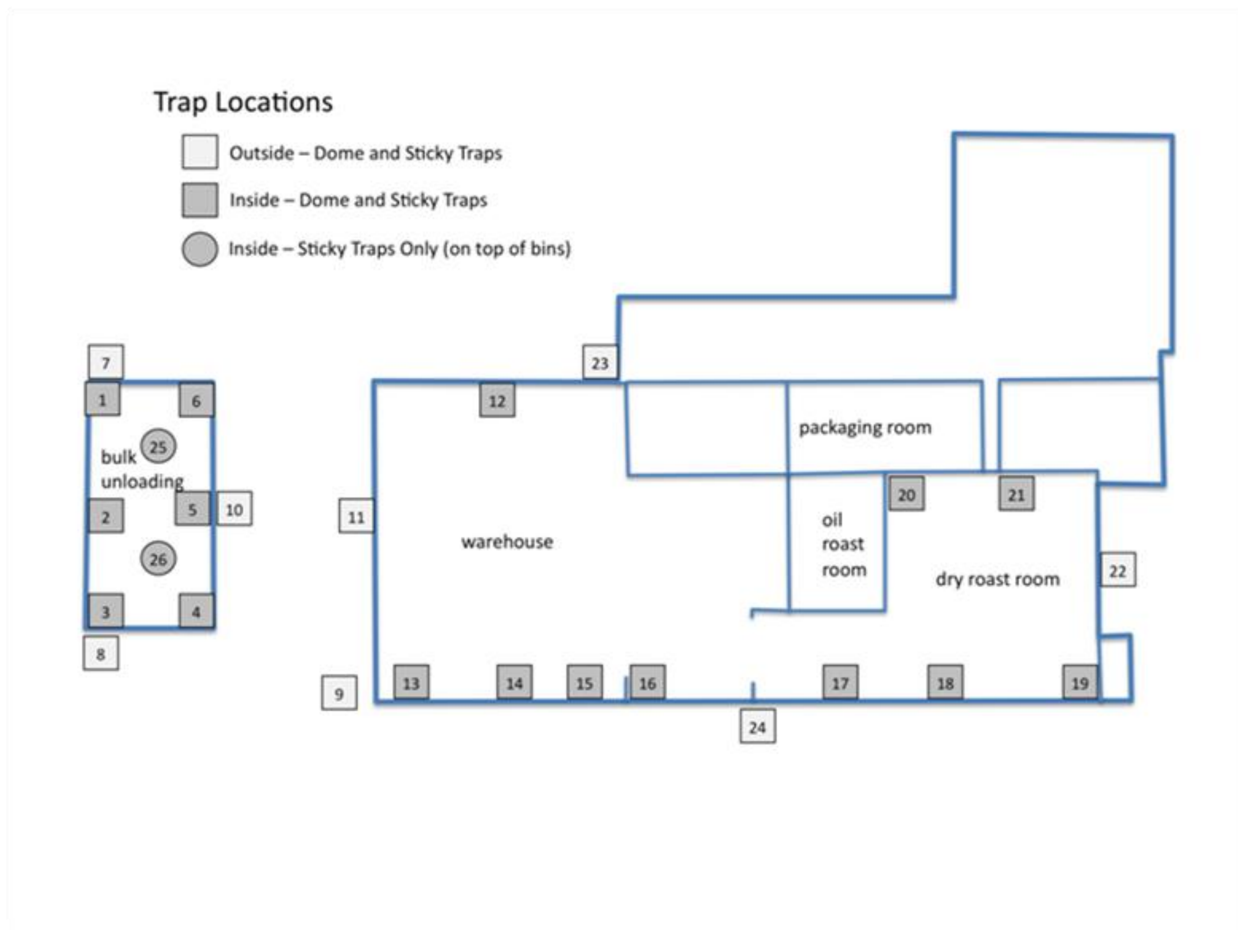


Figure 7. Locations of traps at facility A.

Insect capture results

Prior to the heat treatment in the bulk storage room (BBU), Indianmeal moth was the primary species captured, with some warehouse beetles captured as well (Figure 8). Indianmeal moth was the primary species captured in the dry roast room (DRR) as well, though the numbers were lower than BBU and few red flour beetles were also captured. In the warehouse few insects were captured. Outside the facility, warehouse beetle was found in abundance and few Indianmeal moth.

