Structural heat treatments against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae): effect of flour depth, life stage and floor

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Abstract

The effect of high temperatures (50-60°C) and two levels of sanitation (~0.5 and 43 g of flour), on mortality of eggs, young larvae, old larvae, pupae, and adults of the red flour beetle, *Tribolium castaneum*, were evaluated during heat treatment of a pilot flour mill at Kansas State University. The mill was heated once during 13-14 May 2009 and once during 25-26 August 2009. Each of the heat treatments lasted 24 h. Bioassay boxes, with life stages of *T. castaneum* and temperature sensors confined in small compartments, were placed in 25 locations across all five mill floors. Temperature data showed that the mean time to 50°C based on the two treatments ranged from 10.39 to 17.18 h, and the mean time above 50°C ranged from 6.01 to 13.63 h. The mean maximum temperatures attained ranged from 50.7 to 61.4°C. In general, temperatures were lower in compartments with 43 g of flour when compared with compartments with 0.5 g of flour. Temperatures were also lower on the first floor than on the remaining floors. In box bioassays, essentially none of the life stages survived the 24 h heat treatment (99-100% mortality), except on the first floor. The survival of insects, especially on the first floor, is related to how quickly temperatures reached 50°C and how long temperatures were held between 50 and 60°C, and the maximum temperatures attained at a given location. There were only small differences in mortality between the two levels of sanitation. These results show that heat treatment of flour mills can control all life stages of *T. castaneum* in 24 h.

Keywords**:** *Tribolium castaneum*, Heat treatment, Sanitation, Life stages, Methyl bromide alternatives

1. Introduction

Extreme temperatures cause significant natural mortality in insect populations thus offering a potential tool that can be used as an environmentally benign management strategy (Hallman and Denlinger, 1998). Heat treatment of structures is an age-old technology for managing insects associated with grain-processing facilities such as flour mills (Dean, 1911). Heat treatment involves raising the ambient temperature of a food-processing facility to 50-60°C and holding these temperatures for 24 h or less to kill all life stages of stored-product insects (Dosland et al., 2006). Some companies have been known to extend this time to at least 36 h. There is renewed interest in heat treatments because methyl bromide, a structural fumigant, was phased out in the USA as of 2005 except for certain critical uses. The phase out of methyl bromide is related to its adverse effects on stratospheric ozone (Makhijani and Gurney, 1995).

Grain-processing facilities such as flour mills are dusty, because of continuous production. Flour beetles (*Tribolium spp.)* are the most abundant insect species in flour mills and their life stages are found throughout the milling process (Good, 1937). The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is a major pest of flour mills worldwide (Sinha and Watters, 1985; Hagstrum and Subramanyam, 2009).

Previous research at Kansas State University has shown that the susceptibility of stored-product insects to heat varies among species and within a species among the life stages (Mahroof et al., 2003a; b; Boina and Subramanyam, 2004). In general, it is now well recognized that the minimum temperature for effective disinfestation should be at least 50°C (Dosland et al., 2006). Very little is known about how sanitation influences effectiveness of heat in killing life stages of *T. castaneum*. Flour is a good insulator, and before heat treatments flour mill staff do extensive cleaning to remove flour that can serve as refugia for insects to escape the heat. Therefore, in the present investigation, mortality of eggs, young larvae, old larvae, pupae, and adults of *T. castaneum* during two heat treatments of a pilot flour mill were evaluated. Additionally, during the heat treatment of the flour mill, the effect of different sanitation levels on mortality of *T. castaneum* life stages was assessed.

2. Materials and methods

2.1. Pilot flour mill

The Hal Ross Flour Mill of the Department of Grain Science and Industry, Kansas State University, is a state-of-the-art pilot scale mill. It has five floors and a capacity of 9626 m^3. The mill is used for student and faculty research and teaching, and occasionally for use by grain industry stakeholders.

