Susceptibility of *Tribolium castaneum* Life Stages Exposed to Elevated Temperatures during Heat Treatments of a Pilot Flour Mill: Influence of Sanitation, Temperatures Attained Among Mills Floors, and Costs

MONIKA BRIJWANI,[1] BHADRIRAJU SUBRAMANYAM,[1,2] PAUL W. FLINN,[3] MICHAEL R. LANGEMEIER,[4] MICHELLE HARTZER,[1] AND RAJSHEKHAR HULASARE[5]

J. Econ. Entomol. 105(2): 709–717 (2012); DOI: http://dx.doi.org/10.1603/EC11114

ABSTRACT The influence of sanitation on responses of life stages of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), was investigated in a pilot flour mill subjected to three, 24-h heat treatments by using forced-air gas heaters fueled by propane. Two sanitation levels, dusting of wheat flour and 2-cm-deep flour, were created in 25 plastic bioassay boxes, each holding eggs, young larvae, old larvae, pupae, and adults of *T. castaneum* plus two temperature sensors. Data loggers (48) were placed on the five mill floors to record air temperatures. The time required to reach 50°C, time above 50°C, and the maximum temperature among mill floors and in bioassay boxes were measured. The maximum temperature in bioassay boxes and in the mill was lower on the first floor than on other floors. This trend was apparent in time required to reach 50°C and time above 50°C, especially in compartments with 2-cm-deep flour. The mean ± SE mortality of *T. castaneum* life stages on the first floor was 55.5 ± 12.9–98.6 ± 0.8%; it was 93.2 ± 6.7–100 ± 0.0% on other floors. Adults were the least susceptible stage. Mortality of *T. castaneum* stages in compartments with 2-cm-deep flour was generally lower than those with flour dust. Costs for the three heat treatments ranged from US$27,438 to $28,838. An effective heat treatment can be conducted within 24 h, provided temperatures on mill floors reach 50°C in 8–12 h and are held above 50°C for at least 10–14 h, with maximum temperatures held between 50 and 60°C.

KEY WORDS heat treatment, temperature, sanitation, insect mortality, economics

The use of high temperatures or heat treatments for disinfesting food-processing facilities such as flour mills is not a new concept. In the United States, the practical use of high temperatures, generated from steam, to control flour-mill insects was demonstrated by Dean (1911) and Goodwin (1912). Dean (1913) mentioned that heat treatments were embraced by several mills in Ohio, Illinois, Nebraska, Iowa, Indiana, and southern Canada as an effective alternative to fumigation with hydrogen cyanide, but he did not provide details of temperatures attained, heat treatment duration, and efficacy against insects. In this anecdotal report (Dean 1913), it was assumed that mill insects were incapable of withstanding temperatures of 48.9–50°C for any length of time. These observations have been empirically validated recently by several studies (Wright et al. 2002, Dowdy and Fields 2002; Mahroof et al. 2003a,b; Roesli et al. 2003; Boina and Subramanyam 2004) that reported the temperatures for effective disinfestations to be in the range of 50–60°C. Dean (1911) reported higher temperatures among mill floors and greater efficacy against insects solely based on visual observations, when the heat treatment lasted 24 h as opposed to 7.5 h. Dean (1911) during a 24-h heat treatment and Goodwin (1912) during a 19.5-h heat treatment of separate mills observed temperatures to be consistently lower on the first floor compared with floors above the first floor. Both studies noted temperatures to be lower in grain and flour accumulations than in open areas of the mill.

The earlier work on flour-mill heat treatments focused on temperature profiles observed within mills with anecdotal observations on insects. It was unclear whether fans were used to distribute heat within the mill. Pepper and Strand (1935) reported greater temperature penetration into a concrete block when fans were used to circulate heat than in still air, suggesting air movement to be an important factor in enhancing heat distribution. They recommended the use of fans during mill heat treatments.

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by Kansas State University or the U.S. Department of Agriculture (USDA).

[1] Department of Grain Science and Industry, Kansas State University, Manhattan, KS 66506.
[2] Corresponding author, e-mail: sbhadrir@ksu.edu.
[3] USDA–ARS, Center for Grain and Animal Health Research, 1515 College Ave., Manhattan, KS 66502.
[4] Department of Agricultural Economics, Kansas State University, Manhattan, KS 66506.
[5] Temp-Air, Inc., 3700 W. Preserve Blvd., Burnsville, MN 55337.

Mahroof et al. (2003a), in an unreplicated trial, used eggs, young larvae, old larvae, pupae, and adults of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)—a major pest of flour mills (Good 1937, Hagstrum and Subramanyam 2009)—confined in plastic boxes (4.5 cm in length by 4.5 cm in width by 1.5 cm in height) during heat treatments of pilot flour and feed mills at Kansas State University, Manhattan, KS. No consistent trends were observed among life stages in their susceptibility to heat, despite temperatures being ≥50°C in a majority of the locations examined. However, under laboratory conditions at constant temperatures ranging from 42 to 60°C, Mahroof et al. (2003b) found the young larvae of *T. castaneum* to be more heat tolerant than the other stages. Therefore, there is a need to confirm stage-specific susceptibility of *T. castaneum* life stages during practical flour-mill heat treatments.

None of the studies to date examined how temperatures attained on different mill floors influenced efficacy against mill insects. This is particularly important because the floor in contact with the ground or foundation is not heated on both sides like the floors above it. Roesli et al. (2003), through weekly monitoring of specific stored-product insects in pheromone traps before and after a feed mill heat treatment, observed captures of adults of *T. castaneum* within 2 to 4 wk after a heat treatment on the first floor compared with captures on floors 2–4. This could be due to temperatures not reaching 50°C allowing insects to survive a heat treatment; immigration of insects into the mill through open entrances and windows; insects being brought in on infested raw materials; or a combination of the above (Dean 1911, Roesli et al. 2003).

Unsanitary conditions due to accumulations of flour within equipment and on the floor are common during continuous operation of flour mills. Dean (1911), Goodwin (1912), and Pepper and Strand (1935) have shown that flour accumulations are slow to heat up due to flour being a poor conductor of heat. The influence of sanitation on efficacy of heat treatments against life stages of stored-product insects in general, and *T. castaneum* in particular, is unknown.