Prior to the heat treatments the captures of Indianmeal moth were declining due to seasonal patterns, so when heat treatments were performed there was already low insect activity. The Indianmeal moth activity was found during the monitoring period immediately after the heat treatment, however, in the following two periods there were no captures. Because of the seasonal patterns in insect activity and temperature it is difficult to draw firm conclusions about the impact of the heat treatment on the resident insect populations, but results from bioassays provide direct assessment of effectiveness of the heat treatment. Heat treatment, like fumigants, does not have residual effectiveness. However, depending on the source of insect populations and delays in population rebounds, a period free of insect captures or low captures following a heat treatment was expected. However, the fact that companies keep their doors open allows insects from outside to enter facilities. Additionally, the onset of cooler temperatures in October and November may have resulted in low insect captures observed after the heat treatment.

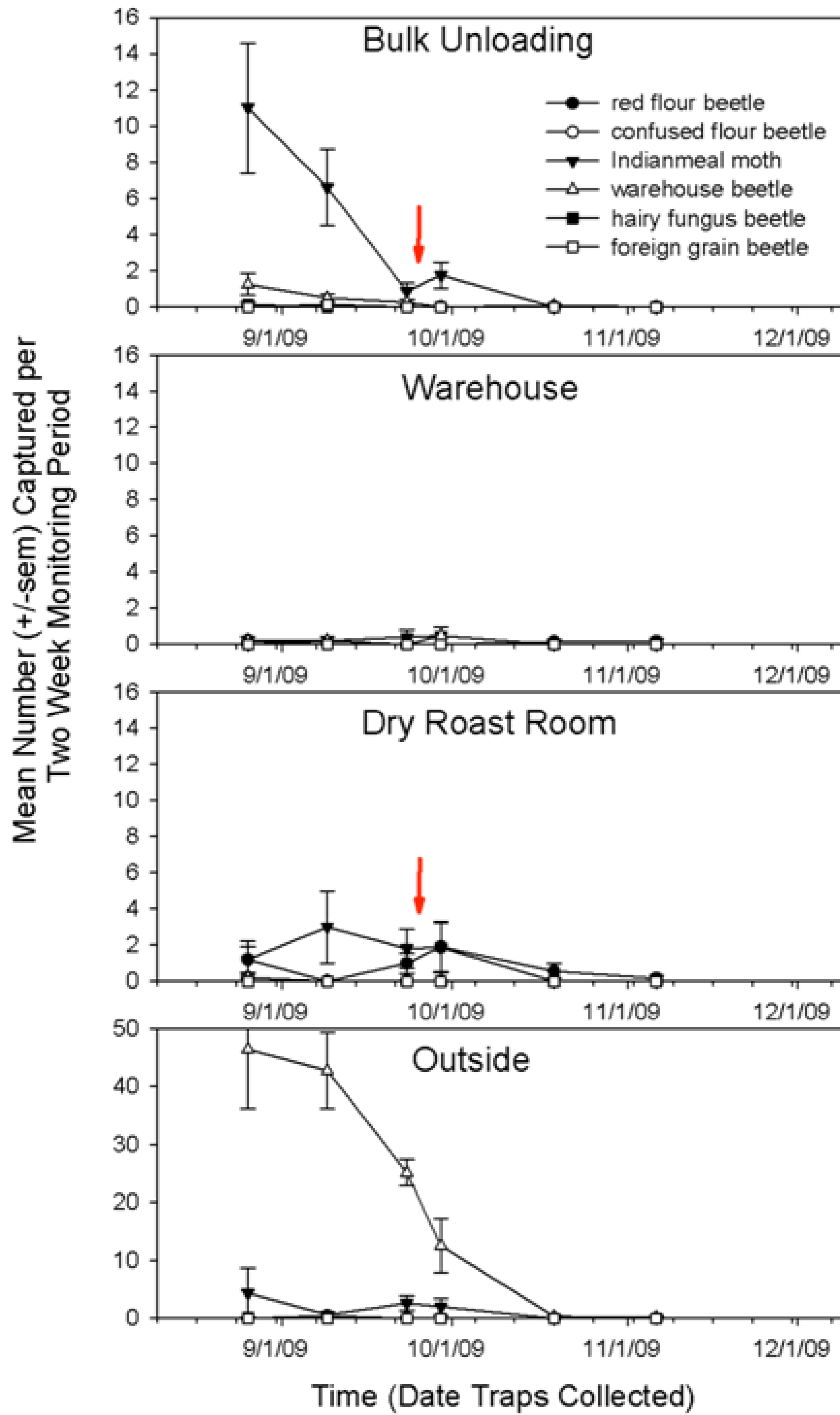


Figure 8. Pheromone trap captures of primary insect species in different zones of the facility A, with red arrow indicating date of heat treatment.

