

EFFECT OF SANITATION ON RESPONSES OF *TRIBOLIUM CASTANEUM* (HERBST)
(COLEOPTERA: TENEBRIONIDAE) LIFE STAGES TO STRUCTURAL HEAT
TREATMENTS

by

MONIKA BRIJWANI

B.S., Dr. Y. S. P. University of Horticulture & Forestry, Solan, India, 1999
M.S., Central Food Technological Research Institute, University of Mysore, Mysore, India, 2002

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Grain Science & Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2011

Approved by:

Major Professor
Dr. Bhadriraju Subramanyam

Copyright

MONIKA BRIJWANI

2011

Abstract

Heat treatment involves raising the ambient temperature of food-processing facilities such as flour mills to 50-60°C for killing stored-product insects. However, very little is known about the influence of sanitation on responses of stored-product insects to structural heat treatments. The impact of sanitation on responses of life stages of the red flour beetle, *Tribolium castaneum*, an economically important pest in flour mills, were investigated during three 24 h structural heat treatments of the Kansas State University pilot flour mill. Two sanitation levels, dusting of wheat flour (~0.5 g) and 2-cm deep flour (~43 g), were created in 25 plastic bioassay boxes each holding 50 eggs, 50 young larvae, 50 old larvae, 50 pupae, and 50 adults of *T. castaneum* in separate compartments. Five boxes were placed on each of five floors of the pilot mill during 13-14 May 2009, 25-26 August 2009, and 7-8 May 2010 heat treatments using forced air gas heaters. During the August 2009 and May 2010 heat treatments, 100 eggs or 100 adults of *T. castaneum* were exposed inside each 20 cm diameter by 15 cm high PVC ring placed only on first and third floors and holding 0.1 (15 g), 0.2 (38 g), 1 (109 g), 3 (388 g), 6 (937 g), or 10 (1645 g) cm deep wheat flour. Among the mill floors, first floor had lower maximum temperature. The first floor rests on a thick concrete foundation, did not get heated from both sides unlike other floors, and had poor air movement resulting in cold pockets (temperatures <50°C). Mortality of life stages was lower on first floor than other floors and adults were less susceptible than other life stages especially on first floor. In general, both these tests have shown that the mortality of *T. castaneum* life stages were influenced by how quickly temperatures reached 50°C, how long temperatures were held above 50°C, and the maximum temperature. Protective effects of sanitation were evident only if temperatures did not reach 50°C. However,

removal of flour accumulations is essential to improve heat treatment effectiveness against all *T. castaneum* life stages during a 24 h treatment.

Table of Contents

List of Figures	vii
List of Tables	viii
Acknowledgements.....	ix
Chapter 1 - Insect management in flour mills using heat treatments.....	1
Abstract.....	1
Insects Associated with Flour Mills.....	2
Methyl Bromide Phase Out.....	4
The Use of Heat for Insect Management	6
Practical Heat Treatment of Flour Mills	9
References Cited.....	13
Chapter 2 - Influence of sanitation on temperatures attained and mortality of <i>Tribolium</i> <i>castaneum</i> life stages in a pilot flour mill subjected to heat treatments	18
Abstract.....	18
Introduction.....	19
Materials and Methods.....	21
Insect Cultures	21
Insect Bioassay Box	22
Pilot Flour Mill	22
Temperatures Monitoring of Mill Floors and in Bioassay Boxes.....	23
Heat Treatment.....	24
Insect Mortality Assessment	26
Heat Treatment Costs.....	26
Data Analysis	26
Results.....	28
Temperature Data Outdoors and Among Mill Floors.....	28
Temperature Data from Bioassay Boxes	28
Mortality of <i>T. castaneum</i> Life Stages in Unexposed and Heat-Exposed Bioassay Boxes..	31
Economic Analysis	32

Discussion.....	33
Acknowledgments	40
References Cited.....	41
Chapter 3 - Impact of varying levels of sanitation on mortality of <i>Tribolium castaneum</i> eggs and adults during heat treatments of a pilot flour mill	53
Abstract.....	53
Introduction.....	54
Materials and Methods.....	55
Insect Cultures	55
Pilot Flour Mill	55
Egg and Adult Bioassays	56
Heat Treatment.....	57
Sample Collection and Insect Mortality Assessment.....	58
Data Analysis	59
Results and Discussion	60
Acknowledgments	65
References Cited.....	66

List of Figures

- Figure 2.1 The 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 $n < 15$ 43
- Figure 3.1. Mean ($n = 2$) temperature profiles observed during 24-h heat treatments at flour depths of 0.1 to 10.0 cm in rings infested with *T. castaneum* eggs and adults placed on the first and third floors of a pilot flour mill. 69
- Figure 3.2. A linear regression model describing mean \pm SE ($n = 2$) egg mortality (A) and an exponential decay model describing adult mortality (B) of *T. castaneum* as a function of flour depth in rings placed on the first floor of a pilot mill subjected to heat treatment. 71

List of Tables

Table 2.1 Bioassay box locations among five floors of the Hal Ross flour mill	45
Table 2.2 Temperature parameters (mean \pm SE) observed at the floor level during heat treatment of the Hal Ross flour mill.....	47
Table 2.3 Mortality of <i>T. castaneum</i> life stages in unheated (control) bioassay boxes	48
Table 2.4 Mortality of <i>T. castaneum</i> life stages in bioassay boxes placed on the five floors of the Hal Ross flour mill subjected to heat treatment.....	49
Table 2.5 Itemized costs for each of the three heat treatments conducted in the Hal Ross flour mill	51
Table 3.1 Temperature parameters and <i>T. castaneum</i> egg and adult mortality (mean \pm SE) inside unheated (control) PVC rings at different flour depths	73
Table 3.2 Temperature parameters (mean \pm SE) outside the PVC rings during pilot flour mill heat treatment	75
Table 3.3 Temperature parameters and corrected <i>T. castaneum</i> egg and adult mortality (mean \pm SE) in PVC rings on the first floor at different flour depths during pilot flour mill heat treatment	76
Table 3.4 Temperature parameters and corrected <i>T. castaneum</i> egg and adult mortality (mean \pm SE) in PVC rings on the third floor at different flour depths during pilot flour mill heat treatment	78

Acknowledgements

I would like to express my heartfelt thanks to all the individuals whose contributions have made my work a success. First, I would like to express my sincere gratitude to my major advisor Dr. Bhadriraju Subramanyam for his constant encouragement, assistance, and valuable inputs throughout my graduate studies, and especially on this research project. It has been a great opportunity to work and learn from him. I am deeply thankful to Dr. Paul W. Flinn, USDA, Agricultural research Service, Center for Grain and Animal health Research, Manhattan, KS, and Dr. Michael R. Langemeier, Department of Agricultural Economics, K-State, for taking great interest on my research progress and encouraging me with ideas and analysis as members of my supervisory committee. I greatly appreciate and thank Dr. Mark West, USDA- Northern Plains Area, Boulder, CO, for help with statistical analysis. I would also like to thank Dr. Raj Hulasare, Temp-Air®, Burnsville, MN, for providing technical information on heat treatments. I am grateful to Ms. Michelle Hartzler for help with the bioassays. I also thank Mr. Sam Hanni for help with mill experiments.

I would also like to thank my laboratory mates for all the good times that I spent with them during my time here, and they include Jim Weaver, Alex Young, Meng Xue, Moses Khamis, Dr. Sara Savoldelli, Dr. Wat Chayaprasert, and Gustavo Correa. It was indeed an honor and privilege to work with the Department of Grain Science faculty, staff, and students. One of my cherished memories would be that I was a graduate student in this department when it was celebrating its centennial year.

My heartfelt gratitude and love goes to my parents Mrs. Sudesh Verma and Late Mr. S. P. Verma for inspiring me by setting their own example of living a beautiful life, and I feel so blessed to be your daughter. I gratefully thank my brother (Akhilesh) for his strong support and

care and putting a smile on my face with his cheerful talks. I thank my sisters (Meenu and Deepa) for their immense love and for being there when I needed them the most. To save the best for the last, I would like to thank my husband, Khushal, for believing in and nurturing my dreams with his love and patience and walking with me all the way through.

Lastly, this project would not be possible without the financial support from the 2008 USDA/CSREES (NIFA) Methyl Bromide Transitions Program and The Propane Education and Research Council (PERC), Washington, D.C.

Chapter 1 - Insect management in flour mills using heat treatments

Abstract

Tactics to manage insect infestations in grain-processing facilities historically included the use of heat treatments and the fumigant methyl bromide. The phase out of methyl bromide in the United States in 2005 because of its adverse effects on stratospheric ozone has generated renewed interest in heat treatments. Scientific literature published during the last decade shed new light on the following areas: differences in susceptibility among different stored-product insect species to heat; identification of a heat tolerant stage of a species based on stage-specific susceptibility at different constant elevated temperatures; confusion on determining a heat-tolerant stage during commercial heat treatments of grain-processing facilities; development and validation of a thermal death kinetic model to predict survival of insect life stages and determine the degree and duration of insect suppression obtained following a heat treatment intervention. It is well known that grain and grain products are poor conductors of heat, based on temperatures measured during facility heat treatments. However, data are lacking on the influence of sanitation on responses of stored-product insects to elevated temperatures, and this is a fruitful area for further research. The research reported here is designed to address this question in a Kansas State University pilot flour mill subjected to three heat treatments during 2009-2010, and the test insect is the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), major pest of flour mills worldwide.