In this study, influence of sanitation, simulated by varying the amount of flour, and temperatures attained on different mill floors on susceptibility of eggs, young larvae, old larvae, pupae, and adults of *T. castaneum* were investigated during three heat treatments of a pilot flour mill. In addition, the itemized costs associated with each of the three heat treatments were determined.

Materials and Methods

Insect Cultures. Cultures of *T. castaneum* were reared on a diet of wheat flour with 5% (by wt) brewer's yeast. Each 0.94-liter glass mason jar with 200 g of the insect diet was seeded with 100 *T. castaneum* adults. After infestation jars were closed with filter paper lids. To obtain eggs and 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 250-μm opening sieve (Seedburo Equipment Company, Chicago, IL). These containers were incubated at 28°C and 65% RH. After 2 d, the adults were removed from the jars using an 841-μm opening sieve. The flour was sifted using a 250-μm sieve to collect the eggs. To obtain young larvae (first instars), infested containers were sifted after 6 d by using a 250-μm sieve. Old larvae (sixth to seventh instars), unsexed pupae, and unsexed adults of mixed ages were separated using an 841-μm sieve directly from culture jars. The ages of eggs, young larvae, old larvae, and pupae from time of infestation of flour with adults were 2, 6, 22, and 26 d, respectively.

Insect Bioassay Box. Individual stages of *T. castaneum* were exposed to heat treatment in a rectangular clear plastic box (27 cm in length by 17.5 cm in width by 4.2 cm in height) with 12 compartments (Subramanyam 2010). Each compartment measured 8.3 cm in length by 4.2 cm in width by 3.7 cm in depth. Two sanitation levels were simulated within the bioassay box. In the top row of compartments within a box, the 2-cm-deep flour (43 g/compartment) simulated "poor sanitation," and the bottom row of compartments with dusting of flour (≈0.5 g/compartment) simulated "good sanitation." All flour used in the bioassay boxes was first sifted through a 250-μm sieve. Only 10 of the 12 compartments were used for confining various insect life stages. In each compartment, 50 individuals of a life stage were introduced. In the remaining two compartments, temperature sensors were placed (see below). The bioassay box and individual compartments had 1-cm-diameter perforations that were covered by wire mesh screens with 177-μm openings. Lids of each compartment also were covered with this mesh. Perforations were made to facilitate heat distribution within bioassay box compartments.

Pilot Flour Mill. The Hal Ross flour mill, affiliated with the Department of Grain Science and Industry, Kansas State University, is a pilot scale mill that opened in October 2006. The mill has five floors occupying a total volume of 9,628 m^3. The foundation, floors, and walls are made of poured concrete with steel reinforcements. The 101.6-cm-thick foundation has 1,486.2 metric tons of poured concrete with 59.9 metric tons of reinforced steel. All the exterior walls and the roof are insulated with 7.62-cm Styrofoam insulation. There are two stairways made of galvanized steel; the stairway on the west side leads from the first floor to the roof, and the stairway on the east side leads from the first floor to the fifth floor. There is a 1,361-kg freight or personnel elevator. The first floor has a control room, manager's office, maintenance office, meeting room, and restrooms in addition to the flour processing area. The dimensions of each floor are 15.3 m in length by 27.5 m in width. Each floor has an extension on the north side that is 7.1 m in length by 7.6 m in width. The ceiling heights of the first, second, third, fourth, and fifth floors are 3.7, 4.4, 4.1, 4.1 and 4.7 m, respectively. A 20.3-cm-thick concrete between floors separates second through fifth floors. The weight of various pieces equipment and supports made of steel among the five mill floors was ≈123

Table 1. Bioassay box locations among five floors of the Hal Ross flour mill

Floor	Bioassay box	Location
First	1	Floor, southwest corner
	2	Floor by door, south
	3	Roller mill, inside motor compartment
	4	Roller mill, on top of second break rolls
	5	Floor, northeast corner
Second	6	Floor, southwest corner
	7	Floor, under the color sorter
	8	Scourer aspirator, inside cylinder screen
	9	Rebolt sifter, in the top screen
	10	Floor, middle of the room
Third	11	Floor, southwest corner
	12	Cylinder separator, inside middle cylinder
	13	Purifier, on top of screens in purifier 1
	14	Mixer
	15	Floor, middle of the room
Fourth	16	Floor, southwest corner
	17	Sifter, between two sieves
	18	Diverter
	19	Combi-cleaner, on middle screen
	20	Floor, middle of the room
Fifth	21	Floor, southwest corner
	22	Carter day screen separator, middle screen
	23	Technovator
	24	Ingredient feeder
	25	Floor, middle of the room

metric tons. The maximum daily flour mill production capacity is 18.2 metric tons or 400 hundred weight.

Five bioassay boxes were placed at various locations on each of the five floors in the flour mill (Table 1). Out of the 25 bioassay boxes, 13 were placed inside pieces of equipment and 12 were on the floor. The bioassay boxes that were not exposed to heat (control treatment) were infested similarly with all the life stages of *T. castaneum* and were placed in a laboratory growth chamber at 28°C and 65% RH.

Temperature Monitoring of Mill Floors and in Bioassay Boxes. To record floor-level temperatures during heat treatment, 48 HOBO data loggers (Onset Computer Corporation, Bourne, MA) were programmed to acquire data every minute. The first floor had eight data loggers, and the second through fifth floors each had 10 data loggers. On each floor, these data loggers were placed in a grid fashion. Humidity levels during heat treatment were not recorded, because previous research has shown that during heat treatments, humidity levels are ≤25% (Mahroof et al. 2003a, Roesli et al. 2003). Data on the minimum and maximum outdoor temperature and humidity during each heat treatment were obtained online from a weather station in Northview, Manhattan, KS (http://www.wunderground.com), which is 1.8 miles from the pilot flour mill.

Temperature in each of 25 bioassay boxes placed within the mill was measured by SmartButton sensors (ACR Systems, Inc., Surrey, BC, Canada). Two sensors were placed in each bioassay box to record temperatures every 2 min during the heat treatment. One sensor was placed in a compartment with flour dust, and the other was placed in compartment with 2-cm-deep flour, and both compartments did not have any insects. In the compartment with flour dust the sensor was visible and in the 2-cm-deep flour the sensor was placed at a depth of 1 cm.

