

# Stewart Postharvest Review

An international journal for reviews in postharvest biology and technology

## Heat treatment of grain-processing facilities for insect management: a historical overview and recent advances

Bhadriraju Subramanyam<sup>1\*</sup>, Rizana Mahroof<sup>2</sup> and Monika Brijwani<sup>1</sup>

<sup>1</sup>Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas, USA

<sup>2</sup>Department of Biological Sciences, South Carolina State University, Orangeburg, South Carolina, USA

### Abstract

**Purpose of review:** Tactics to manage infestations of stored-product insects in grain-processing facilities historically included the use of heat treatments and the fumigant methyl bromide. The phase out of methyl bromide in the USA in 2005, because of its adverse effects on stratospheric ozone, has renewed interest in using heat treatments. In this review, a brief history regarding the use of heat treatments in grain-processing facilities, such as flour mills, is described along with recent research developments and future research needs.

**Findings:** Scientific literature published during the last decade shed new light on the following areas: identification of a heat-tolerant stage of a species based on stage-specific susceptibility at different constant elevated temperatures; differences in susceptibility among stored-product insect species to elevated temperatures; confusion in determining a heat-tolerant stage during commercial heat treatments of grain-processing facilities; impact of sanitation on insect responses; degree and duration of insect suppression obtained following a heat treatment intervention; development and validation of a thermal death kinetic model to predict survival of heat-tolerant insect life stages as a function of time-dependent temperature data; and development of software programs to estimate the heat energy required including costs of a heat treatment based on fuel used and predicting survival of heat-tolerant insect stages in “real time” during heat treatments.

**Directions for future research:** Temperature-time mortality relationships have been developed for a few economically important insect species and additional species should be evaluated in both laboratory and field conditions. Very little is understood about the impact of the lethal and sub-lethal elevated temperatures used during heat treatments on insect behaviour and reproduction. The cost-effectiveness of combining elevated temperatures with other chemical or nonchemical methods in grain-processing facilities also should be explored.

### Abbreviations

CFR	Code of Federal Regulations
CUE	Critical Use Exemption
E.A.R.T.H.	Efficacy Assessment in Real-Time during Heat treatment
HTC	Heat Treatment Calculator
UNEP-MBTOC	United Nations Environment Programme-Methyl Bromide Technical Options Committee
US-EPA	United States-Environmental Protection Agency

**\*Correspondence to:** Bhadriraju Subramanyam, Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas 66506, USA. Tel: +1 785 532 4092; Fax: +1 785 532 7010; email: [sbhadrir@k-state.edu](mailto:sbhadrir@k-state.edu)

**Stewart Postharvest Review 2011, 3:1**

Published online 01 December 2011

doi: 10.2212/spr.2011.3.1

## Introduction

Grain-processing facilities such as flour mills are ideal habitats for supporting infestation by economically damaging stored-product insect pests, because of year-round warm temperatures and constant availability of abundant food resources [1, 2]. Several surveys have documented the presence of stored-product insect species in moving mill stock [1, 3], static mill stock, and within and around milling equipment [4–7]. Insect infestations were detected in mills that were in operation [8–12] or no longer in use [13]. In the USA, Good [3] surveyed 19 flour mills in Kansas, Missouri, and Oklahoma from 1934 to 1935 by taking 227-g (8-oz) samples monthly from 24 elevator boots and mill streams. He reported 30 different insect species from 17 of the 19 mills, representing 15 families in five orders. Four of the eight most abundant stored product insect species made up nearly 97.5% of the 74,175 insects found in the samples; these species were *Tribolium* spp. (84.6% of the total insects), the square-nosed fungus beetle, *Lathridius (Laemophloeus) minutus* (L.); rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (8%); and cadelle, *Tenebroides mauritanicus* (L.) (3.2%). The lesser grain borer, *Rhyzopertha dominica* (F.); rice weevil, *Sitophilus oryzae* (L.); granary weevil, *Sitophilus granarius* (L.); and long-headed flour beetle, *Latheticus oryzae* (Waterhouse), each constituted less than 2% of the total insects found. Salmond [8] sampled mill stocks stored in bags, bulk, and within machines in a flour mill in the United Kingdom on six different occasions from August 1948 to September 1949. He found 85 species of insects, representing the orders Coleoptera, Lepidoptera, Diptera and Hymenoptera. The Mediterranean flour moth, *Ephestia (Anagasta) kuehniella* (Zeller); broad-horned flour beetle, *Gnatoscerus cornutus* (F.); and confused flour beetle, *Tribolium confusum* Jacquelin du Val, were considered the most economically important species, because of their interference with the milling process and contamination of the finished products. Buchelos [11], from October 1978 to November 1980, monitored stored-product Coleoptera in two mills in Greece by using adhesive strips (75 x 5 cm) attached vertically to a wall 1 m off the floor. He reported 23 different insect species, representing 13 different families. The five most abundant stored-product species reported were *T. confusum*; *S. oryzae*; *C. ferrugineus*; the red flour beetle, *Tribolium castaneum* (Herbst); and the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.). In the USA, Campbell and Arbogast [12] monitored stored-product insects inside and outside a flour mill by using both traps (commercial pitfall and sticky traps) and product samples from June 2001 to December 2003. They found 17 insect species, representing 12 families in three orders (Coleoptera, Hymenoptera, and Psocoptera). Captures of *P. interpunctella* adults in sticky traps and adults of the warehouse beetle, *Trogoderma variabile* (Ballion), were greater outside than inside the mill. However, captures of *T. castaneum* adults in pitfall traps were greater inside than outside the mill. They suggested movement of insects between the mill and the outside environment. Lack of an inbound inspection and keeping doors open are some of the factors contributing to re-infestation of

