HEAT TREATMENT OF EMPTY STORAGE BINS AND GRAIN-PROCESSING FACILITIES: FACTORS INFLUENCING EFFICACY AGAINST ADULTS OF THE RED FLOUR BEETLE, *TRIBOLIUM CASTANEUM* (HERBST)

by

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B.S., University of Kentucky, 2010
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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Grain Science and Industry
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Abstract

Heat treatment, a more than 100-year-old technology, involves raising the ambient temperature of a an empty bins/storage space or a clean gran-processing facility to 50-60°C for 24 h or less to kill stored-product insects. Heat treatment is an environmentally benign and a safer alternative to chemical insecticides. The studies reported here were conducted to determine the effect of short-term exposure to sub-lethal temperatures on adults of *Tribolium castaneum* (Herbst) and their subsequent susceptibility to temperatures of 50 and 55°C for 60 and 15 min, respectively; to determine the effect of rearing *T. castaneum* at select elevated temperatures for 10 generations on their subsequent susceptibility to temperatures of 50 and 55°C; to determine the effects of age and sex on susceptibility of *T. castaneum* adults to 50 and 55°C; and to determine the effectiveness of a diatomaceous earth (DE) formulation at several elevated temperatures below 50°C on the mortality of *T. castaneum* adults.

Results of the studies showed that short-term exposure between 24 and 72 h, to sub-lethal temperatures (32, 36, and 40°C) only increased survival for insects acclimated to 32°C. Acclimation at 36 and 40°C resulted in higher mortality after exposure for 24, 48, and 72 h at 50 and 55°C. Rearing insects at 32 and 36°C for 10 generations resulted in the highest survival of adults at 50°C. However, when adults reared at 32°C were exposed during heat treatment of Hal Ross Flour Mill, the adults were least susceptible to dynamically changing temperatures over time. Female *T. castaneum* adults were more heat tolerant than males, and adults 1 d post-emergence were the most heat tolerant when exposed to 55°C for xx minutes compared with adults aged 7-42 d. The efficacy of DE was enhanced at higher constant temperatures, which can lessen energy inputs in order to obtain a complete kill of insects when temperatures do not reach

50°C or greater. Given the changing climate, it is very useful for researchers to understand the implications of increasing temperatures on the heat tolerance of insects.

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Dedication

This work is dedicated to my late grandmother and grandfather, Lois J. Brashear and Leon Brashear. Also, to my husband and kids–always dream big and never stop.

Chapter 1. Introduction

The phase-out of methyl bromide has resulted in exploring alternatives to treat grain-processing facilities and storage bins. One viable alternative to methyl bromide is the use of high temperatures for disinfesting empty storage bins and grain-processing facilities. Heat treatment is a one hundred year old technology, and it is safe and effective for management of stored-product insect pests associated with empty bins and grain-processing facilities (Dosland et al., 2006). Heat treatment of a structure involves raising its inside temperature to 50-60°C and maintaining these temperatures for 24 h or less (Subramanyam et al., 2011). The benefit of conducting heat treatments over the use of fumigants is that it is an environmentally sensitive approach and no special registration or licenses are needed.

A great deal of research has been conducted at Kansas State University over the last 17 years to determine temperatures attained during heat treatment of grain-processing facilities, stored-product insect stages tolerant to elevated temperatures, effect of sanitation on heat treatment effectiveness, and development of thermal death kinetic models to predict insect mortality (Subramanyam et al., 2011). In addition, software programs have been developed to determine the amount of heat energy needed for structural heat treatments and to predict insect mortality, and to analyze and summarize heat treatment data. In this introduction, the rationale for conducting heat treatment research using the red flour beetle, *Tribolium castaneum* (Herbst), as a model insect is presented.

1.1 Methyl bromide

In 1987, the United States signed the Montreal Protocol, which prohibited substances that depleted the stratospheric ozone (Subramanyam et al., 2011). In the United States methyl bromide is listed as a Class I ozone depleting substance and was phased-out by 2005 (40 Code of Federal Regulations (CFR) Part 82, Federal Register Volume 69, No. 246). The United States Environmental Protection Agency allowed a critical use exemption process for continued use of methyl bromide past the phase-out date until viable alternatives are thoroughly tested and proven to be cost effective. In addition, methyl bromide still continues to be used for quarantine or pre-shipment uses. Prior to 2005, methyl bromide was widely used by the grain-processing industry as it is highly effective against stored-product insects.

1.2 *Tribolium castaneum* (Herbst)

T. castaneum is a cosmopolitan pest that is found in different areas of the world that covers both temperate and tropical climates (Sinha and Watters, 1985; Hagstrum et al., 2013). *T castaneum* has been associated with 246 commodities worldwide, including flour, cereals, pasta, beans, and nuts (Hagstrum et al., 2013). It is the most common insect found in flour mills. In raw stored grain, *T. castaneum* is a secondary or external pest because it feeds on broken kernels. The developmental time of *T. castaneum* varies with temperature and relative humidity. A majority of pest management intervention in the grain-processing industry are directed at this common and economically important stored-product insect pest.

1.3 Heat shock proteins

Heat shock proteins are essential for the protection of cellular functions and aid in lessening the removal of denatured proteins from the cell, which is very important when organisms are confronted with environmental stress such as high temperatures (Mahroof et al., 2005). There are different families of heat shock proteins (HSP) based on the molecular weight. The most commonly researched HSPs for insects when looking at environmental stresses, including heat stress, belong to the HSP 70 group with molecular weights that vary between 65 and 75 kDa (Mahroof et al., 2005).

Acclimation of insects to sub-lethal temperatures can induce the expression of heat shock proteins (HSPs), which can subsequently protect cells from heat stress (Parsell and Lindquist, 1993). At the biochemical level, the proteins that became denatured from the initial heat stress are resolubilized due to HSPs, resulting in rapid heat hardening (Chown and Nicholson, 2004; Zhao and Jones, 2012). Xu et al. (2010) showed that the Hsp83 gene, the homologue to Hsp90, in *T. castaneum* could be induced when insects are exposed for 40°C for one hour. Scott et al. (1997) conducted heat acclimation studies on *Trichogramma carverae*, a parasitoid wasp, and found that more adult wasps placed at temperatures of 33°C or 35°C for short durations (1-2 h) survived at 40°C compared to those not acclimated to the higher temperatures.

1.4 Heat treatment temperatures

Heat treatments were first used to control stored-product insects in flour mills in the early 1900's (Dean, 1911). Pepper and Strand (1935) determined that insects can be killed at temperatures between 48.9-54.4°C (120-130°F). They mentioned that temperatures should be measured at the floor level, where adult stages of insects are active. Additionally, Pepper and

Strand (1935) recommended the use of fans to recirculate the hot air within a structure to attain uniform temperatures during structural heat treatments. Research at Kansas State University has shown that temperatures during practical heat treatments should be maintained within the range of 50-60°C for 24 h or less (Dosland et al., 2006; Subramanyam et al., 2011). Spot treatments of empty storage bins, especially those made of steel, can be accomplished in 4 h to kill insects (Tilley et al., 2007).

1.5 Impact of sanitation on heat treatment effectiveness

Time is an important factor in heat treatments to ensure the heat penetrates any cracks or crevices in floor or wall spaces and into any processing equipment to kill insects that may be hiding in such places (Subramanyam et al., 2011). Prior to the heat treatment, sanitation of the facility and removal of grain or grain products is essential as these are typically poor conductors of heat, and insects could escape being exposed to lethal temperatures by seeking refuge in such materials (Subramanyam et al., 2002). Brijwani et al. (2012a) conducted an experiment in which eggs and adult stages *T. castaneum* were placed inside separate PVC rings containing wheat flour at different depths (0.1, 0.2, 1.0, 3.0, 6.0, and 10.0 cm) to simulate different levels of sanitation that could be present in a flour mill. The rings were placed on the first and third floor of a pilot flour mill at Kansas State University subjected to a 24 h heat treatment. Results showed that on the first floor, 50°C was only reached in rings with 0.1 and 0.2 cm deep flour. Both the eggs and adult survival was inversely related to flour depth, and the survival of eggs was greater than that of the adults. On the third floor, 50°C was reached in all of the rings in 15-17 h and temperatures above 50°C were maintained for 6-8 h, resulting in 100% mortality of the eggs and adults of *T. castaneum*. Reaching the target temperature can be more difficult at the ground level

due to the fact that the concrete floor functions as a heat sink (Pepper and Strand, 1935). Jian et al. (2012) took temperature measurements in a flour mill storage building undergoing heat treatment and found that the concrete floor was the coldest spot. Achieving a good level of sanitation is an important factor in ensuring the efficacy of heat treatments.

1.6 Rate of heating and heat tolerance

Mahroof et al. (2003a) conducted a laboratory experiment to determine time-mortality relationships for different life stages (eggs, young larvae, old larvae, pupae, and adults) of *T. castaneum*. Each stage was exposed to constant temperatures of 42, 46, 50, 54, 58, and 60°C in laboratory growth chambers at 22% r.h., typically observed during heat treatments (Mahroof et al., 2003b). They reported young larvae (first instars) to be the most heat-tolerant stage. For example, a minimum of 7.2 h at or above 50°C was needed to kill 99% of the exposed individuals. All other stages required 1.8 h or less to kill 99% of the exposed individuals. Based on these results, it was predicted that the success of a heat treatment could be gauged based on the mortality of the young larvae of *T. castaneum*. In a heat treatment of a pilot flour mill (Mahroof et al., 2003b), old larvae and pupae of *T. castaneum* were observed to be more heat tolerant than the other stages. This finding is different from the results obtained at constant temperatures (Mahroof et al., 2003a). Brijwani et al. (2012b) reported adults to be least susceptible to heat compared with other life stages during heat treatment of a pilot flour mill. The discrepancy between the findings of Mahroof et al. (2003b) and Brijwani et al. (2012b) could be related to differences in heating rates, resulting in differential susceptibility of the exposed life stages. Very little is known about how heating rates influence heat tolerance. This aspect needs further study.

1.7 Short-term and generational adaptation to high temperatures and susceptibility to heat treatments

Insects are frequently exposed to fluctuating temperatures in nature. Their ability to adapt or acclimate to these temperatures has been shown to increase different species' survivorship at lethal temperatures (Scott et al., 1997; Overgaard et al., 2008). Scott et al. (1997) conducted heat acclimation studies on *Trichogramma carverae*, a parasitoid wasp, and found that more adult wasps placed at temperatures of 33°C or 35°C for short durations (1-2 h) survived at 40°C compared to those not acclimated to the higher temperatures. However, the wasps were found to have reduced longevity after exposure to 40°C.

To date, there is no published literature about how acclimation of life stages of *T. castaneum* to sub-lethal temperatures affects their subsequent susceptibility to lethal temperatures during a heat treatment. Survival of acclimated beetles may help mobile life stages (larvae and adults) seek refuge in insulated areas of the mill or leave the heated area and escape a heat treatment.

There are no published data on time-mortality relationships of populations and life stages of *T. castaneum* from different geographical regions of the world. Sourcing insects from different parts of the world may have restrictions. Therefore, this aspect was studied by rearing insects for multiple generations at three temperatures, 28, 32, and 36°C, to determine their subsequent susceptibility to 50 and 55ºC.

1.8 Effect of adult sex and age on susceptibility of *T. castaneum* to high temperatures

Population dynamics are greatly influenced by temperature, which affects many physiochemical and biochemical processes (Nyamukondiwa and Terblanche, 2009). Insects are constantly exposed to varying temperatures in their natural environments, and their ability to adapt to a wide range of temperatures is vital for their ability to survive and reproduce. The effects of age, gender, and feeding status on thermal tolerance have not been clearly documented for *T. castaneum* adults.

1.9 Thermal death kinetic model for adults

An understanding of how elevated temperatures affect stored-product insect species and their life stages is important in developing heat treatments. Heat treatments are common in treating pests found in fresh commodities, such as fresh and dried fruits, tree nuts, and certain grains (Landolt et al., 1984; Neven, 1994; Waddell et al., 2000; Wang et al., 2002a,b; Johnson et al., 2003). Thermal death kinetic (TDK) models were first used to model thermal inactivation of bacteria and have also been used to model the death of insects in fresh commodities. When dealing with these types of insects and commodities, heating rates are much higher than those employed during structural heat treatments; 1-18°C/min versus 0.3-13.7°C/h (Mahroof et al., 2003a; Roseli, 2003; Boina et al., 2008). The time of treatment also varies greatly, from a matter of minutes in fresh commodities, dried fruits, and nuts, to 24-36 h for a structural heat treatment (Johnson et al., 2004; Boina et al., 2008; Subramanyam et al., 2011).

Models to predict insect mortality during a structural heat treatment are important to heat treatment operators so that energy is not wasted due to overheating. Overheating where

temperatures exceed 60ºC results in damage to heat-sensitive equipment and fixtures in the facility and unnecessary expense. On the other hand, underheating where temperatures are below 50°C can result in insect survival. Therefore, it is important to calculate the right amount of heat energy needed, and conduct heat treatment for long enough time for heat to penetrate all areas and also maintain temperatures of 50-60°C throughout the facility (Boina et al., 2008).

1.10 Combination of heat with diatomaceous earth to disinfest empty bins and grain-processing facilities

Stored grain losses result in the loss of millions of dollars for farmers annually (Harein and Meronuk, 1995). Much of this loss can be prevented by removing grain debris from under perforated floors which harbors and feeds stored-product insects prior to storage of newly harvested grain. Approved insecticides and inert dust can be applied to the bin walls and concrete floor to kill live insects present, however, some of these can be very harmful to the environment or those applying the insecticides, thus creating a need for more sustainable pest management methods. Diatomaceous earth (DE) powders contain fossilized skeletons of diatoms with particle sizes ranging from 1- 60 µm. DE abrades the insect's waxy cuticle leading to loss of water from the insect body. Death is due to dehydration (Dowdy and Fields, 2002). Heat treatment of empty bins has been proven effective to kill insects by raising temperatures to 50-60°C for 2-4 hours (Tilley et al., 2006). The benefit of conducting a heat treatment over fumigation is that it is an environmentally conscious approach and no special licenses or complying with federal regulations are needed. Previous research has shown heat treatments in combination with certain chemical insecticides and DE to increase efficacy in laboratory trials and flour milling facilities (Subramanyam and Roesli, 2000; Dowdy and Fields, 2002; Arthur and Dowdy, 2002). The

use of elevated temperatures with low-toxicity insecticides could greatly improve their overall performance and create a more sustainable method for farmers to disinfest their bins. By combining these treatment types, lower energy inputs are required to obtain temperatures lethal to insects. By lowering the temperature needed for insect lethality, it would make treatments a viable option for farmers because they can schedule bin cleaning based on ambient temperatures and not rely on costly external heaters. Eliminating stored-product insects in empty bins prior to storage of new grain, along with additional integrated pest management methods, can increase the profitability and quality of stored grain in a more sustainable and environmentally friendly manner, thereby reducing the need for chemical treatments and energy requirements.

The use of DE in conjunction with heat in a flour mill was found to be effective for controlling the confused flour beetle, *Tribolium confusum* (Jacquelin du Val), in areas where 47°C was not attained (Dowdy and Fields, 2002). They found that when used together, less DE and lower temperatures were effective in reducing *T. confusum* populations similar to the use of higher rates of DE and higher temperatures, respectively. Arthur and Dowdy (2003) found using an insecticide (cyfluthrin and hydroprene) in a flour mill increased toxicity when used in combination with a heat treatment. High temperatures will degrade certain insecticides, but the degradation may be minimal because the treated substrates are only exposed for short periods of time during a structural heat treatment (Arthur and Dowdy, 2003).

1.11 Research objectives

The following are the research objectives for the studies with adults of *T. castaneum* and elevated temperatures.

- To develop and validate a dynamic model for predicting the survival of *T. castaneum* adults to elevated temperatures during heat treatment of grain-processing facilities (Chapter 2),To evaluate heat treatments based on temperature profiles, bioassays, and trapping data (Chapter 3),
- To determine the effects of sex, age, and short-term heat acclimation on the mortality of adults of *T. castaneum* to elevated temperatures (Chapter 4),
- To determine the effect of continuous rearing of *T. castaneum* at elevated temperatures on subsequent thermal tolerance to heat treatments (Chapter 5),
- To investigate the influence of temperature and application rate on efficacy of a diatomaceous earth formulation against *T. castaneum* adults (Chapter 6).

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Chapter 2. A dynamic model for predicting survival of *Tribolium castaneum* adults to elevated temperatures during heat treatment of grain-processing facilities

2.1 Abstract

A dynamic model was developed to predict the survival of adults of the red flour beetle, *Tribolium castaneum* (Herbst), based on the logarithmic survival of adults as a function of time at nine constant temperatures between 42-60°C, and the logarithmic reduction of adult survival as a function of temperature. The developed model was validated using 10 datasets from a pilot feed mill, and two grain-processing facilities. Bioassays included bleached flour in plastic boxes containing 10 or 20 *T. castaneum* adults. Samples were collected at different intervals during the heat treatment of feed mills and grain-processing facilities and adults were sifted into plastic cups with bleached flour and placed in an environmental growth chamber at 28°C and 65% r.h. and assessed for mortality after 24 h. In the seven locations where 50°C was attained, the heating rate to 50°C from the initial ambient temperature ranged from 2.0°C/h to 15.4°C/h, and the time above 50°C until survival reached 0% was between 0.0-2.4 h. Three locations monitored did not reach 50°C before survival reached 0%. Mean absolute deviations between observed and predicted survival ranged from 5.7-39.5%. The dynamic model developed can be used to effectively predict survival of *T. castaneum* adults based only on time-dependent temperature.

2.2 Introduction

With the phase out of methyl bromide, alternatives to manage insects in grain-processing facilities and storage bins must be explored. Heat treatment is a one hundred year old technology that is safe and very effective for management of stored-product insect pests associated with empty bins and food-processing facilities (Dosland et al., 2006). Heat treatment of a structure involves raising its interior temperatures to 50-60°C for 24 h or less (Boina et al., 2008; Subramanyam et al., 2011). The benefit of conducting heat treatments over the use of fumigants is that it is an environmentally sensitive-approach and does not require special training or complying with United States Environmental Protection Agency regulations. Heat treatments are safe to workers and by-standers, and are relatively inexpensive compared to other pest-management interventions.

An understanding of how elevated temperatures affect stored-product insect species and their life stages is important in developing heat treatments. Heat treatments are common in managing insect pests found in fresh commodities, such as fresh and dried fruits, tree nuts, and certain grains (Landolt et al., 1984; Neven, 1994; Waddell et al., 2000; Wang et al., 2002a,b; Johnson et al., 2003). Thermal death kinetic (TDK) models were first used to model thermal inactivation of bacteria and have also been used to model the death of insects in fresh commodities (Neven, 2000). When dealing with these types of insects and commodities, heating rates are much higher (1-18°C/min) than those employed during structural heat treatments (0.3-13.7°C/h) (Mahroof et al., 2003a; Roesli, 2003; Boina et al., 2008). The time of treatment also varies greatly, from a matter of minutes in fresh commodities, dried fruits, and nuts, to 24 h or less for a structural heat treatment (Johnson et al., 2004; Boina et al., 2008; Subramanyam et al., 2011).