Heat Treatment in Facility B

Facility B and heating equipment

The tempering room of dimensions 30.5 x 12.2 x 12.2 m was subjected to the heat treatment. 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 tempering room with 91.4 cm diameter fabric ductwork. Uniform distribution of the heat was ensured with the help of 12 fans, each with a 91.4 cm fan blade diameter, placed in the room to distribute the hot air. Fans were placed in each room in such a fashion to assure uniform circulation of hot air, and during hourly temperature monitoring by the service provider, using a hand-held infrared thermometer, fan locations were changed to allow cool spots (where temperatures were below 50°C) to reach 50°C and above during the heat treatment period.

Insect bioassays

Eggs and adults of the red flour beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), were reared on wheat flour plus 5% (by wt) Brewer's yeast at 28°C and 65% relative humidity (RH) in laboratory. To obtain eggs, 50 unsexed *T. castaneum* adults of mixed ages were introduced into 150 ml plastic containers holding 30 g of flour that was sifted through a 250-µm opening sieve. These containers were incubated at 28°C and 65% RH and were sifted after 2 d to separate adults from eggs using a 250-µm sieve. Plastic vials (2.6 cm inner diameter and 4.9 cm height) were filled with 5 g of bleached wheat flour sifted previously using a 250-µm opening sieve. In each vial, 20 eggs or adults were introduced. These vials were covered with plastic lids that had a mesh to allow air flow but prevent insect escape. Four vials infested with eggs and four vials infested with adults were placed in each of 24 locations throughout the tempering room. Two vials each infested with adults and eggs were placed outside the tempering

room to determine insect mortality of these stages that are not exposed to heat, and these served as the positive controls. Another set of vials (four for each life stage) remained in the laboratory growth chamber set at 28°C and 65% RH served as the negative controls. There were four sets of controls for the four sampling times. The layout of tempering room with heating equipment and insect bioassay locations is shown in Figure 9.