structures disinfested either by a heat treatment or fumigation.

Suggested pest management methods for flour mills involve stock rotation [14], sanitation [15, 16], aspiration, use of impact machines [17, 18], crack and crevice treatment with residual products, and exclusionary tactics such as closing doors, use of air curtains near entrances, and screening windows. Space treatments include the use of the fumigant methyl bromide and fogging with several approved aerosol insecticides [19].

## Methyl bromide phase out

The USA is one of the signatories to the 1987 Montreal Protocol to phase out production and use of ozone-depleting substances to protect stratospheric ozone. Protecting stratospheric ozone is important for reducing the amount of ultraviolet-B radiation reaching the earth and for reducing the incidence of human skin cancers and cataracts [20]. According to the 1990 Clean Air Act Amendments the USA should satisfy its obligations under the Montreal Protocol. Methyl bromide, is listed under the Clean Air Act as a Class I ozone depleting substance, and was intended for phase out by 2005 (40 Code of Federal Regulations (CFR) Part 82, Federal Register Volume 69, No. 246). The schedule for phase out was as follows: 1993 to 1998, freeze production and net imports at 1991 base levels (25,500 metric tons); 1999 to 2000, 25% reduction from baseline levels; 2001 to 2002, 50% reduction from baseline levels; 2003 to 2004, 70% reduction from baseline levels; and 2005, 100% phase out, except for certain critical uses agreed to by the Montreal Protocol Parties. The United States Environmental Protection Agency (US-EPA) has established the critical use exemption (CUE) process to allow methyl bromide use for quarantine, pre-shipment, and certain industry groups, where technically and economically feasible alternatives are non-existent. The CUE process also provided adequate transition time for these groups to embrace and adopt methyl bromide alternatives. The US-EPA has established a framework for assigning allowances for critical use of methyl bromide, and determining the quantities of exempted methyl bromide allowable under the Clean Air Act and the Montreal Protocol. In 2005, the USA nominated about 536 metric tons of methyl bromide for use in food- and feed-processing plants (Federal Register Volume 69, No. 246, p. 76986), and the Montreal Protocol Parties reduced this amount to 483 metric tons, because of the availability of a non-ozone depleting fumigant sulfuryl fluoride (ProFume™), a methyl bromide alternative registered in January 2004 for use in grain-processing facilities. Further reductions were anticipated in the amounts available for 2006 and beyond. The amount of methyl bromide nominated and approved by the Montreal Protocol Parties (United Nation's Environment Programme Methyl Bromide Technical Options Committee, UNEP MBTOC) since methyl bromide phase out has been steadily declining over time. For example, in 2006, 2007, 2008, and 2009, the critical use nominations for methyl bromide granted were 32.03, 26.61, 21.0, and 19.5% of 1991 baseline levels, respectively. The actual

quantities nominated for grain/food-processing facilities in 2006, 2007, 2008, 2009, and 2010 were 529.6, 401.9, 362.9, 291.4, and 191.9 metric tons, respectively. The current nomination allowed in 2011 for use in food-processing facilities is 135.3 metric tons.