A heating block system was used to determine the most heat tolerant stage of *T. castaneum* and to subsequently develop a TDK model for the most heat tolerant stage (Johnson et al., 2004). The heating rate used to determine the thermal death kinetics of the most heat tolerant stage was 15°C/min, which is comparable to the heating rate achieved when treating nuts by radio frequency and microwave energy (Johnson et al., 2004). Insects from each life stage were placed in the heating block system at 48, 50, and 52°C for three different times each to determine which stage was most heat tolerant. Based on the results, it was found that mature larvae were the most heat tolerant, followed by pupae and adults, and then eggs and younger larvae. Mature larvae were used for the TDK experiments, where they were placed in the heating block system at 4-5 exposure times ranging from 0.5-80 min at 48, 50, and 52°C. Findings revealed that it would take 85 min to achieve 100% mortality of mature larvae at 48°C, 12 min at 50°C, and 2 min at 52°C.

Researchers have found differences in the most heat tolerant stages of insect species, in large part due to a large variability in heating conditions. Mahroof et al. (2003a) conducted a laboratory experiment to determine time-mortality relationships for different life stages representing eggs, young larvae, old larvae, pupae, and adults of *T. castaneum*. Each stage was exposed to constant temperatures of 42, 46, 50, 54, 58, and 60°C, temperatures typically observed during heat treatments, in laboratory growth chambers at 22% r.h. (Mahroof et al., 2003b). They reported young larvae or first instars to be the most heat-tolerant. A minimum of 7.2 h at or above 50°C was needed to kill 99% of the exposed individuals. All other stages required 1.8 h or less to kill 99% of the exposed individuals. Based on these results, it was predicted that the success of a heat treatment could be gauged based on the mortality of the young larvae of *T. castaneum*. In a heat treatment of a pilot flour mill (Mahroof et al., 2003b),

old larvae and pupae of *T. castaneum* were observed to be more heat tolerant than the other stages. This finding is at variance from results obtained at constant temperatures (Mahroof et al., 2003a). Brijwani et al. (2012b) reported adults to be the more tolerant to heat compared with other life stages during heat treatment of a pilot flour mill. The discrepancy between the findings of Mahroof et al. (2003b) and Brijwani et al. (2012b) could be related to differences in heat application methods and heating rates, resulting in differential susceptibility of the exposed life stages (Wang et al., 2007). The increase in heat tolerance of certain life stages could be related to the heating rate, which may affect the production of heat shock proteins, or increased respiration due to a higher metabolic rate (Emekci et al., 2002; Mahroof et al., 2003b, 2005a, 2005b; Wang et al., 2007). An increase in heat tolerance may be related to lower heating rates, which can occur during structural heat treatments.

Models to predict insect mortality during a structural heat treatment are important to heat treatment operators so that energy is not wasted due to under-heating or over-heating. Over-heating (>60ºC) results in damage to heat-sensitive equipment and fixtures in the facility and unnecessary expense. Under-heating (<50°C) could result in insects surviving sub-lethal temperatures which can happen if the facility is not heated long enough to allow heat to penetrate areas where insects are present (Boina et al., 2008).

Boina et al. (2008) developed a dynamic model for mature larvae of the confused flour beetle, *Tribolium confusum* (Jacquelin duVal) based on the first-order or Bigelow model commonly used for microbial inactivation (Stumbo, 1973; Van Boekel, 2008):

$$\boldsymbol{S(t) = \exp(-\frac{t}{D})} \qquad \text{Equation 2.1}$$

where $S(t) = N/N_0$, the ratio of the final number of bacteria (N) to the initial number (N_0), t is time (min), and D is the decimal reduction time (min), which is the time required to kill 90%, or 1 log reduction, of the mircroorganisms.

The goal of this research was to apply the dynamic model adapted from Equation 2.1 for mature larvae of *T. confusum* and to develop a similar model for adults of *T. castaneum* so that reliable recommendations can be provided to heat treatment applicators.

2.3 Materials and methods

2.3.1 *Insects*

T. castaneum cultures were reared in 0.94-L glass jars filled with 250 g of a medium consisting of 95% organic, whole wheat flour (Heartland Mills, Marienthal, Kansas, USA) and 5% (by wt) brewer's yeast in growth chambers at 28°C and 65% r.h. Jars were closed with metal lids fitted with filter papers and wire-mesh screens. Cultures were reared in the Stored-Product Insect Research and Education Laboratory at Kansas State University in the Department of Grain Science and Industry, Manhattan, Kansas, USA, where they have been in rearing since 1999.

2.3.2 *Bioassays*

Twenty *T. castaneum* adults of mixed-age and sex were used in experiments. They were separated from diet using a sieve with 841µm openings (Seedburo Equipment Co., Chicago, Illinois, USA). They were placed in plastic boxes (4.5×4.5×1.5 cm) containing 305 ± 3 mg bleached wheat flour, and covered with a wire-mesh screened lid to allow for air diffusion. After insect introduction, boxes were placed inside growth chambers (Model I-36 VL, Percival Scientific, Perry, IA, USA) set at 42, 44, 46, 48, 50, 52, 54, 58, and 60°C. A control treatment

was held in a growth chamber maintained at 28°C and 65% r.h. The temperature and humidity levels were measured using a HOBO® data sensor (Onset Computer Corporation, Bourne, Massachusetts, USA). At elevated temperatures humidity levels typically range from 20-22% (Mahroof et al., 2003a; Subramanyam et al., 2011). At each temperature, five boxes (n = 5) were removed at each of the 7-14 exposure times across the nine temperatures. Five boxes with adults and diet placed at 28°C and 65% r.h., and sampled at times corresponding to each of the elevated temperatures served as the control treatment.

After exposure to different temperatures, adults were placed in 150-ml plastic cups containing 40 g bleached wheat flour plus 5% yeast (by wt). Containers were closed with perforated lids covered with a fine mesh (600-µm aperture) to prevent insect escape but to allow air diffusion. Containers held at 28°C and 65% r.h. for 24 h prior to mortality assessments. To determine mortality, adults were separated from the flour in containers using an 841-µm sieve. Live and dead adults were counted, and percentage survival was calculated based on number of live adults out of the total. There was no adult mortality in any of the controls.

2.3.3 *Model development*

Data were pooled across the five replicates at each collection time to calculate number of adults surviving out of the exposed100 adults. Survival counts were transformed to logarithmic scale and plotted as a function of time (Figure 2.1). At each temperature, the data were fitted to a polynomial equation (2.2) using Table Curve 2D software (Systat Software Inc., Chicago, IL, USA):

$$y = a + bx + cx^2 \qquad \text{Equation 2.2}$$

where, *y* is the $\log_{10}$ of survival, *x* is the exposure time (min), and *a*, *b*, and *c* are the estimated parameters.

From these data, a *D*-value could be calculated for each temperature tested, which represents the time (min) for a one-log reduction in the survival of *T. castaneum* adults. The slope of each line was not constant, so the average slope was calculated using the first derivative of the polynomial equation for every one minute in Microsoft Excel®, and then the inverse of the average of the slopes was calculated to determine the mean instantaneous *D*-value at that temperature. The *D*-values were plotted as a function of temperature and fitted to Equation 2.3:

$$D(T_t) = \exp(a + \frac{b}{T_t^2}) \qquad \text{Equation 2.3}$$

Where, $D(T_t)$ is the *D*-value as a function of time-dependent temperature (T_t), and *a* and *b* are estimated parameter (Figure 2.2).

Boina et al. (2008) developed a novel dynamic model (Equation 2.4) based on first-order kinetics for *T. confusum* old larvae, and the same model was adapted for use with *T. castaneum* adults:

$$N_t = \frac{N_0}{10^{\left(\sum_0^t \frac{\Delta t}{D(T_t)}\right)}} \qquad \text{Equation 2.4}$$

where, N_t is the number of surviving insects at time *t* during heat treatment, N_o is the number of insects initially exposed to the heat treatment (if unknown, it is assumed to be 100), Δt is the incremental time in which temperature was measured in minutes, and $D(T_t)$ is the *D*-value calculated from Equation 2.3.

In order to validate the dynamic model, the survival of *T. castaneum* adults during two facility heat treatments (five different locations) was predicted using Equation 2.4 and the model

was validated using temperature and bioassay data collected during the heat treatments and by comparing the predicted survival to the observed or actual survival.

2.3.4 *Heat treatment datasets*

Ten temperature and bioassay datasets were collected from three facility heat treatments for model validation. The first data set was collected from the Kansas State University pilot feed mill. Natural gas heaters from Temp-Air (Rupp Industries, Inc., Burnsville, MN, USA) were used to heat the feed mill on 5-6 August 2003. Commercial heaters (three THP-550 and one THP-1400) were placed outside the mill and hot air was forced into the mill through nylon ducts (50.8-cm diameter). The maximum heat energy output of the three THP-550 heaters and the THP-1400 heater was 161.19 kW/h and 410.30 kW/h, respectively. Bayley fans (1.5 hp, 391 cm^3/min) from Temp-Air were used inside the mill to more evenly distribute the heat. Two fans were placed on each of the three floors of the feed mill.

Bioassay boxes (n=28) were placed on the first floor of the feed mill, each containing 305 mg of bleached wheat flour and 20 *T. castaneum* adults. A HOBO® temperature sensor was placed next to the boxes and was programmed to take temperature readings every five min. Two boxes were collected at 14 different times between 0-4 h of the heat treatment, and the heat treatment lasted a total of 24 h. After collection, the boxes were taken to the laboratory where insects from both boxes were placed into 150-ml plastic containers containing 40 g of bleached wheat flour, and placed inside the control growth chamber held at 28°C and 65% r.h. for 24 h before samples were assessed for insect survival. A percentage was calculated based on number of surviving insects out of 40. Control mortality was 0%.

Eight datasets (Commercial 22, 24, 25, 27-31) were collected from eight different locations within a grain processing facility in the Midwestern United States subjected to a heat treatment. Heat treatment was conducted 31 August- 2 September 2007. Temp-Air steam heaters, model STHP-650 (maximum heat energy output of 150.23 kW/h), were used to deliver the heat. Portable fans (Aerovent, Piqua, OH, USA) were used in this facility to distribute the heat inside the facility. For this heat treatment, *T. castaneum* adults (*n*=30) were held in round plastic vials (2.6-cm inner diameter, 4.9 cm height) containing 5 g of bleached wheat flour and closed with a 600-µm screened mesh lid. Vials were placed at various locations around the facility and one vial was collected at the beginning of the heat treatment and one each after 2, 3, 4, 5, 6, 7, and 8.2 h into the heat treatment. The heat treatment lasted 24 h, but there were no surviving adult insects after 9 h in any of the locations where bioassays were kept, so the data sets were truncated when 100% mortality of adult insects (0% survival) was observed at each location. HOBO® temperature sensors recorded temperatures every minute. After each collection time, insects were placed in 150-ml plastic containers with 40 g of bleached wheat flour and held for 24 h at 28°C and 65% r.h. before survival was assessed.

The third heat treatment was conducted 18-19 August 2014 at a grain-processing facility in the southeastern United States. The facility was heated using four propane heaters with a heat output of 1318.8 kW/h (THP4500, Rupp Industries Inc., Burnsville, MN). Various fans were used to ensure even heat distribution: 19 Schaffer fans (91.4 cm), 18 box fans (121.9 cm), and 8 Bailey fans (71.1 cm). For this heat treatment, *T. castaneum* adults (*n*=10) were placed in five round plastic vials (2.6-cm inner diameter, 4.9 cm height) containing 5 g of bleached wheat flour and closed with a 600-µm screened mesh lid. Vials were placed in three different locations at varying distances from the heat source on the first floor. Five vials were collected at the

beginning of the heat treatment and five more each after 0.6, 0.9, 1.0, 1.5, 1.7, and 2.0 h into the heat treatment. The heat treatment lasted 26 h, but there were no surviving adult insects after 2.0 h of treatment. HOBO® temperature sensors recorded temperature every minute. After collection, insects were placed in 150-ml plastic containers with 40 g of bleached wheat flour and held for 24 h before survival was assessed.

2.3.5 *Data analysis*

For each of the 10 datasets the following were determined: the starting temperature, time taken to reach 50°C, heating rate to 50°C, time above 50°C, observed time to 0% survival, predicted time to 0% survival, and maximum temperature reached.

The dynamic model (Equation 2.4) was programmed into Microsoft Excel® to obtain predicted survival values based on time and temperature values collected during the heat treatments. The predicted values (y) were compared to observed values (x) and a linear regression was obtained for each data set. The slope of each regression was compared to a deviation from 1 (the expected slope) using a t-test with n-2 degrees of freedom (Zar, 1984). A P-value greater than 0.05 showed that the predicted survival values obtained from the model were not significantly different from the observed survival values. Next, the difference between predicted and observed values at each time of data collection was calculated. The average and standard error of the deviations were calculated for each data set, and the percentage of deviation values within 1% (predicted = observed), greater than 1% (the model over-predicted adult survival), and less than -1% (the model under-predicted adult survival) was determined (Table 2.3).

2.4 Results and Discussion

2.4.1 *Model development at constant temperatures*

The polynomial equation (2.2) was used to fit the curves of the logarithm of adult *T. castaneum* survival versus time at nine constant temperatures, and the r^2 values and parameters are given in Table 2.1. As the fixed temperature increased between 42 and 60°C, the time needed for logarithmic survival decreased. The first derivative of Equation 2.2 was used at each constant temperature to determine the slope of the polynomial curve at each minute. The inverse of the average of the instantaneous slopes was determined to be the mean instantaneous *D*-value, which is the time in minutes needed for one log reduction of insect population at each of the temperatures. This value ranged from 1542.88 min at 42°C, to 10.10 min at 60°C (Table 2.1). The largest *D*-value was obtained at 42°C and decreased by more than ten-fold at 46°C. The *D*-value at 48°C was three times less than the value at 46°C, and the values between 50 and 60°C remained in the range of 9.27 - 20.61 min.

The resulting *D*-values were plotted against temperature (Figure 2.2) and fitted to an exponential decay model (Equation 2.3), where a = -6.81 and b = 24964.9. *D*-values for temperatures in heat treatment data sets less than 42°C were extrapolated in the dynamic model using Equation 2.3. The model was validated based on given recorded values for temperature and time from each heat treatment conducted, and resulting *D*-values plugged into the dynamic model using Microsoft Excel (Equation 2.4).

Temperatures between 45-50°C typically cause death of stored-product insects within 1 d and death in less than 1 h within the range of 50-62°C (Fields, 1992). Survival reached 0% between 25-60 min at temperatures between 50-60°C under laboratory conditions. Researchers

recommend heat treatment temperatures reach 50°C or greater, and for those temperatures to be held for several hours in order to penetrate hard to heat areas, such as doorways, floor-wall junctions, and voids in the floor or walls (Dowdy and Fields, 2002; Roesli et al., 2003; Boina and Subramanyam, 2004).

2.4.2 *Validation datasets*

The starting temperature for the validation datasets ranged between 26.7-40.6°C (Table 2.2). The coolest starting temperature was at the KSU feed mill. It took 3.0 h to heat to 50°C, at a heating rate of 7.8°C/h. Survival of *T. castaneum* adults reached 0% within 3.9 h. The warmest starting temperature was at the grain-processing facility (Commercial 28) where it took 3.4 h to heat to 50°C at a heating rate of 2.8°C/h. Adult survival reached 0% within 4.0 h at this location. Temperatures never reached 50°C at Commercial 22, Commercial 29, and Commercial 30, and the maximum temperatures reached were 48.0, 49.6, and 48.5°C, respectively. However, despite the fact that 50°C was never reached in these locations, 0% survival of *T. castaneum* adults was observed within 9.0, 8.3, and 7.0 h, respectively. Temperatures remained greater than 46°C for 1.6, 2.3, and 1.8 h at Commercial 22, 29, and 30, respectively. Temperatures were greater than 42°C for 4.3, 4.5, and 3.9 h, respectively. The heating rate to the maximum temperature was 1.6°C /h at Commercial 22, 2.0°C/h at Commercial 29, and 1.9°C/h at Commercial 30. These heating rates are consistent with those commonly found during structural heat treatments (Mahroof et al., 2003a; Roesli, 2003; Boina et al., 2008). Heating rates are recommended to be between 3.0-5.0°C/h in order to prevent the development of thermo-tolerance in insects as well as to prevent heat damage to the facility (Subramanyam et al., 2011).

Temperature and survival were plotted against time for each facility and location monitored during heat treatment and the predicted survival at each time and temperature can be seen in the same plot in Figure 2.3. In most cases, the model under-predicted the lag phase, where the insects remained alive until a rapid decline in survival began. This could be due to the fact that the average slope of a non-linear curve was used in calculations of the *D*-value. The rapid decline in survival of *T. castaneum* adults during heat treatment has not been documented with the other life stages, and merits further study.

In seven out of the 10 datasets, the model under-predicted the survival of *T. castaneum* adults in 14.3-71.4% of the observations (Table 2.3). In six of the 10 datasets, the model had over-predictions, ranging from 22.2-66.7% of the observations. Accurate predictions were made between 21.4-50% of the time. Despite the under-predictions, in each case, the predicted time to reach 0% survival was within 0.1-1.6 h of the observed time for 0% survival (Table 2.2). It is important to note that we do not know the exact time that 0% survival was achieved; the observed time to 0% survival was based on the collection time that resulted in no living adult insects.

The mean deviation for each heat treatment and location varied between 5.7-39.5%. It is typically better to over-predict than to under-predict insect survival in the case of heat treatments because it is better to over-heat a facility to ensure a complete kill than to not heat long enough thus allowing some insects to survive and continue to reproduce. A linear regression was performed in SAS to compare the predicted and observed values for each heat treatment and location (r^2 = 0.25-0.91). The slope values ranged from 0.60-1.11. A *t*-test was then conducted to compare deviation of each slope from 1, and the *P*-values ranged from 0.37-0.92, signifying that

each slope was not significantly different than 1. This indicated that the dynamic model developed adequately described the survival of *T. castaneum* adults at varying heating rates.

2.5 Conclusion

The dynamic model developed to predict survival of *T. castaneum* adults during structural heat treatments can be used in conjunction with other dynamic models developed for other life-stages of *T. castaneum* and other insect species. Understanding a species' response to elevated temperatures can be extremely useful when developing heat treatment methodologies in dynamic environments. Normally temperatures of 50-60°C must be reached and held for several hours in order for insects to die, but in the case of three of the validation datasets, 50°C was not reached, yet the *T. castaneum* adults still died. In addition, the developed model under-predicted the initial lag phase of *T. castaneum* adults where there is no decline in survival until a certain point is reached, and a rapid decline takes place. Future research may involve investigation into heat accumulation within the adult insect and how it affects their metabolism or ability to produce heat shock proteins (Mahroof et al., 2005a). The dynamic model used here does not account for development of thermotolerance in insects during structural heat treatments.

2.6 References

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Table 2.1 Parameters of a polynomial equation used to describe the relationship between logarithm of survival of *T. castaneum* adults and exposure time at nine temperatures.

Temp (ºC)	*n*	Parameter ± SE			Adj r^2	Mean ± SE instantaneous *D*-value (min)
		a	*b*	*c*		
42	14	0.85 ± 0.34	0.001 ± 0.000	-3E-7 ± 4.4E-8	0.96	1542.9 ± 23.8 (901)
44	11	-0.81 ± 0.94	0.007 ± 0.002	4E-6 ± 7.8E-7	0.94	440.0 ± 12.8 (321)
46	11	-0.21 ± 0.82	0.017 ± 0.004	-3.1E-5 ± 5.6E-6	0.96	134.1 ± 9.1 (101)
48	7	-1.25 ± 0.93	0.082 ± 0.019	-0.001 ± 8.8E-5	0.94	44.6 ± 7.9 (31)
50	7	-1.00 ± 1.29	0.202 ± 0.067	-0.003 ± 0.001	0.93	14.6 ± 3.2 (11)
52	10	2.20 ± 0.50	0.000 ± 0.029	-0.001 ± 0.000	0.94	20.7 ± 2.1 (16)
54	10	2.04 ± 0.36	-4E-05 ± 0.039	-0.002 ± 0.001	0.87	14.6 ± 1.9 (11)
58	8	1.70 ± 0.08	0.088 ± 0.011	-0.006 ± 0.000	0.99	9.3 ± 2.2 (8)
60	9	2.37 ± 0.25	-0.095 ± 0.038	-0.000 ± 0.001	0.93	10.1 ± 0.1 (9)

Table 2.2 Temperature parameters and observed and predicted survival data obtained from heat treatment datasets.