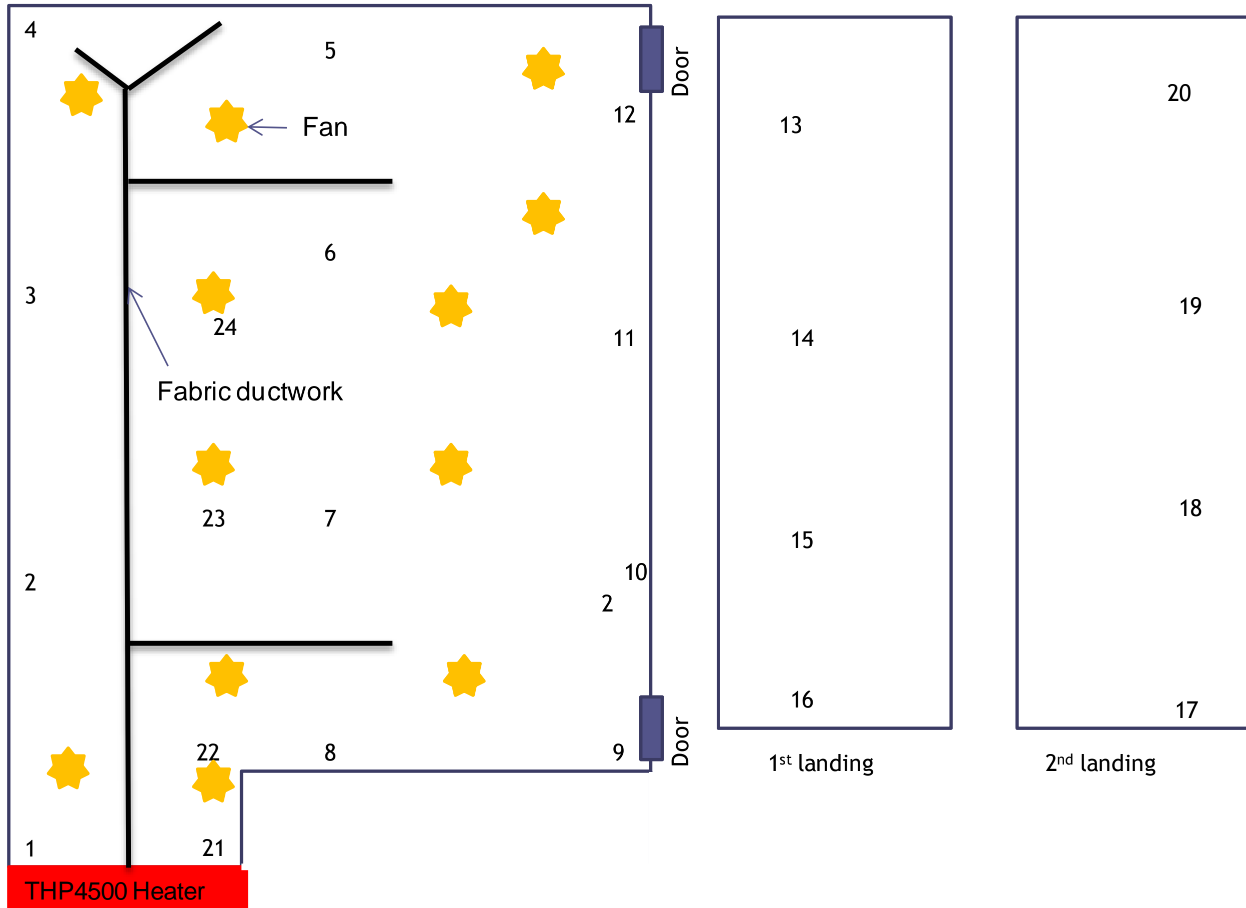


Figure 9. Heater and fan locations and locations of bioassay vials labeled 1 through 24 in the tempering room in facility B.

Vial sampling and mortality assessment

During the heat treatment one vial with eggs and one with adults was collected at 1.5, 3, 5 and 24 h (end of heat treatment) from each of the 24 locations in the tempering room. After the heat treatment, all vials were brought back to the laboratory on September 26, 2010, and mortality of adults determined 24 h after incubation at 28°C and 65% RH. Adult mortality was based on number of dead adults out of the total exposed (20). Vials with eggs were placed in a growth chamber at 28°C and 65% RH 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. Egg-to-adult

mortality was corrected for mortality of eggs in vials not exposed to the heat treatment (negative and positive control vials).

Temperature monitoring

The temperature sensors (SmartButton sensors, ACR Systems, Inc., Surrey, Canada) were launched to record temperature every 2 minutes. These sensors were placed in an additional vial containing 5 g of flour and without any insects. This additional vial containing the temperature sensor was placed at all 24 locations along with the set of vials of eggs and adults to record temperatures at these locations.

Data Analysis

The mean temperature profile across all 24 data loggers within the tempering room was plotted as a function of time using the SigmaPlot®11 software. The mean starting temperature (^{o}C), time to 50°C (h), time above 50°C (h), and the maximum temperature (^{o}C) attained within this room was determined.

The thermal death kinetic (TDK) model, first developed and validated for old larvae of the confused flour beetle (Boina et al. 2008), was developed and validated for the young larvae of the red flour beetle (Subramanyam and Mahroof, unpublished data), to predict insect survival as a function of the time-dependent temperature. These models were developed for the most heat resistant life stages of these insects, because controlling such stages should control all other stages.

For temperature data at each location in the tempering room, the predicted time in hours for 99% mortality (LT_{99}) was obtained based on the TDK model. The temperature data from each data logger was also used to determine the time to 50°C (h), time above 50°C (h), and maximum temperature (^{o}C). The relationship between time to 50°C (h), time above 50°C (h), or

maximum temperature (^{o}C) and predicted LT_{99} for 24 locations within the tempering room were best described by fitting linear or nonlinear regression models (SAS Institute 2002).

Heat treatment Results and Discussion for Facility B

Temperature profiles

The mean temperature over time in the tempering room is shown in Figure 10. The mean time to $50^{o}C$ was within 1.5 h after the start of the heat treatment in the tempering room. This facility heated quickly. Generally, the optimum time to attain lethal temperatures of 50 to $60^{o}C$ within a facility usually should range from 6 to 8 h. During the heat treatment, across all 24 locations, 50.0% of the locations were above $50^{o}C$ in 1.5 h and 91.7% of locations were above $50^{o}C$ in 3 to 6 h. Close to the end of the heat treatment (24 h) there was a temperature drop, and only in 79.2% of the locations temperatures were above $50^{o}C$ (Table 5) because the amount of heat input was lowered prior to terminating the heat treatment.