Presently, CUEs are allowed for rice millers in all locations of the USA who are members of the United States Rice Millers Association, pet food manufacturing facilities within the USA who are active members of Pet Food Institute, Kraft Foods in the USA, and members of the North American Millers' Association and National Pest Management Association, provided that the facilities being fumigated are older structures that cannot be properly sealed to use an alternative (ProFume™), or where the presence of sensitive electronic equipment prohibits use of another alternative (ECO<sub>2</sub>FUME™, which has 2% phosphine and phosphine is corrosive to copper). The US-EPA also determined that the rates required to kill eggs of stored-product insects is cost-prohibitive with alternatives (ProFume™) than with methyl bromide. Therefore, heat appears to be a viable and cost-effective alternative to methyl bromide and other fumigant alternatives. The methyl bromide phase out has renewed interest in the use of elevated temperatures or heat treatments, a technology that is 100 years old [21, 22].

#### **A brief history on the use of elevated temperatures for insect management**

Elevated or high temperatures were suggested as a practical means for managing insects associated with stored grain as early as 1762 [23]. Oosthuizen [24] reviewed the early history of using high temperatures for stored-product insects. In France in the late 1800s, infested grain was exposed to high temperatures to kill insects in rooms equipped with special heating coils. Webster [25] reported that larvae and pupae of the Angoumois grain moth, *Sitotroga cerealella* (Olivier), were cooked when exposed to 60°C for 9 hours. Lintner [26] reported that eggs, larvae, and pupae of *T. castaneum* can be killed when exposed to 49 to 54°C, whereas adults required 66°C. Goodwin [27] reported that 13 stored-product insects in grain were killed after exposure for 1 to 2 hours at 50 to 55°C and 40 to 50% relative humidity. The germination of the seeds was not affected when 50 types of grains and legumes were exposed for 2 hours at 70°C [28]. These early studies did not provide enough detail to determine how the experiments were conducted and analysed. It was Dean [21] that first reported the use of heat for management of insects in flour mills in 1911. In a flour mill in Kansas subjected to a 7.5-hour heat treatment from a steam heater, temperatures were measured at four locations on each floor, some in open areas and others in flour or in locations where flour would normally accumulate such as conveyer belts and spouts. The ambient mill temperature at the start of the heat treatment was 35.5°C. The highest temperature recorded on the first floor was 38°C, and the lowest was 34.4°C at the bottom of an elevator boot. On the second floor a temperature of 37°C was recorded at 8.89 cm in a sack of flour placed at 1.22 m

above the floor, and the highest temperature recorded was 51°C. On the third floor temperatures ranged from 46°C in a flour conveyer 1.83 m above the floor to 52°C in an open area. On the fourth floor a temperature of 42°C was recorded in flour on a conveyer belt at a depth of 5.08 cm; however, the temperature in the open area was 48°C. As temperatures increased the relative humidity in the mill dropped from 93% to 27%. The mill had natural infestation of *T. confusum*. Visual inspections showed that insects on the first floor were not killed while the treatment was partially effective on the other floors. No data were presented on numbers of insects found. Therefore, observations on efficacy against insects are purely qualitative or, at best, speculative.

A second heat treatment was conducted in the same mill three weeks after the first treatment, and the heat treatment lasted 24 hours instead of 7.5 hours [21]. The starting mill temperature was 32.2°C. On the first floor, the temperature measured in an open area was 40.5°C, and at a depth of 10.16 cm in wheat, a temperature of 35.5°C was recorded. On the second floor the maximum temperature recorded was 56.4°C and the temperature at a depth of 7.62 cm in a sack of flour that was 91.4 cm above the floor was 47.5°C. The temperatures on the third floor ranged from 53.8 to 60.5°C. On the fourth floor the temperatures ranged from 47.8 to 53.3°C. The relative humidity for a majority of time during the heat treatment was around 12%. After this treatment no live *T. confusum* were observed, but data on insect numbers were not provided.

Dean [29] reported that several flour mills in Ohio, Nebraska, Illinois, Iowa, Indiana, and southern Canada have embraced heat to control insects. He reported lethal temperatures to be around 47.8 to 50°C. No adverse effects were observed on floors, belts, and the mill machinery. Goodwin [30] was the first to suggest using 50 to 60°C for insect control in mills. He reported that insects subjected to heat treatments died at lower temperatures in dry air than in humid air. He suggested conducting a heat treatment during warmer times of the year because temperatures are already high and attaining 50 to 60°C would be easier. He recommended cleaning the mill prior to heat treatment, removing sacks of flour, and disassembling pieces of equipment for improving heat treatment effectiveness. Additionally, sealing the building gaps, to prevent cold air infiltration, was suggested to retain heat. Goodwin [30] gave four methods for determining heat energy requirements.