Keywords methyl bromide alternative, heat treatment, sanitation, insect mortality

Insects Associated with Flour Mills

Flour mills are ideal habitats for supporting infestation by economically damaging stored-product insect pests, because of year-round warm temperatures and constant availability of patchy food resources (Wagner and Cotton 1935, Smallman and Loschiavo 1952). Several mill surveys have documented the presence of stored-product insect species in moving mill stock (Wagner and Cotton 1935, Good 1937), static mill stock, and within and around milling equipment (Dyte 1965, 1966; Hosny et al. 1968, Rilett and Weigel 1956). Insect infestations were detected in mills that were in operation (Buchelos 1980, 1981; Salmond 1956, Salama and Salem 1973, Campbell and Arbogast 2004) or no longer in use (Williams 1961). In the United States, Good (1937) surveyed 19 flour mills in Kansas, Missouri, and Oklahoma during 1934 to 1935 by taking 227-g (8-oz) samples monthly from 24 elevator boots and mill streams. He reported 30 different species from 17 of the 19 mills, representing 15 families in five orders. Four of the eight most abundant stored product insect species made up nearly 97.5% of the 74,175 insects found in the samples, and these species were *Tribolium* spp. (84.6% of the total insects), the square-nosed fungus beetle, *Lathridius (Laemophloeus) minutus* (L.); rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (8%); and cadelle, *Tenebroides mauritanicus* (L.) (3.2%). The lesser grain borer, *Rhyzopertha dominica* (F.); rice weevil, *Sitophilus oryzae* (L.); granary weevil, *Sitophilus granarius* (L.); and long-headed flour beetle, *Latheticus oryzae* (Waterhouse), each of these constituted less than 2% of the total insects found. Salmond (1956) sampled mill stocks stored in bags, bulk, and within machines in a flour mill in the United Kingdom on six different occasions from August 1948 to September 1949. He found 85 species of insects, representing the orders Coleoptera, Lepidoptera, Diptera, and Hymenoptera. The

Mediterranean flour moth, *Ephestia (Anagasta) kuehniella* (Zeller); broad-horned flour beetle, *Gnathocerus cornutus* (F.); and confused flour beetle, *Tribolium confusum* (Jacquelin du Val), were considered the most economically important species, because of their interference with the milling process and contamination of the finished products. Buchelos (1981), during October 1978 to November 1980, monitored stored-product Coleoptera in two mills in Greece by using adhesive strips (75 x 5 cm) attached vertically to a wall 1 m off the floor. He reported 23 different insect species, representing 13 different families. The five most abundant stored-product species reported were *T. confusum*; *S. oryzae*; *C. ferrugineus*; the red flour beetle, *Tribolium castaneum* (Herbst); and the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L). Campbell and Arbogast (2004) recently monitored stored-product insects inside and outside a flour mill by using both traps (commercial pitfall and sticky traps) and product samples during June 2001 to December 2003. They found 17 insect species, representing 12 families in 3 orders (Coleoptera, Hymenoptera, and Psocoptera). Captures of *P. interpunctella* adults in sticky traps and adults of the warehouse beetle, *Trogoderma variabile* (Ballion), were greater outside than inside the mill. However, captures of *T. castaneum* adults in pitfall traps were greater inside than outside the mill. They suggested movement of insects between the mill and the outside environment. Lack of an inbound inspection and keeping doors open are some of the factors contributing to re-infestation of the treated structures.

Suggested pest management methods for flour mills involve stock rotation (Banks 1986), sanitation (Chowaniec 1986, Foulk 1990), aspiration, use of impact machines (Stratil and Wohlgemuth 1989, Watters 1991), crack and crevice treatment with residual products, and exclusionary tactics such as closing doors, using air curtains near entrances, and screening

windows. Space treatments include the use of the fumigant methyl bromide and fogging with several approved insecticides (Subramanyam et al. 1993).

Methyl Bromide Phase Out

The United States is one of the signatories to the 1987 Montreal Protocol to phase out production and use of ozone-depleting substances to protect the stratospheric ozone. Protecting the stratospheric ozone is important for reducing the amount of UV-B radiation reaching the earth and for reducing the incidence of human skin cancers and cataracts (Makhijani and Gurney 1995). According to the 1990 Clean Air Act Amendments the United States should satisfy its obligations under the Montreal Protocol. Methyl bromide, is controlled under the Clean Air Act as a Class I ozone depleting substance, and was intended for phase out by 2005 (40 CFR Part 82, Federal register Vol. 69, No. 246). The schedule for phase out was as follows: 1993 to 1998, freeze production and net imports at 1991 base levels (25,500 metric tons); 1999 to 2000, 25% reduction from baseline levels; 2001 to 2002, 50% reduction from baseline levels; 2003 to 2004, 70% reduction from baseline levels; and 2005, 100% phase out, except for allowable exemptions such as critical use exemptions agreed to by the Montreal Protocol Parties. The United States Environmental Protection Agency (US-EPA) has established the critical use exemption (CUE) process to allow methyl bromide use for quarantine, pre-shipment, and certain industry groups, where technically and economically feasible alternatives are non-existent. The CUE process also provided adequate transition time for these groups to embrace and adopt methyl bromide alternative. The US-EPA has established a framework for assigning allowances for critical use of methyl bromide, and determining the quantities of exempted methyl bromide allowable under the Clean Air Act and the Montreal Protocol. In 2005, the US nominated about 536 metric tons of

methyl bromide for use in food- and feed-processing plants (40 CFR Part 82, Federal Register Vol. 69, No. 246, p. 76986), and the Montreal Protocol Parties reduced this amount to 483 metric tons, because of the availability of ProFume™, an alternative fumigant registered in January 2004 for use in processing facilities. Further reductions are anticipated in the amounts available for 2006 and beyond. The amount of methyl bromide nominated and approved by the Montreal Protocol Parties (United Nations Environmental Programme Methyl Bromide Transitions Options Committee, UNEP MBTOC) since methyl bromide phase out has been steadily declining over time. For example, in 2006, 2007, 2008, and 2009, the critical use nominations for methyl bromide granted were 32.03, 26.61, 21.0, and 19.5% of 1991 baseline levels, respectively. The actual quantities nominated for food-processing facilities in 2006, 2007, 2008, 2009, and 2010 were 529.6, 401.9, 362.9, 291.4, and 191.9 metric tons, respectively. The current nomination allowed in 2011 for use in the food-processing facilities is 135.3 metric tons.

Presently, critical use exemptions are allowed for rice millers in all locations of the US who are members of the US Rice Millers Association, pet food manufacturing facilities within US who are active members of Pet Food Institute, Kraft Foods in the US, and members of the North American Millers' Association, provided that the facilities being fumigated are older structures that cannot be properly sealed to use an alternative (ProFume™), or the presence of sensitive electronic equipment prohibits use of an alternative (ECO₂FUME™, which has phosphine that is corrosive to copper). The US-EPA also determined that the rates required to kill eggs of stored-product insects is cost-prohibitive with alternatives (ProFume™) than with methyl bromide. Therefore, heat appears to be a viable and cost-effective alternative to methyl bromide and other fumigant alternatives. The methyl bromide phase out has renewed interest in

the use of elevated temperatures or heat treatments, a technology that is 100 years old (Dean 1911, Fields and White 2002).

The Use of Heat for Insect Management

High temperatures were suggested as a practical means to manage insects associated with stored grain as early as 1762 (Monceau and Tillet 1962). Oosthuizen (1935) reviewed the early history of using high temperatures for stored-product insects. In France in the late 1800s, infested grain was exposed to high temperatures to kill insects in rooms equipped with special heating coils. Webster (1883) reported that larvae and pupae of *S. cerealella* were cooked when exposed to 60°C for 9 h. Lintner (1885) reported that eggs, larvae, and pupae of *T. castaneum* can be killed when exposed to 49 to 54°C whereas adults required 66°C. Goodwin (1914) reported that 13 stored-product insects in grain were killed after exposure for 1 to 2 h at 50 to 55°C and 40 to 50% RH. The germination of the seeds was not affected when 50 types of grains and legumes were exposed for 2 h at 70°C (de Ong 1919). These early studies did not provide enough detail to determine how the experiments were conducted and analyzed. It was Dean (1911) that first reported the use of heat for management of insects in flour mills. In a flour mill in Kansas subjected to a 7.5 h heat treatment from a steam heater, temperatures were measured at four locations on each floor, some in open areas and others in flour or in locations where flour would normally accumulate such as conveyer belts and spouts. The ambient mill temperature at the start of the heat treatment was 35.5°C. The highest temperature recorded on the first floor was 38°C, and the lowest was 34.4°C at the bottom of an elevator boot. On the second floor a temperature of 37°C was recorded at 8.89 cm in a sack of flour placed at 1.22 m above the floor, and the highest temperature recorded was 51°C. On the third floor temperatures ranged from 46°C in a

flour conveyer 1.83 m above the floor to 52°C in an open area. On the fourth floor a temperature of 42°C was recorded at 5.08 cm depth of flour in a conveyer belt; temperature in the open area was 48°C. As temperatures increased the relative humidity in the mill dropped from 93% to 27%. The mill had natural infestation of *T. confusum*. Visual inspections showed that insects on the first floor were not killed while the treatment was partially effective on the other floors. No data were presented on numbers of insects found. Therefore, observations on efficacy against insects are purely qualitative or, at best, speculative.

A second heat treatment was conducted in the same mill three weeks after the first treatment, and the heat treatment lasted 24 h (Dean 1911). The starting mill temperature was 32.2°C. On the first floor, temperature measured in an open area was 40.5°C and at a depth of 10.16 cm in wheat a temperature of 35.5°C was recorded. On the second floor the maximum temperature recorded was 56.4°C and temperature at 7.62 cm in a sack of flour that was 91.4 cm above the floor the temperature recorded was 47.5°C. The temperatures on the third floor ranged from 53.8 to 60.5°C. On the fourth floor the temperatures ranged from 47.8 to 53.3°C. The relative humidity for a majority of time was around 12%. After this treatment no live *T. confusum* were observed.