Validation dataset	Starting temp (°C)	Time to 50°C (h)	Heating rate to 50°C (°C/h)	Time above 50°C (h)	Obs. time to 0% survival[b] (h)	Pred. time to 0% survival (h)	Max temp (°C)
KSU feed mill	26.7	3.0	7.8	0.9	3.9	3.8	54.7
Commercial 22	33.6	--[a]	--[a]	--[a]	9.0	10.6	48.0
Commercial 24	38.3	4.6	2.5	2.4	7.0	5.4	54.7
Commercial 25	34.0	7.0	2.3	0.0	7.0	7.3	50.1
Commercial 27	36.6	5.9	2.3	0.1	6.0	6.2	50.1
Commercial 28	40.6	3.4	2.8	0.6	4.0	3.9	51.2
Commercial 29	32.8	--[a]	--[a]	--[a]	8.3	8.9	49.6
Commercial 30	35.3	--[a]	--[a]	--[a]	7.0	8.2	48.5
Commercial 31	38.3	6.0	2.0	0.0	6.0	6.3	50.1
Commercial B	31.5	1.2	15.4	0.8	2.0	1.4	64.5

[a] Temperatures never reached 50°C before 0% survival achieved.
[b] Observed time refers to the time the last sample was taken that resulted in 0% survival.

Table 2.3 Deviation values between predicted and observed survival of *T. castaneum* adults for the 10 datasets.

Validation dataset	*n*	Mean ± SE deviation[a]	Percent observations		
			Within 1% survival[b] (pred=obs)	Greater than 1% survival[c] (pred>obs)	Less than 1% survival[d] (pred<obs)
KSU feed mill	14	16.5 ± 6.2	21.4	57.2	21.4
Commercial 22	9	5.7 ± 4.0	33.3	22.2	44.5
Commercial 24	7	34.2 ± 13.6	28.6	0.0	71.4
Commercial 25	7	26.3 ± 13.1	28.6	0.0	71.4
Commercial 27	6	24.5 ± 12.1	33.3	66.7	0.0
Commercial 28	4	22.2 ± 15.8	50.0	50.0	0.0
Commercial 29	8	12.5 ± 6.1	50.0	50.0	0.0
Commercial 30	7	20.5 ± 11.6	28.6	57.1	14.3
Commercial 31	6	36.7 ± 16.2	33.3	0.0	66.7
Commercial B	7	39.5 ± 15.9	28.6	0.0	71.4

[a]Σ(%predicted survival - %observed survival)/*n*.
[b]Accurate prediction.
[c]Under-prediction of survival.
[d]Over-prediction of survival.

Table 2.4 Parameter estimates from linear regressions of predicted versus observed survival of *T. castaneum* adults during a facility heat treatments.

Validation data set	r^2	Mean ± SE parameter estimate		*t*-value[a] (df)	*P*-value
		Intercept	Slope		
K-State feed mill	0.76	-23.61 ± 13.03	1.11 ± 0.18	0.628 (12)	0.5420
Commercial 22	0.91	1.36 ± 9.07	0.91 ± 0.11	-0.867 (7)	0.4147
Commercial 24	0.43	-10.03 ± 28.13	0.66 ± 0.34	-0.983 (5)	0.3709
Commercial 25	0.42	-7.24 ± 36.61	0.77 ± 0.41	-0.563 (5)	0.5975
Commercial 27	0.62	-6.41 ± 24.17	0.74 ± 0.29	-0.873 (4)	0.4319
Commercial 28	0.64	-6.49 ± 27.54	0.72 ± 0.38	-0.731 (2)	0.5409
Commercial 29	0.82	-10.93 ± 16.04	0.98 ± 0.19	-0.106 (6)	0.9194
Commercial 30	0.48	2.58 ± 31.63	0.73 ± 0.34	-0.787 (5)	0.4670
Commercial B	0.25	-6.52 ± 42.78	0.60 ± 0.47	-0.835 (5)	0.4418

[a] *t*-test for H_o: slope = 1

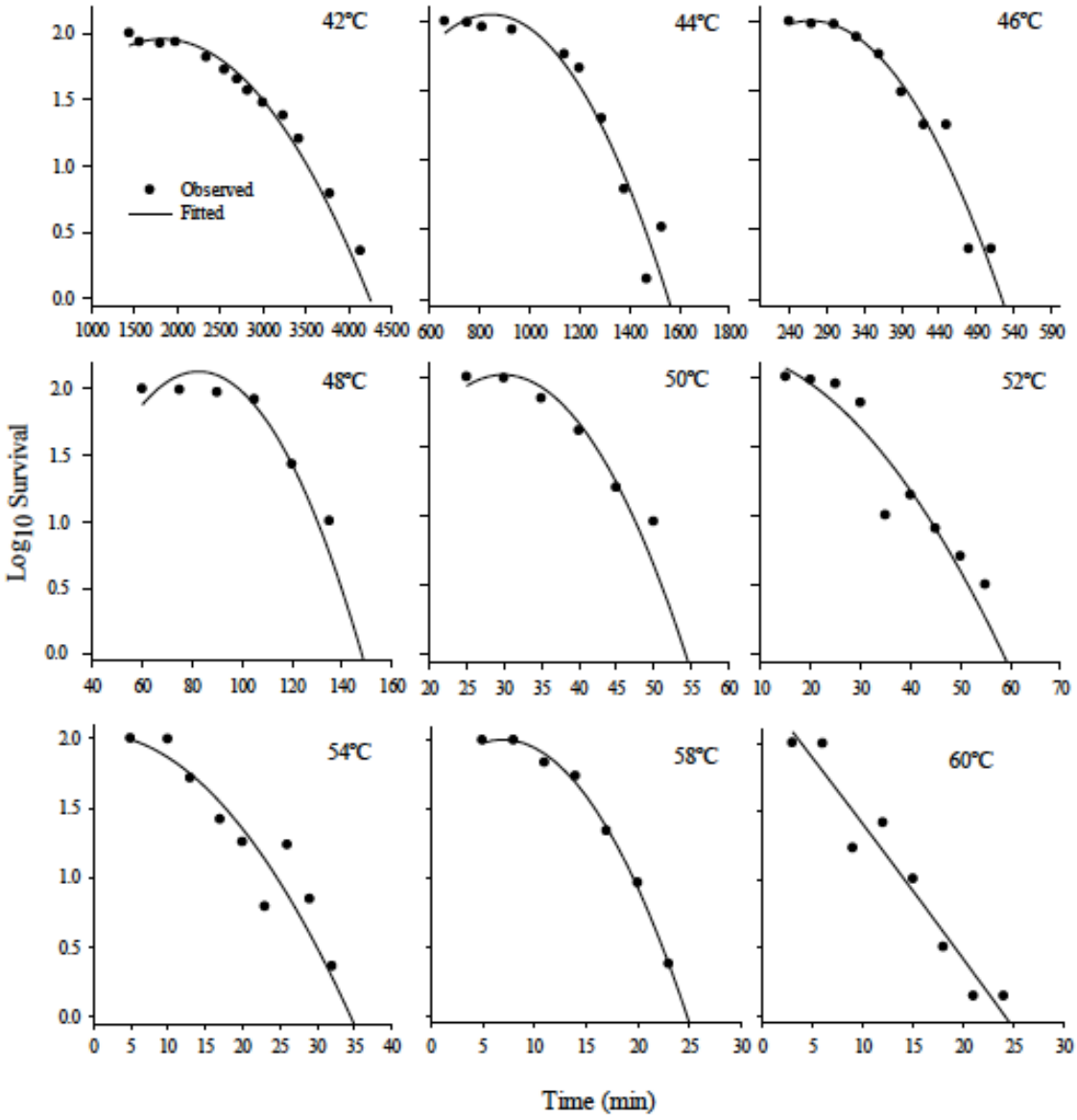

Figure 2.1 Time-dependent logarithmic survival plots for *T. castaneum* adults exposed to nine constant temperatures.

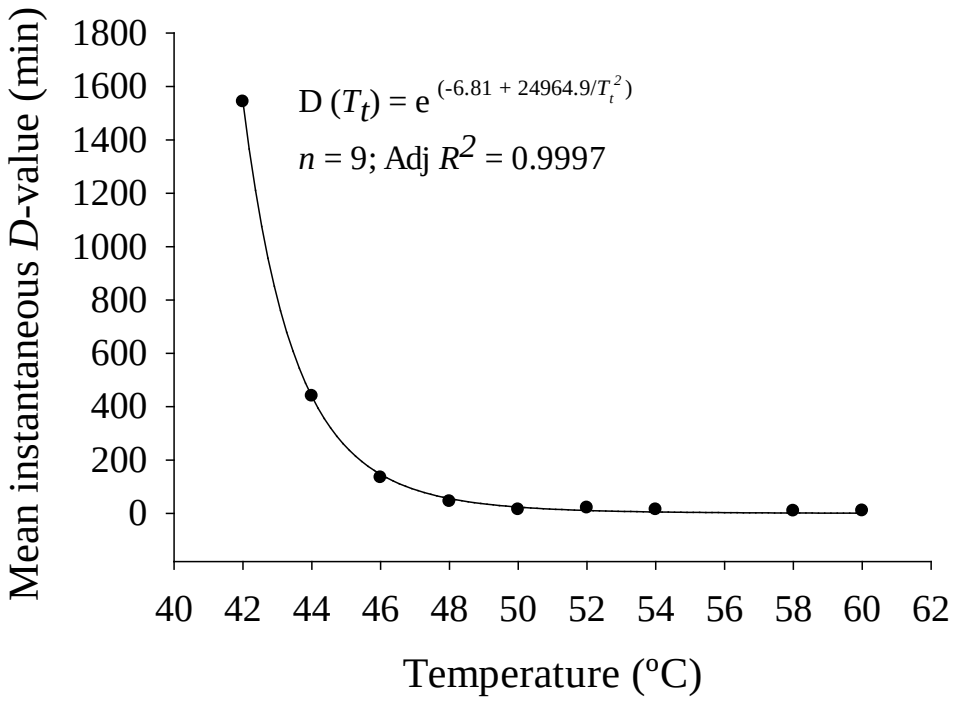

Figure 2.2 Mean instantaneous *D*-value as a function of temperature.

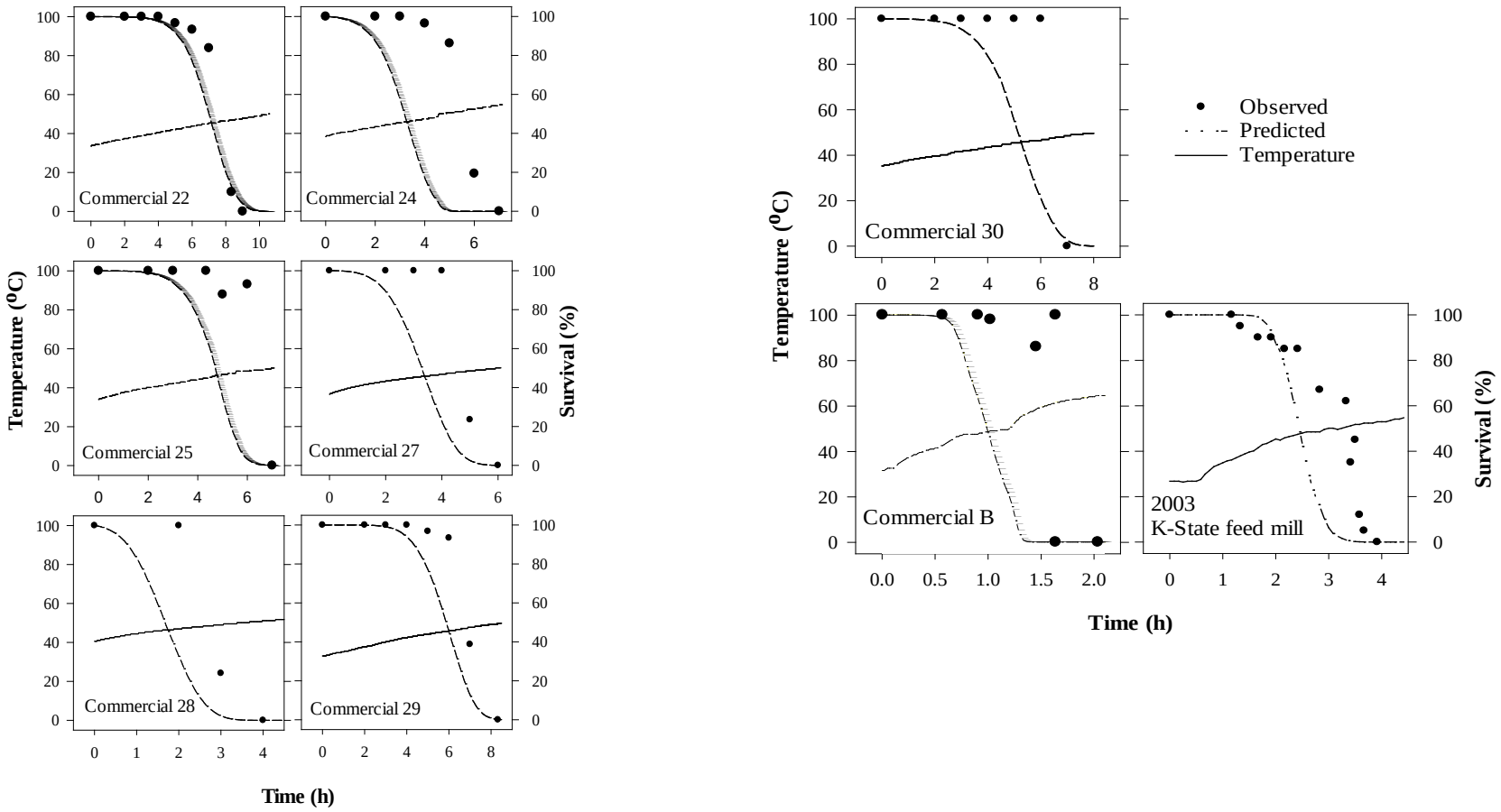

Figure 2.3 Validation data sets showing observed (filled circle) and predicted (dashed line) insect survival during facility heat treatments. The solid line shows time-dependent temperature.

Chapter 3. Evaluation of commercial heat treatments based on trapping data, temperature profiles, and bioassays

3.1 Abstract

Two rooms in a pasta manufacturing facility were subjected to a steam heat treatment, which was generated by a natural gas fueled boiler. The first room, the bin room, contained empty steel storage bins, and the second room, the pasta press room, contained various pieces of equipment. Before being heated, the rooms were cleaned and the steel bins and equipment were opened to allow heat penetration. Temperature sensors were placed in 49 total locations during a 16 h treatment of the bin room, and a 17 h treatment of the press room during 1-2 July 2006. Temperatures reached 50°C in 3.5 to 5 h in most locations and stayed above 50°C for 12.5 to 14 h. In addition, 45 pitfall traps were placed inside the facility to determine captures of adults of the red flour beetle, *Tribolium castaneum* (Herbst), both before and after the heat treatment. There was an 86-100% reduction in the numbers of *T. castaneum* adults in the bin and pasta press rooms immediately after the heat treatment, and trap captures were kept below pre-heat treatment levels by good exclusion and sanitation practices for another two months. Bioassays of adults of *T. castaneum* were placed in the heat treated rooms at a total of four different locations. There was 100% mortality of *T. castaneum* within 5.7 h in the bin room; in the press room, 100% mortality was obtained by the end of the 24 h heat treatment in all three locations where *T. castaneum* bioassays were placed.

The second heat treatment took place in a rice mill in the southeastern United States. Temperature sensors were placed in three locations with bioassays of *T. castaneum* eggs, young larvae, old larvae, and adults. Samples were taken 0.6, 0.9, 1.0, 1.5, 1.7, 2.0, 4.1, 6.2, and 26 h

into the heat treatment. Based on bioassay results, adults appeared to be the most heat tolerant stage, while eggs appeared to be the least heat tolerant stage. All life stages were dead within 6.2 h of heat treatment. These results confirm that heat treatments are an effective tool to reduce or eliminate populations of *T. castaneum* in structures.

3.2 Introduction

The use of elevated temperatures or heat treatments to manage stored-product pests associated with grain-processing facilities is an effective alternative method to using fumigants such as methyl bromide and sulfuryl fluoride (Brijwani et al., 2012). Methyl bromide, an ozone-depleting fumigant, was phased out in the United States in 2005. Sulfuryl fluoride, a non-ozone depleting fumigant was registered by United States Environmental Protection Agency in 2004. It is not effective against eggs of stored-product insects, especially at temperatures below 27ºC (Lawrence et al., 2012). Heat treatment involves raising the ambient temperature of clean, empty grain-processing facilities or empty storage bins to 50-60ºC and holding these temperatures for 24 h or less (Dowdy and Fields, 2002; Wright et al., 2002; Dosland et al., 2006; Subramanyam et al., 2011). Insects exposed to 50ºC will die within minutes to an hour (Fields, 1992). Heat treatments are environmentally sensitive and safe for workers. The effectiveness of heat treatments against stored-product insects depends on how quickly temperatures reach 50ºC from the ambient, how long temperatures are held above 50ºC, and the maximum temperature attained (Subramanyam et al., 2011; 2012). The predicted time to kill 99% of young larvae of the red flour beetle, *Tribolium castaneum* (Herbst), which is the most heat tolerant stage when compared with eggs, old larvae, pupae, and adults at 50-60°C, was positively related to time to 50ºC and negatively related to time above 50ºC and the maximum temperature, based on tests conducted in commercial facilities (Mahroof et al., 2003; Subramanyam et al., 2012).

In the present investigation the effectiveness of a heat treatment of a pasta manufacturing facility (Facility A) and rice mill (Facility B) were evaluated. In facility A, the effectiveness was evaluated by examining temperatures attained, number of adults of *T. castaneum* captured several weeks before and after the heat treatment intervention, and mortality of insects in

bioassay boxes. The effectiveness at facility B was evaluated based on temperature profiles and mortality of different *T. castaneum* life stages (eggs, young larvae, old larvae, and adults) in bioassay vials collected at different times during heat treatment.

3.3 Materials and Methods

3.3.1 *Facility A*

Facility A is a pasta processing facility located in the southeastern region of the United States. The heat treatment took place in two rooms, the press room and the bin room. The press room is where pasta is pressed, and the bin room is where the flour is stored. The heat treatment took place July 1-2, 2006, and the data presented were obtained by Dr. Bhadriraju Subramanyam in the Department of Grain Science and Industry at Kansas State University, Manhattan, Kansas, USA.

3.3.1.1 Heat treatment

The press room had a volume of 43,891 m^3 with a floor area of 4,343 m^2. The bin room volume was 3,398 m^3 with a floor area of 335 m^2. To uniformly circulate hot air within the heated rooms, five drum fans were placed throughout the bin room, and seven drum fans, 10 pedestal fans, and four heat buster fans (TempAir, Burnsville, Minnesota, USA) were placed throughout the larger press room. Room schematics and placement of bioassays are shown in Figure 3.1.