Relative humidity appears to influence time to death in insect life stages exposed to high temperatures. Oosthuizen [24] examined effects of high temperatures on *T. confusum* life stages at different relative humidities. At 44°C, all 3-day-old eggs were killed in 19 hours at 0% humidity, but complete mortality at 75% humidity took 24 hours. He also found sex related differences in susceptibility at 44°C and 0% humidity, with virgin females being less susceptible than males. At 44 and 46°C he reported pupae to be more tolerant than eggs, old larvae, and adults. However, at 48 and 50°C adults were more

tolerant than eggs, old larvae, and pupae. The oviposition rate and fertility were adversely affected by exposure of larvae and pupae, while adverse reproductive effects in adults were temporary. At approximately 41°C effect on fertility was directly related to the exposure time. Oosthuizen [24] also showed that when 150 g of flour forming a depth of 10.2 cm was placed in a water bath at 51°C, about 2.5 hours were needed for the centre of the flour to reach the target temperature of 51°C. This suggested flour to be a heat insulator or a poor conductor of temperature.

Pepper and Strand [31] empirically measured temperatures attained at the surface and at various depths (2.54 to 22.86 cm) of a 0.372 m<sup>2</sup> concrete block that was 25.4-cm thick. The surface temperature attained by the block when 1.6 kW/h heat energy was applied for 10 hours was 65.5°C. The temperatures were inversely related to the depth of concrete. For every 1-cm depth of the concrete (as measured from the surface) the temperature dropped by 1°C. They reported higher temperatures at all depths when fans were used to circulate the hot air than in still air. Pepper and Strand [31] measured temperatures in two mills subjected to a 12- and 20-hour heat treatment. They showed temperature differences between the north, center, and south ends of each of the mill floors. Generally, temperatures were lower on the first and second floors compared with other floors. They suggested a starting ambient mill temperature of 32.2°C for conducting a successful heat treatment to reduce the heat energy required to reach lethal temperatures. The target temperature during heat treatment was suggested to be around 54.4 to 60°C. Based on heat-loss calculations, Pepper and Strand [31] suggested the heat energy requirement to be 0.86 kW/h/m<sup>3</sup>. Current heat treatments at commercial facilities are done using 0.10 kW/h/m<sup>3</sup>. The high heat energy required in the former case could be due to leaks in the mill or mills made predominantly of wood. Pepper and Strand [31] suggested measuring temperatures at the floor level and the use of fans to distribute heat. They also suggested that the maximum ceiling temperature should not exceed 71.1°C. In addition, the sprinkler heads installed should be able to withstand a temperature of 100°C. Pepper and Strand [31] were also the first to report on structural damage due to heat treatments such as cracks in wooden spouts. Like Dean [21] they did not show any data on the efficacy of heat treatments on insects associated with mills.

#### **Heat treatment of grain-processing facilities: Basics**

Heat treatment involves raising the ambient temperature of the whole or a portion of the facility to 50 to 60°C and maintaining these elevated temperatures for 24 to 36 hours [32–34]. Temperatures between 50 and 60°C should be attained in all critical locations where insects are usually observed to be present. The long heat treatment period (24 to 36 hours) is necessary for heat to penetrate wall voids and equipment to kill insects harbouring in them. Facility heat treatments are labour intensive, because grain and grain product residues within the facility should be thoroughly cleaned and raw and finished products removed as they are poor conductors of

heat. Insects may hide in these materials and escape the heat treatment. In the USA, in addition to flour mills, many grain-processing companies such as General Mills, ConAgra Foods, Cargill Inc., Kraft Foods, Quaker Oats (PepsiCo), New World Pasta, and Nestle Purina, among others, have been using heat treatments, on a limited basis, as an alternative to methyl bromide for more than a decade. In addition to grain/food-processing facilities, heat treatment can also be used in empty storage structures (bins, silos) [35], warehouses, and bakeries. A discussion on these aspects is beyond the scope of this article.

Electric heaters, forced air gas heaters, or steam heaters can be used to conduct a heat treatment. With the forced air gas heaters the facility is placed under positive pressure during a heat treatment, and the entire air within the building is exchanged several times. The number of air exchanges when using electric and steam heaters may be one or two per hour. With forced air there are about four air exchanges per hour, and forcing the air allows heat to reach gaps in the building and equipment much better than electric or steam heaters. The forced air gas heaters can use natural gas or propane as fuel. Since these heaters have an open flame they are placed outside a facility, and nylon ducts are placed within the facility to introduce heated air.

During facility heat treatments, horizontal and vertical stratification of temperatures results in non-uniform distribution of heat [36, 37]. Therefore, fans are used in strategic locations to minimise temperature stratification, and to uniformly heat facilities to ensure effective insect kill. The placement of fans is the art part of heat treatment, and during heat treatments, fans need to be moved to improve heat circulation and to eliminate cool spots (locations where temperatures are less than 50°C). This can only be accomplished with proper temperature monitoring throughout the heat treatment period.

