

Impact of Varying Levels of Sanitation on Mortality of *Tribolium castaneum* Eggs and Adults During Heat Treatment of a Pilot Flour Mill

MONIKA BRIJWANI,¹ BHADRIRAJU SUBRAMANYAM,^{1,2} AND PAUL W. FLINN³

J. Econ. Entomol. 105(2): 703–708 (2012); DOI: <http://dx.doi.org/10.1603/EC11115>

ABSTRACT The influence of sanitation on responses of life stages of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), an economically important pest in flour mills, was investigated in a pilot flour mill subjected to two, 24-h heat treatments. One hundred eggs or 100 adults of *T. castaneum* were exposed inside each 20-cm diameter by 15-cm high PVC rings holding 0.1-, 0.2-, 1.0-, 3.0-, 6.0-, and 10.0-cm-deep wheat flour to simulate different sanitation levels that may exist in a flour mill. These rings were placed on the first and third floors of a pilot flour mill. On the first floor, temperatures inside rings with eggs reached 50°C in 7–11 h only in 0.1- and 0.2-cm-deep flour treatments. In all other treatments the maximum temperatures attained generally were below 50°C and inversely related to flour depth. Adults of *T. castaneum* on this floor were less susceptible than eggs. The egg mortality decreased linearly with an increase in flour depth, whereas that of adults decreased exponentially. All eggs and adults in rings on the third floor were killed irrespective of flour depth, because temperatures inside rings reached 50°C in 15–17 h and were held above 50°C for 6–8 h with the maximum temperatures ranging between 55.0 and 57.0°C. Although the protective effects of flour on survival of *T. castaneum* eggs and adults were evident only if temperatures did not reach 50°C, removal of flour accumulations is essential to improve heat treatment effectiveness.

KEY WORDS heat treatment, temperature, sanitation, red flour beetle, efficacy assessment

In the United States, Canada, Europe, and Australia, elevated temperatures (50–60°C) or heat treatments have been used for managing stored-product insects associated with flour mills and bakeries (Dean 1911, Goodwin 1912, Beckett et al. 2007). Heat treatment is a viable alternative to methyl bromide, a structural fumigant that was phased out in the United States in 2005 because of its adverse effects on stratospheric ozone (Norman et al. 2008). During heat treatments, all accessible areas of flour mill and equipment should be thoroughly cleaned to remove any accumulated flour, because these accumulations serve as insect harborage sites. Many stored-product insects have been reported within moving mill stocks, static mill stocks, and within pieces of equipment where products accumulate (Wagner and Cotton 1935; Good 1937; Rilett and Weigel 1956; Dyte 1965, 1966; Hosny et al. 1968). Grain and flour accumulations are poor conductors of heat (Dean 1911, Pepper and Strand 1935), and during

heat treatments, insects can seek refuge in these accumulations and escape the lethal effects of high temperatures (Goodwin 1922). Limited data are available on the impact of sanitation on survival of insect life stages during structural heat treatments. Brijwani (2011) reported lower temperatures and greater survival of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) life stages in 2-cm-deep flour (43 g) than in flour dust (≈ 0.5 g) during heat treatments of a pilot flour mill. The survival of *T. castaneum* life stages was greater in bioassay boxes placed on the first floor of the mill than those placed on second through fifth floors, because temperatures were lower on the first floor compared with the other floors (Brijwani 2011). Additionally, on the first floor, adults of *T. castaneum* were found to be less susceptible to heat when compared with eggs, young larvae, old larvae, and pupae. Dean (1911) and Goodwin (1912) also reported temperatures during heat treatments to be generally lower on the first floor than those observed on floors above it.

In the current study the influence of six flour depths between 0.1 and 10.0 cm on the mortality of an immobile stage (eggs) and mobile stage (adults) of *T. castaneum* was evaluated in a pilot flour mill subjected to two heat treatments during 2009–2010. Because temperatures attained vary between the first floor and floors above it, the influence of the six flour depths on

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. Kansas State University and USDA are equal opportunity providers and employers.