3.3.1.2 Temperature monitoring

Temperatures were recorded at one minute intervals using HOBO® data loggers (Onset Computers Corporation, Bourne, MA, USA). There were 37 data loggers placed in the press

room, and 12 data loggers in the bin room. A data logger was placed with the insect bioassays (see below) in each of the three locations in the press room and in one location in the bin room so that temperature, time, and mortality data could be correlated.

3.3.1.3 Bioassays

Cultures of *T. castaneum* from the Stored-Product Insect Research and Education Laboratory, Department of Grain Science and Industry, Kansas State University, were reared on organic whole wheat flour plus 5% by weight brewer's yeast diet. All cultures were reared in a growth chamber at 28°C and 65% r.h. Unsexed adults (50) of mixed ages of *T. castaneum* were placed in plastic bioassay boxes (4.5 cm×1.5 cm high) containing 5 g of whole wheat flour. Plastic boxes were placed in three locations in the press room (A, B, and C) to get a representative sampling of the effects of temperature differences within the room. In the bin room one location (D) had insect bioassays which consisted of 50 *T. castaneum* adults in each 0.45-L glass jars with 50 g of flour. In the press room, bioassay boxes with *T. castaneum* were collected at the following times after heat treatment started: 1.2, 2.8, 4.1, 5.8, 6.6, 7.5, 8.0, 8.3, 8.7, 8.8, 9.1, 9.5 and 12.4 h. In the bin room, bioassay boxes were collected after 2.6, 3.4, 4.1, 4.7, 5.2, 5.7, 6.2, 6.7, 7.5, and 8.3 h into the heat treatment because of temperatures reaching lethal levels (≥50°C) quickly. Counts were taken of live and dead insects on July 2, 2006 on site, and the percent survival was calculated. Control insects were held in an unheated room with an average temperature of 20ºC. None of the control insects died during the heat treatment period.

3.3.1.4 Trapping

Commercial food- and pheromone-baited pitfall traps (Trécé, Enid, Oklahoma, USA) were used to sample adults of *T. castaneum* in 35 locations in the press room, 10 locations in the

bin room, and in five locations outside the facility. The receptacle of the bottom portion of pitfall trap was fitted a filter paper and 15 drops of a food attractant oil was added. The lid for the trap on the inside was fitted with an aggregation pheromone (4,8-dimethyldecanol) lure to capture red flour beetles, *Tribolium castaneum* (Herbst). Traps were placed on the floor of each room in a grid-like fashion (Campbell et al., 2002). Trapping was conducted for approximately six weeks prior to the heat treatment (May 16 through June 28, 2006) and for an additional seven weeks after (July 3 through August 23, 2006). Trap captures were counted at approximately biweekly intervals. New traps and lures were placed after the heat treatment. Trap capture of adult insects before and after heat treatment intervention were used to determine the degree of suppression of resident populations and duration of effectiveness of a single heat treatment.

3.3.2 *Facility B*

Facility B is a rice mill in the southeastern region of the United States. The heat treatment at this facility was conducted August 18-19, 2014, and the data were collected by the dissertation author, Dr. Jennifer Frederick.

3.3.2.1 Heat treatment

The rice processing facility had a volume of 26,051 m^3. Four propane heaters with a heat output of 1318.8 kW/h (THP4500, Rupp Industries Inc., Burnsville, Minnesota, USA) were placed around the facility for heating. Additionally, 19 Schaefer fans (91.4 cm, Pinnacle Climate Technologies, Sauk Rapids, Minnesota, USA), 18 box fans (121.9 cm), and 8 Bayley fans (71.1 cm, Bayley Fan, Lebanon, Indianapolis. USA) were set up to help evenly distribute the heat (Figure 3.2). The heat treatment lasted for 26 h.

3.3.2.2 Temperature monitoring

HOBO® data loggers recorded the temperature every minute. TempAir had 62 temperature sensors set up around the facility, but for this analysis, only six sensors were monitored, and they were placed in front of and behind each of the bioassay sampling areas.

3.3.2.3 Bioassays

For this heat treatment, *T. castaneum* eggs, young larvae, old larvae, and adults (*n*=10 each) were collected from rearing jars and each life stage was placed in five round plastic vials (2.6-cm inner diameter, 4.9 cm height) containing 5 g of bleached wheat flour and closed with a 600-µm screened mesh lid. Adults and old larvae were separated from growth medium using an 841-µm aperture sieve and young larvae and eggs were separated using a 250-µm aperture sieve (Seedburo Equipment Co., Chicago, Illinois, USA). Five vials of each life stage were collected at the beginning of the heat treatment and five more each after 0.6, 0.9, 1.0, 1.5, 1.7, 2.0, 4.1, 6.2, and 26 h into the heat treatment. Collection times were selected to get samples before temperatures reached 50°C, close to 50°C, and above 50°C. After collection, insects were placed in 150-ml plastic containers with 40 g of bleached wheat flour and held for 24 h before adult survival was assessed. For immature life stages, the containers were held in a laboratory growth chamber maintained at 28°C and 65% r.h. until emergence of adults. Mortality was assessed based on number of adults that emerged out of the total immatures that were exposed. Control containers were held in the chamber throughout the duration of the heat treatment to correct for control mortality.

3.3.3 *Data analysis*

The time-dependent temperature data from each location was used to determine the starting ambient temperature, time required to reach 50ºC and rate of heating to 50ºC, time temperature was held above 50ºC, and the maximum temperature attained.

Trap capture data at facility A taken immediately before and immediately after a heat treatment in each of the two rooms were subjected to a two-sample *t*-test (SAS Institute, 2008), and means were considered significant at $\alpha = 0.05$. Trapped insect data were transformed to log(x+1) scale (Roesli et al., 2003) for SAS analysis. Percent reduction in *T. castaneum* adults was calculated using non-transformed data as:

$$\%\,\boldsymbol{reduction} = \left(\mathbf{1} - \frac{\boldsymbol{A}}{\boldsymbol{B}}\right) * \mathbf{100} \qquad \text{Equation 3.1}$$

where, *A* is the mean number of *T. castaneum*/trap/week immediately after heat treatment, and *B* is the mean number of *T. castaneum*/trap/week immediately before heat treatment (Roesli et al., 2003).

3.4 Results and Discussion

3.4.1 *Results and discussion for Facility A*

3.4.1.1 Temperature measurements

Temperatures in both the bin room (Table 3.1) and press room (Table 3.2) at the start of the heat treatment ranged from 23.2-39.7°C. The time to reach 50°C varied from 1.1 to 12.2 h. The rate of increase varied from location to location. Therefore, time above 50ºC also varied by location. Three locations in the press room did not reach 50°C due to inadequate heat flow to these areas. However, in other locations (1, 4, 6-12, 14, 16-18, 20, 23, 26-28, 30-33, 35, 42-45,

and 47-49) temperatures attained were greater than 60ºC. Temperature control should be implemented so that temperatures do not exceed 60ºC for extended periods of time which may cause structural damage. On average, temperatures in the press and bin rooms reached 50ºC within 4-5 h, and temperatures were held above 50ºC for 12-14 h, and the average maximum temperatures were 61-65ºC.

3.4.1.2 Bioassays

Adults of *T. castaneum* died within 12.4 h, 16.5 h, and 5.8 h in locations A, B, and C, respectively, in the press room (Figure 3.3). Location C had the highest rate of heating to 50ºC at 6.6ºC/h, followed by 2.8ºC/h at location B, and 2.3ºC/h at location A. In the bin room, 100% mortality was achieved within 5.2 h, where the heating rate to 50ºC was 9.2ºC/h. The results are consistent with data from other grain-processing facilities where bioassays of adults of *T. castaneum* were placed (Brijwani et al., 2012, Subramanyam et al., 2012). Mortality is positively correlated to a higher heating rate, or where temperatures above 50°C were maintained for a longer time, and negatively correlated to the time taken to reach 50ºC.

3.4.1.3 Trapping

Trapping results showed 86% reduction immediately after the heat treatment in the bin room, and despite this level of reduction, the mean number of adults of *T. castaneum* captured after the heat treatment were not significantly different from those captured prior to the heat treatment (t = 1.66; df = 11.56; P = 0.1233). In the press room, there were no captures (100% reduction) after the heat treatment, and this reduction was significant (t = 2.05; df = 34; P = 0.048) (Table 3.3). Higher numbers of *T. castaneum* were captured outside the facility. Outside the facility, insects were commonly found at the entryway to the storage area of raw ingredients

and the door leading to the area for natural gas service. Inside the facility, adult captures were higher in the bin room compared to the press room. This could be due to the fact that the semolina and durum flour are stored in the bin room. Insect populations were kept at low levels as inferred by trap captures for two months through effective sanitation and exclusion (closing doors) practices by facility sanitarians.

3.4.2 *Results and discussion for Facility B*

3.4.2.1 Temperature measurements

Inside the rice mill at the bioassay locations, the initial temperatures measured were 29.5, 28.5, and 31.0ºC at locations 1, 2, and 3, respectively (Table 3.4). Once heating started, it took between 1.3 and 2.1 h for temperatures to reach 50ºC. The heating rate to 50ºC was highest at location 2, where it was 15.6ºC/h, and lowest at location 1, at 9.9ºC/h. Heat treatments have varying rates of heating, but typically it is recommended that they be between 3.0-5.0ºC to protect the facility from heat damage and to prevent thermo-tolerance in insects (Subramanyam et al., 2011). In this case, heating rates were relatively high due to the high ambient temperatures when the heat treatment began, and lack of technical issues with the propane-fueled heaters upon start up. Maximum temperatures attained were 58.5, 58.0, and 66.5ºC. Temperatures greater than 60ºC can cause structural damage over time if not monitored (Imholte and Imholte-Tauscher, 1999). TempAir, the company performing the heat treatment, carefully monitored temperatures at 62 locations and had the ability to re-position fans so that no locations remained over-heated for too long.

3.4.2.2 Bioassays

At locations 1 and 2, all eggs died within 4.1 h, and young larvae, old larvae, and adults were all dead within 6.2 h (Tables 3.5 and 3.6). At location 3, no eggs survived 1 h into heat treatment, and the other life stages were dead within 2 h (Table 3.7). Location 3 took the shortest amount of time to reach 50°C, 1.3 h, and the heating rate at location 3 was high (14.8°C/h). This rapid exposure to heat caused the insects to die more quickly than the other two locations. It is interesting to note that the adults at Location 3 were all alive 1.6 h into the heat treatment, but within a period of 24 min, when the next sample period occurred, they had all died. This is consistent with previous findings concerning the mortality of adults. There is typically a sharper decline in adult mortality than with other life stages.

3.5 Conclusions

Our results suggest that heat treatment is an effective tool to manage *T. castaneum* life stages in grain-processing facilities provided temperatures reach 50ºC and are held above 50ºC for several hours. The bioassay data collected at each facility confirmed this, and the trapping data collected from facility A revealed that one heat treatment could help keep resident insect populations low for up to two months. Heat treatment is a viable alternative to structural fumigants, and an effective heat treatment can be conducted in less than 24 h.

3.6 References

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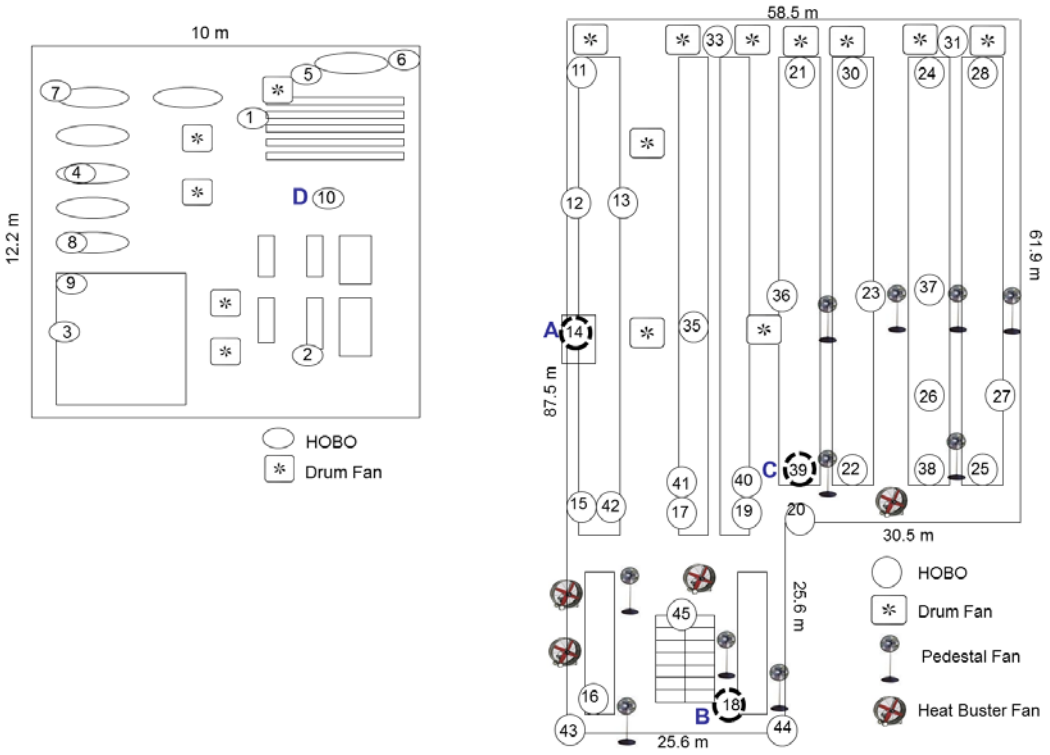

Figure 3.1 A schematic of the pasta facility (bin and press rooms) showing locations of fans, HOBO® temperature loggers, and insect bioassays.

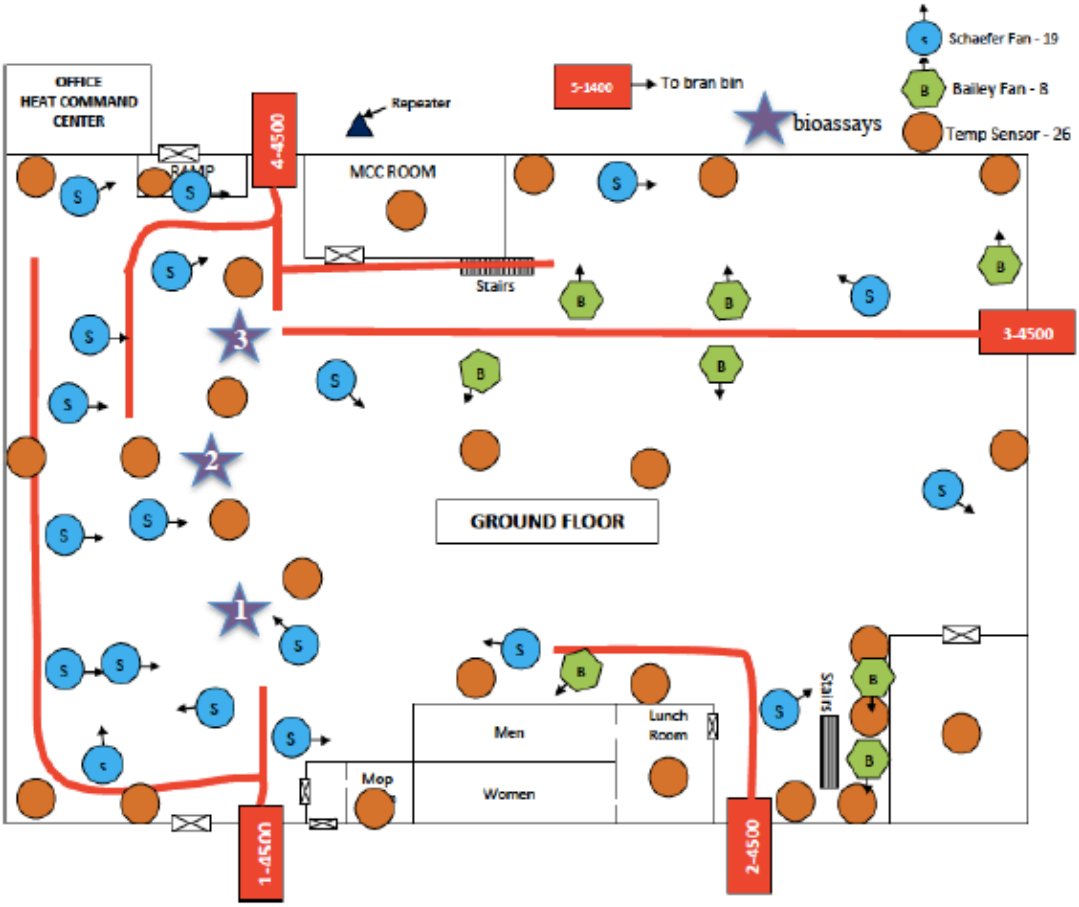

Figure 3.2 Layout of rice processing facility with bioassay, fan, and temperature sensor locations during heat treatment.

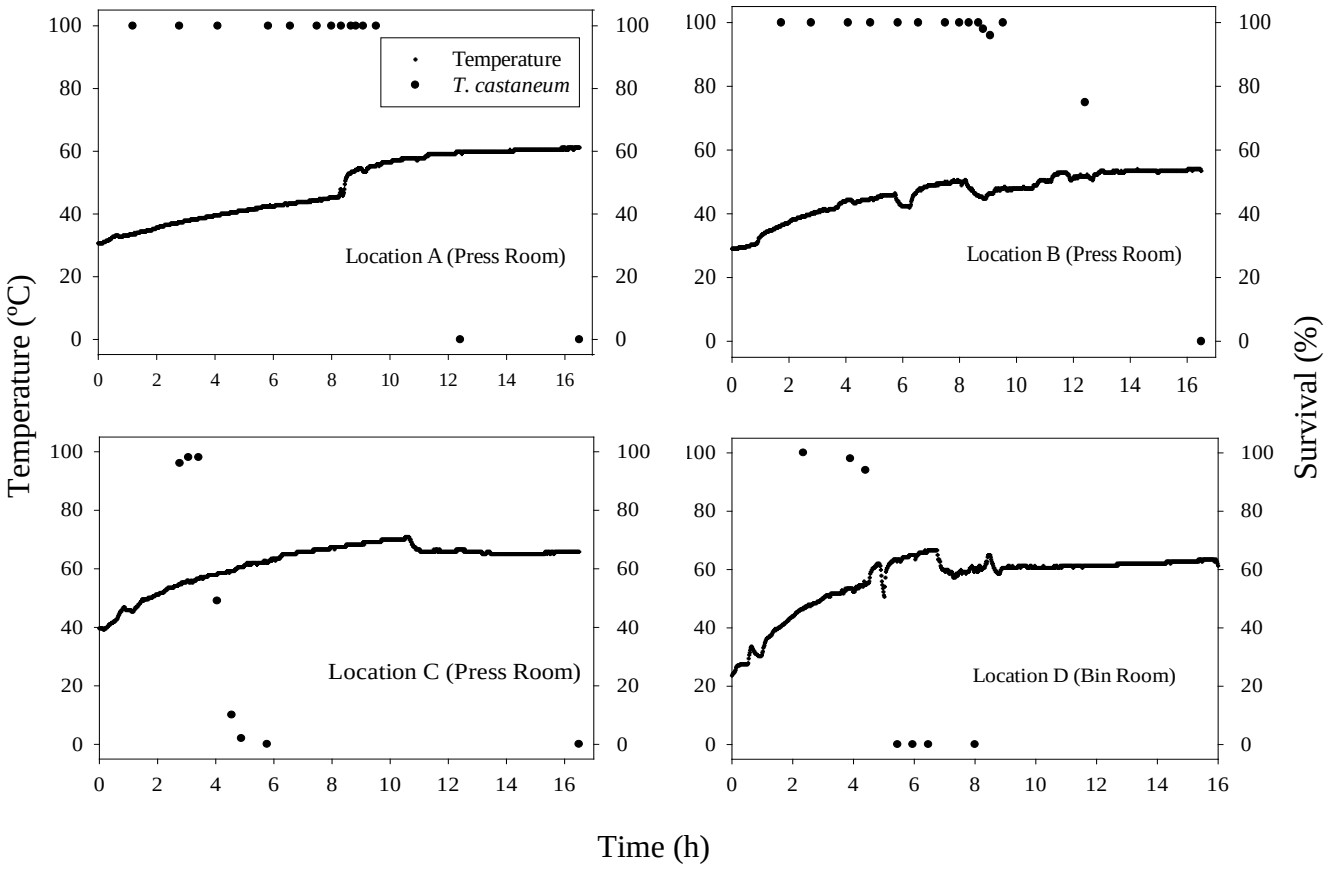

Figure 3.3 Temperature profiles and percent survival of *T. castaneum* adults as a function of time at locations A, B, and C in the press room, and location D in the bin room of the pasta facility.