Dean (1913) reported that several flour mills in Ohio, Nebraska, Illinois, Iowa, Indiana, and southern Canada have embraced heat to control insects. He reported lethal temperatures to be around 47.8 to 50°C. No adverse effects were observed on floors, belts, and the mill machinery. Goodwin (1922) was the first to suggest using 50 to 60°C for insect control in mills. He reported that insects die at low temperatures in dry air than in humid air. He suggested conducting a heat treatment during warmer times of the year, and recommended cleaning the mill prior to heat treatment, removing sacks of flour, and disassembling pieces of equipment for

improving heat treatment effectiveness. Additionally, sealing the building gaps, to prevent cold air infiltration, was suggested to retain heat. Goodwin (1922) gave four methods for determining heat energy requirements. Oosthuizen (1935) examined effects of high temperatures on *T. confusum* life stages at different relative humidities. At 44°C, all 3-d-old eggs were killed in 19 h at 0% humidity, but complete mortality at 75% humidity took 24 h. At 46°C at 0, 30, and 75% humidity complete egg mortality occurred in 150 minutes whereas at 100% it took slightly less than 50 min. Also, at 46°C mature larvae did not pupate. He also found sex related differences in susceptibility at 44°C and 0% RH, with virgin females being less susceptible than males. At 44 and 46°C he reported pupae to be more tolerant than eggs, old larvae, and adults. However, at 48 and 50°C adults were more tolerant than eggs, old larvae, and pupae. The oviposition rate and fertility were adversely affected by exposure of larvae and pupae, while adverse reproductive effects in adults were temporary. At approximately 41°C effect on fertility was directly related to the exposure time. Oosthuizen (1935) also showed that when 150 g of flour of 10.2 cm flour depth was placed in a water bath at 10.2 cm depth at 51°C, about 2.5 h were needed for the center of the flour to reach the water bath temperature. This suggested flour to be a heat insulator or a poor conductor of temperature.

Pepper and Strand (1935) empirically measured temperatures attained at the surface and at various depths (2.54 to 22.86 cm) of a 0.372 m² concrete block that was 25.4-cm thick. The surface temperature attained by the block when 1.6 kW/h heat energy was applied for 10 h was 65.5°C. The temperatures were inversely related to the depth of concrete. For every 1-cm depth of the concrete the temperature dropped by 1°C. They reported higher temperatures at all depths when fans were used to circulate the hot air than in still air. Pepper and Strand (1935) measured temperatures in two mills subjected to a 12 and 20 h heat treatment. They showed temperature

differences between the north, center, and south ends of each of the mill floors. Generally, temperatures were lower on the first floor and second floor compared with other floors. Pepper and Strand (1935) suggested an ambient mill temperature of 32.2°C for conducting a heat treatment, to reduce the heat energy required. The target temperature during heat treatment was suggested to be around 54.4 to 60°C. Based on heat-loss calculation, Pepper and Strand (1935) suggested the heat energy requirement to be 0.86 kW/h/m³. Current heat treatments at commercial facilities are done using 0.10 kW/h/m³. The high heat energy required in the former case could be due to leaks in the mill or mills made predominantly of wood. Pepper and Strand (1935) suggested measuring temperatures at the floor level and the use of fans to distribute heat. They suggested that the maximum ceiling temperature should not exceed 71.1°C. In addition, the sprinkler heads installed should be able to withstand a temperature of 100°C. Pepper and Strand (1935) were also the first to report on structural damage due to heat treatments such as cracks in wooden spouts. Like Dean (1911) they did not show any data on the efficacy of heat treatments on insects associated with mills.

Practical Heat Treatment of Flour Mills

Heat treatment involves raising the ambient temperature of the whole or a portion of the facility to 50 to 60°C and maintaining these elevated temperatures for 24 to 36 h (Imholte and Imholte-Tauscher 1999, Dowdy and Fields 2002, Wright et al. 2002). In facility heat treatments, gas, electric, or steam heaters are used to slowly heat the ambient air. The long heat treatment period (24 to 36 h) is necessary for heat to penetrate wall voids and equipment to kill insects harboring in them. Facility heat treatments are labor intensive, because grain and grain products within the facility should be thoroughly cleaned and products removed as they are poor

conductors of heat. Insects may hide in these materials and escape the heat treatment. In the United States, many grain-processing companies such as General Mills, ConAgra, Cargill Inc., Kraft Foods, Quaker Oats (PepsiCo), New World Pasta, and Nestle Purina, among others, have been using heat treatments, on a limited basis, as an alternative to methyl bromide for more than a decade.

During facility heat treatments, horizontal and vertical stratification of temperatures (Roesli et al. 2003, Mahroof et al. 2003a) results in non-uniform heating of various portion of the facility. Therefore, fans are used in strategic locations to minimize temperature stratification, and to uniformly heat facilities to ensure complete insect kill. The placement of fans is the art part of heat treatment, and during heat treatments, fans need to be moved to improve heat circulation and to eliminate cool spots (locations at $<50^{\circ}\text{C}$). This can only be accomplished with temperature monitoring throughout the heat treatment period.

The effectiveness of heat treatments against insects is determined by using specific life stages of stored-product insects in plastic vials or containers with some food (Mahroof et al. 2003a). An indirect method involves using traps with lures or food-baits to determine insect population numbers, primarily of adults, before and after a heat treatment. This method would not only indicate the degree of suppression obtained after a heat treatment but also indicate the duration of effectiveness of a single heat treatment (Roesli et al. 2003).

Mahroof et al. (2003a) exposed eggs, young larvae, old larvae, pupae, and adults of *T. castaneum* during heat treatment of Kansas State University pilot flour and feed mills. There were no clear cut trends of a particular life stage being heat tolerant, but a few adults and pupae survived the heat treatment compared with the other stages. However, in the laboratory at constant temperatures, Mahroof et al. (2003b) showed that the young larvae were more heat-

tolerant than the other stages. Boina and Subramanyam (2004) showed the old larvae of *T. confusum* to be heat-tolerant based on tests in the laboratory at constant temperatures. A thermal death kinetic model was developed and validated to predict survival of *T. confusum* old larvae during facility heat treatments (Boina et al. 2008). This model can be used to predict survival of old larvae of *T. confusum* based on time-dependent temperature data. Mahroof and Subramanyam (2006) showed the old larvae (fifth instars) of *P. interpunctella* to be the heat tolerant stage, whereas in *L. serricornis*, eggs were found to be the heat-tolerant stage (Yu et al. 2011). These two studies were also conducted at constant temperatures. In the drug store beetle, *Stegobium paniceum* (L.), the young larvae were found to be heat-tolerant based on tests at constant temperatures (Hulasare et al. 2010).

Identification of the heat tolerant stage is important, because these stages should be used in bioassays during facility heat treatments. Controlling the heat tolerant stage should ensure control all other stages; however, this assumption may not always be true. At 50 to 60°C the young larvae of *T. castaneum* appears to be the most heat-tolerant of the species studied to date. Mahroof et al. (2003a) found young larvae to be susceptible during actual heat treatment of facilities whereas laboratory tests showed this stage to be more tolerant than eggs, old larvae, pupae, and adults. Similarly, Yu et al. (2011) were unable to find a heat tolerant stage of *L. serricornis* during heat treatment of a commercial facility. Such anomalous results can be a result of heat tolerance being influenced by heating rates (Beckett et al. 2007), and the influence of heating rates on stage-specific tolerance has been well studied during thermal disinfestations of stored grains (Beckett and Morton 2003) and not during heat treatments of structures. The impact of varying heating rates on heat tolerance among stages of a given insect species during heat treatments is a fruitful area for further research.

Roesli et al. (2003) used adult trapping data to indirectly determine susceptibility of different stored-product insect species. Following a heat treatment of a feed mill, adults of *L. serricornis* were not captured in traps; however adults of *T. castaneum* were captured within 2-4 weeks. Trap capture data can indirectly indicate which of the species is more susceptible to high temperatures.

Previous research has shown that flour is a poor conductor of temperature. However, there are no studies on the influence of sanitation or flour accumulations on efficacy of heat treatments against stored-product insects. Therefore, experiments were conducted in 2009 and 2010 during three heat treatments of the Hal Ross flour mill at Kansas State University, Manhattan, Kansas, to evaluate the influence of sanitation on heat treatment effectiveness against life stages of *T. castaneum*—a pest that is of economic importance in flour mills.

Specific objectives of this research were:

1. To evaluate the efficacy of three heat treatments of the Hal Ross flour mill at Kansas State University and two simulated sanitation levels against eggs, young larvae, old larvae, pupae, and adults of *T. castaneum*.

2. To evaluate the influence of two heat treatments and six flour depths on mortality of eggs and adults of *T. castaneum* on two mills floors.

3. To determine the economics of three heat treatments of the Hal Ross flour mill.

References Cited

- Banks, H. J. 1986.** Impact, physical removal and exclusion for insect control in stored products, pp. 165-184. *In* E. Donahayae and S. Navarro (eds.), Proceedings of 4th International Working Conference on Stored-Product Protection. Tel Aviv, Israel.
- Beckett, S. J., and R. Morton. 2003.** Mortality of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) at grain temperatures ranging from 50°C to 60°C obtained at different heating rates in a spouted bed. *J. Stored Prod. Res.* 39: 313-332.
- 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, U. K.
- Boina, D. A., and Bh. Subramanyam. 2004.** Relative susceptibility of *Tribolium confusum* life stages exposed to elevated temperatures. *J. Econ. Entomol.* 97: 2168-2173.
- Boina, D. A., Bh. Subramanyam, and S. Alavi. 2008.** Dynamic model for predicting survival of mature larvae of *Tribolium confusum* during facility heat treatments. *J. Econ. Entomol.* 101: 989-997.
- Buchelos, C. T. 1980.** Moth populations at a typical flour mill. *Annls. Inst. Phytopath. Benaki, (N.S.)* 12: 188-197.
- Buchelos, C. T. 1981.** Coleoptera populations at flour mills and related areas. *Annls. Inst. Phytopath. Benaki, (N.S.)* 13: 6-29.
- Campbell, J. F., and R. T. Arbogast. 2004.** Stored-product insects in a flour mill: population dynamics and response to fumigation treatments. *Entomol. Exp. Appl.* 112: 217-225.