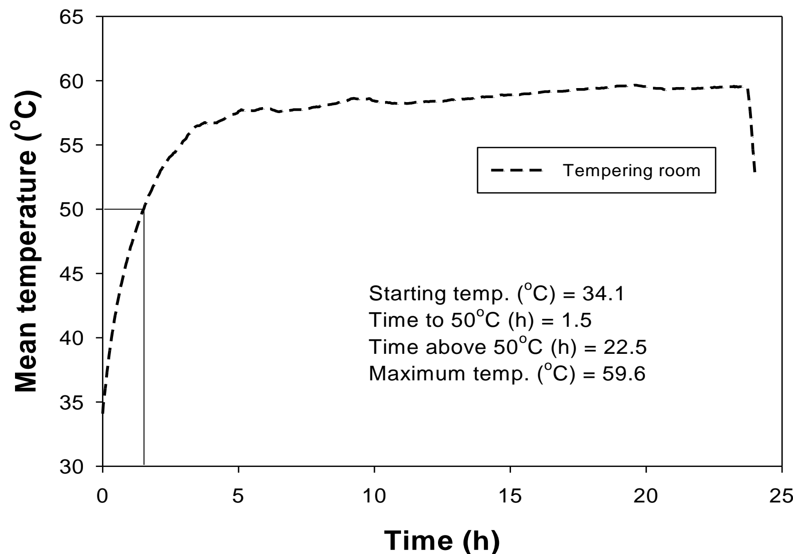


Figure 10. Mean temperature observed in the tempering room in facility B. The black line shows time taken to reach $50^{o}C$. Other temperature data are shown in the inset box.

Table 5. Number of locations with temperatures below and above 50°C at the four pick-up times in tempering room in facility B subjected to heat treatment during September 25-26, 2010.

Time (h)	No. locations	Temp. (^{o}C) (Mean)[a]	No. locations	Temp. (^{o}C) (Mean)[a]
1.5	12	46.5	12	53.5
3.0	2	47.8	22	56.0
6.0	2	47.8	22	56.0
24.0	5	47.7	19	54.2

Insect mortality

Mortality of adults that were not exposed to heat was 0%, whereas mortality for eggs (based on number of adults that emerged out of the total exposed) ranged from 15 to 55% for vials placed outside the heated site which was in the range observed for vials in the growth chamber (10 to 50%) placed at 28°C and 65% RH.

The mean mortality of adults and egg-to-adult mortality is shown in Table 6. One hundred percent of egg mortality at all locations was observed after 3 and 6 h into the heat treatment where mean temperatures attained were 50.0 and 55.4^{o}C, respectively. However, adults had a lower mortality compared to eggs for all the pick-up times except for the last pick-up time at 24 h, where it was 100% for both life stages. After 1.5 h of the treatment, 7 out of 24 locations (29.1%) and 12 out of 24 locations (50.0%) had 100% adult mortality and 100% egg-to-adult mortality and the mean temperatures were 54.5 and 53.0^{o}C, respectively. In general, commercial

kill of eggs and adults of red flour beetles were observed during this heat treatment at 3.0 and 24.0 h, respectively.

Table 6. Mean temperature and mean corrected egg-to-adult and adult mortality of the red flour beetle in tempering room in facility B.

Time (h)	Mean temp (°C)	Mean mortality (%)[a] of:	
		Egg-to-adult[b]	Adult[c]
1.5	50.0	74.5 ± 10.0	33.3 ± 9.7
3.0	55.4	100.0 ± 0.0	56.7 ± 9.9
6.0	55.4	100.0 ± 0.0	78.3 ± 8.0
24.0	52.9	100.0 ± 0.0	100.0 ± 0.0

Each mean is based on mortality in n = 20 vials, and each vial contained 20 eggs or adults. Separate vials were used for each life stage.

[a]Mean mortality and standard error were determined using the binomial distribution.

[b]The mortality of egg-to-adult in control vials at 1.5, 3.0, 6.0 and 24.0 h into the heat treatment were 32.5% (39/120), 34.2% (41/120), 30.0% (36/120) and 33.3% (40/120), respectively.

[c]Mortality of adults (n = 20/vial) in control vials at each pick-up time was 0%.

Relation between temperature variables and predicted time to 99% mortality (LT_{99})

In the tempering room, the time required to reach 50°C was positively related to the predicted time for 99% mortality and this was described by a two parameter power model ($y = a*x^b$) ($r^2 = 0.987$) (Figure 11A). The mean ± SE for parameters *a* and *b* values for this model were 6.88 ± 0.15 and 0.37 ± 0.02, respectively. The time above 50°C was inversely related to the predicted time for 99% mortality and this was described by a two parameter power equation ($y = a + bx^c$) ($r^2 = 0.973$) (Figure 11B). The mean ± SE for parameters, *a*, *b*, and *c* were 19.93 ± 0.54, and 0.001 ± 0.00005, respectively. The maximum temperature (°C) was also inversely related to the predicted time for 99% mortality and this was described by an exponential decay model ($y = a*e^{-bx}$) ($r^2 = 0.972$) (Figure 11C). The mean ± SE for parameters *a* and *b* were 122122.79 ± 5046.83 and -0.12 ± 0.007, respectively.