Table 3.1 Temperatures measured in the bin room of the pasta facility during heat treatment.

Location	Initial temp (°C)	Time to 50°C (h)	Rate of increase (°C/h)	Time above 50°C (h)	Max temp (°C)
1	26.7	4.9	4.8	4.1	61.3
2	26.3	1.8	13.3	15.1	59.9
3	26.3	5.3	4.4	11.8	57.9
4	27.5	5.2	4.3	11.3	73.7
5	27.5	6.8	3.3	9.6	53.5
6	27.1	2.6	9.0	14.0	65.0
7	27.5	1.1	19.9	15.5	83.8
8	26.7	3.5	6.6	13.5	62.7
9	27.5	3.0	7.5	13.9	62.7
10	23.2	2.9	9.2	13.6	67.4
11	23.6	3.6	7.3	13.3	66.6
12	23.6	3.0	8.9	13.5	66.6
Average	26.2	3.6	8.2	12.4	65.1

[a](50°C – initial temperature, °C)/Time to 50°C (h).

Table 3.2 Temperatures measured in the press room during heat treatment.

Location	Initial temp (°C)	Time to 50°C (h)	Rate of increase (°C/h)	Time above 50°C (h)	Max temp (°C)
13	28.3	12.2	1.8	4.5	50.7
14	36.1	1.4	10.3	17.9	71.8
16	39.7	1.7	6.2	15.9	66.6
17	30.3	2.2	8.8	15.4	66.6
18	32.8	2.5	6.9	15.2	65.8
19	35.3	6.0	2.5	11.7	59.2
20	30.2	2.0	9.9	15.4	64.2
21	30.7	16.5	1.2	0.3	50.7
22	27.5	5.3	4.2	3.0	51.2
23	31.1	5.4	3.5	12.0	60.6
24	34.0	6.9	2.3	10.7	54.7
25	29.9	10.8	1.9	4.5	53.0
26	29.9	3.4	5.9	14.2	61.3
27	29.9	4.4	4.6	13.7	60.6
28	35.7	2.0	7.1	16.6	70.9
29	29.9	3.6	5.6	13.8	59.2
30	29.9	3.3	6.0	14.4	62.0
31	33.2	1.9	9.1	16.1	68.3
32	29.1	2.6	8.0	15.3	65.8
33	31.9	4.4	4.1	14.8	62.7
34	29.5	7.9	2.6	9.6	57.9
35	32.8	2.2	8.0	11.8	60.6
36	29.5	8.3	2.5	8.7	56.0
37	29.5	7.0	2.9	9.8	56.0
38	30.3	5.0	3.9	12.5	58.6
41	29.1	7.5	2.8	0.5	50.7
42	31.9	3.6	5.1	16.4	67.4
43	31.5	1.4	12.9	20.0	72.7
44	31.5	5.2	3.6	50.1	62.7
45	34.0	1.4	11.4	18.0	73.7
46	30.7	5.2	3.7	2.0	53.5
47	30.7	8.5	2.3	9.0	61.3
48	31.5	3.6	5.2	14.4	65.0
49	29.5	2.5	8.2	50.1	65.8
Average	31.4	4.9	5.4	14.1	61.4

[a] (50°C - starting temperature, °C)/time to 50°C (h)

Table 3.3 Trap captures of *T. castaneum* adults before and after a heat treatment.

Date of collection (2006)	Mean no. adults/trap/week		
	Press room (*n*=35)	Bin room (*n*=10)	Outside (*n*=5)
30-May	0.46	0.40	0.50
14-Jun	0.20	0.42	0.65
28-Jun	0.32	0.65	0.00
1-2 July	Heat treatment		
11-Jul	0.00	0.09	0.00
25-Jul	0.03	0.10	0.38
8-Aug	0.00	0.05	0.50
23-Aug	0.01	0.05	0.20

Table 3.4 Temperatures measured in the rice processing facility during heat treatment.

Location	Initial temp (°C)	Time to 50°C (h)	Rate of increase (°C/h)	Time above 50°C (h)	Max temp (°C)
1	29.5	2.1	9.9	23.9	58.5
2	29.0	1.4	15.6	24.7	58.0
3	31.0	1.3	14.8	24.7	66.5
Average	29.8	1.6	13.4	24.4	61.0

Table 3.5 Bioassay data collected from location 1 at a rice-processing facility during heat treatment.

Sampling time (h)	Temp (°C)	Mortality (%)			
		Adults	Old Larvae	Young Larvae	Eggs
0.6	38.5	0	0	13	0
0.9	41.5	0	0	29	3
1.0	42.5	0	0	24	12
1.5	47.0	0	0	19	3
1.6	47.5	0	0	34	29
2.0	49.5	0	17	20	29
4.1	57.5	2	79	19	100
6.2	57.0	100	100	100	100

Table 3.6 Bioassay data collected from location 2 at a rice-processing facility during heat treatment.

		Mortality (%)			
Sampling time (h)	Temp (°C)	Adults	Old Larvae	Young Larvae	Eggs
0.6	40.0	0	0	24	12
0.9	46.0	2	0	36	25
1.0	47.0	0	0	8	9
1.5	50.5	0	19	36	12
1.6	51.5	0	19	31	84
2.0	53.5	0	25	29	94
4.1	58.0	0	90	36	100
6.2	57.0	100	100	100	100

Table 3.7 Bioassay data collected from location 3 at a rice-processing facility during heat treatment.

		Mortality (%)			
Sampling time (h)	Temp (°C)	Adults	Old Larvae	Young Larvae	Eggs
0.6	43.5	0	0	13	16
0.9	47.5	0	42	24	64
1.0	48.5	2	23	34	100
1.5	59.5	14	90	24	100
1.6	61.0	0	92	87	100
2.0	64.0	100	100	100	100

Chapter 4. Effect of sex, age, and short-term heat acclimation on the mortality of adults of *Tribolium castaneum* exposed to elevated temperatures

4.1 Abstract

Heat treatments of grain-processing facilities and empty storage bins have been implemented to kill stored-product insects, including the red flour beetle, *Tribolium castaneum* (Herbst). However, there are many factors that influence an insect's ability to tolerate high temperatures, including insect sex, age, and acclimation to sub-lethal temperatures. In this study, we examined the effect survival of male and female *T. castaneum* adults at temperatures of 50 and 55°C. We observed that females of *T. castaneum* were more tolerant than males. We examined effect of adult age on susceptibility to elevated temperatures. One-day-old insects were relatively more heat tolerant compared to insects at other ages, which supports the developmental carry-over hypothesis whereby newly emerged adults have the same thermal tolerance as the early pupal stage. Insects of mixed age and sex were acclimated at 32, 36, and 40°C for 24, 48, and 72 h before undergoing heat treatments in another experiment. No consistent trends were observed, but it appeared that insects maintained at 28°C (the control, or rearing temperature) and 32°C were more thermos-tolerant than those acclimated short-term at the higher temperatures.

4.2 Introduction

The phase out of methyl bromide, along with consumer demand for less toxic methods to disinfest food products, has resulted in exploring alternatives in grain treatments in food-processing facilities against several species of stored-product insects. One viable alternative to chemical methods is the use of lethal temperatures for disinfesting empty storage bins and grain-processing facilities (Fields, 1992; Fields et al., 1997; Mahroof et al., 2003; Dosland et al., 2006; Subramanyam et al., 2011). Heat treatment, documented in literature since the early 1900's, is a safe and effective treatment method for the management of stored-product insects associated with empty bins and grain-processing facilities (Dean, 1911; Dosland et al., 2006). Heat treatment of a structure involves raising its inside temperatures to 50-60°C and maintaining those temperatures for 24 h or less (Subramanyam et al., 2011). Stored-product insects in grain and food-processing facilities are especially susceptible to temperature manipulations.

Insects are frequently exposed to fluctuating temperatures in nature. Their ability to adapt or acclimate to these temperatures has been shown to increase different species' survivorship at lethal temperatures (Scott et al., 1997; Overgaard et al., 2008). Two types of acclimation are documented in the literature: physiological acclimation and developmental acclimation (Gonen, 1977). Physiological acclimation refers to the ability of insects exposed to sub-lethal temperatures for short periods of time to survive later exposure to lethal temperatures, whereas developmental acclimation refers to insects developing a tolerance to elevated temperatures due to exposure to sub-lethal temperatures during their development (Baldwin, 1954; Maynard, 1957; Gonen, 1977). Physiological acclimation is also referred to as heat hardening if there is a brief exposure to sub-lethal temperatures (Scott et al., 1997; Bowler, 2005), and can vary among developmental stages of beetles (Mahroof et al., 2003). Survival at temperatures of 40-50°C can

increase by heat hardening, however, researchers suggest that at lethal temperatures (>55°C), survival is not affected by hardening (Tuda, 2011). At lethal temperatures the enzyme pyruvate kinase, which is essential for glycolosis, is denatured and leads to rapid insect death (Tuda, 2011).

Acclimation of insects to sub-lethal temperatures can induce the expression of heat shock proteins (HSPs), which can subsequently protect cells from heat stress (Parsell and Lindquist, 1993). On a biochemical level, the proteins that became denatured from the initial heat stress are resolubilized due to heat shock proteins (HSPs), resulting in rapid heat hardening (Chown and Nicholson, 2004; Zhao and Jones, 2012). Xu et al. (2010) showed that the Hsp83 gene, the homologue to Hsp90, in the red flour beetle, *Tribolium castaneum* (Herbst), could be induced at 40°C for one hour. Scott et al. (1997) conducted heat acclimation studies on *Trichogramma carverae*, a parasitoid wasp, and found that more adult wasps placed at temperatures of 33°C or 35°C for short durations (1-2 h) survived at 40°C compared to those not acclimated at the higher temperatures.

Population dynamics are greatly influenced by temperature, which affects many physiochemical and biochemical processes (Nyamukondiwa and Terblanche, 2009). Insects are constantly exposed to varying conditions in their natural environments, and their ability to adapt to a wide range of temperatures is critical for their ability to survive and reproduce (Tuda, 2011). Physiological acclimation of stored-product insects is of particular interest in the research of structural heat treatments of grain-processing facilities, where heat is applied to raise temperatures inside the facility to 50-60°C, a range which leads to insect death in as little as one hour (Fields, 1992), even though commercial heat treatments typically last 24 h or less for heat to penetrate harder to reach areas, such as voids in the floor or walls and equipment (Dosland et al.,

2006; Subramanyam et al., 2011). The survival of acclimated *T. castaneum* may help mobile life stages (larvae and adults) seek refuge in insulated areas of the facility or flee the heated area and escape a heat treatment. The effects of adult insect age, sex, and short-term exposure to sub-lethal temperatures have not been clearly documented for *T. castaneum* adults during exposure to lethal temperatures (50 and 55°C). The objectives of this research were to determine effect of sex (male vs. female) on adult *T. castaneum* susceptibility to when exposed to 50 and 55ºC; determine the effect of adult *T. castaneum* age, between 1 d post-pupal emergence, 7, 14, 21, 28, 35, and 42 d on their susceptibility to heat treatment at 50 and 55ºC; and to determine the effect of short-term exposure (24, 48, and 72 h) of adult *T. castaneum* at temperatures of 28, 32, 36, and 30ºC, and their subsequent susceptibility to heat treatment at 50 and 55ºC.

4.3 Materials and Methods

4.3.1 *Insects*

Cultures of *T. castaneum* from the Stored-Product Entomology Research and Education Laboratory, Department of Grain Science and Industry, Kansas State University, which have been in rearing since 1999, were used for all experiments. *T. castaneum* were reared on organic whole wheat flour (Heartland Mills, Marienthal, Kansas, USA) plus 5% by weight brewer's yeast diet. Glass jars, 0.94-L, were filled with 250 g of the flour and yeast diet and were closed with metal lids with a wire-mesh screen and filter paper. Jars were kept in a growth chamber held at 28°C and 65% r.h. Temperature and relative humidity were monitored using HOBO® sensors (Onset Computer Corp., Bourne, Massachusetts, USA).

4.3.2 *Bioassays for sex study*

Sieves with 841µm openings (Seedburo Equipment Co., Chicago, Illinois, USA) were used to separate *T. castaneum* pupae from the growth medium. Pupae were evaluated under a stereoscopic microscope (Nikon SMZ 100 Model, Nikon Instruments, Inc., Melville, NY, USA) to determine sex, and were separated into male and female (Beeman et al., 2009). Pupae were then placed in 150-ml plastic containers and placed back in the growth chamber and monitored daily for emergence into adults. Upon emergence, adults were placed in containers with 30 g of whole wheat flour diet, and placed back in the growth chamber for one week until experimentation

One-week-old adults were placed individually by sex in wells of 24-cell well plates (Corning Glass Works, Corning, NY, USA) with approximately 254 mg of whole wheat flour per well. Five plates were used to achieve a total of 50 males and 50 females. Plates containing 50 males and 50 females were placed in two incubators (Isotemp Standard Lab Incubator, Fisher Scientific, Denver, Colorado, USA) at 50 or 55°C and 18-21% r.h. Heat treatment times were 60 min at 50°C and 15 min at 55°C. There were four replicates per sex and temperature combination.

4.3.3 *Bioassays for age study*

Adults of mixed-sex were separated from growth medium post-adult emergence to monitor their age. Insects were subjected to heat treatment at 50 and 55°C and 18-21% r.h. at 1 day post-emergence and at weekly intervals for 6 weeks. Ten adults were placed in five or six 4.5 cm^2 -plastic boxes, depending on available insects, with 5 g whole wheat flour and a wire

mesh lid, and placed into heat treatment chambers at 50 or 55°C for 60 or 15 min, respectively. There were six replicates per age.

4.3.4 *Bioassays for short-term acclimation study*

Ten adults of mixed sex and age (2-4 weeks post-adult emergence), were placed in 4.5 cm^2 -plastic boxes with 5 g whole wheat flour. Ten boxes per treatment were placed in chambers held at the following temperatures; 28 (control), 32, 36, or 40°C. The control chamber's relative humidity was maintained at 65% r.h. while in the incubators it varied between 19 and 22%. Boxes were held at a constant temperature for 24, 48, and 72 h, immediately followed by a heat treatment at 50 or 55°C for 60 or 15 min respectively. There were three replicates per temperature and time combination.

4.3.5 *Data analysis*

Treatment means were calculated using the PROC MEANS procedure in SAS (SAS Institute, 2008). Data were transformed to angular values and were analyzed using the general linear model in SAS to run one-way analyses of variance (ANOVA) to determine mortality differences between treatments. Based on significance at $\alpha = 0.05$, a one-way ANOVA was then run to further analyze any differences at heat treatment temperatures of either 50 or 55°C. To determine differences between means, the Ryan-Einot-Gabriel-Welsch test (REGWQ) was conducted. To compare the treatment means to the control temperature for acclimation studies, the Dunnett test was used (SAS Institute, 2008).

4.4 Results and Discussion

4.4.1 *Sex studies*

The mean ± SE mortality of female insects subjected to 50°C was 28.0 ± 1.2%, and for males it was 48.7 ± 3.5%. The mean ± SE mortality of insects subjected to heating at 55°C was 12.8 ± 6.7% for females, and 20.2 ± 8.9% for males. Mortality was higher for males at both 50 and 55°C compared to that of females. A one-way ANOVA looking at male and female adults heat-treated at 50°C revealed significance at $\alpha = 0.05$ ($F = 37.98$; $df = 1, 4$; $P = 0.0035$), showing that females were more tolerant to heat than males, but there was no significant difference between sexes when exposed to 55°C ($F = 0.54$; $df = 1, 4$; $P = 0.5023$).

These results are consistent with the literature regarding male versus female survival at high temperatures. Tuda (2011) found that temperatures of 55°C and greater reduced an insect's ability to adapt to high heat due to the rapid denaturing of the enzyme pyruvate kinase, essential for glycolosis. Li et al. (2011) found that 3-day old females of western flower thrips, *Frankliniella occidentalis* (Pergande),were more tolerant when exposed to 40°C than their male counterparts. Two whitefly species, *Trialeurodes vaporariorum* (Westwood) and *Bemisia tabaci* (Gennadius), were exposed to temperatures between 37-45°C for one hour, and females of both species had higher survival than males at 39°C and higher.

Female invertebrates are generally larger than males (Prenter et al., 1998; Teder and Tammaru, 2005). Shape, size, and surface area are all factors that influence an insect's ability to withstand convective and radiation heat exchange (Casey, 1992). A smaller insect has a higher surface area to volume ratio making it generally less heat tolerant compared to an insect with a larger body size and volume (Calder, 1984; Piyaphongkul et al., 2012). Malek et al. (2015)

reported that *T. castaneum* females had a significantly higher mass compared with males at constant and variable temperatures during rearing, which is consistent with our finding that adult females of *T. castaneum* appear to be more heat tolerant than males, at least when raised at 28°C. Scharf et al. (2014) found that at 26°C, females of *T. castaneum* resisted heat stress longer than males, while for those raised at 34°C, the opposite was true.

4.4.2 *Age study*

One-way ANOVA showed that the mortality of various ages of *T. castaneum* adults was significant when exposed to 50 and 55°C ($F = 2.41$; $df = 6, 241$; $P = 0.0282$ at 50°C and $F = 14.30$; $df = 6, 236$; $P < 0.0001$ at 55°C). Mortality data at 50 and 55°C, presented in Figures 4.1 and 4.2, show similar non-linear trends between 1-42 d. The average mortality of *T. castaneum* adults heat-treated at 50°C was lowest at 21 d of age (70.0 ± 6.1%), although it was not significantly different compared to 1, 7, 14, 28, and 35 d means. The highest mortality occurred at 42 d (93.1 ± 2.0%), but it was not significantly different than mean mortalities at 1, 7, 14, 28, and 35 d. The lowest mortality of adults heat-treated at 55°C was at 1 d post-eclosion (32.9 ± 4.9%) which was not significantly different than mean mortalities at 14, 21, and 28 d. The highest mortality was seen at 42 d (93.5 ± 2.9%), which was statistically significant.