- Chowaniec, T. 1986.** Sanitation costs—the bottom line. Assn. Operative Millers Bull., December, pp. 4867-4871.
- de Ong, E. R. 1919.** Effect of excessive sterilization measures on the germination of seeds. J. Econ. Entomol. 12: 343-345.
- Dean, G. A. 1911.** Heat as a means of controlling mill insects. J. Econ. Entomol. 4: 142-158.
- Dean, G. A. 1913.** Milled and stored grain insects. Kansas State Agri. College. 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.
- 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.
- Fields, P. G., and N. D. G. White. 2002.** Alternatives to methyl bromide treatments for stored-product and quarantine insects. Annu. Rev. Entomol. 47: 331-359.
- Foulk, J. D. 1990.** Pest-related aspects of sanitation audits. Pest Control Tech. 18: 32.
- Good, N. E. 1937.** Insects found in the milling streams of flour mills in the southwestern milling area. J. Kansas Entomol. Soc. 10: 135-148.
- Goodwin, W. H. 1914.** Some factors affecting results in the use of high temperature for the control of insects injuring cereal products. J. Econ. Entomol. 7: 313-322.
- Goodwin, W. H. 1922.** Heat for control of cereal insects. Ohio Agr. Expt. Sta. Bull. 354: 1- 18.

- 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.
- Hulasare, R., Bh. Subramanyam, P. G. Fields, and A. Y. Abdelghany. 2010.** Heat treatment: a viable methyl bromide alternative for managing stored-product insects in food-processing facilities, pp. 661 - 667. In M. O. Carvalho, P. G. Fields, C. S. Adler, F. H. Arthur, C. G. Athanassiou, J. F. Campbell, F. Fleurat-Lessard, P. W. Flinn, R. J. Hodges, A. A. İşikber, S. Navarro, R. T. Noyes, J. Riudavets, K. K. Sinha, G. R. Thorpe, B. H. Timlick, P. Trematerra, and N. D. G. White (eds.), Proceedings of the 10th International Working Conference on Stored-Product Protection, 27 June - 2 July, 2010, Estoril, Portugal. Julius-Kühn-Archiv, 425, 2010, <http://pub.jki.bund.de/index.php/JKA/issue/view/719>.
- Imholte, T. J., and T. Imholte-Tauscher. 1999.** Engineering for food safety and sanitation. Technical Institute of Food Safety, Woodinville, Washington DC.
- Lintner, J.A. 1885.** *Tribolium ferrugineum* (Fabr.) 2nd Reprint. Injur. and other Insects of New York, pp. 136-139.
- 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.
- Makhijani, A., and K. R. Gurney. 1995.** Mending the ozone hole: science, technology and policy. MIT Press, Cambridge, MA.
- Monceau, D. H. L., and M. Tillet. 1762.** Histoire d'un insect qui devore les grains de l'Angoumois, avec les moyens que l'on peut employer pour le detruire. Paris. pp. 314.
- Oosthuizen, M. J. 1935.** The effect of high temperature on the confused flour beetle. University of Minnesota Agri. Expt. Sta. Bul. 107:1-45.
- Pepper, J.H., and A. L., Strand. 1935.** Superheating as a control for cereal-mill insects. Montana State College Agri. Expt. Sta. Bul. 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.
- Salama, H. S., and S. A. Salem. 1973.** Seasonal fluctuations in the populations of insect fauna in flour mills. *Z. Angew. Entomol.* 74: 160-169.
- Salmond, K. F. 1956.** The insect and mite fauna of a Scottish flour mill. *Bull. Entomol. Res.* 47: 621-630.
- Smallman, B. N., and S. R. Loschiavo. 1952.** Mill sanitation studies. I. Relative susceptibilities of mill stocks to infestation by the confused flour beetle. *Cereal Chem.* 29: 431-440.

- Stratil, V. H., and R. Wohlgemuth. 1989.** Untersuchungen zum Wirkungsmechanismus von Prallmaschinen auf vorratsschädliche Insekten. *Anz. Schadlinskd., Pflanzen-schutz, Umweltschutz.* 62: 41-47.
- Subramanyam, Bh., J. Heaps, and P. Harein. 1993.** Insecticide recommendations for food-processing plants. University of Minnesota Extension Service FS-3921-A.
- Wagner G. B., and R. T. Cotton. 1935.** Factors affecting insect abundance in flour mills. *Northwestern Miller*, November, pp. 522-523.
- Watters, F. L. 1991.** Physical methods to manage stored-food pests, pp. 399-414. *In* J. R. Gorham (ed.), *Ecology and management of food-industry pests.* Association of Official Analytical Chemists, Arlington, VA.
- Webster, F. M. 1883.** Observations on the Angoumois grain moth and its parasites. 12th Report of the State Entomologist on Noxious and Beneficial Insects of Illinois, pp. 144-154.
- Williams, G. C. 1961.** Infestation of a disused flour mill. *Milling*, January, pp.92.
- 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 serricornis* (F.) life stages to elevated temperatures used during structural heat treatments. *J. Econ. Entomol.* 104: 317-324.

Chapter 2 - Influence of sanitation on temperatures attained and mortality of *Tribolium castaneum* life stages in a pilot flour mill subjected to heat treatments

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 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 \pm SE mortality of *T. castaneum* life stages on the first floor was 55.5 ± 12.9 to $98.6 \pm 0.8\%$; it was 93.2 ± 6.7 to $100 \pm 0.0\%$ 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 \$ 25,605 to 38,005. An effective heat treatment can be conducted within 24 h, provided the mill is sanitary and temperatures reach 50°C and are held above 50°C for several hours.

Keywords heat treatment, temperatures, sanitation, insect mortality, economics

Introduction

The use of high temperatures or heat treatments for disinfecting 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 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 to 50°C for any length of time. These observations have been empirically validated recently by several researchers (Wright et al. 2002, Dowdy and Fields 2002, Mahroof et al. 2003a, b; Roesli et al. 2003, Boina and Subramanyam 2004), who reported the temperatures for effective disinfestations to be in the range of 50 to 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. For example, except for the first floor, temperatures on second, third, and fourth floors ranged from 47.5 to 60°C during a 24-h treatment, but on these same three floors temperatures of 37 to 52°C were observed during a 7.5-h treatment. 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 when compared with floors above it. Both authors observed 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.

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 long, 4.5 cm wide, and 1.5 cm high) during heat treatments of pilot flour and feed mills at Kansas State University, Manhattan, KS. In the feed mill, mortality of old larvae and pupae at the end of the heat treatment ranged from 30 to 50% only at two of the 10 locations. In 9 out of the 10 locations in the flour mill mortality of pupae was 78 to 95%, while that of other stages was 97 to 100%. No consistent trends were observed among life stages in their susceptibility to heat, despite temperatures being $\geq 50^{\circ}\text{C}$ in a majority of the locations. 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 since 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 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 two to four weeks after a heat treatment on the first floor than floors above it. 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, and/or insects being brought in on infested raw materials (Dean 1911, Roesli et al. 1993).

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 the fact that flour is a poor conductor of heat. The influence of sanitation or depth of flour on efficacy of heat treatments against life stages of stored-product insects in general, and *T. castaneum* in particular, is unknown.

In the replicated trials reported here, temperatures attained on different mill floors and influence of sanitation (depth of flour) 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.

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-L 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- μ m 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 retain the eggs. To obtain young larvae (first instars), infested containers were sifted after 6 d using a 250- μm sieve. Old larvae (sixth to seventh instars), pupae, and mixed age adults were separated using an 841- μm sieve directly from culture jars.

Insect Bioassay Box

Individual stages of *T. castaneum* were exposed to heat treatment in a rectangular clear plastic box that was 27 cm long, 17.5 cm wide, and 4.2 cm high with 12 compartments (Subramanyam 2010). Each compartment measured 8.3-cm long, 4.2-cm wide, and 3.7-cm deep. 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 m³. 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 one on the west side leads from the first floor to the roof while one 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 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 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 the milling equipment, predominantly made of steel, among the five mill floors is approximately 123-metric tons. The maximum daily flour mill production capacity is 18.2- metric tons or 400 hundred weight (cwt).

Five insect boxes were placed at various locations on each of the five floors in the flour mill (Table 2.1). 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.

Temperatures 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 8 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 $\leq 25\%$ (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, Canada). Two sensors were placed in each bioassay box to record temperatures every two minutes 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, 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 were used to heat the facility. Two heaters (THP-4500), with a maximum heating capacity of 1318.8 kW/h (4.5 million BTU/h), and one heater (THP-1400) 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 towards the end of the heat treatment. All gas heaters were fueled by two 3,785-L propane tanks filled to 80% of the total capacity. Liquid propane from each tank passed through a vaporizer before it was utilized by the heaters. The THP-4500 and THP-1400 heaters can utilize 183.9 and 57.1 L/h of propane, respectively. 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 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, Burnsville, MN), 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 m³/min. The fan power requirements were 115V, 60 Hz, 8 Amps, and 1 Phase. Power load centers with a power capacity of 460V, 60 Hz, 60 Amps, 3 Phase were used to plug in the fans. A total of 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. Percentage 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. The fixed costs for each heat treatment comprised of the forklift rental, generator rental, diesel usage 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 US \$1,000/d for 3 d, which included set up prior to heat treatment, monitoring the heat treatment, tear down at the end of heat treatment, and the travel time. The variable costs included a nominal consulting fee for the Temp-Air's supervisor, and quantity of the 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 (ANOVA) and Fisher's protected least significant difference (LSD) 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 mean \pm 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 mean \pm SE 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 x flour depth was sliced by stage using the GLIMMIX procedure. All statistical differences were considered significant at the $\alpha = 0.05$ level.