Heat Treatment. The Hal Ross flour mill was subjected to three heat treatments, each lasting 24 h, during 13–14 May 2009, 25–26 August 2009, and 7–8 May 2010, by using forced-air gas heaters. All treatments were performed by a commercial heat treatment service provider (Temp-Air, Inc., Burnsville, MN). The mill vents were not sealed for heat treatments. Three direct-fired, forced-air gas heaters from Temp-Air, Inc. were used to heat the facility. Two heaters (THP-4500), each with a maximum heating capacity of 1,318.8 kW/h (4.5 million BTU/h), and one heater (THP-1400) each with a maximum heating capacity of 410.3 kW/h (1.4 million BTU/h), were used for each heat treatment. The power requirement for THP-4500 heater was 460 V, 60 Hz, 30 Amp, and 3 Phase, whereas that for THP-1400 heater was 460 V, 60 Hz, 10 Amp, and 3 Phase. The maximum discharge temperature at the outlet of the heaters was 93.3°C with a minimum discharge temperature of 60°C toward the end of the heat treatment. All gas heaters were fueled by two 3,785-liter propane tanks filled to 80% of the total capacity. Liquid propane from each tank passed through a vaporizer before it was used by the heaters. The THP-4500 and THP-1400 heaters can use 170.3 and 53.0 liter/h of propane, respectively. The airflow rate for THP-4500 and THP-1400 heaters was 708 and 212.4 m^3/min, respectively. The heaters were located outside the mill because of an open flame. The heaters, when ignited, heat the cold air outside and force it into the mill via a network of polyurethane fabric ducts. The THP-4500 heater was connected to 91.4-cm-diameter ducts and the THP-1400 heater to 60.9-cm-diameter ducts. These ducts were placed from first to fifth floors and along both the stairways. The ducts had 15.3-cm-diameter openings at regular intervals to serve as hot air outlets. There were a total of six air exchanges an hour during each heat treatment.

Eight fans (Temp-Air, Inc.), with a fan blade diameter of 91.4 cm, were placed on each of the five floors for heat distribution. Each fan had an airflow rate of 311.5 m^3/min. The fan power requirements were 115 V, 60 Hz, 8 Amps, and 1 Phase. Power load centers with a power capacity of 460 V, 60 Hz, 60 Amps, and 3 Phase were used to plug in the fans. In total, 24 fans were plugged into each load center. The fan locations were changed as the heat treatment progressed to ensure uniform heat distribution by eliminating cool spots (locations where temperatures were <50°C).

Insect Mortality Assessment. After 24 h of heat treatment, all bioassay boxes from the mill were brought to the laboratory and incubated at 28°C and 65% RH. The number of live and dead adults was determined 24 h after collecting all bioassay boxes, and the mortality was expressed as a percentage based on the number dead adults divided by the total number of insects exposed (50). Immature stages were transferred into 150-ml plastic containers and reared to the adult stage. Into each of the 150-ml containers holding immature stages from compartments with flour dust we added 10 g of flour as food. Percentage of mortality of immature stages was calculated based on number of individuals that did not emerge as adults divided by the total number of individuals exposed. Mortality of *T. castaneum* life stages in bioassay boxes for the control treatment was determined similarly.

Heat Treatment Costs. An economic analysis of the heat treatment was based on the actual fixed and variable costs incurred from billing records; all values are in U.S. dollars. The fixed costs for each heat treatment comprised of the forklift rental, generator rental, diesel use charges for the generator used to power the heaters, fan rental charges, cost of the fabric ducts, transportation costs for delivering heating equipment to the flour mill, and charges for four service technicians estimated at $1,000/d for 3 d, and included set up before heat treatment, monitoring the heat treatment, tear down at the end of heat treatment, and the travel time. The fabric duct ($11,000) can be used for six heat treatments in total, so the cost for each of the three heat treatments was ≈$1,833. The variable costs included a consulting fee for the Temp-Air, Inc.'s engineer/supervisor for managing the heat treatment, and the fee was ≈$100/h, and the quantity of actual fuel consumed for each heat treatment and current market price for the fuel.

Data Analysis. The time-dependent temperature data of each of the 48 HOBO data loggers for each of the heat treatments was downloaded to a computer. From these data, the starting ambient temperature, time in hours required to reach 50°C from the starting ambient temperature, number of hours temperatures were maintained above 50°C, and the maximum temperature attained during the 24-h heat treatment were determined. These values were averaged by floor across all three heat treatments. Differences among floors in the time required to reach 50°C, time above 50°C, or the maximum temperature were determined by one-way analysis of variance and Fisher's protected least significant difference test using the GLM procedure (SAS Institute 2002).

The starting temperature, time to 50°C, time above 50°C, and maximum temperature were also excerpted from the time-dependent temperature data within the bioassay boxes for each location and sanitation level for each heat treatment. The starting temperature, time to 50°C, time above 50°C, and the maximum temperature by floor and sanitation level, across all three heat treatments, for boxes placed within equipment and those placed on the mill floor were compared using two-sample *t*-tests (SAS Institute 2002). The means ± SE for time to 50°C, time above 50°C, and the maximum temperature across all three heat treatments by floor and sanitation level, irrespective of location, were plotted by floor and sanitation level.

The mortality of *T. castaneum* life stages was corrected for mortality in corresponding control treatments (Abbott 1925). The corrected mortality data for eggs, young larvae, old larvae, pupae, and adults were calculated by floor and sanitation level. These data were subjected to the GLIMMIX procedure (SAS Institute 2002) to determine differences among the fixed effects (life stage, flour depth, time to 50°C, time above 50°C, and maximum temperature) and an interaction effect (life stage and flour depth). Box within a treatment was the random effect for this analysis. Mortality differences between the two sanitation levels for the stage × flour depth was sliced by stage by using the GLIMMIX procedure. All statistical differences were considered significant at the $\alpha = 0.05$ level.