Thermal resistance does not remain constant during the life-cycle of an insect, and can dramatically change even within specific life-stages. For many insect species, thermal resistance has been shown to be the highest at eclosion, from the pupal to adult stages (Bowler and Terblanche, 2008), which our study confirms for *T. castaneum* adults. Baldwin (1954) found that for the parasitic chalcid (*Dahlbominus fuscipennis* Zett.) there was a decrease in its thermal tolerance between 1-4 d of adult life. Pappas et al. (2007) found that the expression of Hsp70

was reduced between eclosion and 3 d for *Drosophila melanogaster* Meigen. Our results are consistent with these findings as the mortality of *T. castaneum* adults slightly increased at 7 d when heat-treated at 50°C, and significantly increased when heat-treated at 55°C. Davison (1968) found that the heat tolerance of 1-d old adults of *Calliphora erythrocephala* Meigen was the same as the 1-d old pupa, and that the high tolerance was lost in the first five days after emergence from pupal to adult stage. This finding was consistent with the developmental carry-over hypothesis where the high heat tolerance in young adult insects can be attributed to carry-over tolerance from the pupal stage (Hollingsworth and Bowler, 1966; Bowler et al., 2008). In *C. erythrocephala*, the pupal stage is the most heat resistant stage, and heat tolerance provides an advantage to immobile life stages. Mahroof et al. (2003) found that the pupae and old instars were the most heat tolerant life stages of *T. castaneum* in structural heat treatments. The developmental carry-over hypothesis appears to fit well with *T. castaneum* young adults.

There was a decrease in mortality, although not statistically significant, between 14-21 d for 50°C, and 21-28 d for 55°C, after which mortality increased until 42 d, where mortality was the highest. The changes in thermal tolerance could be related to the activity of certain enzymes during development or changes in physiological resistance. Chaudhary et al. (1966) found that there was a change in aldolase activity during the metamorphosis of the confused flour beetle, *Tribolium confusum* (Jacquelin du Val) that decreased with adult age. Sun (1947) found that *T. confusum* became less resistant to carbon disulphide with increased age. With some insect species, systematic patterns of variation exist, while for others, there appear to be no generalized patterns (Bowler and Terblanche, 2008).

4.4.3 *Short-term acclimation study*

Adults of *T. castaneum* were acclimated for 24-72 h at temperatures between 32-40°C and then subjected to heat treatment at 50 and 55°C (Figures 4.3 and 4.4). A two-way ANOVA of the data for insects heat-treated at 50°C revealed that the acclimation time, and the interaction between acclimation time and temperature were not significant (Table 4.1). However, the acclimation temperature was significant ($P = 0.0134$). A Dunnett *t*-test was run to compare the treatment mortality means for each acclimation time (24, 48, or 72 h) to the control temperature mortality for insects with no acclimation time above their rearing temperature of 28°C. The only significant difference was found at 72 h acclimation time at 32°C, where the mortality was significantly lower than the control mortality (82.8 ± 5.4% versus 98.5 ± 0.9%). Mortality of adults heat-treated at 50°C for 60 min was high across all acclimation temperatures and times (>82.8%), and the averages were not significantly different from each other, or the control except for those acclimated at 32°C for 72 h, which had the lowest mortality.

The two-way ANOVA for the data collected from the 55°C heat treatment revealed significance for acclimation time and temperature, and their interaction (Table 4.2). The Dunnett *t*-test showed that the treatment means that differed from the control were at 36°C for 24 h, 36 and 40°C for 48 h, and 40°C for 72 h. In each case, the mortalities were higher than the control. Goodman et al. (2012) exposed *T. castaneum* adults to 40°C for 20 min and found that the expression of hsp68a was significantly induced. They suggest that acclimation occurs rapidly in *T. castaneum*, so it is possible that the length of acclimation times used in the current study were too long to see any benefits from the expression of certain heat shock proteins.

Insect responses to acclimation may occur over a long or very short interval of time depending on the species (Watson and Hoffmann, 1996). Kellett et al. (2005) found that pre-

treating *D. melanogaster* adults at 31, 33, or 35°C each resulted in an equally increased thermo-tolerance of 25% compared to the 25°C control. Based on the results from the current study, 32°C appears to be the optimal temperature for *T. castaneum* acclimation at each of the temperatures tested at 24, 48, and 72 h, though not statistically different from insects which were not acclimated at 28°C, except those held at 32°C for 72 h when heat treated at 50°C. From our results, it does not appear that acclimating adults to 36 or 40°C benefits their thermal tolerance, at least at the time scale between 24 and 72 h. Acclimation temperatures >40°C may be needed for shorter periods of time in order to induce an increase in thermal tolerance for *T. castaneum* adults.

4.5 Conclusions

Various aspects affect an insect's ability to withstand elevated temperatures including sex, age, and acclimation. Our study showed that when reared at 28°C, females were more heat tolerant than males at heat treatment temperatures of 50 and 55°C, which is most likely due to the larger size of females. One-day-old insects were found to be fairly heat tolerant, and thermos-tolerance decreased between 7 and 14 d, and increased again between 14 and 28 d. The highest mortality at both heat treatment temperatures was seen at 42 d. The developmental carry-over hypothesis appears to hold true for *T. castaneum* adults, and then varies non-linearly over the following 6 weeks post-eclosion. Finally, short-term acclimation studies revealed very little variation in mortality for insects heat-treated at 50°C, while at 55°C, insects were more heat tolerant when maintained at 28°C, or acclimated at 32°C.

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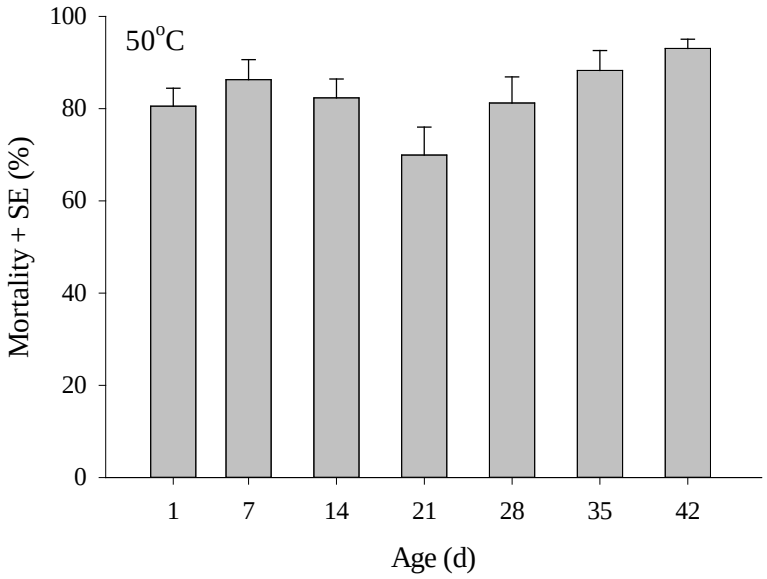

Figure 4.1 Mortality of *T. castaneum* adults between ages 1-42 d heat-treated at 50°C.

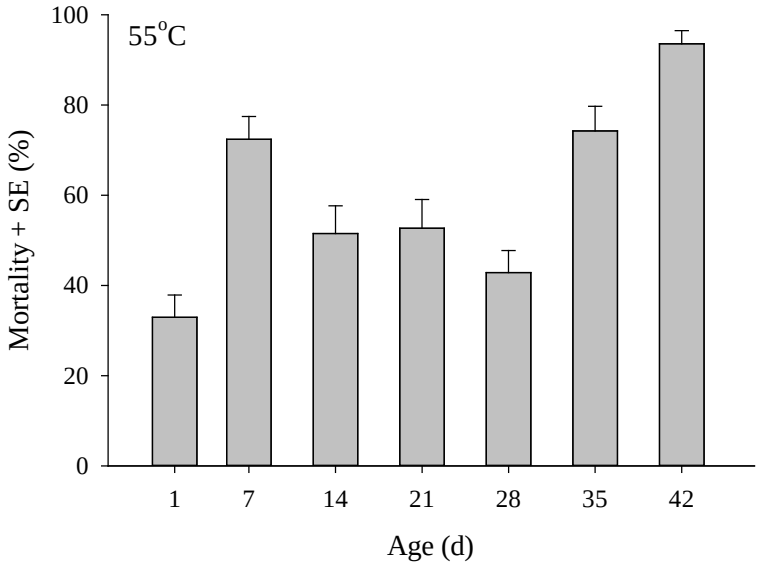

Figure 4.2 Mortality of *T. castaneum* adults between ages 1-42 d heat-treated at 55°C.

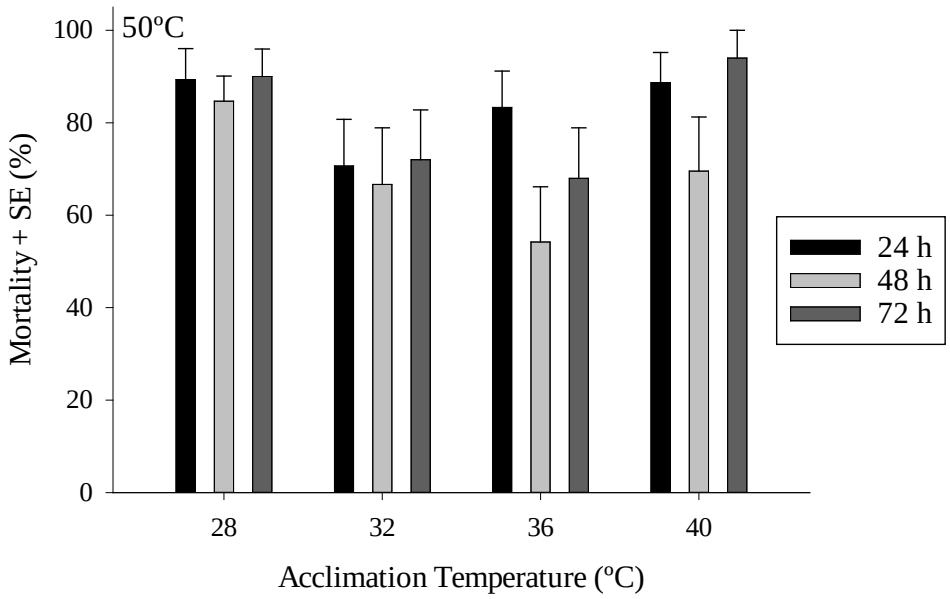

Figure 4.3 Mortality of *T. castaneum* adults after acclimation at four temperatures, for 24, 48, or 72 h and heat treatment at 50°C.

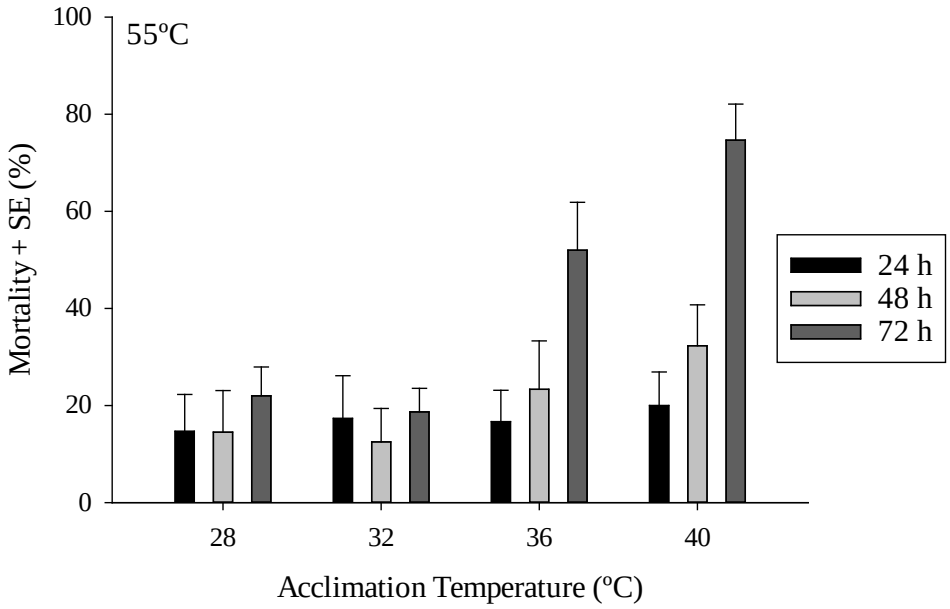

Figure 4.4 Mortality of *T. castaneum* adults after acclimation at four temperatures, for 24, 48, or 72 h and heat treatment at 55°C.

Table 4.1 Two-way ANOVA statistics showing main and interactive effects of acclimation time and heat treatment temperature on corrected mortality of *T. castaneum* adults heat treated at 50°C.

Source	df	Mean Square	*F*-value	*P*-value
Acclimation time	2	0.170	2.55	0.0788
Acclimation temperature	3	0.239	3.61	0.0134*
Time*temperature	6	0.122	1.83	0.0908
Error	468	0.066		

*****Significant ($P < 0.05$).

Table 4.2 Two-way ANOVA statistics showing main and interactive effects of acclimation time and heat treatment temperature on corrected mortality of *T. castaneum* adults heat treated at 55°C.

Source	df	Mean Square	*F*-value	*P*-value
Acclimation time	2	1.975	15.51	<0.0001*
Acclimation temperature	3	3.084	24.22	<0.0001*
Time*temperature	6	10.940	7.38	<0.0001*
Error	468	0.127		

*****Significant ($P < 0.05$).

Chapter 5. The effect of continuous rearing of *Tribolium castaneum* at elevated temperatures on subsequent thermal tolerance to heat treatments

5.1 Abstract

Red flour beetles, *Tribolium castaneum* (Herbst), are exposed to dynamic environments in storage conditions or inside grain and food-processing facilities. They are also cosmopolitan pests adapted to many different climatic conditions. Little is known about the effect of developmental acclimation over various generations of *T. castaneum* at elevated temperatures. In this research, *T. castaneum* were reared at 28, 32, and 36°C for ten generations, and after each generation, a sample was exposed to temperatures of 50°C or 55°C for 60 or 15 min, respectively. Data showed that developmental acclimation occurred after the second generation, and continued on to the tenth generation for insects reared at both 32 and 36°C. Insects from the tenth generation were also exposed to heat treatment at four locations in a flour mill. Temperatures did not reach 50°C due to inefficiency of the heaters, and heating rates to maximum temperatures varied between 0.26 and 0.30°C /h. Bioassays of insects reared at 32°C resulted in the lowest mortality, while those reared at 36°C appeared to be the most susceptible to heat. These results are consistent with previous research showing differences in experiments at fixed versus dynamic temperatures.

5.2 Introduction

The ability of insects to adapt to their dynamic surroundings is essential for their survival. Acclimation is one phenotypic response insects employ to adapt to temperature changes in the environment (Huey et al., 1999). Leroi et al. (1994) developed the Beneficial Acclimation Hypothesis (BAH) that states that insects acclimated to a certain environment perform better at that environment than insects acclimated to other environments. From the BAH, the prediction can be made that insects acclimated at higher temperatures will be more tolerant of higher temperatures (Huey et al., 1999), and there has been some research that supports the hypothesis and some that has not.

Two species of *Drosophila* flies were reared at 28°C and when exposed to a temperature gradient ranging from 18-40°C, they preferred lower temperatures when compared to the flies which were reared at either 19 or 25°C (Krstevska and Hoffmann, 1994). Another study, however, found that *Drosophila* flies reared at 30°C for several generations were attracted to higher temperatures than those reared at 25°C (Good, 1993). This discrepancy could be due to a difference in methodologies between the two experiments. Similarly, in a study by Baldwin (1954), *Dahlbominus fuscipennis* (Zett.), a parasitic insect, was found to be more tolerant of temperatures between 40 and 46°C when reared at 29°C compared to when they were reared at 17°C. Scott et al. (1997) determined that the developmental acclimation of *Trichogramma carverae* (Oatman and Pinto) at elevated temperatures negatively impacted its fitness, although survival was increased after heat stress. *T. castaneum* reared at 34°C were better able to resist heat stress when exposed to 43.5°C compared to those reared at 26°C (Scharf et al., 2004).

The objectives of this research were to determine the effects of continuous rearing of *T. castaneum* cultures at 32 and 36°C for ten generations compared to the control rearing

temperature of 28°C on their susceptibility to lethal temperatures of 50 and 55°C, and to determine the susceptibility to dynamic temperatures of the F_{10} generation in a pilot flour mill heat treatment.

5.3 Materials and Methods

5.3.1 *Insects*

Cultures of *T. castaneum* were reared in 0.94-L glass jars filled with 250 g of a medium consisting of 95% organic, whole wheat flour (Heartland Mills, Marienthal, Kansas, USA) and 5% (by wt.) brewer's yeast. To start the cultures for generational tests, 200 adults of *T. castaneum* were placed in each jar (three jars per temperature) and placed in incubators with a volume of 0.14 m^3 (Isotemp Standard Lab Incubator, Fisher Scientific, Denver, Colorado, USA) set at three temperatures: 28, 32, or 36°C. The relative humidity averaged between 35-45%. Cultures were left alone for three days to allow the female adults to lay eggs, and then adults were sifted out of the growth medium using a sieve with 841µm openings (Seedburo Equipment Co., Chicago, Illinois, USA) and discarded. Jars were then placed back in their respective incubators until adult emergence, and these adults were the F_1 generation. Cultures were reared in the Stored-Product Insect Research and Education Laboratory at Kansas State University in the Department of Grain Science and Industry, Manhattan, Kansas, USA.

For the next generation, clean jars were filled with the whole wheat flour and yeast mixture. Adults (n=200) from the F_1 generation were placed in each jar and allowed to mate at their respective rearing temperatures for 72 h before being removed from the growth medium and discarded. The adults which emerged were labeled as the F_2 generation, and this procedure continued until the F_{10} generation.

5.3.2 *Bioassays*

T. castaneum adults of mixed sex were collected from jars for heat treatment at approximately one week post-pupal emergence. Adults (*n*=20) from each rearing temperature were placed in five plastic boxes (4.5×4.5×1.5 cm) containing 305 ± 3 mg bleached wheat flour, and covered with a wire-mesh screened lid to allow for air diffusion. One hundred adults were heat treated from each temperature.

5.3.3 *Laboratory heat treatment*

Plastic boxes containing one-week-old adults were exposed to 50 and 55°C for 60 or 15 min, respectively. After heat treatment, the plastic boxes were moved to the incubators where the insects were reared and held for 24 h before being assessed for mortality. Mortality was calculated as: [(total number treated – number alive) / total number treated] x 100%.

5.3.4 *Flour mill heat treatment*

The Hal Ross Flour Mill at Kansas State University (Manhattan, Kansas, USA) was heat treated by the flour mill manager July 15-19, 2015. The flour mill has five floors and a total volume of 49,611 m^3. Each floor measures 15.4 m in length and 27.5 m in width. Bioassay samples were placed in four total locations, two on the second floor and two on the third floor. One group of samples was placed near the floor wall junction furthest away from the heat source, and the other group was placed in the middle of the floor.

Twenty-five boxes containing adults of the F_{10} generation from the 28, 32, and 36°C incubators were placed at four locations in the flour mill, and collected at 18.6, 27.5, 34.7, 47.5, and 68.0 h into the heat treatment. After collection, arenas were taken to the Stored Product Insect Research and Education Laboratory in the Department of Grain Science and Industry at

Kansas State University (Manhattan, Kansas, USA), where insects were placed in a growth chamber held at 28°C and 65% r.h. Insects were sifted from the flour after 24 h and counts were taken of number of alive and number of dead insects. Insects were determined to be dead when there was no movement after being gently prodded with a camel's hair brush. Temperature and relative humidity were monitored at each location where bioassays were placed using HOBO® data-loggers (Onset Computer Corp., Bourne, Massachusetts, USA).

5.3.5 *Data analysis*

Data for generational studies were analyzed in SAS (SAS Institute, 2008). Two-way ANOVA statistics were obtained in SAS showing main and interactive effects of generation and rearing temperature on the mortality of *T. castaneum* adults during heat treatment. The general linear model statement was implemented at each generation, F_1-F_{10}, and the Dunnett *t*-test was used to compare the means of insect mortality obtained from each rearing temperature to the control mortality from cultures reared at 28°C and 65% r.h., and exposed to 50 and 55°C.