¹ Department of Grain Science and Industry, Kansas State University, Manhattan, KS 66506.

² Corresponding author, e-mail: sbhadrir@ksu.edu.

³ USDA, Agricultural Research Service, Center for Grain and Animal Health Research, 1515 College Ave., Manhattan, KS 66502.

mortality of *T. castaneum* eggs and adults was verified on the first and third mill floors.

Materials and Methods

Insect Cultures. Cultures of *T. castaneum* were reared on a diet of wheat flour with 5% (by wt) brewer's yeast at 28°C and 65% RH. Each 0.94-liter glass mason culture jar with 200 g of the insect diet was seeded with 100 *T. castaneum* adults. After infestation, jars were closed with lids fitted with filter papers. To obtain eggs, 50 unsexed *T. castaneum* adults of mixed ages were introduced into 150-ml plastic containers holding 50 g of flour that was sifted through a 250- μm opening sieve (Seedburo Equipment Company, Chicago, IL). These containers were held at 28°C and 65% RH for 2 d after which the adults were removed from the jars by using an 841- μm opening sieve. The flour was sifted using a 250- μm sieve to retain the eggs. Unsexed, mixed-age adults were separated directly from culture jars by using an 841- μm sieve. The collected eggs and adults were used in heat treatment experiments.

Pilot Flour Mill. The Hal Ross pilot flour mill, belonging to the Department of Grain Science and Industry, Kansas State University, has five floors occupying a total volume of 9,628 m³. The foundation, floors, and walls are made of poured concrete with steel reinforcements. The foundation is 101.6-cm thick and has 1,486.2-metric tons of poured concrete and 59.9-metric tons of reinforced steel. All exterior walls and the roof have a 7.62-cm Styrofoam insulation. There are two stairways made of galvanized steel; the one on the west side leads from first floor to the roof while one on the east side leads from first floor to the fifth floor. The dimensions of each floor are 15.3 m long and 27.5 m wide. Each floor has an extension on the north side that is 7.1 m long and 7.6 m wide. The ceiling height of the first, second, third, fourth and fifth floors is 3.7, 4.4, 4.1, 4.1 and 4.7 m, respectively. A 20.3-cm-thick concrete floor separates second through fifth floors. The milling equipment made of steel among the mill floors weighs \approx 123 metric tons. The maximum daily flour mill production capacity is 18.2 metric tons (400 cwt).

Egg and Adult Bioassays. Polyvinyl chloride (PVC) pipes of 20 cm in diameter and 0.82 cm in thickness (Ferguson, Manhattan, KS) were cut to provide cylinders of 15-cm height. In total, 36 rings were used in experiments. Before heat treatment, 12 rings each were placed on the first and third floors of the flour mill subjected to heat treatments. Another 12 rings were placed on the fourth floor of an old pilot flour mill located in Shellenberger Hall, Department of Grain Science and Industry, Kansas State University, to serve as the control (unheated) treatment. Elmer's clay (Elmer's Products Inc., Columbus, OH) was used at base-floor junctions of rings to prevent insect escape. The rings on a given floor were placed at a distance of \approx 40 cm from one another. A temperature sensor (Smart-Button; ACR Systems, Inc., Surrey, Canada) was placed on the floor at the center on the inside of each

ring to record temperatures at 1-min intervals. Temperatures were also recorded at 1-min intervals, outside the rings on both the first and third mill floors. Six sensors were placed next to the rings on each floor. Temperatures were recorded similarly inside and outside rings in the unheated floor of the old mill that served as the control treatment.