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 32 to 87%, respectively. During the 25-26 August 2009 treatment, temperatures ranged from 21 to 34.2°C and the humidity from 46 to 89%. Temperatures 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 \pm 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 2.2). Temperatures among mill floors were held above 50°C for 10.1 ± 1.4 to 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), and 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 ($t = -0.98$ to 1.27; $P \geq 0.2276$),

fourth ($t = -1.69$ to -0.21 ; $P \geq 0.1140$), and fifth ($t = -0.64$ to 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 \pm 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 to 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 \pm SE ($n = 15$, except for 2-cm deep flour treatment on the first floor where $n = 14$), starting temperatures observed among mill floors in compartments with dusting of flour ranged from $24.7 \pm 1.3^{\circ}\text{C}$ (first floor) to $25.9 \pm 0.6^{\circ}\text{C}$ (third floor), and in compartments with 2-cm deep flour the temperatures ranged from $23.5 \pm 0.9^{\circ}\text{C}$ (first floor) to $25.3 \pm 0.5^{\circ}\text{C}$ (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 Figure 2.1. On the first floor, the time to reach 50°C was longest in compartments with dusting of flour (mean \pm SE, 15.5 \pm 1.7 h), whereas it was longest in compartments with 2-cm deep flour on the fourth (16.2 \pm 1.4 h), followed by those in the first floor (15.7 \pm 2.1 h). In general, compartments with 2-cm deep flour took 2 to 5 h longer to reach 50°C than those with dusting of flour. Also, 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. For example, 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 failed to reach 50°C, respectively.

Generally, temperatures were held above 50°C for 0.3 to 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 \leq 6.6 h on the first floor compared to 7.1 to 13.7 h in second through fifth floors. The maximum temperatures attained ranged from 53.9 \pm 1.7°C to 60.0 \pm 0.7°C between the sanitation levels and among mill floors. In compartments with 2-cm deep flour, temperatures were 0.5 to 2.0°C lower than those with dusting of flour. Irrespective of the sanitation level, the maximum temperatures attained were lower in compartments on the first floor by 1.0 to 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 \pm SE mortality of old larvae, pupae, and adults in bioassay boxes that were not exposed to the heat treatment (control treatment) was $\leq 1.7\%$, but that of eggs and young larvae ranged from 12.0 ± 3.5 to $48.0 \pm 6.1\%$ (Table 2.3). Eggs and young larvae in compartments with dusting of flour had higher mortality than those in 2-cm deep flour.

The mean \pm SE mortality of each *T. castaneum* life stage at the two sanitation levels ranged from 55.5 ± 12.9 to $98.6 \pm 0.8\%$ on the first floor (Table 2.4). On the first floor, 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 $100.0 \pm 0.0\%$. The two exceptions were on the fourth floor where the mortality of old larvae and adults in 2-cm deep flour was $93.2 \pm 6.7\%$ and $95.6 \pm 4.4\%$, respectively. The mortality of different stages on second through fifth floors was 98 to 100%, so it was statistically impractical to compare floors or sanitation levels. 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 were 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 to the May 2009 and May 2010 treatments (Table 2.5), because temperatures were warmer in August relative to May. The propane cost was six 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 was also applied for delivery and pickup of the propane tank for each treatment. All other fixed charges for each heat treatment were for Temp-Air 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 customized fabric ducts was a one-time cost, and this was included for the very first heat

treatment. The same ducts were used for the next two treatments, and hence, the treatments costs for August 2009 and May 2010 were less. The \$12,000 for service personnel per heat treatment was for 4 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 supervisor from Temp-Air was compensated for time spent in setting-up and monitoring the heat treatment, and these charges varied by heat treatment and ranged from \$687 to \$2,613 per treatment. 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 \$25,605 to 38,005, or \$2.66 to \$3.95/m³ interior building space.

Given that 7.12 kW/h of 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 37,750, 34,787, and 39,182 kW/h or 0.16, 0.15, and 0.17 kW/h/m³, respectively.

Discussion

The mill has its own boiler which 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, and 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. Additionally, 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 when compared with other floors. The reason for this may be that the first floor rests on a foundation made up of 1,486.2-metric tons of concrete, and unlike the second through fifth floors, is not heated from both sides (top and bottom). A linear regression analysis of Pepper and Strand's (1935) data on heating of a 0.372 m² concrete block of 25.4 cm thickness using 1.57 kW/h of heat for 10 hours, 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 \pm SE y-intercept of the regression was 62.98 \pm 0.48; the slope of -1.03 \pm 0.43, indicated for every 1-cm depth of concrete below the surface the temperature dropped by \sim 1°C.

Another plausible reason is that the low maximum temperature observed could be due to loss of heat to unheated rooms on the first floor (office rooms and the mill control room). 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 to 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 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 while 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* life stages in the control treatment was high for eggs and young larvae, especially in compartments with dusting of flour, because very little food was available for these stages to survive to the adult stage. The corrected mortality of life stages was 93 to 100% on second through fifth floors, except for the first floor where all stages survived. The survival of all stages was statistically related to the maximum temperature, which in general, was lower on the first floor compared to 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 to other floors.

In the current study, 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 $\geq 82\%$. 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 (LT₉₉). Heat tolerance among insect life stages of *T. castaneum*, the 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 is also 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 time periods, 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 to 60°C (Mahroof et al. 2003b). However, as mentioned above, 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 to 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 to 0.10 kW/h/m³ (Bh. S., unpublished data), but in our pilot flour mill, the heat energy used was 1.5 to 2.4 times greater than this figure (0.15 to 0.17 kW/h/m³). 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 cost for the first heat treatment was higher, primarily because of the extra expense incurred for the fabric ducts. 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 pre-installed 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 utilized would be the same. The fuel costs, in addition to being related to differences in outside and inside temperatures, were also related to temperature monitoring data. The service provider used 76 to 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 these were provided gratis by the service provider. The cost of the first heat treatment was about \$4.00/m³ of the facility while that of the other two was \$3.00/m³. This is the first report itemizing heat treatment costs of three heat treatments in the same facility.

In summary, the results presented in this study show that effective heat treatments can be conducted within 24 h, provided temperatures reach 50°C and are held above 50°C for at least 12 hours, 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. This finding

reinforces the need to conduct thorough sanitation prior to 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 to 4.00/m³ observed in this study was slightly higher compared to commercial facilities, because the mill was air-tight and the lack of free air movement did not allow mill floors to reach 50°C within a short period of time. 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 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 number 2008-51102-04583. The Propane Education and Research Council (PERC), Washington, D. C., underwrote all heat treatment expenses, including a hands-on heat treatment workshop (http://www.ksre.ksu.edu/grsc_subi/Conference/conference.htm). This paper is contribution number 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 structure 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, UK.
- Boina, D. A., 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.** Milled and stored grain insects. *Kansas State Agri. College 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. Kansas Entomol. Soc.* 10: 135-148.
- Goodwin, W. H. 1912.** Flour mill fumigation. *Ohio Agri. Expt. Station 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. Amer.* 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. Montana State College Agri. Expt. Station 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 Journal, Fourth Quarter, pp. 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.

Figure 2.1 The 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 $n < 15$.