5.4 Results and Discussion

5.4.1 *Generational heat treatments*

T. castaneum adults reared at 28, 32, and 36°C all died when heat treated at 55°C for 15 min, so the only data considered from here forward are from the insects heat treated at 50°C. A two-way ANOVA (Table 5.1) showed that generation, rearing temperature, and their interaction were all significant ($P<0.0001$). The data from generations F_1 and F_2 showed no significant differences in mortality between the 28°C cultures and those reared at 32 and 36°C (F_{range} = 0.95-2.99; df = 2, 12; P_{range} = 0.0882 – 0.4140). Differences between mortalities by rearing

temperature began to be seen at the F_3 generation and continued to the F_{10} generation (Table 5.2). At all generations beyond F_2 except the F_4 and F_6 generations, the heat treatment mortality from insects reared at 32 and 36°C were both significantly lower compared to the mortality of insects reared at the control temperature. These data suggest that an irreversible acclimation due to rearing *T. castaneum* at higher temperatures may result after the F_2 generation, and affirms previous research supporting an increased thermo-tolerance when insects are reared at higher temperatures compared with those reared at lower temperatures (Baldwin, 1954; Scott et al., 1997; Scharf et al., 2004).

5.4.2 *Flour mill temperatures*

During the flour mill heat treatment, temperatures rose slowly, but steadily throughout the 68 h in which bioassays were exposed to heat, however, in the four locations monitored, the temperature never reached 50ºC. The initial temperatures measured at each location were 27.4, 27.2, 27.1, and 27.7ºC for locations 1-4, respectively. There was a decrease in temperature after 48 min when the heaters were temporarily turned off, and then restarted after roughly 2.5 h due to malfunction. Maximum temperatures reached were 45.4ºC and 47.5ºC at locations 1 and 2 on the second floor, and 47.3ºC and 45.3ºC at locations 3 and 4 on the third floor. The rate of temperature increase to the maximum temperature at each location was 0.26ºC/h at locations 1 and 4, and 0.30ºC/h at locations 2 and 3. Heating rates to 50ºC recommended for structural facilities are between 0.30 and 13.7ºC/h (Mahroof et al., 2003a), but in this case, 50ºC was not attained at any of the monitored locations in the first 68 h of treatment. Heat treatments typically last 24 h (Subramanyam et al., 2011), but due to various issues with the steam heaters in the flour mill, and little air movement due to lack of fans, it took much longer to heat the facility.

5.4.3 *Flour mill bioassays*

Bioassays were collected five times during the heat treatment (18.6 h, 27.5 h, 34.7 h, and 68.0 h) at the four locations where samples were placed. No mortality was seen from any of the bioassays at 18.6 h into the heat treatment, but the highest temperature recorded at that time was 34ºC at locations 1 and 3. After 47.5 h, most insects in each location from rearing temperatures of 28 and 32ºC were still alive, however, insects reared at 36ºC started dying after 27.5 h (Tables 5.3-5.5). Locations 1 and 4 had the least mortality after the last bioassays were collected at 68 h, where temperatures were 45.4 and 45.3ºC, respectively. At locations 1 and 4, the mortality was highest for insects reared at 36ºC, 26.4 and 16%, respectively. Location 2 had 100% mortality for insects reared at 28, 32, and 36ºC after 68 h, and the temperature reached 47.5ºC. However, for samples taken at 47.5 h, 14% of the insects reared at 36ºC were dead compared to 0% for those reared at 28 and 32ºC. At location 3, the highest mortality of 92% was seen for insects reared at 36ºC, followed by 72% mortality for insects reared at 28ºC, and then 32% mortality for insects reared at 32ºC.

Rearing insects at 36ºC did not appear to have an advantage during the pilot flour mill heat treatment, at least with such low heating rates. This could be attributed to lower body mass that may occur for insects reared above or below the optimal temperature (Malek et al., 2015). Due to the potentially smaller mass, the insect would have a higher surface area to volume ratio making it less heat tolerant compared to an insect with increased body mass (Calder, 1984; Casey, 1992; Piyaphongkul et al., 2012).

5.5 Conclusions

The results from the laboratory study and field study are at slight variance with one another. From the laboratory studies, we found that rearing *T. castaneum* at both 32 and 36ºC increased their heat tolerance compared with the insects reared at 28ºC. In the flour mill heat treatment, the insects reared at 36ºC in general had the highest mortality. Those insects reared at 32ºC were the ones with the most apparent thermo-tolerance.

It has been established that laboratory trials at fixed temperatures versus situations where temperatures are dynamic can result in different conclusions. Mahroof et al. (2003a) conducted a laboratory experiment to determine time-mortality relationships for different life stages representing eggs, young larvae, old larvae, pupae, and adults of *T. castaneum*. Stages were exposed to constant temperatures in laboratory growth chambers at 22% r.h. (Mahroof et al., 2003b). Neonate larvae were found to be the most heat-tolerant. In a heat treatment of a pilot flour mill, old larvae and pupae of *T. castaneum* were observed to be more heat tolerant than the other stages (Mahroof et al., 2003b). In a pilot flour mill, Brijwani et al. (2012) reported adults to be the more tolerant to heat. The increase in heat tolerance of certain life stages could be related to the heating rate, which may affect the production of heat shock proteins, or increased respiration due to a higher metabolic rate (Emekci et al., 2002; Mahroof et al., 2003b, 2005a, 2005b). Increased heat tolerance could be related to lower heating rates, typical of structural heat treatments, and was the case for the flour mill examined in this research.

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Table 5.1 Two-way ANOVA statistics showing main and interactive effects of generation and rearing temperature on the mortality of *T. castaneum* adults during laboratory heat treatment.

Source	df	Mean	*F*-value	*P*-value
Generation	9	1.279	9.96	<0.0001*
Rearing temperature	2	7.258	56.49	<0.0001*
Generation*rearing	18	0.445	3.47	<0.0001*
Error	120	0.129		

*****Significant ($P < 0.05$).

Table 5.2 *T. castaneum* mortality (out of 100 insects) during laboratory heat treatment by generation and rearing temperature.

Generation	Rearing Temp	% Mean*
F_1	28	100.0
	32	100.0
	36	87.9
F_2	28	80.0
	32	59.0
	36	43.0
F_3	28	90.0
	32	11.0 *
	36	16.0 *
F_4	28	91.0
	32	94.0
	36	58.0 *
F_5	28	97.0
	32	33.0 *
	36	67.0 *
F_6	28	54.0
	32	7.0 *
	36	58.0
F_7	28	81.0
	32	3.0 *
	36	19.0 *
F_8	28	100.0
	32	50.0 *
	36	21.0 *
F_9	28	92.0
	32	27.0 *
	36	6.0 *
F_{10}	28	100.0
	32	32.0 *
	36	45.0 *

* There were significant differences between control and 32 or 36ºC (F_{range} = 4.91 – 39.83; df = 2, 12; P_{range} = <0.0001 – 0.0277; by one-way ANOVA).

Table 5.3 Mortality of *T. castaneum* adults reared at three different temperatures (F_{10} generation) collected from location 1 during heat treatment.

Time (h)	Temperature during heat treatment (ºC)	Heat treatment mortality (%)		
		28°C	32°C	36°C
18.6	34.2	0.0	0.0	0.0
27.5	37.7	0.0	0.0	4.3
34.7	38.9	0.0	2.0	16.0
47.5	41.3	2.0	0.0	20.0
68.0	45.4	6.2	4.0	26.4

Table 5.4 Mortality of *T. castaneum* adults reared at three different temperatures (F_{10} generation) collected from location 2 during heat treatment.

Time (h)	Temperature during heat treatment (ºC)	Heat treatment mortality (%)		
		28°C	32°C	36°C
18.6	30.5	0.0	0.0	0.0
27.5	38.1	0.0	0.0	20.0
34.7	40.2	0.0	2.0	8.2
47.5	43.5	0.0	0.0	14.0
68.0	47.5	100.0	100.0	100.0

Table 5.5 Mortality of *T. castaneum* adults reared at three different temperatures (F_{10} generation) collected from location 3 during heat treatment.

Time (h)	Temperature during heat treatment (ºC)	Heat treatment mortality (%)		
		28°C	32°C	36°C
18.6	34.1	0.0	0.0	0.0
27.5	38.1	2.0	0.0	4.0
34.7	40.0	2.1	0.0	14.0
47.5	43.0	0.0	0.0	12.2
68.0	47.3	72.0	32.0	92.0

Table 5.6 Mortality of *T. castaneum* adults reared at three different temperatures (F_{10} generation) collected from location 4 during heat treatment.

Time (h)	Temperature during heat treatment (ºC)	Heat treatment mortality (%)		
		28°C	32°C	36°C
18.6	31.4	0.0	0.0	0.0
27.5	36.5	0.0	0.0	12.0
34.7	38.0	0.0	0.0	6.2
47.5	41.5	0.0	0.0	16.0
68.0	45.3	0.0	0.0	16.0

Chapter 6. Influence of temperature and application rate on efficacy of a diatomaceous earth formulation against *Tribolium castaneum* adults

6.1 Abstract

Unsanitary storage bins can harbor grain-infesting insects, including the red flour beetle, *Tribolium castaneum* (Herbst). In a previous study involving heat treatment of empty bins, temperatures in the range of 50-55°C for 2-4 h were effective in completely killing stored-product insects. Previous research in flour mills showed improved efficacy in killing stored-product insects by using diatomaceous earth (DE) dusts at temperatures below 50°C. In the current study, the efficacy of a diatomaceous earth formulation (DiaFil® 610) applied to concrete arenas, to simulate floor of empty bins, was examined at three application rates (0, 2.5 and 5.0 g/m^2) to control *T. castaneum* adults at five constant temperatures (28, 36, 42, 44, and 46°C). Ten adults of *T. castaneum* were placed on individual untreated and DE-treated concrete arenas for 4, 8, 12, and 24 h at each of the five temperatures. The efficacy of DE against *T. castaneum* adults increased with an increase in temperature and exposure time. Generally more adults died at 5.0 g/m^2 when compared with 2.5 g/m^2. In 2.5 and 5.0 g/m^2 DE treatments, exposure for 12 h at a temperature of 42°C resulted in 73-77% mortality of adults with 100% mortality observed after 24 h. At 44 and 46°C, 100% mortality of adults was observed after 24 h of exposure at both DE rates. At these two temperatures, the high mortality in untreated arenas (controls) at 8, 12, and 24 h exposures ranged from 27-100% confounding the true effects of DE. Our results suggest that combined use of DE and temperatures below 50°C can be used as an

integrated approach for controlling insects in empty bins prior to storage of newly-harvested grain.

6.2 Introduction

Several species of stored-product insects have been reported from empty bins (Chao, 1954; Wright, 1991; Reed et al., 2003; Arthur et al., 2006; Hagstrum et al., 2008). Removal of grain and grain debris from empty bins prior to storing newly-harvested grain can help in reducing insect numbers in stored grain (Reed et al., 2003; Arthur et al., 2006). Some stored-product insects are long-lived, and removal of residual grain and grain debris, which serves as their food, may not be sufficient to control them. The use of an approved insecticide (Arthur and Subramanyam, 2012) after sanitation of empty bins is shown to provide effective control of insects. Bridgeman (1994) conducted tests with an amorphous silica (Dryacide®, A & R McLaughlin Private Limited, Wembley Downs, Western Australia) applied to storage surfaces at 6-8 g/m^2 in four 100 m long x 15 m wide x 5 m high rectangular storage structures in Australia. Treatment efficacy was verified by using 30 flour-baited cardboard traps (Wright, 1991) in each storage facility. Trapping three weeks before sanitation and three weeks after sanitation showed no significant difference in the percentage of traps with beetles and psocids. However, after application of Dryacide®, trapping over the next 11 weeks showed a decrease in the percentage of traps with beetles and psocids from 18 to 3% and from 90 to 40%, respectively.

Clean, empty bins can also be treated with several alternatives to chemical insecticides. Two approved alternatives to chemical insecticides include the use of diatomaceous earth or DE and the use of high temperatures (Subramanyam and Roesli, 2000; Tilley et al., 2007; Subramanyam et al., 2011), or a combination of DE and heat (Dowdy and Fields, 2002).

There are numerous studies documenting the effectiveness of DE dusts against stored-product insects, mostly on grains (McGaughey, 1972; Korunić et al., 1996; Subramanyam and Roesli, 2000; Kavallieratos et al., 2005; Vardeman et al., 2007; Kavallieratos et al., 2010). There

are limited published studies examining the efficacy of heat treatment of empty bins against adults of stored-product insects. Tilley et al. (2007) reported 100% mortality of adults of the red flour beetle, *Tribolium castaneum* (Herbst), lesser grain borer, *Rhyzopertha dominica* (F.), and rice weevil, *Sitophilus oryzae* (L.), by raising temperatures of the bin's floor to a minimum of 50°C for up to 2-4 h. Moog and Maier (2007) reported 77-91% mortality of adults of the maize weevil, *Sitophilus zeamais* (Motschulsky), when exposed for 3 h at 55°C in the plenum area of empty bins. The mortality of *T. castaneum* adults in the plenum area at this temperature and exposure time was 72-87%. However, similar exposure in areas 1.83 m above the plenum resulted in 100% mortality of both species. The authors inferred that the lack of uniform distribution of hot air at the plenum may have resulted in less than 100% mortality of beetles.

Previous research has shown that heat treatments in combination with DE increased mortality of stored-product insects. Dowdy (1999) reported mortality of unfed and fed adults of *T. castaneum* adults exposed to untreated glass Petri dishes and dishes treated with 5 g/m^2 of four DE dusts at 34 and 50°C and 65% r.h. The DE formulations used were Concern® (Necessary Organics, Inc., New Castle, Virginia, USA), Natural Guard® (VPG Co-op Gardening Group, Inc., Bonham, Texas, USA), Insecto® (Natural Insecto Products, Inc., New Castle, Virginia, USA), and Protect-It® (Headley Technologies, Vancouver, British Columbia, Canada). Exposure of unfed insects for 15-30 minutes to 34°C alone resulted in 0-1.3 and 42.5-55.0% mortality, when mortality assessments were made 1 d and 7 d after exposure, respectively (Dowdy, 1999). A similar exposure to 50°C resulted in 1.3-28.8 and 51.3-65.0% when assessments were made 1 and 7 d after exposure, respectively. Adults that were fed or had access to food showed reduced mortalities that ranged from 0-1.3% and 0-56.3%, irrespective of whether observations were made 1 or 7 d after exposure. Protect-It® was the most efficacious

dust producing 91.3-100% mortality of unfed adults after a 15-30 minute exposure to 34 and 50°C when mortality was assessed 1 d after exposure. The mortality with the other three DE dusts was greater at 50°C compared to 34°C, and the mortality of adults ranged from 8.9-76.3% based on mortality 1 d after exposure. However, all dusts produced 97.5-100% mortality at both temperatures when mortality of adults was assessed after 7 d. Mortality of adults that were unfed never reached 100%, irrespective of the temperature, exposure time, and post-mortality assessment time, except for adults exposed for 30 minutes to Protect-It® at 50°C. These results suggest that sanitation, in conjunction with heat and DE, is more effective than heat alone or DE plus heat. Additionally, this study also showed delayed mortality effects associated with heat alone and heat plus DE. Fields et al. (1997) reported that in an oat mill, completely mortality of adults of the confused flour beetle, *Tribolium confusum* Jacquelin du Val, occurred when temperatures reached 47°C after 32-38 h, but in the presence of DE complete mortality of adults occurred when temperatures reached 41ºC. Dowdy and Fields (2002) evaluated heat in combination with application of Protect-It® applied at 0.3 g/m^2 to second and third floor surfaces of a pilot flour mill subjected to a heat treatment against *T. confusum* adults. The benefits of DE were only evident on the south side of the second floor where temperatures did not quickly reach 47°C. At the end of the heat treatment, adults exposed to partially and fully treated DE floor surfaces had 50 and 75% mortality, respectively, compared to 15% mortality of those exposed to heat alone. Ebeling (1994) showed that the time required for 100% mortality of the German cockroach, *Blatella germanica* (L.), at a temperature of 43.3°C in the presence of a silica aerogel, a synthetic silica, was reduced from 147 to 41 minutes.

To our knowledge, there are no published studies that investigated the combined efficacy of DE and a range of temperatures on concrete surfaces, such as those found in empty bins. The

combination of these treatment methods would involve lower energy inputs to obtain temperatures lethal to insects (Fields et al., 1997). Eliminating stored-product insects in empty bins prior to storage of newly-harvested grain, along with additional integrated pest management methods, such as bin sanitation, can increase the profitability and quality of stored grain in a more sustainable and environmentally friendly manner.

In the present investigation, laboratory experiments were designed to examine the influence of five temperatures below 50°C, two DE application rates to concrete arenas, and four exposure times on mortality of *T. castaneum* adults. Concrete arenas in 9-cm Petri dishes simulated the floor of empty bins.

6.3 Materials and Methods

6.3.1 *Insects*

Cultures of *T. castaneum* were reared in the Stored-Product Insect Research and Education Laboratory at Kansas State University in the Department of Grain Science and Industry, Manhattan, Kansas, USA. Cultures were reared in 0.94-L glass jars filled with 250 g of a medium consisting of 95% organic, whole wheat flour (Heartland Mills, Marienthal, Kansas, USA) and 5% (by wt) brewer's yeast in growth chambers at 28°C and 65% r.h. Jars were closed with metal lids fitted with filter papers and wire-mesh screens.

6.3.2 *Concrete arenas*

Ready-mix concrete (Rockite, Hartline Products Co., Inc., Cleveland, Ohio, USA) was mixed with tap water to make a slurry. The slurry was poured into plastic Petri dishes (Fisher Scientific, Denver, Colorado, USA) with a diameter of 9 cm, height of 1.5 cm, and surface area

of ~62 cm^2. The ratio of grams of concrete mix to milliliters of water used to fill the Petri dishes was 2:1. The slurry was allowed to dry in the Petri dishes for 48 h before dishes were used in experiments.

6.3.3 *Diatomaceous earth application*

The DE formulation used for experiments was DiaFil610® (Imerys Minerals California, Inc., San Jose, California, USA). DiaFil® 610 is natural fresh water DE, white in color, and has an average particle size of 10 μm (Korunić, 1997). It has a surface area of 26-28 m^2/g. The DE moisture content was 3-5%. DE was applied at either 2.5 or 5.0 g/m^2, the recommended rate for application to empty storage bins for insect management, directly onto the concrete arenas. After adding DE, the Petri dishes were gently shaken in a counter clockwise manner to evenly distribute DE on concrete arenas. Control treatment (0 g/m^2) included concrete arenas that were not treated with DE.

6.3.4 *Bioassays*

Adults of *T. castaneum* used in experiments were separated from diet using a sieve with 841 μm openings (Seedburo Equipment Co., Chicago, Illinois, USA). Ten adults of 1-4-weeks of age and of mixed sex were aspirated and placed on untreated and DE-treated concrete arenas. After insect introduction, concrete arenas were placed inside incubators with a volume of 0.14 m^3 (Isotemp Standard Lab Incubator, Fisher Scientific, Denver, Colorado, USA) set at 28, 36, 42, 44, and 46°C. The temperature and humidity levels were measured using HOBO® data loggers (Onset Computer Corporation, Bourne, Massachusetts, USA). Humidity levels at 28, 36, 42, 44, and 46°C were an average of 65, 21, 20, 19, and 18% r.h., respectively. Generally, at elevated temperatures humidity levels are around 22-25% (Mahroof et al., 2003; Subramanyam et al.,

2011). At each temperature, 20 untreated concrete arenas and 20 arenas each treated with DE at 2.5 or 5.0 g/m^2 were placed in incubators. At each of the temperatures, a replication consisted of five arenas representing a DE application rate of 0, 2.5, or 5.0 g/m^2 that were sampled after 4, 8, 12, and 24 h of exposure. At each exposure time, all adults from five arenas (50 adults) were pooled and placed in 150 ml round plastic containers holding 30 g of *T. castaneum* diet. Containers were closed with perforated lids covered with a fine mesh to prevent insect escape but allow air diffusion. Care was taken when picking adults from DE-treated concrete arenas so as to not transfer any DE to insect diet in containers. Containers were then held at 28°C and 65% r.h. for an additional 24 h before mortality assessments were made. To determine mortality, adults were separated from the flour in containers using an 841-µm sieve. Live and dead adults were counted, and percentage mortality was calculated based on number of dead adults out of the total. Each temperature, DE rate, and exposure time combination was replicated six times.