#### **Heat treatment of facilities versus commodities**

Heat has also been used to disinfest perishable and dry, durable food products [38]. High temperature treatments are used for disinfestation of dried fruits and nuts, perishable commodities (fruits) [39], and grains [40]. Facility heat treatments are distinctly different from heat treatment of fresh fruits, nuts, or grains. In facility heat treatments, heaters are used to slowly heat the ambient air. A long heat treatment period is necessary for the heat to penetrate wall voids and equipment to kill insects harbouring in them. A typical heat treatment may last 24 to 36 hours [36, 37]. In heat treatments of fresh commodities, nuts, dried fruits, or grains, high temperatures of 60 to 85°C are used for short time periods (in minutes). Typical heating rates during heat treatment of perishable commodities, nuts, dried fruits, and grains range from 1 to 15°C per minute, whereas during facility heat treatments heating rates should generally be around 3 to 5°C per hour for effective disinfestation. However, in both cases the products or the facility subjected to high temperatures are allowed to cool to ambient temperature, and this may take several hours.

### Heat energy required for facility heat treatments

Dosland *et al.* [41] gave detailed step-by-step procedures for conducting and evaluating a facility heat treatment. One important aspect of conducting an effective heat treatment involves calculating how much heat energy is required after accounting for heat losses due to exposed surfaces, equipment, and infiltration. There are well known equations to do heat loss-calculations [42]. Research at Kansas State University and discussions with heat treatment service providers showed that the amount of heat energy should range from 0.074 to 0.102 kW per cubic meter of the facility per hour, and during a 2009 heat treatment of a flour mill at Kansas State University, the heat energy used was as high as 0.16 kW per cubic meter per hour. An indirect method of determining whether or not adequate heat energy is being used is by observing how quickly ambient temperatures reach 50°C. In effective heat treatments, the time to reach 50°C from an ambient temperature should usually take about 8–10 hours, and depending on the time of year and the leakiness of a structure, this time can take as long as 15 hours.

### Characterising temperature profiles

It is important to monitor temperatures in as many locations as possible, either continuously using micro-processor based temperature sensors, or at hourly intervals using thermocouples and or infrared thermometers. A typical temperature profile during heat treatment is shown in Figure 1. From the temperature data, the following information should be extracted: number of hours required to reach 50°C from an ambient temperature, number of hours temperatures were maintained above 50°C and the maximum temperature [36, 37]. The time to reach 50°C is important to determine the heating rate, which is calculated as the difference between 50°C and the ambient temperature at the start of the heat treatment divided by the time to 50°C. This rate should be between 3 and 5°C per hour in properly conducted heat treatments for effective disinfection. Temperatures should be held at least for several hours above 50°C to kill insects. The maximum temperature should not exceed 60°C to prevent any structural damage or damage to equipment. Information broken down in this fashion can be related to insect mortality if live insects confined in cards or vials are used to gauge the effectiveness of a heat treatment.

### Effects of heat on insects

Lethality in insects at high temperatures depends on both the temperature and time of exposure [43–46]. Temperature and exposure time to achieve a certain percentage of insect kill are inversely related. At high temperatures insect cuticular wax becomes compromised allowing loss of water. This affects water balance in insects, leading to death by desiccation [47]. High temperature exposure denatures proteins, affects hemolymph ionic balance and pH, and adversely affects enzyme activity [43, 48].

High temperatures that do not kill insects can adversely affect their reproduction. Recently, Mahroof *et al.* [49] have shown

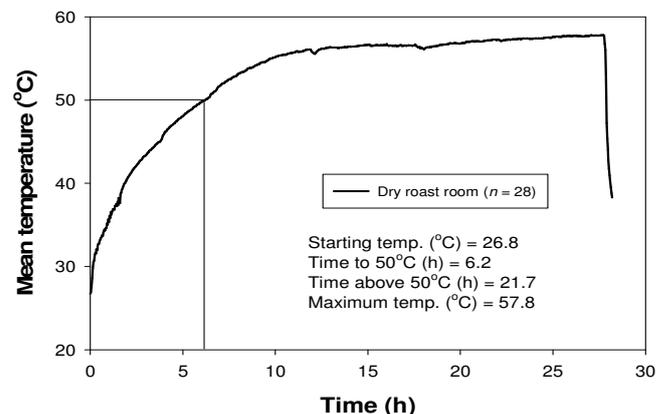
that when pupae and adults of *T. castaneum* were exposed to 50°C for 39 and 60 min, respectively, the surviving adults from these insects showed significant reduction in oviposition, egg-to-adult survival rate, and adult progeny production. Such effects could delay population rebounds following a heat treatment.