Six sanitation levels were simulated within these rings by adding a known quantity of wheat flour with 5% (by wt) brewer's yeast (flour from now on). Approximately 15, 38, 109, 388, 937, and 1,645 g of the flour were held in suitable size jars of 0.2–1.9-liter capacity. One hundred eggs or 100 unsexed adults of mixed ages of *T. castaneum* were added on top of the flour in separate jars. These jars were carried to the flour mill where contents were gently transferred from the jars into the bioassay rings to create flour depths of 0.1, 0.2, 1.0, 3.0, 6.0, and 10.0 cm, respectively. On each floor, six rings were used for eggs and six for adults. After introduction of flour and insects inside rings, the top ends of rings were closed with mesh screens with 0.6-mm² openings to prevent insect escape. To hold the mesh cover in place, Elmer's clay was used at the top end of each ring.

Heat Treatment. The pilot flour mill was subjected to a total of three, 24-h heat treatments between 2009 and 2010 using forced-air gas heaters that were fueled by propane (Brijwani 2011). The first, second, and third heat treatments were conducted during 13–14 May 2009, 25–26 August 2009, and 7–8 May 2010, respectively. The experiments to evaluate the impact of varying flour depths on mortality of *T. castaneum* eggs and adults were conducted during the second and third heat treatments ($n = 2$), because during the first heat treatment, preliminary experiments were done to establish and fine tune experimental procedures. All treatments were performed by a commercial heat treatment service provider (Temp-Air Inc., Burnsville, MN). Two THP-4500 heaters (Temp-Air, Burnsville, MN) with 1318.8 kW/h heating capacity and one heater with 410.3 kW/h heating capacity were used for each heat treatment (Brijwani 2011). The maximum discharge temperature at the outlet of the heaters was 93.3°C with a minimum discharge temperature of 60°C at the end of the heat treatment. The airflow rate for THP-4500 and THP-1400 heaters was 708 m³/min and 212.4 m³/min, respectively. The heaters were located outside the mill because of an open flame. The hot air from the heaters was channeled throughout the flour mill by means of 91.4- and 60.9-cm fabric ducts with 15.3-cm-diameter openings at regular intervals. These ducts were placed on the first to fifth floors and along both stairways. Heat distribution was facilitated by use of eight fans (Temp-Air Inc., Burnsville, MN) on each floor. The fan had a blade diameter of 91.4 cm, with an airflow rate of 311.5 m³/min. Fan locations were changed as the heat treatment progressed to ensure uniform heat distribution by eliminating cool spots (locations at $<50^\circ\text{C}$).

Sample Collection and Insect Mortality Assessment. At the end of each heat treatment, the rings from the first and third floors of the pilot flour mill (heat-

exposed) and from the fourth floor of the old flour mill (unexposed, control) were lifted off the floor and all of the flour with insects was gently collected into a pan with the help of a soft brush and immediately transferred into labeled polyethylene bags. In the laboratory the contents of each bag were transferred into 0.2–1.9-liter jars, labeled and incubated in the growth chamber at 28°C and 65% RH. Mortality of *T. castaneum* adults was determined 24 h after incubation, and expressed as a percentage based on number of dead adults out of the total exposed (100). The eggs were reared to the adult stage (45 d) in the growth chamber, and egg mortality was based on number of adults that failed to emerge out of the total exposed. Mortality of eggs and adults in the control samples was determined similarly.

Data Analysis. In the control treatment, mean \pm SE for minimum, maximum, and average temperature at different flour depths were summarized. The mean time-dependent temperature data at each flour depth for heat-exposed eggs and adults on the first and third floors of the pilot flour mill were plotted as a function of time to show variations in temperature profiles observed. Temperature data from six sensors placed outside the rings on the first or third mill floors were pooled for the second and third heat treatments. Differences in the mean \pm SE starting temperature, time (h) required to reach 50°C from the starting temperature, time (h) above 50°C, or the maximum temperature observed outside the rings between the first and third floor were determined using two-sample *t*-tests at the $\alpha = 0.05$ level (SAS Institute 2002). Inside the rings, the mean \pm SE starting temperature, time (h) required to reach 50°C, time (h) above 50°C, and the maximum temperature were determined by floor and insect stage for each flour depth. On first or third floor, significant differences ($\alpha = 0.05$) in each of these variables among flour depths was determined using one-way analysis of variance (ANOVA) and Ryan-Einot-Gabriel-Welch test (SAS Institute 2002). The mortality of *T. castaneum* eggs and adults at each flour depth on first and third mill floors were corrected for corresponding control mortality (Abbott 1925). The relationship between corrected mean mortality of *T. castaneum* eggs or adults and flour depth were described using regression models, where mortality of these stages was <100%. The corrected mean egg mortality as a function of flour depth was described by a linear regression ($y = a - bx$), whereas that of adults was described by an exponential decay model ($y = Ax^{-b}$) (SAS Institute 2002).