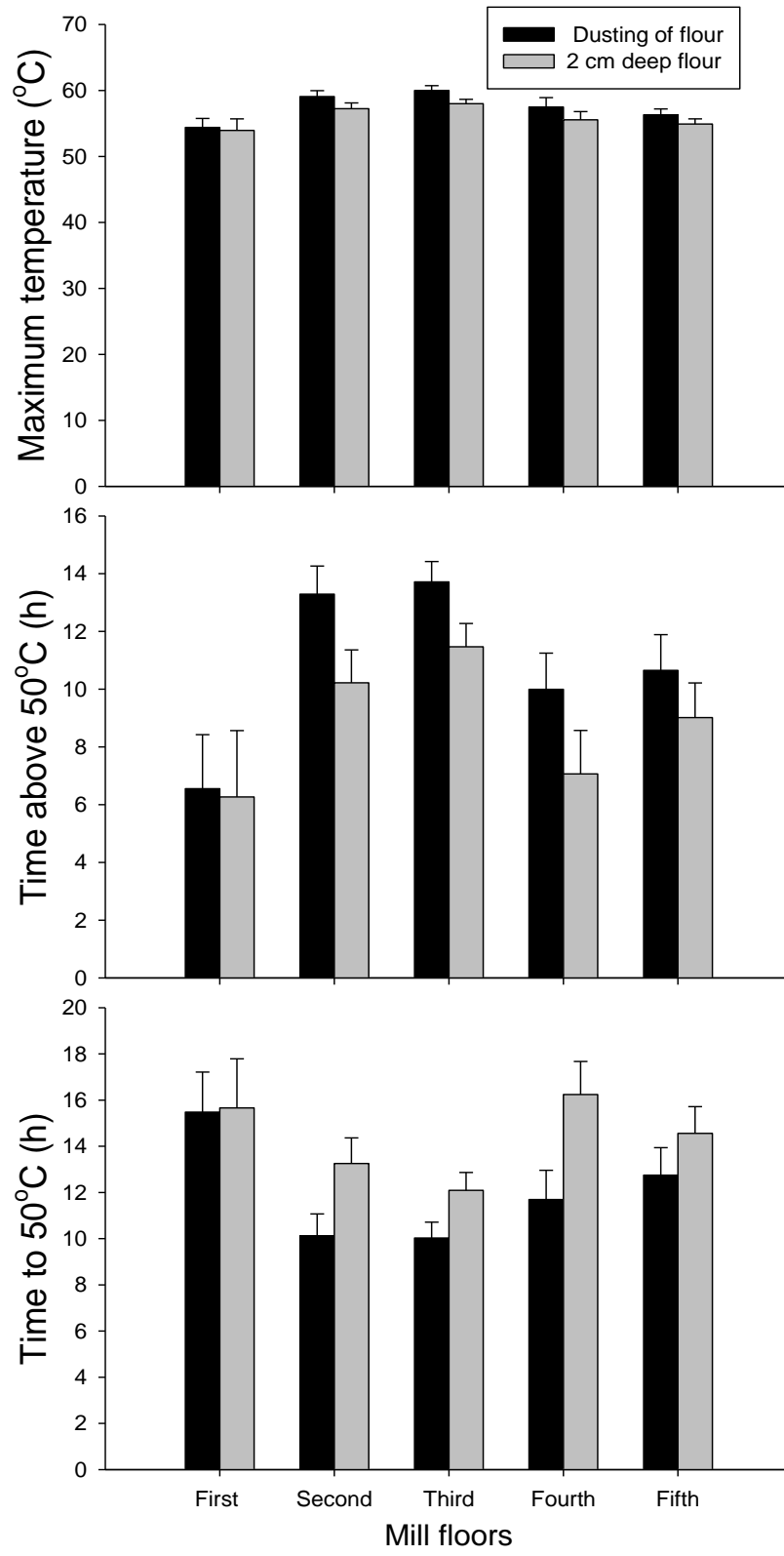


Table 2.1 Bioassay box locations among five floors of the Hal Ross flour mill

Floor	Bioassay box number	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

Floor	Bioassay box number	Location
	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

Table 2.2 Temperature parameters (mean \pm 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)	Maximum temperature (°C)
First ^a	8.9 \pm 1.3	10.1 \pm 1.4	53.6 \pm 2.7
Second	8.5 \pm 0.9	13.6 \pm 1.0	58.3 \pm 2.2
Third	10.0 \pm 0.6	13.5 \pm 0.6	59.3 \pm 0.6
Fourth	12.0 \pm 1.0	11.2 \pm 1.0	57.4 \pm 0.8
Fifth ^b	9.7 \pm 1.0	12.3 \pm 1.0	56.8 \pm 0.8

Each mean is based on $n = 30$ unless otherwise indicated.

^a $n = 24$.

^b $n = 29$, because one temperature data logger failed to record temperatures.

Table 2.3 Mortality of *T. castaneum* life stages in unheated (control) bioassay boxes

Life stage	Mean \pm SE mortality (%)	
	Dusting of flour	2-cm deep flour
Eggs	48.0 \pm 6.1	18.7 \pm 2.6
Young larvae	42.0 \pm 14.5	12.0 \pm 3.5
Old larvae	0.7 \pm 0.4	0.0 \pm 0.0
Pupae	1.6 \pm 0.3	1.4 \pm 0.4
Adults	0.8 \pm 0.3	1.7 \pm 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.

Table 2.4 Mortality of *T. castaneum* life stages in bioassay boxes placed on the five floors of the Hal Ross flour mill subjected to heat treatment

Floor	Life stage	Mean \pm SE mortality (%) ^a	
		Dusting of flour	2-cm deep flour
First	Eggs	98.6 \pm 0.8	97.2 \pm 1.6
	Young larvae	98.0 \pm 1.1	82.0 \pm 6.9
	Old larvae	93.1 \pm 6.6	85.8 \pm 6.7
	Pupae	94.8 \pm 6.7	85.0 \pm 8.0
	Adults	93.3 \pm 6.7	55.5 \pm 12.9
Second	Eggs	100.0 \pm 0.0	99.8 \pm 0.2
	Young larvae	99.3 \pm 0.5	100.0 \pm 0.0
	Old larvae	100.0 \pm 0.0	100.0 \pm 0.0
	Pupae	100.0 \pm 0.0	100.0 \pm 0.0 ^b
	Adults	100.0 \pm 0.0	100.0 \pm 0.0
Third	Eggs	100.0 \pm 0.0	98.4 \pm 1.6
	Young larvae	99.3 \pm 0.7	99.8 \pm 0.2
	Old larvae	100.0 \pm 0.0	99.7 \pm 0.3
	Pupae	100.0 \pm 0.0	100.0 \pm 0.0
	Adults	100.0 \pm 0.0	100.0 \pm 0.0

Floor	Life stage	Mean \pm SE mortality (%) ^a	
		Dusting of flour	2-cm deep flour
Fourth	Eggs	100.0 \pm 0.0	100.0 \pm 0.0
	Young larvae	100.0 \pm 0.0	99.2 \pm 0.8 ^b
	Old larvae	99.0 \pm 1.0	93.2 \pm 6.7
	Pupae	100.0 \pm 0.0	98.9 \pm 1.1
	Adults	100.0 \pm 0.0	95.6 \pm 4.4
Fifth	Eggs	100.0 \pm 0.0	100.0 \pm 0.0
	Young larvae	99.8 \pm 0.2	99.5 \pm 0.5
	Old larvae	99.5 \pm 0.3	99.3 \pm 0.5
	Pupae	100.0 \pm 0.0	100.0 \pm 0.0
	Adults	100.0 \pm 0.0	100.0 \pm 0.0

^aEach mean is based on $n = 15$ unless otherwise indicated.

^b $n = 14$.

Table 2.5 Itemized costs for each of the three heat treatments conducted in the Hal Ross flour mill

Cost item	Costs (\$) for heat treatment conducted during:			Total cost (\$)
	13-14 May 2009	25-26 Aug 2009	7-8 May 2010	
Propane consumed (L)	5,299	4,883	5,500	15,682
Price/L	0.38	0.38	0.44	
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	11,000	None	None	11,000
Service personnel ^c	12,000	12,000	12,000	36,000
Supervisor's fee ^d	2,454	2,613	687	5,754
Total cost	38,005	27,005	25,605	90,615

^aFor delivering, recharging, and picking up the propane tanks.

^bA one-time charge for the customized-ducts. The same ducts were used for the second and third heat treatments.

^cFour technicians were paid \$1,000/d each for 3 d. Costs included travel, lodging, and time spent on site during heat treatments (12 h/shift).

^dNegotiable consulting fee.

Chapter 3 - Impact of varying levels of sanitation on mortality of *Tribolium castaneum* eggs and adults during heat treatments of a pilot flour mill

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, were 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 ring holding 0.1-, 0.2-, 1.0-, 3.0-, 6.0-, or 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 to 11 h only in 0.1- and 0.2-cm deep flour treatments. In all other treatments the maximum temperatures attained were generally 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 to 17 h and were held above 50°C for 6 to 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.

Keywords heat treatment, temperatures, sanitation, red flour beetle, efficacy assessment

Introduction

In the United States, elevated temperatures (50 to 60°C) or heat treatments have been used for managing stored-product insects associated with flour mills (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 due to its adverse effects on stratospheric ozone (Dosland et al. 2006, Beckett et al. 2007, Hulasare et al. 2010, Subramanyam 2010). 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). 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 (Brijwani 2011). Of the temperature variables studied (time to 50°C, time above 50°C, and maximum temperature), only the maximum temperature significantly influenced survival of *T. castaneum* life stages. Additionally, on the first floor, adults were found to be less susceptible to heat when compared with eggs, young larvae, old larvae, and pupae.

The present study was designed to characterize the influence of six flour depths between 0.1 and 10.0 cm, to simulate unsanitary conditions, on the mortality of an immobile stage (eggs) and mobile stage (adults) of *T. castaneum*, an economically important pest of flour mills (Good 1937, Hagstrum and Subramanyam 2009). The impact of flour depths on survival of *T. castaneum* eggs and adults was evaluated on the first and third flour mill floors, because Dean (1911) and Goodwin (1912) reported temperatures during heat treatments to be generally lower on the first floor than those observed on floors above it.

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-L 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, 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- μ m 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 using an 841- μ m opening sieve. The flour was sifted using a 250- μ m sieve to retain the eggs. Mixed age adults were separated directly from culture jars using an 841- μ m 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 1486.2-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 about 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 diameter and 0.82-cm thickness (Ferguson, Manhattan, KS) were cut to provide cylinder heights of 15-cm. A total of 36 rings were used in experiments. Prior to heat treatment, 12 rings each were placed on the first and third floors of the pilot flour mill. 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 the ring base-floor junctions to prevent insects from escaping from underneath the rings. The rings on a given floor were placed at a distance of ~40 cm from one another. The same clay was used at the top end of each ring to hold in place a black cloth mesh cover with 0.6-mm² openings. A temperature sensor (SmartButton; ACR Systems, Inc., Surrey, Canada) was placed

at the center of each ring on the floor to record temperatures at 1-min intervals. Temperatures were also recorded at 1-min intervals, outside the PVC rings on both the first and third floor. Six SmartButton sensors were placed next to the ring on each floor.

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- to 1.9-L 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. To prevent insects from escaping the top ends of rings were closed with mesh screens.

Heat Treatment

The Hal Ross flour mill was subjected to a total of three, 24-h heat treatments between 2009 and 2010 (Brijwani 2011), but 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 ($n = 2$). The second heat treatment was performed on 25-26 August 2009 and the third on 7-8 May 2010 using forced-air gas heaters that were fueled by propane. 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 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°C).