2.2. Box bioassays

Cultures of *T. castaneum* were reared on wheat flour plus 5% (by wt) Brewer's yeast diet at 28°C and 65% r.h. Eggs, young larvae, old larvae, pupae, and unsexed adults of mixed ages were collected from cultures following procedures described by Mahroof et al. (2003a;b). Each life stage was confined in plastic compartments inside a rectangular plastic box that was 27 cm long, 17.5 cm wide, and 4.2 cm high. There were a total of 12 small compartments within this box, and each compartment measured 8.3 cm long, 4.2 cm wide, and 3.7 cm deep. The top row of the compartments had 2 cm deep flour (43 g/compartment), and the bottom row had a dusting of flour (~0.5 g/compartment). Ten of the 12 compartments were used for confining insects and the other two were used for placing small sensors (SmartButton; ACR Systems Inc., Surrey, Canada) for measuring temperatures during heat treatment. In each compartment, 50 individuals of a life stage were introduced. Compartments with 0.5 g of flour represented or simulated "good" sanitation in food-processing facilities and the 43 g of flour simulated "poor" sanitation. Infested boxes were placed in 25 preselected mill locations. There were five boxes on each floor. Boxes among floors were placed within equipment or on the floor. The control bioassay box, with all life stages of *T. castaneum* was placed in a growth chamber in the laboratory at 28°C and 65% r.h. There were four such control boxes. After 24 h of heat treatment, all bioassay boxes were brought to the laboratory and incubated at 28°C and 65% r.h. The mortality of adults was measured 24 h after incubation at 28°C and 65% r.h. Immature stages were reared until emergence of adults and the number of adults counted.

2.3. Mill heat treatment

The structural heat treatments were conducted on 13-14 May 2009 and on 25-26 August 2009. Each treatment was performed for 24 h using forced-air gas heaters and the treatments were done by a professional heat treatment service provider (Temp-Air, Burnsville, MN, U.S.A.). Propane was used as the fuel for the gas heaters. Uniform distribution of heat was ensured by placing 8 fans on each of the five floors. The real-time temperature distribution during heat treatment within the mill was monitored by the facilitator using Temp-Air wireless sensors, and these data are not reported here.

Two SmartButton sensors were used in each bioassay box. One sensor was placed in a compartment with 0.5 g of flour and the other was used in the compartment with 43 g of flour. In compartments with 43 g of flour, the sensor was placed at a depth of 1 cm. Sensors recorded temperature every 2 min during a heat treatment. The 24 h time-dependent temperature profile for the two treatments was averaged by location and flour depth to determine time to reach 50°C, time above 50°C, and the maximum temperature.

2.4. Data analysis

The mean mortality data of *T. castaneum* life stages from the two treatments was subjected to factorial analysis of variance (ANOVA) to determine differences among the main (stage, flour depth, and floor) and interactive effects using the GLM procedure of SAS (SAS Institute, 2002). The relationship between mean mortality of each life stage and time to 50°C, time above 50°C, and maximum temperature was determined by subjecting data to the CORR procedure of SAS. All differences were considered significant at the $\alpha = 0.05$ level.

3. Results

The outside temperature during May and August within the bioassays ranged from 20.5 to 25.5°C and 24.5 to 33.5°C, respectively. During the May treatment 5,306 L of propane was consumed, and the total heat energy generated was 37,748 kW. During August treatment 4,889 L of propane was consumed, and the total heat generated was 34,782 kW. The total heat energy per cubic meter of the mill space per hour during the May and August treatment was 0.16 kW/m^3/h and 0.15 kW/m^3/h, respectively.

The mean time to reach 50^{o}C based on the two treatments ranged from 10.39 to 17.18 h (Table 1). Similarly, temperatures above 50^{o}C were maintained for a mean time period of 6.01 to 13.63 h. The mean maximum temperatures attained ranged from 50.6 to 61.4^{o}C. In general, the time to reach 50^{o}C took longer in compartments with 43 g of flour when compared with compartments with 0.5 g of flour (Fig. 1), whereas the time above 50^{o}C and maximum temperatures attained were slightly lower in compartments with 43 g of flour when compared with compartments with 0.5 g of flour. Temperatures in general were lower on the first floor than on the remaining floors above it.

Table 1 Summary of temperatures observed in bioassay boxes by mill floor based on May and August, 2009 heat treatments of a small flour mill.

	0.5 g of flour			43 g of flour		
Floor	**Time to 50^{o}C (h)**	**Time above 50^{o}C (h)**	**Maximum temp. (^{o}C)**	**Time to 50^{o}C (h)**	**Time above 50^{o}C (h)**	**Maximum temp. (^{o}C)**
First	17.17	6.85	51.95	17.18	6.01	50.65
Second	10.39	13.63	59.55	13.45	10.57	57.70
Third	10.54	13.49	61.35	12.20	11.83	60.05
Fourth	12.56	11.46	58.85	15.39	8.63	56.15
Fifth	12.02	11.99	57.70	14.94	9.07	55.35

Each mean is based on n = 2 replications.