Results and Discussion

In the control (unheated) treatment, mean \pm SE temperatures inside rings with eggs and adults and on the floor next to the rings ranged from 26.0 \pm 2.5 to 26.9 \pm 2.6. In the control treatment the mean \pm SE mortality of *T. castaneum* eggs was 14.5 \pm 4.5 to 24.0 \pm 2.0%, whereas that of adults ranged from 0 to 1.5 \pm 1.5%. The egg mortality is generally around 10% (Sokoloff 1974), but 16% mortality is not uncommon

(Howe 1956). Xue (2010) reported *T. castaneum* egg mortality to be around 25%, which was the highest mortality we observed in life history studies. The egg mortality data includes not only the mortality of the egg stage but also that of the larval and pupal stages, because eggs were reared to adulthood.

The time-dependent temperature profiles within the rings on the first floor were more variable among flour depths than on the third floor, irrespective of the insect stage (Fig. 1). In general, irrespective of the floor and insect stage, the lowest temperatures were observed in rings with 10.0-cm deep flour. Temperatures did not reach 50°C in a majority of the rings on the first floor compared with the third floor. On the first floor, variation in temperature profiles among flour depths was smaller in rings with adults than in rings with eggs. This difference is probably a location effect, because the rings were placed \approx 40 cm from one another. Additionally, the distribution of heat by fans could have affected some of the differences in temperature profiles observed within the rings.

The starting temperatures observed on the first and third floors outside the rings before heat treatment of the pilot flour mill were \approx 30–31°C, and these minor differences were not statistically significant ($P > 0.05$) (Table 1). The time required to reach 50°C, time above 50°C, and the maximum temperature were significantly different between the third and first floors ($P < 0.05$). The third floor reached 50°C \approx 4 h faster than the first floor. As a result temperatures above 50°C were maintained for 4.7 h longer on the third floor compared with the first floor. The maximum temperature attained on the third floor was 6.6°C higher than on the first floor. Dean (1911) during a 24-h heat treatment and Goodwin (1912) during a 19.5-h heat treatment reported lower temperatures on the first floor of a mill than on floors above it. Goodwin (1912) measured temperatures in four to five different locations on each of three floors. Irrespective of the locations measured, average temperatures attained at the end of the heat treatment on the first, second, and third mill floors were 48, 56, and 58°C, respectively. Mahroof et al. (2003a) and Roesli et al. (2003) reported differences in temperatures attained in different locations of flour and feed mill floors, primarily because of vertical and horizontal stratification of temperatures.

On the first floor, mean \pm SE starting temperatures inside the rings tended to be lower with an increase in flour depth, but differences (df = 5, 6) were not significant among flour depths for eggs ($F = 2.47$; $P = 0.1508$) and adults ($F = 3.95$; $P = 0.0624$). Except for flour depths of 0.1- and 0.2 cm with eggs, on this floor, temperatures did not reach 50°C in any of the other flour depths. In the case of eggs, the time required to reach 50°C in 0.1-cm-deep flour was 1.6 times faster and temperatures above 50°C were held for 17 times longer than in 0.2-cm-deep flour. The maximum temperatures were inversely related to flour depth, and significant differences were not observed in rings with eggs ($F = 3.14$; df = 5, 6; $P = 0.0982$), but were apparent in rings with adults ($F = 104.80$; df = 5, 6; $P <$