Sample Collection and Insect Mortality Assessment

At the end of each heat treatment, the rings from the first and third floors of the Hal Ross flour mill (heat-exposed) and from the fourth floor of Shellenberger Hall 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- to 1.9-L jars, labeled and incubated in the growth chamber at 28°C and 65% RH. Mortality of *T. castaneum* adults was determined 24 h after incubating the samples at 28°C and 65% RH, 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 Hal Ross flour mill were plotted as a function of time to show variations in temperature profiles observed. Data from six SmartButton sensors placed outside PVC rings on the first and third mill floors were averaged separately for the 25-26 August 2009 and 7-8 May 2010 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). In the PVC 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 (REGWQ) 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

The mean \pm SE, minimum, maximum, and average temperatures observed on the floor next to PVC rings in the control treatment were 26.2 ± 2.7 , 27.8 ± 1.7 , and $26.8 \pm 2.5^{\circ}\text{C}$, respectively. In the same treatment, the minimum, maximum, and average temperatures varied very little and ranged within 26.0 ± 2.5 to $27.5 \pm 2.0^{\circ}\text{C}$ (Table 3.1). In the control treatment the mean \pm SE mortality of *T. castaneum* eggs was 14.5 ± 4.5 to $24.0 \pm 2.0\%$, whereas that of adults ranged from 0 to $1.5 \pm 1.5\%$ (Table 3.1). 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. The egg mortality data includes not only the mortality of the egg stage but also that of the larval and pupal stages.

The starting temperatures observed on the first and third floors outside the PVC rings prior to heat treatment of the pilot flour mill were $\sim 30\text{-}31^{\circ}\text{C}$, and these minor differences were not statistically significant ($P > 0.05$) (Table 3.2). The third floor reached 50°C in 3.9 h faster than the first floor. As a result temperatures above 50°C were maintained for 4.7 h longer on the third floor when compared with the first floor. The maximum temperature attained on the third floor was 6.6°C higher than on the first floor. The time required to reach 50°C , time above 50°C , and the maximum temperature were significantly different between the third and first floors ($P < 0.05$). 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, the 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 due to vertical and horizontal stratification of temperatures.

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. 3.1). 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, since the rings were placed ~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.

On the first floor, mean \pm SE starting temperatures 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 ($F = 3.95$; $P = 0.0624$) (Table 3.3). 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 ($F = 104.80$; $df = 5, 6$; $P < 0.0001$). The significant difference observed in rings with adults was a result of little or no variation in mean maximum temperatures among flour depths.

The maximum temperatures among flour depths were more variable in rings with eggs and hence the lack of any significant differences. The slow heating of the first floor and lower temperatures observed on the floor relative to the third floor (Table 3.2) combined with the insulating effect of flour did not result in temperatures reaching 50°C in rings with 1.0- to 10.0-cm deep flour. The low temperatures observed on the first floor could be due to 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. In Dean's study (1911), temperature measured in an open area on the first floor was 40.5°C, and at a depth of 10.16-cm in wheat a temperature of 35.5°C was recorded. On the second floor the maximum temperature recorded was 56.4°C in an open area; however, temperature at a depth of 7.62-cm in a sack of flour that was 91.4-cm above the floor a temperature of 47.5°C was recorded.

Both the *T. castaneum* egg and adult mortality were inversely related to flour depth (Table 3.3). The mean \pm SE mortality of eggs on the first floor at six flour depths ranged from 44.1 ± 25.7 to $91.2 \pm 8.8\%$ and that of the adults ranged from 1.0 ± 0.0 to $94.4 \pm 5.6\%$. The egg mortality was satisfactorily described ($r^2 = 0.912$) by a linear regression (Fig. 3.2A). The mean \pm SE intercept and slope values of the linear regression were 88.21 ± 3.18 and -4.15 ± 0.64 , respectively. The adult mortality as a function of flour depth was best described ($r^2 = 0.972$) by an exponential decay model (Fig. 3.2B), and the mean \pm SE of parameter *A* was 21.75 ± 4.28 and parameter *b* was -0.64 ± 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 the stage is immobile and therefore the adverse

effects as a function of flour depth tended to be linear. In contrast, adults are highly mobile and are capable of tunneling into flour (Hagstrum and Smittle 1980). 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 and escape the heat treatment, and as the flour depth or amount increased more adults may have escaped the adverse effects of the heat treatment by tunneling. For example, as the flour depth or amount increased from 0.2 to 10.0 cm (38 to 1,645 g), *T. castaneum* adult density per unit ranged from 2.6 to 0.06 adults/g of flour. The dispersive behavior of *T. castaneum* by tunneling deeper into the flour may have resulted in mortality decreasing exponentially with an increase in flour depth or amount.

On the third floor, the starting temperatures, time to 50°C, time above 50°C, and the maximum temperature among flour depths varied very little (Table 3.4), and each of these variable was not significantly different ($df = 5, 6$) among flour depths in rings with eggs (F , range among variables = 0.07 - 0.75; $P \geq 0.6137$) and adults (F , range = 0.24 - 3.42; $P \geq 0.0831$). The time to 50°C took 15 to 17 h, and temperatures above 50°C were held for 6 to 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 (Table 3.2), 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 3.4). Complete control of eggs and adults on the third floor at all flour depths can be attributed to the high temperatures attained. Although all eggs and adults were killed at all flour depths, it is still necessary during heat treatment of a flour mill to remove any spilled flour

accumulations from all accessible areas. At temperatures $\geq 54^{\circ}\text{C}$, susceptibility differences among *T. castaneum* life stages tend to disappear (Mahroof et al. 2003b).

In this study, 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 Hal Ross flour mill in bioassay boxes when compared with other life stages (Brijwani 2011), but inconsistent with an unreplicated trial of Mahroof et al. (2003a). In Mahroof et al. (2003a), eggs, young larvae, old larvae, pupae, and adults of *T. castaneum* were exposed to heat treatments in pilot flour and feed mills, and no consistent trends were observed in identifying a heat tolerant stage despite survival of old larvae and pupae in a few locations. 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. Yu et al. (2011) were unable to identify a heat tolerant stage of the cigarette beetle, *Lasioderma serricornis* (L.), during heat treatment of a commercial facility, but experiments at constant temperatures of 46, 50, and 54°C revealed eggs to be the most heat tolerant stage when compared with young larvae, old larvae, pupae, and adults. Additional work is needed to determine heat tolerance of stored-product insect life stages during heat treatments of facilities where temperatures are dynamically changing over time rather than at constant temperatures (Yu et al. 2011).

In summary, the susceptibility of *T. castaneum* eggs and adults was inversely related to flour depths or sanitation levels, only when temperatures did not reach 50°C. Although sanitation did not influence mortality of *T. castaneum* eggs and adults on the third floor, it is important to remove flour accumulations to improve heat distribution and effectiveness against *T. castaneum* life stages.

Acknowledgments

We are grateful to David Hagstrum for reviewing an earlier draft of this manuscript. 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), Washington, D. C. 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, U.K.
- 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, KS.
- Dean, D. A. 1911.** Heat as a means of controlling mill insects. J. Econ. Entomol. 4: 142-158.
- Dosland, O., Bh. Subramanyam, K. Sheppard, and R. Mahroof. 2006.** Temperature modification for insect control, pp. 89-103. *In* J. W. Heaps. (ed.), Insect management for food storage and processing, second edition. American Association of Cereal Chemists, St. Paul, MN.
- 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. Kansas Entomol. Soc. 10: 135-148.

- Goodwin, W. H. 1912.** Flour mill fumigation. Ohio Agri. Expt. Station Bull. 234: 171-184.
- Goodwin, W. H. 1922.** Heat for control of cereal insects. Ohio Agr. Expt. Station 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. Amer. 73: 11-13.
- Hagstrum, D. W., and Bh. Subramanyam. 2009.** Stored-Product Insect Resource. American Association of Cereal Chemists International, St. Paul, MN.
- Hulasare, R., Bh. Subramanyam, P. G. Fields, and A. Y. Abdelghany. 2010.** Heat treatment: a viable methyl bromide alternative for managing stored-product insects in food-processing facilities, pp. 661 - 667. In M. O. Carvalho, P. G. Fields, C. S. Adler, F. H. Arthur, C. G. Athanassiou, J. F. Campbell, F. Fleurat-Lessard, P. W. Flinn, R. J. Hodges, A. A. Işikber, S. Navarro, R. T. Noyes, J. Riudavets, K. K. Sinha, G. R. Thorpe, B. H. Timlick, P. Trematerra, and N. D. G. White (eds.), Proceedings of the 10th international working conference on stored-product protection, 27 June - 2 July, 2010, Estoril, Portugal. Julius-Kühn-Archiv, 425, 2010,
<http://pub.jki.bund.de/index.php./JKA/issue/view/719>.
- 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.
- Pepper, J. H., and A. L. Strand. 1935.** Superheating as a control for cereal-mill insects. Montana State College Agri. Expt. Station 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.
- 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.
- Subramanyam, Bh. 2010.** Controlling red flour beetle. Milling Journal, Fourth Quarter, pp. 32-34.
- Wagner G. B., and R. T. Cotton. 1935.** Factors affecting insect abundance in flour mills. Northwestern Miller, November, pp. 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, KS.
- Yu, C., Bh. Subramanyam, P. W. Flinn, and J. A. Gwartz. 2011.** Susceptibility of *Lasioderma serricorne* (F.) life stages to elevated temperatures used during structural heat treatments. J. Econ. Entomol. 104: 317-324.

Figure 3.1. Mean ($n = 2$) temperature profiles observed during 24-h heat treatments at flour depths of 0.1 to 10.0 cm in rings infested with *T. castaneum* eggs and adults placed on the first and third floors of a pilot flour mill.