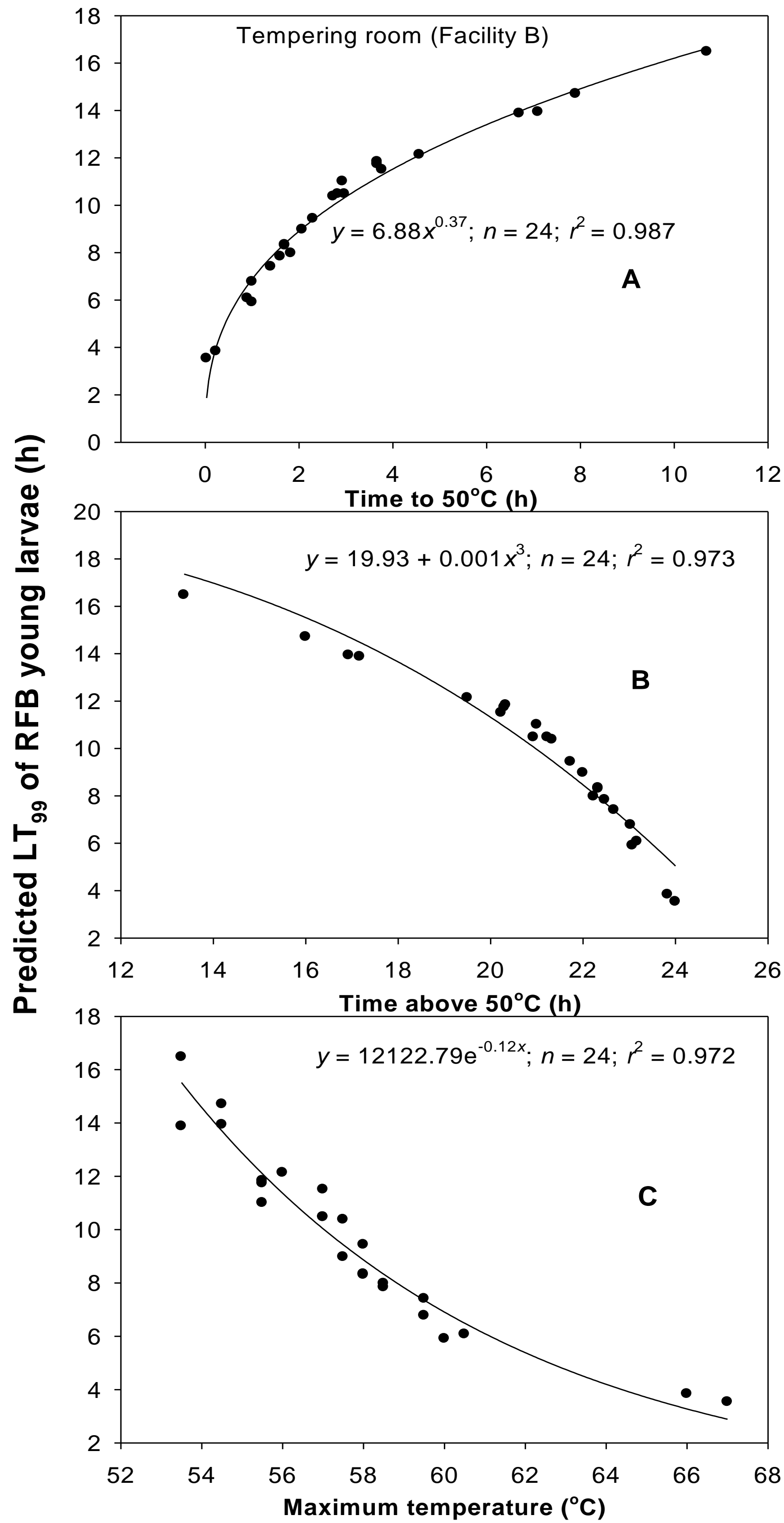


Figure 11. Regression models for temperature variables and predicted time to 99% mortality of red flour beetle in tempering room in facility B.

Insect Monitoring Data in Facility B (*Provided by Drs. James Campbell/Paul Flinn*)

Trap locations and set-up

Twenty Pherocon II traps (Trece, Adair OK) were placed at the location (Figure 12), primarily in the tempering room, which was the location that was scheduled for heat treatment, but also in the adjacent warehouse and processing area and outside the facility. Outside trap locations were initially near the building but were moved further away after the first round of monitoring due to concerns raised by the collaborators at the facility. Cigarette beetle was the primary pest reported prior to start of the study, so traps were baited with pheromone lures for cigarette beetle, as well as lures for commonly occurring species the Indianmeal moth and warehouse beetle.