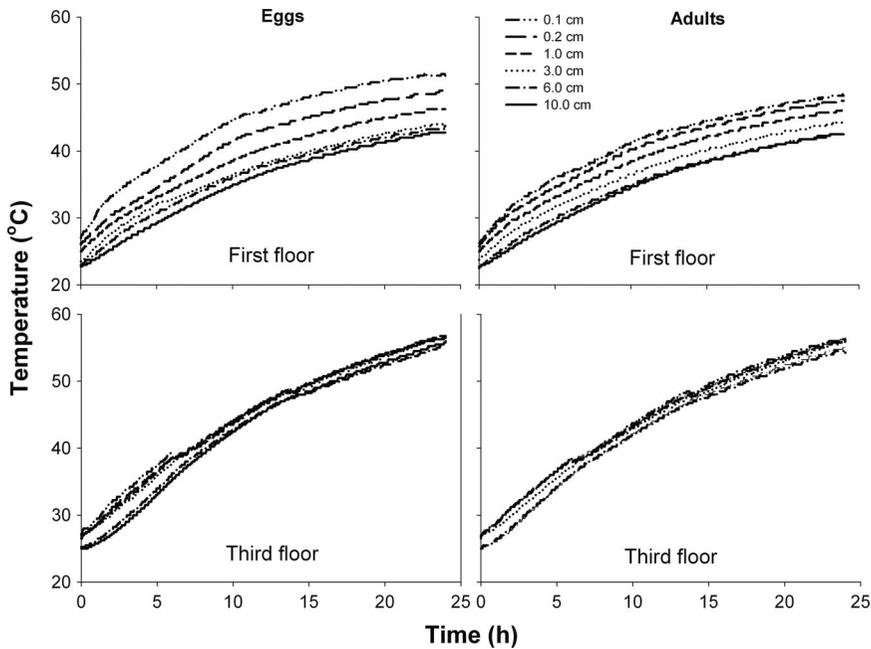


Fig. 1. Temperature profiles observed during 24-h heat treatments at flour depths of 0.1–10.0 cm in rings infested with *T. castaneum* eggs and adults placed on the first and third floors of a pilot flour mill. Each temperature profile represents a mean of two heat treatments.

0.0001). The slow heating of the first floor and significantly lower temperatures ($P < 0.05$) observed on this floor relative to the third floor combined with the insulating effect of flour resulted in temperatures not reaching 50°C in rings with 1.0–10.0-cm-deep flour. The low temperatures observed on the first floor could be because of loss of heat to the concrete foundation which is in contact with the first floor (Pepper and Strand 1935). Pepper and Strand (1935) reported an inverse relationship between concrete depth and temperatures attained. They reported that for every 1 cm below the surface of concrete the temperature dropped by 1°C.

Both the *T. castaneum* egg and adult mortalities were inversely related to flour depth (Table 2). The mean \pm SE mortality of eggs on the first floor at six flour depths ranged from 44.1 \pm 25.7 to 91.2 \pm 8.8% and that of the adults ranged from 1.0 \pm 0.0 to 94.4 \pm 5.6%. The egg mortality was satisfactorily described ($r^2 = 0.912$) by the linear regression. The mean \pm SE in-

tercept (a) and slope (b) values of the linear regression were 88.21 \pm 3.18 and 4.15 \pm 0.64, respectively. The adult mortality as a function of flour depth was best described ($r^2 = 0.972$) by an exponential decay model, and the mean \pm SE of parameter A was 21.75 \pm 4.28 and parameter b was 0.64 \pm 0.09. The inverse relationship between flour depth and mortality of *T. castaneum* eggs and adults shows the protective effect of flour on insect survival. This protective effect was linear for eggs because this stage is immobile. In contrast, adults are highly mobile and are capable of tunneling into flour (Hagstrum and Smittle 1980), thereby escaping the adverse effect of high temperatures. The highest adult mortality was observed in 0.1-cm-deep flour because there was very little flour (15 g) for the adults to tunnel down and escape the heat treatment. With increased flour depth more adults may have escaped higher temperatures by moving down in the flour toward the cooler floor. The dispersive behavior of *T. castaneum* by tunneling