Table 2. Temperature parameters (mean ± SE) observed at the floor level during heat treatment of the Hal Ross flour mill

Floor	Time to 50°C (h)	Time above 50°C (h)	Max. temp. (°C)
First[a]	8.9 ± 1.3	10.1 ± 1.4	53.6 ± 2.7
Second	8.5 ± 0.9	13.6 ± 1.0	58.3 ± 2.2
Third	10.0 ± 0.6	13.5 ± 0.6	59.3 ± 0.6
Fourth	12.0 ± 1.0	11.2 ± 1.0	57.4 ± 0.8
Fifth[b]	9.7 ± 1.0	12.3 ± 1.0	56.8 ± 0.8

Each mean is based on $n = 30$ unless otherwise indicated.
[a] $n = 24$, because on this floor there were eight rather than 10 HOBO data-logging units.
[b] $n = 29$, because one temperature data logger failed to record temperatures.

Results

Temperature Data Outdoors and Among Mill Floors. The minimum and maximum temperature and humidity during the 13–14 May 2009 treatment ranged from 10.2 to 27.5°C and from 32 to 87%, respectively. During the 25–26 August 2009 treatment, temperature ranged from 21 to 34.2°C and humidity from 46 to 89%. Temperature during the 7–8 May 2010 treatment ranged from 5.1 to 20.8°C and the humidity from 28 to 76%. Despite variations in outdoor temperatures, mean ± SE ambient temperature among mill floors based on HOBO data loggers at the floor level was essentially similar ($F = 0.34$; df = 4, 138; $P = 0.8501$) and ranged from 27.2 ± 1.2 to 29.1 ± 2.3. The time to 50°C among the mill floors varied by 3.5 h, but these differences were not significant ($F = 2.03$; df = 4, 138; $P = 0.0937$) (Table 2). Temperatures among mill floors were held above 50°C for 10.1 ± 1.4–13.6 ± 1.0 h, and these minor differences were not statistically significant ($F = 2.09$; df = 4, 138; $P = 0.0849$). The first floor attained lowest maximum temperature (53.6 ± 2.7) and the third floor attained the highest temperatures (59.3 ± 0.6); differences among floors were not significant ($F = 1.69$; df = 4, 138; $P = 0.1565$).

Temperature Data From Bioassay Boxes. The starting temperature, time to 50°C, time above 50°C, and the maximum temperature data in compartments with dusting of flour for bioassay boxes within equipment were not significantly different (df = 13) from data in bioassay boxes placed at floor level on the first (t, range among variables = −1.55 to −0.25; $P \geq 0.1440$), second ($t = -0.98$–1.27; $P \geq 0.2276$), fourth ($t = -1.69$ to −0.21; $P \geq 0.1140$), and fifth ($t = -0.64$–0.84; $P \geq 0.4168$) floor. On the third floor, 50°C was attained in the compartments with dusting of flour in bioassay boxes within equipment in a mean ± SE ($n = 9$) time of 8.8 ± 0.6 h, whereas those in boxes at the floor level ($n = 6$) reached 50°C in 11.9 ± 1.2 h. This time difference was significant ($t = -2.53$; df = 13; $P = 0.0252$). Consequently, temperatures in these compartments within equipment were held above 50°C for 15.0 ± 0.6 h compared with 11.9 ± 1.2 h for those at floor level ($t = 2.57$; df = 13; $P = 0.0234$). Both the starting temperature and the maximum temperature did not differ from one another in compartments with dusting of flour inside and outside the equipment (t, range between variables = −0.24 to 1.60; df = 13; $P > 0.1325$). In compartments with 2-cm-deep flour in bioassay boxes placed within and outside equipment, the starting temperature, time to 50°C, time above 50°C, and the maximum temperature were not significantly different (df = 13) on the first (t, range among variables = −1.65 to −1.08; $P \geq 0.1206$), second ($t = -1.26$ to 1.36; $P \geq 0.1963$), third ($t = -1.52$ to 1.57; $P \geq 0.1415$), fourth ($t = -1.62$ to 0.18; $P \geq 0.1286$), and fifth ($t = -0.60$ to 1.70; $P \geq 0.1138$) floor. These results suggested that, with the exception of the third floor mentioned above, temperature variables measured in compartments with dusting of flour or 2-cm-deep flour within and outside equipment were essentially similar. Therefore, further data analyses were conducted using information from all bioassay boxes without reference to their position within or outside equipment.

The mean ± SE ($n = 15$, except for 2-cm-deep flour treatment on the first floor where $n = 14$ because of a faulty sensor), starting temperatures observed among mill floors in compartments with dusting of flour ranged from 24.7 ± 1.3°C (first floor) to 25.9 ± 0.6°C (third floor), and in compartments with 2-cm-deep flour the temperatures ranged from 23.5 ± 0.9°C (first floor) to 25.3 ± 0.5°C (third floor). The time to 50°C, time above 50°C, and the maximum temperature in compartments with dusting of flour and 2-cm-deep flour are shown in Fig. 1. On the first floor, the time to reach 50°C was longest in compartments with dusting of flour (mean ± SE, 15.5 ± 1.7 h), whereas it was longest in compartments with 2-cm-deep flour on the fourth (16.2 ± 1.4 h), followed by those on the first floor (15.7 ± 2.1 h). Compartments with 2-cm-deep flour took 2–5 h longer to reach 50°C than those with dusting of flour. There were differences among floors in the time required to reach 50°C, and this effect was not consistent between the two sanitation levels. In several locations on each floor, and at the two sanitation levels, temperatures did not reach 50°C. In two compartments with dusting of flour on the first floor and two compartments on the fifth floor, temperatures failed to reach 50°C. Similarly, in compartments with 2-cm-deep flour, on the first, fourth, and fifth floors six, one, and three locations, respectively, failed to reach 50°C.