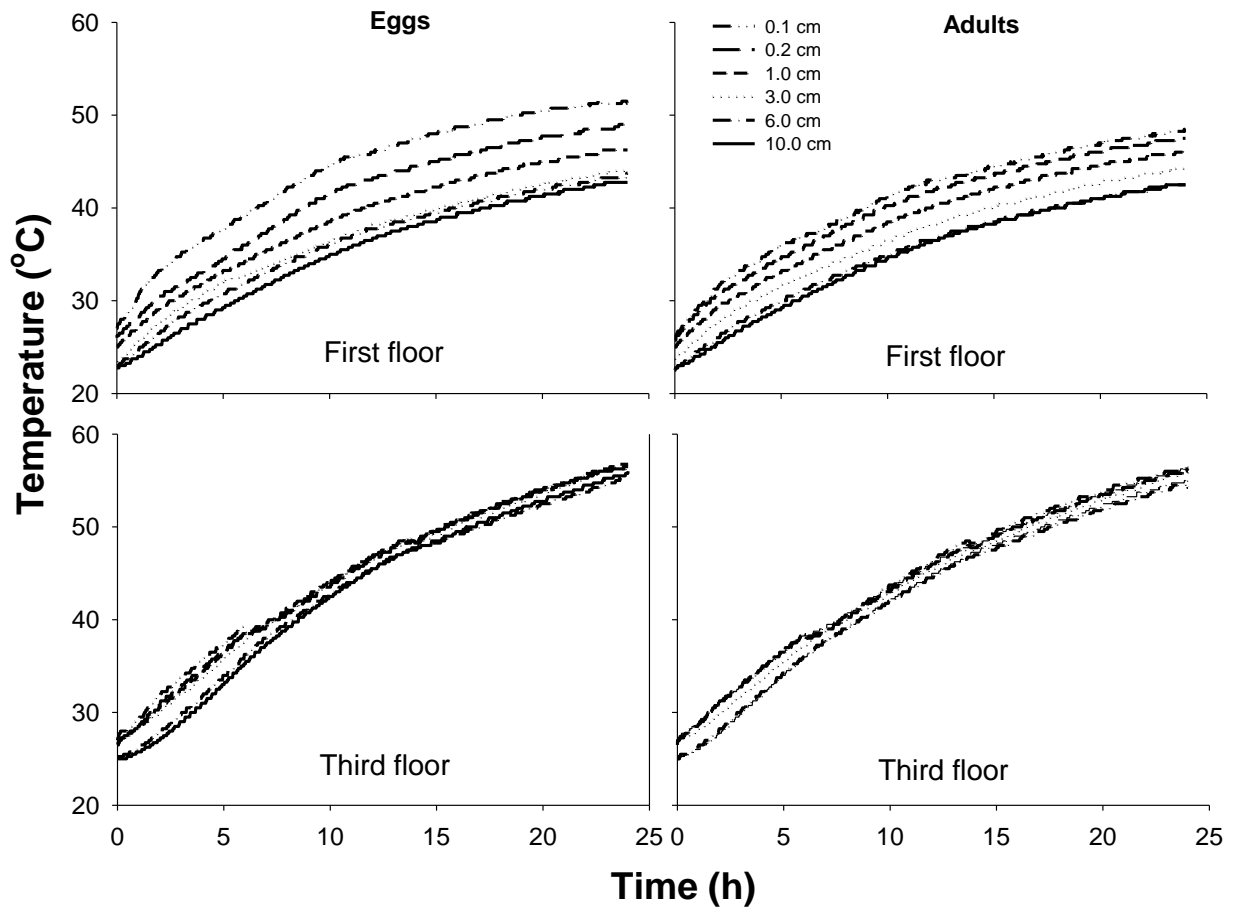


Figure 3.2. A linear regression model describing mean \pm SE ($n = 2$) egg mortality (A) and an exponential decay model describing adult mortality (B) of *T. castaneum* as a function of flour depth in rings placed on the first floor of a pilot mill subjected to heat treatment.

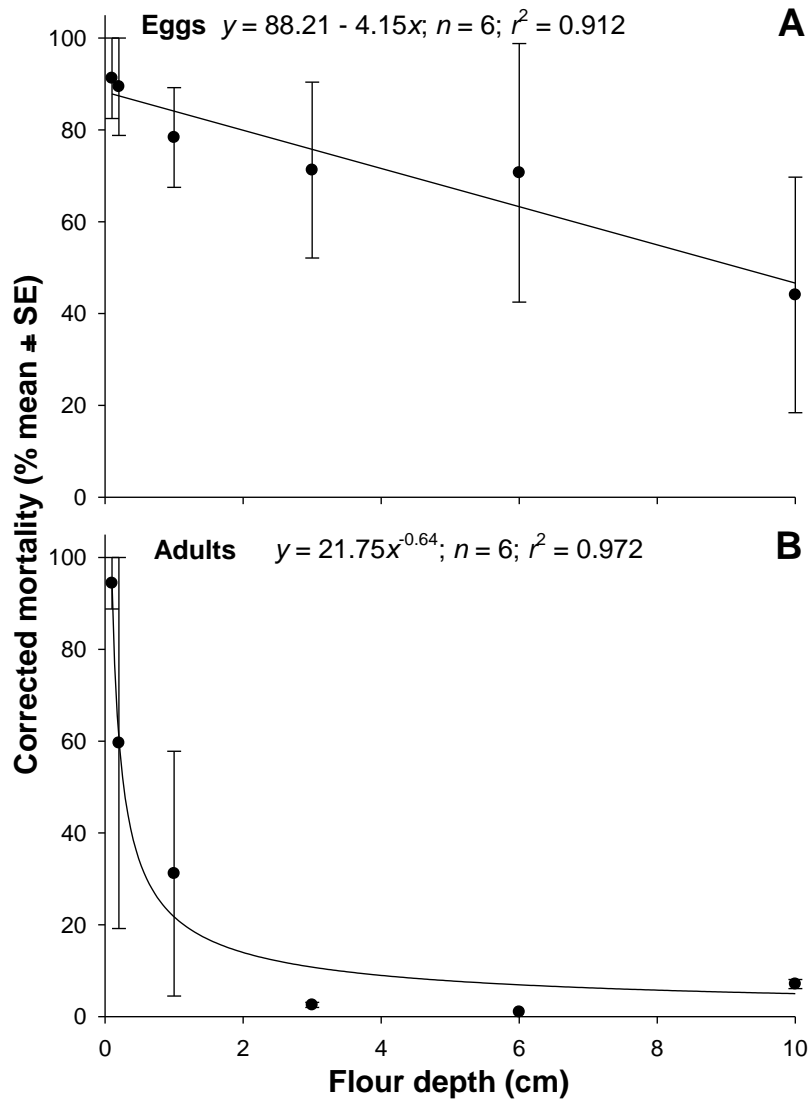


Table 3.1 Temperature parameters and *T. castaneum* egg and adult mortality (mean \pm SE) inside unheated (control) PVC rings at different flour depths

Flour depth (cm)	Temperature ($^{\circ}$ C) ^a			Mortality (%)
	Minimum	Maximum	Average	
Eggs				
0.1	26.0 \pm 2.5	27.3 \pm 1.8	26.6 \pm 2.3	14.5 \pm 4.5 0.2
	26.3 \pm 2.8	27.0 \pm 2.5	26.6 \pm 2.7	20.0 ^b
1.0	26.3 \pm 2.8	27.3 \pm 2.3	26.6 \pm 2.7	17.0 ^b
3.0	26.3 \pm 2.8	27.5 \pm 2.0	26.8 \pm 2.5	16.5 \pm 0.5
6.0	26.5 \pm 2.5	27.5 \pm 2.0	27.0 \pm 2.4	20.0 ^b
10.0	26.3 \pm 2.3	27.0 \pm 2.0	26.7 \pm 2.2	24.0 \pm 2.0
Adults				
0.1	26.3 \pm 2.3	27.0 \pm 2.0	26.6 \pm 2.3	1.5 \pm 1.5
0.2	26.3 \pm 2.3	27.0 \pm 2.0	26.5 \pm 2.3	1.0 \pm 1.0
1.0	26.3 \pm 2.8	27.5 \pm 2.0	26.8 \pm 2.5	0.5 \pm 0.5
3.0	26.3 \pm 2.8	27.3 \pm 2.3	26.9 \pm 2.6	0.0 \pm 0.0
6.0	26.3 \pm 2.3	27.3 \pm 2.3	26.7 \pm 2.2	1.0 \pm 1.0
10.0	26.3 \pm 2.8	27.3 \pm 2.3	26.8 \pm 2.5	1.0 \pm 1.0

Each mean is based on $n = 2$, unless otherwise indicated.

^aThe mean \pm SE for minimum, maximum, and average temperatures outside the unheated (control) PVC rings at floor level were 26.2 ± 2.7 , 27.8 ± 1.7 , and $26.8 \pm 2.5^{\circ}\text{C}$, respectively.

^b $n = 1$. Certain rings were infested by resident *T. castaneum* populations in the mill resulting in greater than 100 adults. Data from these rings were therefore discarded.

Table 3.2 Temperature parameters (mean \pm SE) outside the PVC rings during pilot flour mill heat treatment

Floor	Starting temperature ($^{\circ}$ C)	Time to 50 $^{\circ}$ C (h)	Time above 50 $^{\circ}$ C (h)	Maximum temperature ($^{\circ}$ C)
First	29.8 \pm 4.6	15.1 \pm 3.1	7.9 \pm 2.4	52.0 \pm 0.4
Third	30.8 \pm 4.2	11.2 \pm 0.8	12.6 \pm 1.0	58.6 \pm 2.1
<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*	0.0198*	<0.0001*

Each mean is based on $n = 2$.

^aVariances between the two groups were unequal ($P < 0.05$).

*Significant ($P < 0.05$).

Table 3.3 Temperature parameters and corrected *T. castaneum* egg and adult mortality (mean \pm SE) in PVC rings on the first floor at different flour depths during pilot flour mill heat treatment

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

Each mean is based on $n = 2$.

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

^bDifferences 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 ($P < 0.05$; REGWQ test).

^cTemperatures did not reach 50°C .

Table 3.4 Temperature parameters and corrected *T. castaneum* egg and adult mortality (mean \pm SE) in PVC rings on the third floor at different flour depths during pilot flour mill heat treatment

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

Each mean is based on $n = 2$.

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

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

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

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