Table 1. Temperature parameters (mean \pm SE) measured outside rings on the first and third pilot flour mill floors during heat treatment

Floor	Starting temp (°C)	Time to 50°C (h)	Time above 50°C (h)	Max temp (°C)
First	29.8 \pm 1.4	15.0 \pm 1.1	8.3 \pm 1.2	52.1 \pm 0.4
Third	30.2 \pm 1.1	11.7 \pm 0.3	11.7 \pm 0.3	58.3 \pm 0.8
<i>t</i> -value	-0.26	2.70	-2.70	-6.63
df	22.0	11.0 ^a	11.6 ^a	15.5 ^a
<i>P</i> value	0.8010	0.0206 ^b	0.0198 ^b	<0.0001 ^b

Each mean is based on $n = 12$; means on first floor for time to 50°C and time above 50°C were based on $n = 11$ because temperature in one sensor did not reach 50°C.

^a Variances between the two groups being compared were unequal ($P < 0.05$).

^b Significant ($P < 0.05$).

Table 2. Temperature parameters measured and corrected *T. castaneum* egg and adult mortalities (mean ± SE) in rings placed on the first floor during pilot flour mill heat treatment

Flour depth (cm)	Starting temp (°C) ^a	Time to 50°C (h)	Time above 50°C (h)	Max temp (°C) ^b	Mortality (%)
Eggs					
0.1	27.0 ± 1.0	6.6 ± 6.6	5.1 ± 5.1	51.5 ± 4.0	91.2 ± 8.8
0.2	26.0 ± 0.0	11.0 ± 11.0	0.3 ± 0.3	49.0 ± 1.5	89.4 ± 10.6
1.0	25.0 ± 0.0	– ^c	–	46.3 ± 0.3	78.3 ± 10.8
3.0	23.5 ± 1.0	–	–	44.0 ± 1.0	71.3 ± 19.2
6.0	23.0 ± 1.5	–	–	43.8 ± 1.3	70.6 ± 28.1
10.0	22.8 ± 1.8	–	–	42.8 ± 1.3	44.1 ± 25.7
Adults					
0.1	26.3 ± 0.3	–	–	48.5 ± 0.0a	94.4 ± 5.6
0.2	25.8 ± 0.8	–	–	47.5 ± 0.5a	59.6 ± 40.4
1.0	25.0 ± 0.0	–	–	46.3 ± 0.3b	31.2 ± 26.6
3.0	23.8 ± 0.8	–	–	44.3 ± 0.3c	2.5 ± 0.5
6.0	22.8 ± 1.3	–	–	42.5 ± 0.0d	1.0 ± 0.0
10.0	22.5 ± 1.0	–	–	42.5 ± 0.0d	7.1 ± 1.0

Each mean is based on *n* = 2.

^a Differences among flour depths were not significant for eggs (*F* = 2.47; *df* = 5, 6; *P* = 0.1508, one-way ANOVA) and adults (*F* = 3.95; *df* = 5, 6; *P* = 0.0624).

^b Differences among flour depths were not significant for eggs (*F* = 3.14; *df* = 5, 6; *P* = 0.0982), but significant for adults (*F* = 104.80; *df* = 5, 6; *P* < 0.0001). For adults, means followed by different letters are significantly different (*P* < 0.05; Ryan-Einot-Gabriel-Welch test).

^c Temperatures did not reach 50°C.

deeper into the flour may have resulted in mortality decreasing exponentially with an increase in flour depth or amount.