Temperatures were generally held above 50°C for 0.3–3.5 h longer in compartments with dusting of flour than those with 2-cm-deep flour, and temperatures were held above 50°C only for ≤6.6 h on the first floor compared with 7.1–13.7 h in second through fifth floors. The maximum temperatures attained ranged

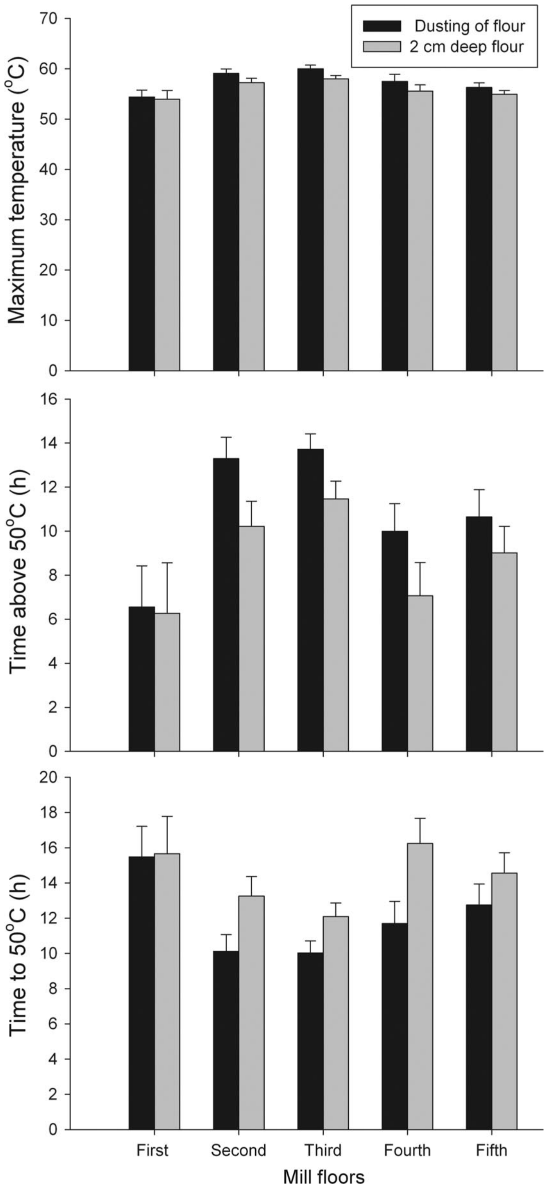

Fig. 1. Mean + SE time to 50°C, time above 50°C, and the maximum temperature observed by floor in compartments of bioassay boxes holding dusting of flour or 2-cm-deep flour during each of the three heat treatments. Each mean is based on $n = 15$/compartment unless otherwise indicated. On the first floor, $n = 13$ for compartments with dusting of flour and $n = 9$ for compartments with 2-cm-deep flour. On the fourth floor, $n = 14$ for compartments with 2-cm-deep flour. On the fifth floor, $n = 13$ for compartments with dusting of flour and $n = 12$ for compartments with 2-cm-deep flour. Temperatures failed to reach 50°C in certain compartments and hence $n < 15$.

from 53.9 ± 1.7°C to 60.0 ± 0.7°C between the sanitation levels and among mill floors. In compartments with 2-cm-deep flour, temperatures were 0.5–2.0°C lower than those with dusting of flour. Irrespective of the sanitation level, the maximum temperatures at-

Table 3. Mortality of *T. castaneum* life stages in unheated (control) bioassay boxes held in a laboratory growth chamber at 28°C and 65% RH

Life stage	Mean ± SE mortality (%)	
	Dusting of flour	2-cm deep flour
Egg	48.0 ± 6.1	18.7 ± 2.6
Young larva	42.0 ± 14.5	12.0 ± 3.5
Old larva	0.7 ± 0.4	0.0 ± 0.0
Pupa	1.6 ± 0.3	1.4 ± 0.4
Adult	0.8 ± 0.3	1.7 ± 0.4

Each mean is based on $n = 3$. At each n, 50 insects of each life stage were placed in individual compartments in a bioassay box.

tained were lower in compartments on the first floor by 1.0–5.6°C compared with those on second through fifth floors.

Mortality of *T. castaneum* Life Stages in Unexposed and Heat-Exposed Bioassay Boxes. The mean ± SE mortality of old larvae, pupae, and adults in bioassay boxes that were not exposed to the heat treatment (control treatment) was ≤1.7% but that of eggs and young larvae ranged from 12.0 ± 3.5 to 48.0 ± 6.1% (Table 3). Eggs and young larvae in compartments with dusting of flour had higher mortality than those in 2-cm-deep flour.

On the first floor, mean ± SE mortality of each *T. castaneum* life stage at the two sanitation levels ranged from 55.5 ± 12.9 to 98.6 ± 0.8% (Table 4). The mortality for each stage was lower in compartments with 2-cm-deep flour than in compartments with dusting of flour, and the greatest magnitude of difference between the sanitation levels was observed only for adults. On second through fifth floors, except in two cases, the mortality of all life stages at the two sanitation levels ranged from 98.4 ± 1.6 to 100.0 ± 0.0%. The two exceptions were on the fourth floor where the mortality of old larvae and adults in 2-cm-deep flour was 93.2 ± 6.7 and 95.6 ± 4.4%, respectively. Therefore, only data from the first floor, where mortality of the different stages varied from 55 to 98%, was used to examine influence of sanitation on mortality of *T. castaneum* life stages.

On the first floor, mortality differences among *T. castaneum* life stages were not significant ($F = 2.20$; df = 4, 63; $P = 0.0796$), but the mortality of the life stages between the two sanitation levels was significantly different ($F = 5.86$; df = 1, 63; $P = 0.0184$). The life stage and sanitation level interaction was not significant, however ($F = 1.97$; df = 4, 63; $P = 0.1101$). Therefore, differences between the two sanitation levels for each life stage were analyzed further. The mortality of eggs, young larvae, old larvae, and pupae on the first floor was not significantly different between the sanitation levels (F, range among stages = 0.34–1.72; df = 1, 63 for each stage; P, range = 0.1944–0.5048). Only the adult mortality was significantly higher in compartments with dusting of flour when compared with mortality in compartments with 2-cm-deep flour ($F = 10.66$; df = 1, 63; $P = 0.0018$).

On the first floor, the mortality of *T. castaneum* life stages at the two sanitation levels was not influenced by the number of hours required to reach 50°C ($\chi^2 = 0.39$, df = 1, $P = 0.5326$) or number of hours temperatures were held above 50°C ($\chi^2 = 0.02$, df = 1, $P = 0.8970$) but was influenced significantly by the maximum temperatures attained ($\chi^2 = 16.27$, df = 1, $P < 0.0001$).