### Gauging heat treatment effectiveness

To gauge heat treatment effectiveness, it is important to identify critical areas in the facility. These areas are usually places where insects can hide and breed or places where temperatures cannot penetrate or reach at least 50°C. Such places are usually identified through inspections. Temperature sensors should be placed in these areas to measure temperatures. Cards with insects such as those marketed by Alteca ([www.alteca.com](http://www.alteca.com)) or life stages of insects in vials with food (5 g of flour and 20 insects/vial) should be placed in critical areas and examined during or after a heat treatment to determine effectiveness against insects. However, it should be noted that insects in the cards are usually without food and therefore these stressed or starved insects tend to succumb quickly to a heat treatment, and may falsely indicate that the treatment was effective, when in fact it was not. For example, in a pet food facility, the mortality of *T. castaneum* adults in cards and bioassay vials with flour (3 g) was compared. Results showed that during the 23-hour-heat treatment, all insects in the cards died at 15 hours, whereas in the vials at the end of the heat treatment, the mortality of adults was below 20%. The use of live insects to gauge heat treatment effectiveness provides valuable information, but in some facilities bringing live insects may be prohibited.

Resident insect populations within a facility should be monitored before and after a heat treatment. At least thirty five traps should be used inside the facility and five outside the facility. In some facilities such as flour mills, it is possible to

Figure 1. An average temperature profile from 28 temperature sensors placed throughout a dry roast room of a food-processing facility in the USA subjected to heat treatment using forced air gas heaters.



**Table 1. Captures of *T. castaneum* adults in pitfall traps before and after a heat treatment conducted on July 4, 2006, of a pasta facility in the USA.**

Date	Mean number of adults/trap/week <sup>a</sup>		
	Press room	Flour room	Outside
May 30	0.46	0.40	0.50
June 14	0.20	0.42	0.65
June 28	0.32	0.65	0
July 4	Heat treatment <sup>b</sup>		
July 11	0 (100%) <sup>c</sup>	0.09 (86%) <sup>c</sup>	0
July 25	0.03	0.10	0.38
August 8	0	0.05	0.50
August 23	0.01	0.05	0.20

**Source:** Bhadriraju Subramanyam (unpublished data).

<sup>a</sup>The number of traps in the press room, flour room and outside the facility was 35, 10, and 5, respectively.

<sup>b</sup>Traps were replaced immediately after the heat treatment was done.

<sup>c</sup>Percentage reduction in trap catch, based on catch just prior to the heat treatment.

sample tailings to determine insect load. These observations should occur every week and should be resumed soon after a heat treatment. The trapping or visual observations of products/tailings following a heat treatment should be done at least on a daily basis for the first week and should continue weekly for at least 8 to 16 weeks. These data provide valuable information on the degree and duration of control obtained after a heat treatment intervention. Table 1 shows mean trap captures of *T. castaneum* adults in food- and pheromone-baited pitfall traps before and after heat treatment of a pasta facility in the USA. These data show that a single treatment's effectiveness lasted close to two months, because the facility managers use excellent sanitation and exclusion tactics. Similar heat treatments in Canada have shown effectiveness of a single treatment to last about 5 months [50]. The doors and windows should be tightly closed to prevent insects from outside coming into a facility. Insects can be brought into a facility on raw materials, and care must be taken to inspect all materials to ensure that they are insect-free. Inspection, sanitation and exclusion practices can help extend the degree and duration of insect suppression obtained with a heat treatment.

### Recent advances

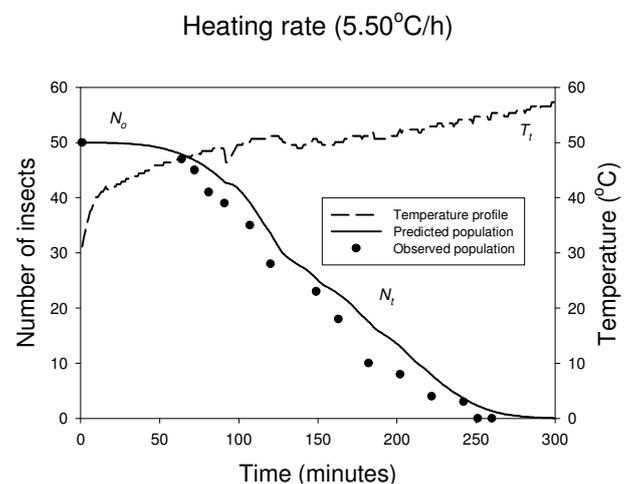
Stage-specific susceptibility of stored-product insects to elevated temperatures varies with the species. Mahroof *et al.* [36] exposed eggs, young larvae, old larvae, pupae, and adults of *T. castaneum* during heat treatment of Kansas State University's pilot flour and feed mills. There were no clear cut trends of a particular life stage being heat tolerant, but a few adults and pupae survived the heat treatment compared with the other stages. However, in the laboratory at six constant elevated temperatures between 42 and 60°C, Mahroof *et al.* [46] showed that the young larvae (first instars) were