On the third floor, inside the rings, the starting temperatures, time to 50°C, time above 50°C, and the maximum temperature among flour depths varied very little (Table 3), and each of these variables was not significantly different (among flour depths in rings with eggs (*F*, range among variables = 0.07–0.75; *df* = 5, 6; *P* ≥ 0.6137) and adults (*F*, range = 0.24–3.42; *df* = 5, 6; *P* ≥ 0.0831). The time to 50°C took 15–17 h, and temperatures above 50°C were held for 6–8 h, and the maximum temperatures were between 55 and 57°C, and these minor differences did not show any trends among flour depths. Higher temperatures were observed on this floor compared with the first floor, because this floor is heated from both the top and

bottom. As mentioned previously, higher temperatures are generally recorded on floors above the first floor (Dean 1911, Goodwin 1912).

On the third floor, the mortality of eggs and adults was 100%, irrespective of the flour depth (Table 3). Complete control of eggs and adults on the third floor at all flour depths can be attributed to the high temperatures attained. At temperatures ≥54°C, susceptibility differences among *T. castaneum* life stages tend to disappear (Mahroof et al. 2003b).

Adults of *T. castaneum* were observed to be less susceptible than eggs only on the first mill floor. This finding is consistent with previous observations in replicated heat treatment trials that adults of *T. castaneum* are less susceptible to heat on the first floor of the pilot flour mill in bioassay boxes when compared with other life stages, including eggs (Brijwani 2011).

Table 3. Temperature parameters and corrected *T. castaneum* egg and adult mortalities (mean ± SE) in rings placed on the third floor during pilot flour mill heat treatment

Flour depth (cm)	Starting temp (°C) ^a	Time to 50°C (h) ^b	Time above 50°C (h) ^c	Maximum temp (°C) ^d	Mortality (%)
Eggs					
0.1	27.0 ± 1.0	15.0 ± 0.2	8.2 ± 0.4	56.5 ± 1.5	100.0 ± 0.0
0.2	26.0 ± 0.0	15.2 ± 0.4	7.8 ± 0.0	56.5 ± 1.5	100.0 ± 0.0
1.0	26.5 ± 0.5	15.3 ± 0.6	7.8 ± 0.3	56.8 ± 2.3	100.0 ± 0.0
3.0	23.5 ± 1.0	15.3 ± 1.8	7.3 ± 0.1	56.8 ± 2.8	100.0 ± 0.0
6.0	23.0 ± 1.5	16.9 ± 1.5	6.0 ± 0.8	55.3 ± 2.3	100.0 ± 0.0
10.0	22.8 ± 1.8	16.5 ± 1.7	6.4 ± 0.8	56.0 ± 2.5	100.0 ± 0.0
Adults					
0.1	26.5 ± 0.5	15.3 ± 0.3	8.2 ± 0.4	56.5 ± 1.5	100.0 ± 0.0
0.2	25.8 ± 0.3	15.7 ± 0.1	7.8 ± 0.1	56.3 ± 1.3	100.0 ± 0.0
1.0	27.0 ± 0.0	15.7 ± 0.3	7.9 ± 0.3	56.5 ± 1.5	100.0 ± 0.0
3.0	27.0 ± 0.0	16.2 ± 0.2	7.3 ± 0.1	56.3 ± 1.3	100.0 ± 0.0
6.0	25.0 ± 1.0	17.4 ± 0.7	6.0 ± 0.8	54.8 ± 1.8	100.0 ± 0.0
10.0	25.0 ± 1.0	17.1 ± 0.8	6.4 ± 0.8	55.3 ± 1.8	100.0 ± 0.0

Each mean is based on *n* = 2.

^a Differences among flour depths were not significant for eggs (*F* = 0.75; *df* = 5, 6; *P* = 0.6137; one-way ANOVA) and adults (*F* = 2.36; *df* = 5, 6; *P* = 0.1625).

^b Differences among flour depths were not significant for eggs (*F* = 0.42; *df* = 5, 6; *P* = 0.8208) and adults (*F* = 3.42; *df* = 5, 6; *P* = 0.0831).