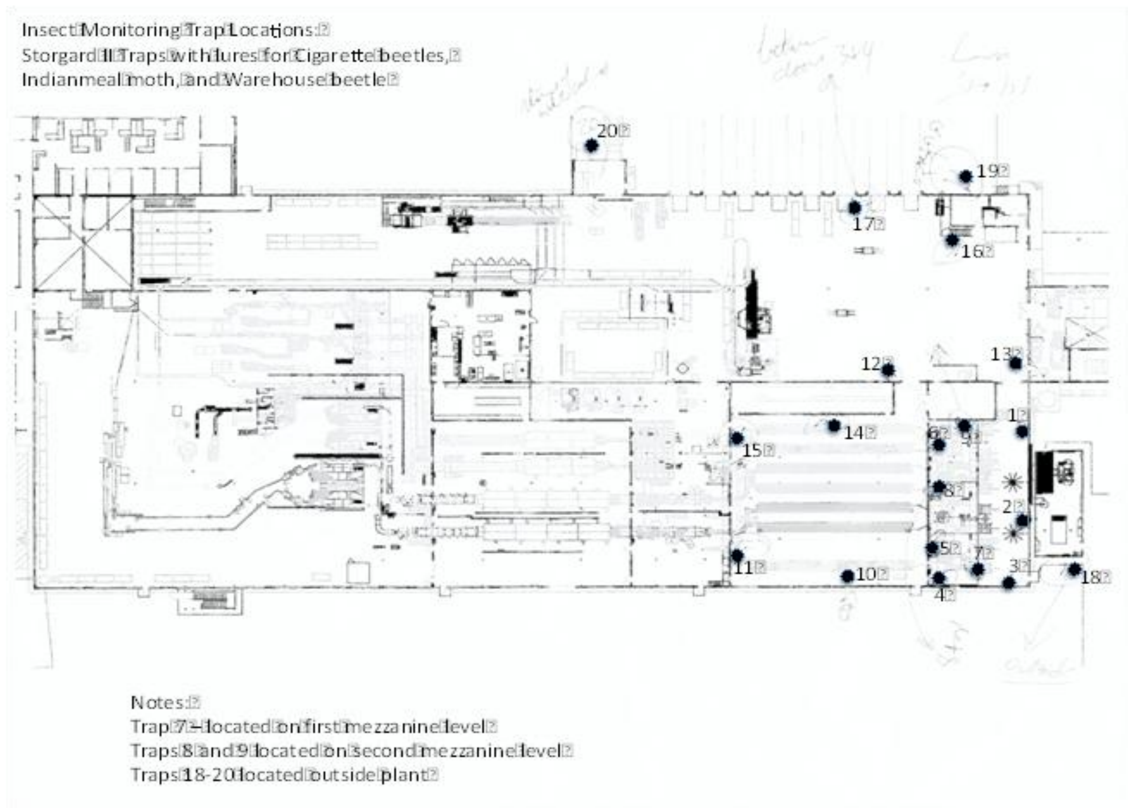
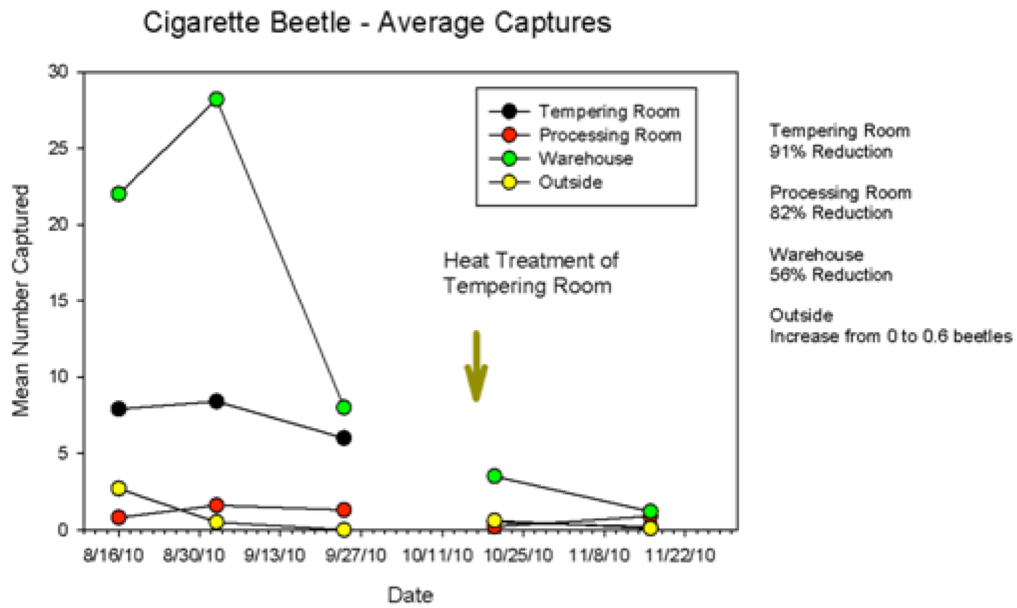