more heat-tolerant than the other stages. Boina and Subramanyam [51] showed the old larvae of *T. confusum* to be heat-tolerant based on tests in the laboratory at constant elevated temperatures. A thermal death kinetic model was developed and validated to predict survival of *T. confusum* old larvae during facility heat treatments [52]. This model can be used to predict survival of old larvae of *T. confusum* based on only the time-dependent temperature data (Figure 2). In a separate study, Mahroof and Subramanyam [53] showed the old larvae (fifth instars) of *P. interpunctella* to be the heat-tolerant stage, whereas in the cigarette beetle, *Lasioderma serricorne* (F.), eggs were found to be the heat-tolerant stage [54]. These two studies were also conducted at constant temperatures. In the drug store beetle, *Stegobium paniceum* (L.), the young larvae were found to be heat-tolerant based on tests at constant temperatures [55]. At 50 to 60°C, among all species and stages tested, the young larvae of *T. castaneum* are the most heat tolerant (Table 2).

The heat tolerance in young larvae of *T. castaneum* was due to increased expression of heat-shock protein, HSP 70 [56, 57]. Time and temperature-dependent expression of HSP 70 showed that the increased heat tolerance in young larvae lasted only as long as 8 hours at 40°C or 30 minutes at 46°C [56]. The production of heat-shock proteins may confer some survival value for insects at temperatures less than 50°C.

Identification of the heat-tolerant stage is important because these stages should be used in bioassays during facility heat treatments. Controlling the heat-tolerant stage should ensure control of all other stages; however, this assumption may not

**Figure 2. Observed and predicted survival of *T. confusum* old larvae during heat treatment of Kansas State University feed mill. The predicted survival of larvae ( $N_t$ ) was based on a thermal death kinetic model [52], which uses only time-dependent temperature data ( $T$ ).  $N_o$  is the initial population of larvae. The closed circles are the actual larvae that survived over time. The heating rate was calculated as beginning temperature minus the end temperature divided by the total time.**



**Table 2. Time in minutes for 99% mortality (LT<sub>99</sub>) of heat-tolerant stages of four stored-product insect species at constant temperatures between 50 and 60°C.**

Species	Stage	Temp. (°C)	LT <sub>99</sub> (95% CL) <sup>a</sup>	Reference
<i>T. castaneum</i>	Young larvae	50	433 (365-572)	[36]
		54	82 (60-208)	
		58	38 (29-76)	
		60	42 (34-66)	
<i>T. confusum</i>	Old larvae	50	90 (82-102)	[51]
		54	56 (49-67)	
		58	38 (30-71)	
		60	24 (20-33)	
<i>P. interpunctella</i>	Old larvae <sup>b</sup>	50	34 (29-43)	[53]
		52	34 (26-67)	
<i>L. serricorne</i>	Eggs <sup>c</sup>	50	190 (170-220)	[54]
		54	39 (36-43)	
<i>S. paniceum</i>	Young larvae	50	234 (176-387) <sup>d</sup>	[55]
		55	11 (7 -14)	
		60	5 (4 - 5)	

<sup>a</sup>Values were rounded off to the nearest integer.

<sup>b</sup>Fifth instars

<sup>c</sup>Time-mortality relationships were based on egg hatchability data.

<sup>d</sup>These values are LT<sub>90s</sub> (95% CL) (Abdelghany AY, Awadalla SS, Abdel-Baky NF, EL-Syrafy HA and Fields PG. 2010 (unpublished data).

always be true. At 50 to 60°C the young larvae of *T. castaneum* appears to be the most heat-tolerant of the species studied to date. Mahroof *et al.* [36] found young larvae to be susceptible during actual heat treatment of facilities whereas laboratory tests showed this stage to be more tolerant than eggs, old larvae, pupae, and adults. Similarly, Yu *et al.* [54] were unable to find a heat-tolerant stage of *L. serricorne* during heat treatment of a commercial facility. Such anomalous results can be due to heat tolerance being influenced by heating rates [38], and the influence of heating rates on stage-specific tolerance has been well studied during thermal disinfestations of stored grains [40] and not during heat treatments of structures. The impact of varying heating rates on heat tolerance among stages of a given insect species during heat treatments is a fruitful area for further research.