Economic Analysis. The propane consumed for heat treatments was lower during the August 2009 treatment compared with the May 2009 and May 2010 treatments (Table 5), because temperatures were warmer in August relative to May. The propane cost was 6 cents higher in May, and the fuel costs for the heat treatments ranged from $1,871 to $2,397. The labor charge of $480 for each heat treatment includes delivery, pickup and emptying the tank by a local company. The mileage charge of $72 also was applied for delivery and pickup of the propane tank for each treatment. All other fixed charges for each heat treatment were for Temp-Air, Inc. and included the following: forklift rental ($1,193), generator rental ($1,244), diesel for the generator ($532), transportation costs for delivering and picking-up the heaters ($1,725), and fan rental ($5,275). The $11,000 for the fabric ductwork was spread over six heat treatments; therefore, for each heat treatment the actual cost was $1,833. The $12,000 for service personnel per heat treatment was for four technicians at the rate of $1,000/d for 3 d and included travel, lodging, and time spent on site during heat treatments (12 h/shift). The engineer/supervisor from Temp-Air, Inc. was compensated for time spent in managing the setting-up

Table 4. Mortality of *T. castaneum* life stages in bioassay boxes placed among the five floors of the Hal Ross flour mill subjected to heat treatments

Floor	Life stage	Mean ± SE mortality (%)[a]	
		Dusting of flour	2-cm-deep flour
First	Egg	98.6 ± 0.8	97.2 ± 1.6
	Young larva	98.0 ± 1.1	82.0 ± 6.9
	Old larva	93.1 ± 6.6	85.8 ± 6.7
	Pupa	94.8 ± 6.7	85.0 ± 8.0
	Adult	93.3 ± 6.7	55.5 ± 12.9
Second	Egg	100.0 ± 0.0	99.8 ± 0.2
	Young larva	99.3 ± 0.5	100.0 ± 0.0
	Old larva	100.0 ± 0.0	100.0 ± 0.0
	Pupa	100.0 ± 0.0	100.0 ± 0.0[b]
	Adult	100.0 ± 0.0	100.0 ± 0.0
Third	Egg	100.0 ± 0.0	98.4 ± 1.6
	Young larva	99.3 ± 0.7	99.8 ± 0.2
	Old larvae	100.0 ± 0.0	99.7 ± 0.3
	Pupae	100.0 ± 0.0	100.0 ± 0.0
	Adults	100.0 ± 0.0	100.0 ± 0.0
Fourth	Eggs	100.0 ± 0.0	100.0 ± 0.0
	Young larvae	100.0 ± 0.0	99.2 ± 0.8[b]
	Old larvae	99.0 ± 1.0	93.2 ± 6.7
	Pupae	100.0 ± 0.0	98.9 ± 1.1
	Adults	100.0 ± 0.0	95.6 ± 4.4
Fifth	Eggs	100.0 ± 0.0	100.0 ± 0.0
	Young larvae	99.8 ± 0.2	99.5 ± 0.5
	Old larvae	99.5 ± 0.3	99.3 ± 0.5
	Pupae	100.0 ± 0.0	100.0 ± 0.0
	Adults	100.0 ± 0.0	100.0 ± 0.0

[a] Each mean is based on $n = 15$ (5 boxes/floor × 3 heat treatments) unless otherwise indicated.

[b] $n = 14$, because of a missing value.

Table 5. Itemized costs for each of the three heat treatments of the Hal Ross flour mill

Cost item	Costs (US$) for heat treatment conducted during:			Total cost (US$)
	13–14 May 2009	25–26 Aug. 2009	7–8 May 2010	
Propane consumed (liters)	5,299	4,883	5,500	15,682
Price/liter	0.3831	0.3831	0.4358	
Propane cost	2,030	1,871	2,397	6,298
Labor charge[a]	480	480	480	1,440
Mileage charge[a]	72	72	72	216
Forklift rental	1,193	1,193	1,193	3,579
Generator rental	1,244	1,244	1,244	3,732
Diesel (for generator)	532	532	532	1,596
Transportation cost	1,725	1,725	1,725	5,175
Fan rental	5,275	5,275	5,275	15,825
Fabric ducting[b]	1,833	1,833	1,833	5,499
Service personnel[c]	12,000	12,000	12,000	36,000
Engineer/supervisor fee[d]	2,454	2,613	687	5,754
Total cost	28,838	28,838	27,438	85,114

[a] For delivering, recharging, and picking up the propane tanks.
[b] The total cost of the ductwork customized for the mill was US$11,000. The same ductwork can be used for up to six heat treatments; therefore, the cost per treatment was ≈ US$1,833.
[c] Four technicians were paid US$1,000/d each for 3 d. Costs included travel, lodging, and time spent on site during heat treatments (12 h/shift).
[d] The engineer/supervisor from Temp-Air, Inc. charged ≈US$100/h for management of each heat treatment and the costs reflect the actual time spent.

and monitoring the heat treatment and making necessary corrections as needed. These costs were billed at approximately $100/h, based on time invested. The supervisor decided to charge us only for a portion of his time during the last heat treatment. The actual costs incurred by Kansas State University for the heat treatments ranged from $27,438 to $28,838, or from $2.96 to $3.11/m^3 interior building space.

Given that 7.12 kW/h energy is produced from a liter of propane, the total energy required for the 24-h heat treatment of the mill during May 2009, August 2009, and May 2010 was 38,206, 35,206, and 39,655 kW, or 0.17, 0.16, and 0.18 kW/h/m^3, respectively.

Discussion

The mill has its own boiler that allowed the ambient mill temperatures among the five floors to be maintained between 27 and 29°C at the beginning of each heat treatment. Therefore, temperature fluctuations outdoors only affected the amount of heat energy needed; consequently, the amount of propane consumed, during the three heat treatments. The ambient mill temperatures at the beginning of the heat treatment were essentially similar. Also, the time to 50°C, time above 50°C, and the maximum temperature were not significantly different among the mill floors suggesting that the heat was uniformly distributed across all mill floors. This was accomplished by proper placement of the fabric ducts and strategic positioning of fans. In addition, air movement was facilitated by opening the hatch door to the roof and louvers in the mill's ventilation shafts. Vertical and horizontal stratification of temperatures among mill floors is imminent during heat treatments based on observations from past studies (Mahroof et al. 2003a, Roesli et al. 2003), and this study. The time to 50°C observed in our study was comparable to that reported by Mahroof et al. (2003a) during a 35-h heat treatment of a feed mill (6–19 h) but lower than that reported by Roesli et al. (2003) during a 37-h heat treatment of a feed mill (2–8 h).