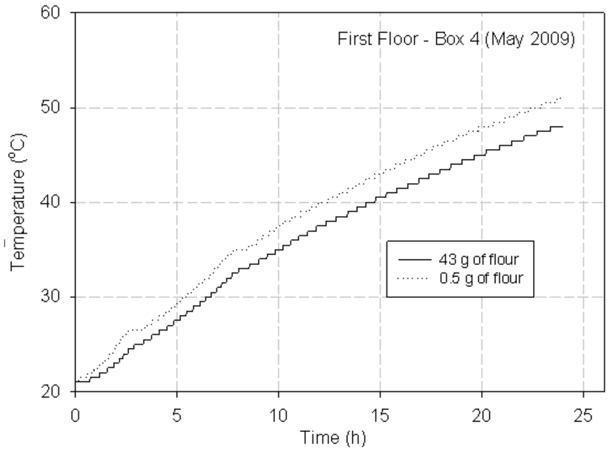

Figure 1 Temperature profiles in compartments with 0.5 g and 43 g of flour. These data are from a bioassay box placed on the first floor of the mill during May heat treatment.

The mean mortality of eggs in unheated compartments with 43 g of flour and 0.5 g of flour was 24 and 57%, respectively. High mortality (37%) was also observed with young larvae in compartments with 0.5 g of flour. Mortality of all other stages in unheated compartments ranged from 0 to 7%. The lack of food in compartments with 0.5 g of flour may have contributed to the high mortality observed.

The mortality of life stages of *T. castaneum* ranged from 99 to 100% on second through fifth floors of the mill (Table 2). On the first mill floor, only the egg stage was completely controlled, especially in compartments with 0.5 g of flour. Mortality of all other stages on this floor ranged from 51 to 99%, with greater survival in compartments with 43 g of flour than in compartments with 0.5 g of flour. In second through fifth floors, very few young larvae and occasionally old larvae in compartments survived, however in all these cases, mortality ranged from 99.4 to 99.8%.

Table 2 Mortality of *T. castaneum* life stages exposed to elevated temperatures during May and August, 2009 heat treatments of small flour mill.

Floor	Life stage	Mean mortality (%)	
		0.5 g of flour	43 g of flour
First	Egg	99.2	98.0
	Young larva	99.0	84.4
	Old larva	89.8	83.4
	Pupa	89.8	90.8
	Adult	90.0	50.6
Second	Egg	100.0	99.8
	Young larva	99.4	100.0
	Old larva	100.0	100.0
	Pupa	100.0	100.0
	Adult	100.0	100.0
Third	Egg	100.0	100.0
	Young larva	99.4	99.8
	Old larva	100.0	99.6
	Pupa	100.0	100.0
	Adult	100.0	100.0
Fourth	Egg	100.0	100.0
	Young larva	100.0	100.0
	Old larva	100.0	99.8
	Pupa	100.0	100.0
	Adult	100.0	100.0
Fifth	Egg	100.0	100.0
	Young larva	99.8	99.4
	Old larva	99.2	99.5
	Pupa	100.0	100.0
	Adult	100.0	100.0

Each mean is based on $n = 2$ replications.

Mortality observed in bioassay boxes varied significantly ($P < 0.05$) among *T. castaneum* life stages, floors, and between compartments with 0.5 and 43 g of flour. Except for stage x depth interaction, all other two and three way interactions were all significant (Table 3).

Table 3 Analysis of variance statistics showing factors affecting *T. castaneum* mortality.

Source of variation	df	Mean square	*F*-value	*P*-value
Stage	4	87.36	3.82	0.0088
Floor	4	614.39	26.84	<0.00001
Depth	1	146.41	6.40	0.0146
Stage*Floor	16	89.64	3.92	0.0001
Stage*Depth	4	53.21	2.32	0.0692**
Floor*Depth	4	147.03	6.42	0.0003
Stage*Floor*Depth	16	53.91	2.36	0.0108
Error	50	22.89		

**Not significant ($P > 0.05$); all other main and interactive effects are significant ($P < 0.05$).

The mortality of each life stage was inversely related to how quickly temperatures in box bioassays reached 50°C, but positively related to time above 50°C and the maximum temperature. All correlation coefficients were significantly different from 0 ($P < 0.05$), except the coefficients relating mortality of adults and young larvae and time to reach 50°C, and a coefficient relating young larvae and time above 50°C (Table 4).