6.3.5 *Data analysis*

Mortality in DE treatments was corrected for control mortality using Abbott's formula (1925). Corrected mortality data were transformed to angular values and analyzed using the general linear model procedure in SAS (SAS Institute, 2008). A three-way analysis of variance (ANOVA) was run to determine significant differences ($P < 0.05$) in mortality due to the main and interactive effects of temperature, DE rate, and exposure time. Corrected mortality data by temperature and DE rate were analyzed using one-way ANOVA to determine significant differences ($P < 0.05$) among exposure times. If ANOVA was significant, the Ryan-Einot-Gabriel-Welsch (REGWQ) step-down, pairwise multiple comparison procedure was used for mean separation (SAS Institute, 2008).

6.4 **Results**

The mortality of *T. castaneum* adults on untreated arenas at 28, 36, and 42°C was <4% after 4-24 h of exposure (Table 6.1). At 44°C, the mortality of adults was 1% at a 4 h exposure, but was between 26-63% at exposures of 8-24 h. Similarly, at 46°C, mortality of adults was 19% at 4 h but was 48, 76, and 100% at 8, 12, and 24 h exposures, respectively. There were no significant differences in control mortality among exposure times at 28 and 36°C (F_{range} = 0.95-1.00; df = 3, 20; $P \geq 0.4133$). However, significant differences were observed among exposure times at temperatures of 42, 44, and 46°C (F_{range} = 4.04-7.34; df = 3, 20; P_{range} = 0.0017-0.0212).

The corrected mortality of *T. castaneum* adults exposed to 2.5 and 5.0 g/m^2 increased with an increase in temperature and exposure time. Three-way ANOVA (Table 6.2) showed that temperature, DE rate, and exposure time were significant ($P < 0.05$). Generally more adults died at 5.0 g/m^2 than at 2.5 g/m^2 (Figures. 6.1 and 6.2). At 44°C, after 8 and 24 h, mortality in 2.5 g/m^2 treatment was 0.3-1.5% greater than in 5.0 g/m^2 treatment. Similarly, at 46°C a 24 h exposure at both DE rates resulted in 100% mortality of adults. Except for the temperature and exposure time interaction, all two and three way interactions were not significant. The significant temperature and exposure time interaction indicated that the mortality responses over time at the different temperatures were not consistent.

One-way ANOVA of *T. castaneum* corrected mortality over time at 2.5 g/m^2 DE treatment at 28, 36, 42, 44, and 46ºC was significant (F_{range} = 4.32-35.82; df = 3, 20; P_{range} = <0.0001-0.0168). Similarly, *T. castaneum* corrected mortality at 5.0 g/m^2 DE treatment over time at each of the five temperatures was significant (F_{range} = 3.56-38.78; df = 3, 20; P_{range} = < 0.0001-0.0326). The trends observed in mortality of adults at each of the five temperatures at 2.5 g/m^2

and 5.0 g/m^2 were similar (Figures 6.1 and 6.2). Only the 24 h exposure at both DE rates produced significantly greater ($P < 0.05$) mortality at 28, 36, and 42°C. Adult mortality at 44 and 46°C at both the DE rates reached 99-100% after a 24 h exposure. However, the high control mortality at 44 and 46°C in DE exposures, especially at 12 and 24 h (Table 6.1) confounded from truly gauging the effect of DE at these temperatures. Nevertheless, the laboratory results support that a combination of heat plus DE can increase mortality of *T. castaneum* adults at temperatures below 50°C.

6.5 Discussion

DE dusts are typically composed of 80-93% silicon dioxide, as well as varying amounts of organic matter, clay minerals, magnesium carbonate, among others (Antonides, 1998; Subramanyam and Roesli, 2000; Shah and Khan, 2014). DE works by abrading the insect's cuticle, interfering with the water retention ability of the insect, and results in death through desiccation (Korunić, 1998; Dowdy and Fields, 2002).

The use of elevated temperatures (50-60°C) is a long-standing technology that is a safe and proven method to manage stored-product insects in empty bins and grain-processing facilities (Dosland et al., 2006; Subramanyam et al., 2011). Lethality in insects at high temperatures depends on both the temperature and exposure time (Evans and Dermott, 1981; Fields, 1992; Denlinger and Yocum, 1999; Mahroof et al., 2003). Death in insects exposed to elevated temperatures is due to quicker formation of lethal lesions, where the healing process that counters the lesions become less operative (Denlinger and Yocum, 1999). At elevated temperatures, insects' cuticular wax becomes more fluid, allowing loss of water, leading to death by desiccation (Hepburn, 1985). Additionally, insect's respiration, an indicator of overall

metabolic rate, is adversely affected at elevated temperatures (Neven, 1998). At the cellular level, exposure of insects to elevated temperatures decreases hemolymph pH and ion concentration, denatures lipids, carbohydrates, proteins and nucleic acids, inactivates major glycolysis enzymes, and disrupts plasma membrane (Hochachka and Somero, 1984; Denlinger and Yocum, 1999; Neven, 2000).

In our study, the mortality of *T. castaneum* adults increased with increasing temperatures and exposure times. At temperatures of 28, 36, 42, and 44°C the combined effect of DE and heat was better than heat alone. The use of DE at temperatures between 36 and 46ºC was shown to increase mortality of *T. castaneum* adults on concrete arenas compared to heat alone or DE treatment at 28°C and 65% r.h. A DE treatment of 5.0 g/m^2 produced slightly greater mortality that varied with temperature and exposure time, than at 2.5 g/m^2, but the differences observed were not large enough to justify using the higher DE rate.

At 44°C after a 24 h exposure, the DE treatments contributed to an additional 35-36% mortality of adults, because temperature alone provided 63% mortality. Unlike observations made by Tilley et al. (2007) at 50°C, we observed 100% mortality of *T. castaneum* adults after a 24 h exposure to 46°C alone on untreated arenas. Insects succumb to elevated temperatures greater than 35°C, and at more extreme temperatures, the death of the insect occur quicker (Fields, 1992). Our results support previous studies conducted at a few constant temperatures that the insecticidal effects of DE increase as the temperature increases (Arthur, 2000; Dowdy and Fields, 2002). The addition of DE to heated environments can result in a more rapid water loss from insects, especially at low humidities, resulting in faster death of insects (Mahroof et al., 2003). Furthermore, increased activity of insects at higher temperatures to seek cooler environments (Fields, 2006) may result in greater pick-up of DE particles, leading to rapid

desiccation effects due to the combined effect of DE and heat. Our results suggest that it is possible to obtain effective control of *T. castaneum* adults by combining DE with temperatures of 44 or 46°C. In practical heat treatments of empty bins (Tilley et al., 2007), unlike the laboratory experiments where temperatures remain constant, temperatures are dynamically changing over time. However, temperatures in the range of 44-46°C can be maintained for several hours to obtain an effective kill of stored-product insects in the presence of DE when disinfesting empty bins. Additional studies are warranted in empty bins to determine minimum temperature-time combinations, with and without food, to show how the combined effect of DE and heat can be used for effective disinfestation under practical field conditions.

6.6 Acknowledgements

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6.7 References

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Table 6.1 Mortality of *T. castaneum* adults on untreated concrete areas (control treatment) corresponding to elevated temperature and exposure time treatments.

Temperature (°C)	Exposure time (h)	Mean mortality ± SE (%)[3]
28[1]	4	0.0 ± 0.0
	8	0.0 ± 0.0
	12	0.0 ± 0.0
	24	0.3 ± 0.3
36[2]	4	0.8 ± 0.7
	8	0.3 ± 0.3
	12	0.0 ± 0.0
	24	1.2 ± 0.8
42[3]	4	0.7 ± 0.7b
	8	0.7 ± 0.7b
	12	0.7 ± 0.4b
	24	3.7 ± 1.0a
44[3]	4	1.0 ± 1.0b
	8	26.7 ± 12.9ab
	12	44.0 ± 16.7a
	24	63.4 ± 12.2a
46[3]	4	19.3 ± 10.7c
	8	47.7 ± 17.8bc
	12	76.3 ± 16.5ab
	24	100.0 ± 0.0a

[1]There were no significant differences among exposure times ($F = 1.00$; df = 3, 20; $P = 0.4133$; by one-way ANOVA).
[2]There were no significant differences among exposure times ($F = 0.95$; df = 3, 20; $P = 0.4339$; by one-way ANOVA).
[3]At each temperature, means among exposure times followed by a different letter are significantly different ($P < 0.05$, by REGWQ test).

Table 6.2 Three-way ANOVA statistics showing main and interactive effective of temperature, DE rate, and exposure time on corrected mortality of *T. castaneum* adults.

Source	df	Mean Square	*F*-value	*P*-value
Temperature	4	6.054	81.81	<0.0001*
Rate	1	0.340	4.59	0.0334*
Exposure time	3	7.660	103.51	<0.0001*
Temperature × rate	4	0.051	0.69	0.5997
Temperature × time	12	0.282	3.81	<0.0001*
Rate × time	3	0.008	0.11	0.9542
Temperature × rate × time	12	0.031	0.42	0.9545
Error	200	0.074		

*****Significant ($P < 0.05$).

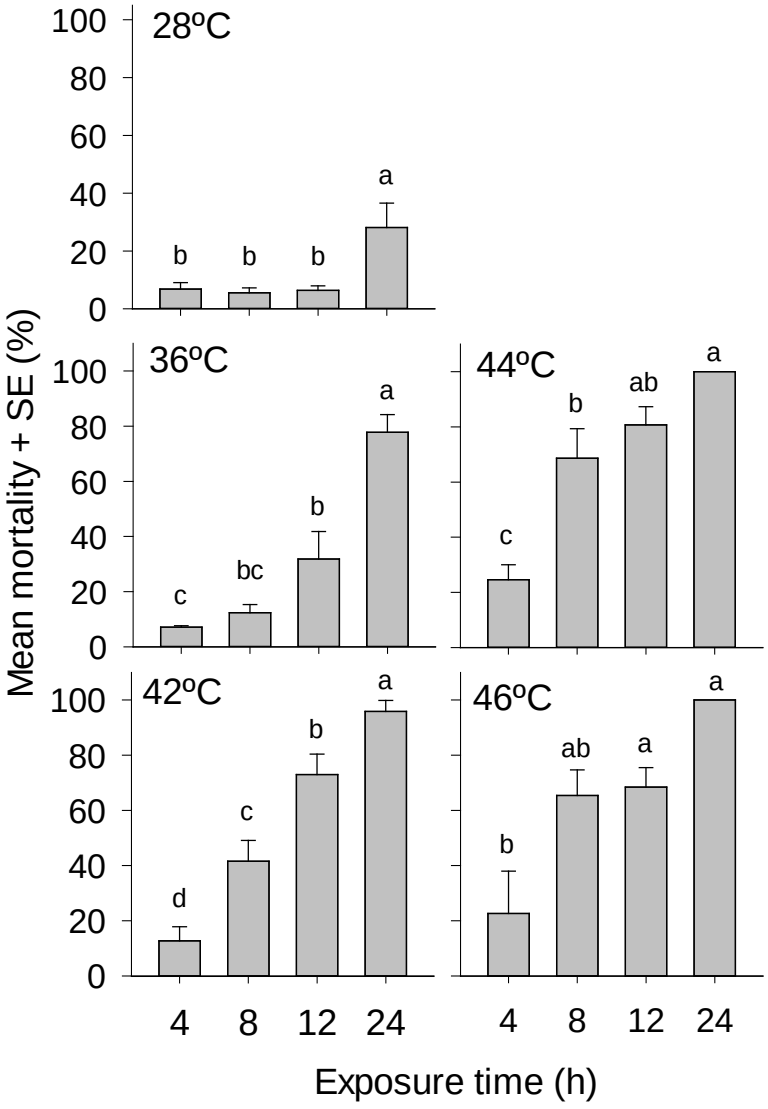

Figure 6.1 Mean + SE corrected mortality of *T. castaneum* adults exposed for 4-24 h to 2.5 g/m^2 of DE on concrete arenas. At each temperature, means among exposure times followed by different letters are significantly different ($P < 0.05$, by REGWQ test).

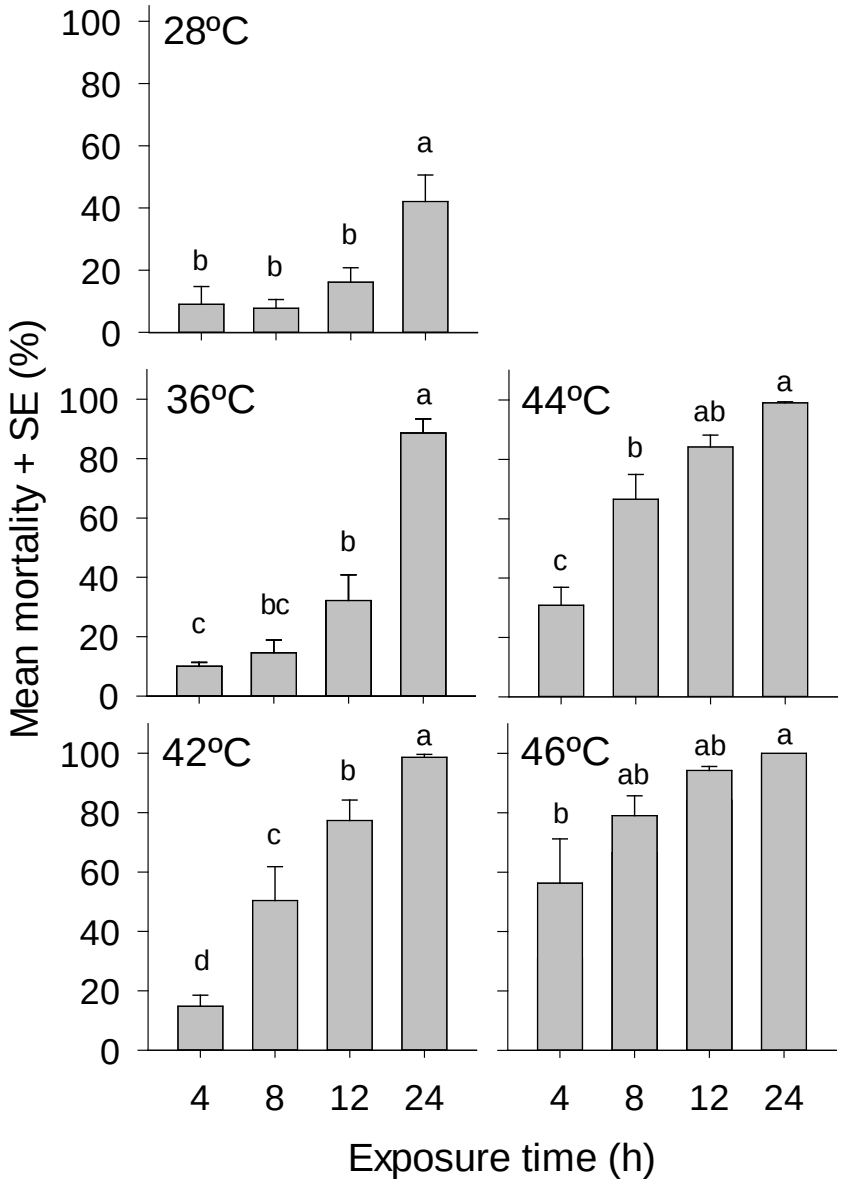

Figure 6.2 Mean + SE corrected mortality of *T. castaneum* adults exposed for 4-24 h to 5.0 g/m^2 of DE on concrete arenas. At each temperature, means among exposure times followed by different letters are significantly different ($P < 0.05$, by REGWQ test).

Chapter 7. Overall Conclusions

7.1 A dynamic model for predicting survival of *Tribolium castaneum* (Herbst) adults to elevated temperatures during heat treatment of grain-processing facilities

A dynamic model for predicting the survival of *T. castaneum* adults was developed and validated using commercial data collected at grain-processing facilities. This model under-predicted the initial lag phase of adult survival, when survival stays constant until a certain critical point is reached wherein a rapid decline in survival occurs. Also notable is that temperatures of 50-60°C are typically targeted in commercial heat treatments, however, in some of the datasets, the maximum temperature never reached 50°C, yet mortality of insects was still 100%.

7.2 Evaluation of heat treatments based on trapping data, temperature profiles, and bioassays

Based on the evaluation conducted at a pasta processing facility and rice mill, the results confirmed that heat treatment is an effective tool to manage stored produce insects. Trapping data were collected at the pasta processing facility and results showed that one heat treatment could help keep insect populations low for up to two months. Additionally, a heat treatment can be conducted in less than 24 h.

7.3 Effect of sex, age, and short-term heat acclimation on the mortality of adults of *Tribolium castaneum* (Herbst) to elevated temperatures

Based on our studies, *T. castaneum* females are more heat tolerant than males when reared at 28°C and heat-treated in the laboratory at 50 and 55°C. The larger body size of female insects could be a reason for this. Adult age was also investigated between 1-42 d of age. While mortality results from heat treatment were not consistent from 1 d to 42 d, 1-d-old insects were found to be the most heat tolerant and mortality was highest at 42 d. The results from the short-term acclimation studies showed very little variation in mortality for insects heat-treated at 50°C, and at 55°C, the insects were more heat tolerant when held at 28 or 32°C.

7.4 The effect of continuous rearing of *Tribolium castaneum* (Herbst) at elevated temperatures on subsequent thermal tolerance to heat treatments

From the laboratory studies performed which involved rearing red flour beetles at different temperatures for 10 generations, we found that rearing *T. castaneum* at both 32 and 36ºC increased their heat tolerance compared with the insects reared at the control temperature of 28ºC. In a pilot flour mill heat treatment, however, the insects reared at 36ºC from the F_{10} generation in general had the highest mortality. Insects reared at 32ºC had the most apparent thermo-tolerance in the field study.

7.5 Influence of temperature and application rate on efficacy of a diatomaceous earth formulation against *Tribolium castaneum* (Herbst) adults

The mortality of *T. castaneum* adults increased as both the temperature and exposure times increased. At temperatures of 28, 36, 42, and 44°C the combined effect of DE and heat was better than heat alone. The use of DE at temperatures between 36 and 46ºC was shown to increase mortality of *T. castaneum* adults on concrete arenas compared to heat alone or DE treatment at 28°C and 65% r.h. A DE treatment of 5.0 g/m^2 produced slightly greater mortality than the dose of 2.5 g/m^2, but the differences observed were not large enough to justify using the higher DE rate.

7.6 Future studies

Future studies should examine more closely the effects of continuous rearing of *T. castaneum* at temperatures greater than 32°C. It would be interesting to see if the developed thermo-tolerance after the second generation is reversible or not.

Additional studies are warranted in grain-processing facilities and empty bins to determine minimum temperature-time combinations, with insects exposed with and without food, to show how the combined effect of DE and heat can be used for effective disinfestation under practical field conditions.