Although there were no significant differences, minor differences were observed in temperatures among the mills floors, especially with respect to the maximum temperature attained, which was lower on the first floor compared with other floors. Dean (1911) during a 7.5-h heat treatment of the flour mill observed that the temperature attained in an elevator boot on the first floor (34°C) was lower than temperatures attained at 1.83-m above the second (51°C), third (52°C), and fourth (48°C) floors. During a 24-h heat treatment of the same flour mill, Dean (1911) reported temperatures of 36–40°C at 1.83 m above the first floor; temperatures at the same height above the second, third, and fourth mill floors were 57, 54–60, and 53°C, respectively. Goodwin (1912), during a 19.5-h heat treatment of a flour mill, measured temperatures in four to five different locations on each of three floors, including deep within flour. Irrespective of the locations measured, the average temperature attained at the end of the heat treatment on the first, second, and third floor was 48, 56, and 58°C, respectively. The wide variation in temperatures observed among mill floors reported by Dean (1911) and Goodwin (1912) were not observed in our study, because fans were not used by these authors. Fans aid in uniform heat distribution as observed in this study, and as previously noted by Pepper and Strand (1935) in their laboratory study.

The reason for lower temperatures attained on the first floor could be due to this floor resting on a foundation made up of 1,486.2 metric tons of concrete, and unlike second through fifth floors it is not heated from both sides (top and bottom). A linear regression analysis of the data of Pepper and Strand (1935) on heating of a 0.372-m^2 concrete block of 25.4-cm thickness by using 1.57 kW/h heat for 10 h showed an inverse relationship between depth of the concrete and temperatures attained ($r^2 = 0.978$). Temperature at the surface of the concrete was 66°C, and at a depth of 22.86 cm below the surface it was 40°C. The mean ± SE y-intercept of the regression was 62.98 ± 0.48; the slope of −1.03 ± 0.43, indicated for every 1-cm depth of concrete below the surface the temperature dropped by ≈1°C.

The uniform heating of mill floors perhaps resulted in a more uniform heating observed in compartments with dusting of flour or 2-cm-deep flour in four of the five floors, where lack of differences in temperature variables were observed in bioassay boxes placed within and outside equipment. The temperatures observed in bioassay boxes cannot be directly compared with mill floor level temperatures, because bioassay boxes, especially those within pieces of equipment, were above the floor level and generally temperatures above the floor tend to be higher than those observed at the floor level (Dean 1911, Goodwin 1912).

Sanitation had an impact on the time to 50°C, time above 50°C, and the maximum temperature. This is expected, because flour is a poor conductor of heat. Dean (1911) at the end of a 7.5-h heat treatment of a flour mill reported that on the second floor, the temperature measured at 8.9 cm in a sack of flour was 41°C, whereas that of the ambient temperature in the center of the room 1.83 m above the floor was 51°C. Similarly, Goodwin (1912) reported that the temperature on the first floor at the end of a 19.5-h heat treatment of a flour mill was 44°C in flour at a depth of 12.7 cm, whereas the temperature at 91.4 cm above the same floor was 61°C.

The mortality of *T. castaneum* in the control treatment was high for eggs and young larvae, only in compartments with dusting of flour. The reasons for such high mortality of young larvae could be due to cannibalism before addition of flour as food. The reasons for high egg mortality are unclear. The survival of all stages was statistically related to the maximum temperature that in general was lower on the first floor compared with the other floors. Adult survival on the first floor was greater in compartments with 2-cm-deep flour than in compartments with dusting of flour. This can be attributed to six compartments out of 15 in 2-cm-deep flour, across all three heat treatments on the first floor, in which temperatures did not reach 50°C. Another reason for insect survival could be that temperatures above 50°C were maintained for ≤6.6 h on the first floor compared with other floors.

Here, we found that adults on the first floor in 2-cm-deep flour were the most heat-tolerant stage. Adults averaged 55% mortality, whereas the other stages had mortalities of ≥82%. In an unreplicated heat treatment trial, where temperatures are dynamic over time, Mahroof et al. (2003a) found *T. castaneum* pupae in general to be more heat tolerant than adults. In bioassays at constant temperatures of 42, 46, 50, 54, 58, and 60°C, Mahroof et al. (2003b) found *T. castaneum* pupae to be more heat tolerant than adults only at 42 and 50°C based on time for 99% mortality (LT_{99}). Heat tolerance among insect life stages of *T. castaneum;* confused flour beetle, *Tribolium confusum* Jacquelin du Val; and the Indianmeal moth, *Plodia interpunctella* (Hübner) varies with temperature (Mahroof et al. 2003b; Boina and Subramanyam 2004; Mahroof and Subramanyam 2006). Heat tolerance among insect life stages also is influenced by heating rates (Beckett et al. 2007), although majority of these data are based on heat treatment with grain and not facility heat treatments. During heat treatment of a food-processing facility, Yu et al. (2011) exposed eggs, young larvae, old larvae, and adults of the cigarette beetle, *Lasioderma serricorne* (L.), in bioassay boxes and could not consistently find a heat-tolerant stage based on mortality responses. However, by exposing eggs, young larvae, old larvae, pupae, and adults of *L. serricorne* at constant temperatures of 46, 50, and 54°C in the laboratory for variable times, Yu et al. (2011) consistently found eggs to be the heat-tolerant stage.

In case of *T. castaneum,* the young larvae are heat tolerant at constant temperatures of 50–60°C (Mahroof et al. 2003b). However, as mentioned, during heat treatment where temperatures are dynamic over time, identifying a heat-tolerant stage becomes difficult (Mahroof et al. 2003a), because the rate of heating or behavioral responses of insects may influence heat tolerance. These aspects warrant further study.