Table 4 Correlation between percentage mortality of *T. castaneum* life stages and temperature variables.

Variables	Adult	Pupa	Old larva	Young larva	Egg
Time to 50°C (h)	-0.61 (0.060)	-0.76 (0.011)	-0.74 (0.014)	-0.52 (0.124)	-0.69 (0.027)
Time above 50°C (h)	0.68 (0.032)	0.78 (0.008)	0.78 (0.007)	0.59 (0.073)	0.75 (0.013)
Maximum temperature (°C)	0.74 (0.014)	0.85 (0.002)	0.86 (0.001)	0.66 (0.039)	0.81 (0.004)

Value in parenthesis is probability that the correlation coefficient is significantly different from zero. All *P*-values are significant ($P < 0.05$), except for the coefficient between adult/young larval mortality and time to 50°C, and for the coefficient between young larval mortality and time above 50°C.

4. Discussion

The mill at Kansas State University is a new facility that opened in 2006, and it is relatively an air tight building. Therefore, distributing the hot air by using fans was not as efficient, especially on the first floor which has large pieces of equipment which hindered air movement. Also, hot air rises up and this vertical stratification resulted in lower temperatures on the first floor. As a result mortality of *T. castaneum* life stages was less than 100% on this floor.

There were mortality differences among *T. castaneum* life stages, and most of these differences were obvious on the first floor. Laboratory tests at constant temperatures between 50 and 60°C (Mahroof et al., 2003b) revealed young larvae of *T. castaneum* to be the most heat-tolerant stage. However, during treatment of a pilot flour mill, where temperatures are dynamic (change over time), pupae were found to be the most heat-tolerant stage (Mahroof et al., 2003a). No consistent trend is obvious in our data, but on second through fifth floors, very few young larvae survived.

Significant differences among floors in mortality of life stages can be attributed to differences found between the first floor and all other floors. On second through fifth floors mortality was essentially between 99 and 100%. Differences in mortality between the two flour depths/quantities were more evident on the first floor, and generally mortality was slightly lower in compartments with 43 g of flour than in compartments with 0.5 g of flour. This effect is related to three factors. The mortality of each stage was influenced by how quickly temperatures reached 50°C, and the number of hours temperatures were held above 50°C within the 24 h window of time, and the maximum temperature.

In summary, data from the two heat treatments proved that heat is a viable alternative to methyl bromide, and effective heat treatments can be conducted within 24 h, provided lethal temperatures (50-60°C) are attained quickly. In our mill, lethal temperatures were attained within 15 to 16 h. Typically, lethal temperatures should be attained within 8 to 10 h so that these lethal temperatures can be held for 14 to 16 h for effective disinfestation. Our results also reinforce the need for sanitation in the mill environment for effective kill of *T. castaneum* life stages.

❖ Mention of product or trade names in this paper do not constitute an endorsement for its use by Kansas State University or USDA.

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References

Boina, D. A., Subramanyam, Bh., 2004. Relative susceptibility of *Tribolium confusum* life stages exposed to elevated temperatures. Journal of Economic Entomology 97, 2168-2173.

Dean, D. A., 1911. Heat as a means of controlling mill insects. Journal of Economic Entomology 4, 142-158.

Dosland, O., Subramanyam, Bh., Sheppard, K., Mahroof, R., 2006. Temperature modification for insect control. In: Heaps J.W. (Ed), Insect Management for Food Storage and Processing. 2nd Ed., AACC International. St. Paul, USA, pp. 89-103.

Good, N.E., 1937. Insects found in the milling streams of flour mills in the South-western milling Area. Journal of the Kansas Entomological Society 10, 135-148.

Hagstrum, D.W., Subramanyam, Bh., 2009. Stored-Product Insect Resource. AACC International, St. Paul, USA.

Hallman, G. J., Denlinger, D. L., 1998. Introduction: temperature sensitivity and integrated pest management. In: Hallman, G.J., Denlinger, D.L. (Eds), Temperature Sensitivity in Insects and Application in Integrated Pest Management , Westview Press, Boulder, USA, pp. 1-5.

Makhijani, A., Gurney. K. R.,1995. Mending the Ozone Hole: Science, Technology and Policy. MIT Press, Massachusetts.

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

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

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

Sinha R.N., Watters, F.L.,1985. Insect Pests of Flour Mills, Grain Elevators, and Feed Mills and Their Control. Agriculture Canada Publication 1776, Canadian Government Publishing Centre, Ottawa, Canada.