Figure 12. Locations of traps at facility B.

Insect capture results

In the monitoring periods prior to the heat treatment, cigarette beetle captures were primarily in the warehouse and the tempering room (Figure 13). The traps in the warehouse with high captures of cigarette beetle tended to around the adjacent walls of the tempering room and the warehouse. Captures of cigarette beetle outside and in the processing area were very low. This pattern of capture is consistent with tempering room being the source of cigarette beetles. Warehouse beetle was also captured at the facility, but primarily outside and some inside the warehouse building – trap located near outside trash bins had the highest captures. Pattern for warehouse beetles was that it was primarily of outside origin with occasionally some immigration into the warehouse. Some Indianmeal moths were captured outside, but none inside the facility.

Following the heat treatment, there was a 91% reduction in cigarette beetle captures in the tempering room immediately following the heat treatment. There were also corresponding decreases in the processing room and warehouse as well which were not treated which is consistent with the tempering room serving as the source for the populations.



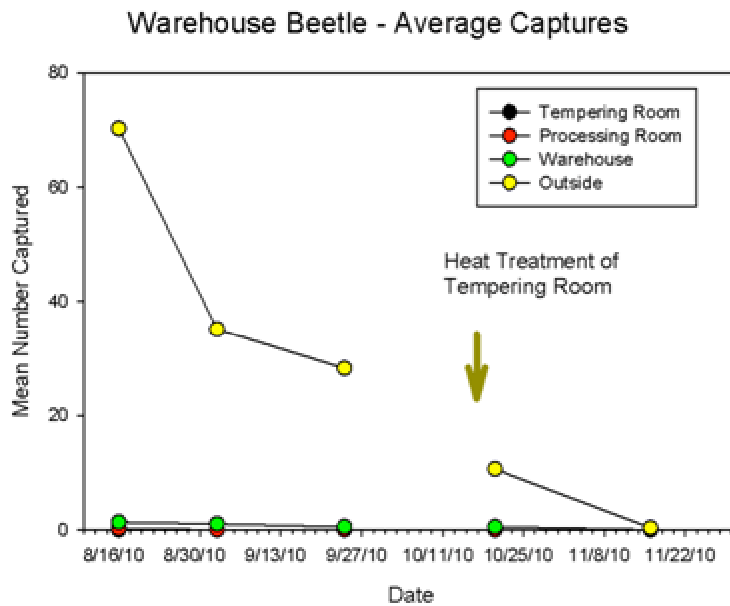


Figure 13. Trends in captures of cigarette beetle and warehouse beetle in pheromone traps placed in facility B.

Summary

Heat treatment is an effective environmentally benign pest management tactic to kill life stages of the red flour beetle within food-processing facilities. The results show that attaining lethal temperature of 50°C and above, and holding these temperatures for a period of 24 to 27 h can control red flour beetle eggs, young larvae, and adults. Young larvae of the red flour beetle are the most heat tolerant stage among all of the insect species and stages tested at high temperatures. Therefore, the predicted survival of this stage, based on the TDK model was used to generate relationships between time to 50°C, time above 50°C, and maximum temperature and time to 99% mortality. The relations show that the time to 50°C and the maximum temperature are positively related to time to 99% mortality and negatively related to time to 99% mortality. The inverse relationship between time above 50°C and time to 99% mortality is expected because time to 50°C was positively related to time to 99% mortality. The data presented here show that successful commercial heat treatment can be conducted within 24 h if the lethal temperatures are achieved. Both the facilities were heated for different durations. Facility B attained lethal temperatures more quickly than facility A and this has to do with the size and pieces of equipment within the heated rooms. No adverse effects on the electrical or structural components were reported. Trap captures showed a suppression of insects in facility B immediately after the heat treatment. The field trials indicated heat to be a viable alternative to methyl bromide for managing stored-product insects.

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Summarized by Dr. Bhadriraju Subramanyam