The antennal segments of *T. castaneum* adults can sense temperature gradients (Holsapple and Florentine 1972), and it is plausible that during heat treatment, the adults were able to sense the high temperatures and tunnel deep into 2-cm-deep flour enabling greater survival compared with adults in compartments with dusting of flour on the first floor. This observation is supported by the fact that the mortality of adults in compartments with dusting of flour on the first floor was comparable to, or slightly lower than that of, other *T. castaneum* stages, whereas the mortality of adults in 2-cm-deep flour on the first floor was only 55%.

In commercial heat treatments, the amount of heat energy used is typically 0.07–0.10 kW/h/m^3 (Bh.S., unpublished data), but in our pilot flour mill, the heat energy used was 1.6–2.6 times greater than this figure (0.16–0.18 kW/h/m^3). The reasons are related to the mill being air tight (no windows) resulting in poor air circulation within the mill, and the only outlet for the air within the building was through the hatch door leading to the roof and the ventilation louvers.

The costs reported here are for hiring an outside service provider for conducting heat treatments and are not typical of expenses incurred when facilities have preinstalled heaters for regular heat treatments. The costs for service personnel would be independent of the facility size, because for any heat treatment, the number of people used would be the same. The fuel costs, in addition to being related to differences in outside and inside temperatures, also were related to temperature monitoring data. The service provider used 76–79 wireless sensors among the mill floors for temperature monitoring to ensure that temperatures have reached 50°C, were held above 50°C for several hours, and did not exceed 60°C. This proprietary data are therefore not mentioned in this paper. The cost of heaters was not included in the total costs, as the heaters were provided free-of-cost by the service provider. This is the first report itemizing heat treatment costs of the three heat treatments in a pilot flour mill.

In summary, the results presented here show that effective heat treatments can be conducted within 24 h, provided temperatures reach 50°C in 8–12 h and are held above 50°C for at least 10–14 h, while avoiding temperatures exceeding 60°C. Unlike previous experiments (Mahroof et al. 2003a), in this study, the adults of *T. castaneum* were found to be the most heat-tolerant stage, and adult survival was positively influenced by the presence of flour. These results reinforce the need to conduct thorough sanitation before a heat treatment to improve heat treatment effectiveness against *T. castaneum* eggs, larvae, and pupae in general, and adults in particular. The cost of ≈$3.00/m^3$ observed for heat treating the pilot flour mill was slightly higher than similar treatments of commercial facilities, because the mill was air-tight as a result of very few windows or openings to the exterior, and the lack of free air movement did not allow mill floors to reach 50°C within a short period. Despite a few limitations, our replicated trials provided new insights on factors affecting heat treatment effectiveness against *T. castaneum* life stages, along with detailed itemized heat treatment costs for propane-fueled forced air gas heaters.

Acknowledgments

We thank Jim Weaver, Alex Young, Xue Meng, Sara Savoldelli, and Khamis Moses for help with the insect bioassays. We are grateful to Mark West for statistical help and to David Hagstrum for reviewing an earlier draft of this manuscript. Research reported here was supported by a grant from USDA–CSREES (NIFA) Methyl Bromide Transitions Program under agreement 2008-51102-04583. The Propane Education and Research Council (PERC), Washington, DC, underwrote all heat treatment expenses, including a hands-on heat treatment workshop (http://www.ksre.ksu.edu/grsc_subi/Conference/confrence.htm). This paper is contribution 11-310-J of the Kansas State University Agricultural Experiment Station.

References Cited

Abbott, W. S. 1925. A method of comparing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Beckett, S. J., P. G. Fields, and Bh. Subramanyam. 2007. Disinfestation of stored products and associated structures using heat, pp. 182–237. *In* J. Tang, E. Mitcham, S. Wang, and S. Lurie (eds.), Heat treatments for postharvest pest control: theory and practice. CAB International, Oxon, United Kingdom.

Boina, D. R., and Bh. Subramanyam. 2004. Relative susceptibility of *Tribolium confusum* life stages exposed to elevated temperatures. J. Econ. Entomol. 97: 2168–2173.

Dean, G. A. 1911. Heat as a means of controlling mill insects. J. Econ. Entomol. 4: 142–158.

Dean, G. A. 1913. Mill and stored grain insects. Kans. State Agric. Col. Tech. Bull. 189: 139–176.

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.

Good, N. E. 1937. Insects found in the milling streams of flour mills in the southwestern milling area. J. Kans. Entomol. Soc. 10: 135–148.

Goodwin, W. H. 1912. Flour mill fumigation. Ohio Agric. Exp. Stn. Bull. 234: 171–184.

Hagstrum, D. W., and Bh. Subramanyam. 2009. Stored-product insect resource. AACC International, St. Paul, MN.

Holsapple, J. G., and G. J. Florentine. 1972. Thermal perception by the red flour beetle, *Tribolium castaneum.* Ann. Entomol. Soc. Am. 65: 1388–1390.

Mahroof, R., Bh. Subramanyam, and D. Eustace. 2003a. Temperature and relative humidity profiles during heat treatment of mills and its efficacy against *Tribolium castaneum* (Herbst) life stages. J. Stored Prod. Res. 39: 555–569.

Mahroof, R., Bh. Subramanyam, J. E. Throne, and A. Menon. 2003b. Time-mortality relationships for *Tribolium castaneum* (Coleoptera: Tenebrionidae) life stages exposed to elevated temperatures. J. Econ. Entomol. 96: 1345–1351.

Mahroof, R., and Bh. Subramanyam. 2006. Susceptibility of *Plodia interpunctella* (Lepidoptera: Pyralidae) developmental stages to high temperatures used during structural heat treatments. Bull. Entomol. Res. 96: 539–545.

Pepper, J. H., and A. L. Strand. 1935. Superheating as a control for cereal-mill insects. Mont. State Col. Agric. Exp. Stn. Bull. 297: 1–26.

Roesli, 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. J. Stored Prod. Res. 39: 521–540.

SAS Institute. 2002. SAS/STAT user's guide, version 9.1. SAS Institute, Cary, NC.

Subramanyam, Bh. 2010. Controlling red flour beetle. Milling J. Fourth Quarter, 32–34.

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. J. Stored Prod. Res. 38: 147–155.

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

Received 8 April 2011; accepted 27 January 2012.