^c Differences among flour depths were not significant for eggs (*F* = 0.49; *df* = 5, 6; *P* = 0.7714) and adults (*F* = 2.97; *df* = 5, 6; *P* = 0.1090).

^d Differences among flour depths were not significant for eggs (*F* = 0.07; *df* = 5, 6; *P* = 0.9947) and adults (*F* = 0.24; *df* = 5, 6; *P* = 0.9325).

In conclusion, the susceptibility of *T. castaneum* eggs and adults was inversely related to flour depths or sanitation levels, only when temperatures failed to reach at least 50°C. Although sanitation did not influence mortality of *T. castaneum* eggs and adults on the third floor where temperatures were at least 50°C and were held between 50 and 60°C for 13 h, it is important to remove flour accumulations to improve heat distribution and effectiveness against *T. castaneum* life stages.

Acknowledgments

Research reported here was supported by grants from USDA/CSREES (NIFA) Methyl Bromide Transitions Program grant under agreement number 2008-51102-04583, and from The Propane Education and Research Council (PERC), WA, DC. This paper is contribution number 11-311-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. 182–236. *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.
- Brijwani, M. 2011. Effect of sanitation on responses of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) life stages to structural heat treatments. M.S. thesis, Kansas State University, Manhattan.
- Dean, D. A. 1911. Heat as a means of controlling mill insects. *J. Econ. Entomol.* 4: 142–158.
- Dyte, C. E. 1965. Studies on insect infestation in the machinery of three English flour mills in relation to seasonal temperature changes. *J. Stored Prod. Res.* 1: 129–144.
- Dyte, C. E. 1966. Studies on the abundance of *Cryptolestes turcicus* (Grouv.) (Coleoptera, Cucujidae) in different machines of an English flour mill. *J. Stored Prod. Res.* 1: 341–352.
- Good, N. E. 1937. Insects found in the milling streams of flour mills in the south-western milling Area. *J. Kans. Entomol. Soc.* 10: 135–148.
- Goodwin, W. H. 1912. Flour mill fumigation. *Ohio Agric. Exp. Stn. Bull.* 234: 171–184.
- Goodwin, W. H. 1922. Heat for control of cereal insects. *Ohio Agric. Exp. Stn. Bull.* 354: 1–18.
- Hagstrum, D. W., and B. J. Smittle. 1980. Age- and sex-specific tunneling rates of adult *Tribolium castaneum*. *Ann. Entomol. Soc. Am.* 73: 11–13.
- Hosny, M. M., M. H. Hassanein, and A. H. Kamel. 1968. Ecological studies on *Anagasta kuehniella* and *Corcyra cephalonica* infesting flour mills in Cairo. *Bull. Soc. Entomol. Egypte* 52: 445–456.
- Howe, R. H. 1956. The effect of temperature and humidity on the rate of development and mortality of *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae). *Ann. Appl. Biol.* 44: 356–368.
- 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.
- Norman, C. S., S. J. DeCanio, and L. Fan. 2008. The Montreal Protocol at 20: ongoing opportunities for integration with climate protection. *Glob. Environ. Change* 18: 330–340.
- Pepper, J. H., and A. L. Strand. 1935. Superheating as a control for cereal-mill insects. *Montana State College Agric. Exp. Stn. Bull.* 297: 1–26.
- Rilett, R. O., and R. D. Weigel. 1956. A winter survey of Coleoptera in feed and flour mills. *J. Econ. Entomol.* 49: 154–156.
- 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.
- Sokoloff, A. 1974. The biology of *Tribolium*, with special emphasis on genetic aspects, vol. II. Oxford University Press, Ely House, London, United Kingdom.
- Wagner, G. B., and R. T. Cotton. 1935. Factors affecting insect abundance in flour mills. *Northwestern Miller* (November) 522–523.
- Xue, M. 2010. Development, relative retention, and oviposition of the red flour beetle, *Tribolium castaneum* (Herbst), on different starches. M.S. thesis, Kansas State University, Manhattan.

Received 8 April 2011; accepted 23 January 2012.