Trap capture data can indirectly indicate which of the species is more susceptible to high temperatures. Roesli *et al.* [37] used adult trapping data to indirectly determine susceptibility of different stored-product insect species. Following a heat treatment of the Kansas State University feed mill, adults of *L. serricorne* were not captured in traps; however adults of *T. castaneum* were captured within 2 to 4 weeks. This indicated that heat treatment was more effective against *L. serricorne* than *T. castaneum*, and these data are consistent with differences among species in their susceptibility at constant elevated temperatures (Table 2).

Previous research has shown that flour is a poor conductor of temperature. However, there are no studies systematically documenting the influence of sanitation or flour accumula-

tions on efficacy of heat treatments against stored-product insects. Therefore, experiments were conducted in 2009 and 2010 during three heat treatments of the Hal Ross flour mill at Kansas State University, Manhattan, Kansas, to evaluate the influence of sanitation on heat treatment effectiveness against eggs and adults of *T. castaneum* [58]. These experiments showed that on the first floor where temperatures were generally below 50°C, egg and adult mortalities were inversely related to flour depth. The response with immobile eggs was linear but exponential with adults (Figure 3), because of adult tunnelling behaviour in flour. Adults were able to tunnel deep into flour especially at higher flour depths thereby escaping the lethal effects of high temperatures. In these same experiments, 100% of eggs and adults died at all flour depths on the third mill floor, because temperatures within flour depths were between 50 and 60°C.

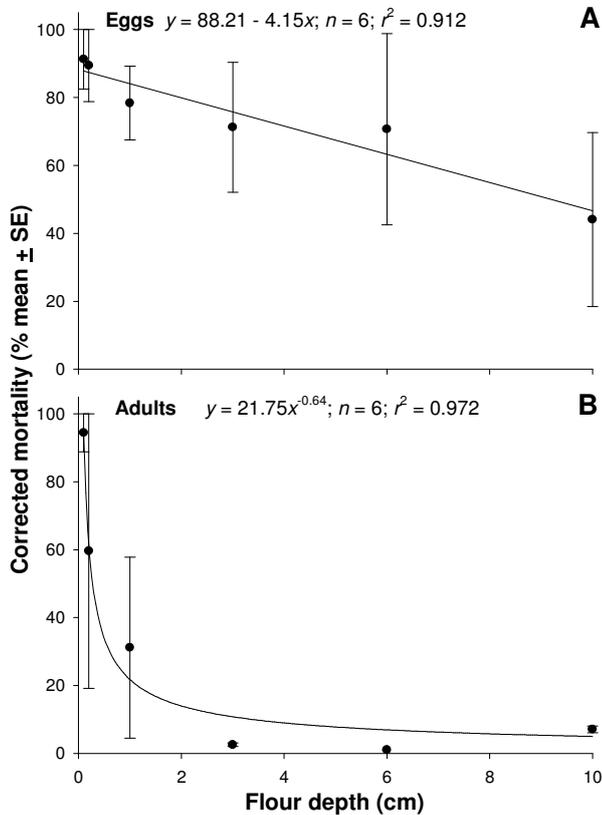
At Kansas State University, the effectiveness of several commercial heat treatments was validated by measuring temperatures at several locations in areas being heated. In addition, bioassays were conducted using eggs, young larvae, and adults of *T. castaneum*. In most locations eggs and adults were used. All nine commercial heat treatments indicated that insects can be easily killed within 24 hours.

Several heat treatment workshops have been held at Kansas State University since 1999 to demonstrate how heat treatments are conducted using electric, forced air, and steam heaters, and to provide hands-on experience to grain/food industry stakeholders and pest management professionals. The pilot flour and feed mills at Kansas State University were subjected to heat treatments by commercial heat treatment service providers. Participants had the opportunity to see how the facility was prepared prior to a heat treatment, and there were several opportunities for participants to walk through mills during a heat treatment. These practical sessions also included classroom lectures by researchers and heat treatment practitioners from the grain and food industry. More than 400 people from different parts of the world attended the six workshops held since 1999. All workshop presentations and information can be accessed at [http://www.ksre.ksu.edu/grsc\\_subi/](http://www.ksre.ksu.edu/grsc_subi/) under the Conferences & Workshops link.

#### Software programs and models to optimise heat treatments

At Kansas State University, a Heat Treatment Calculator (HTC) coded in Visual Basic using Microsoft Visual Studio.NET was developed in 2002 to estimate the heat energy required as well as costs of doing a heat treatment using various energy/fuel sources [42]. The calculator also allows the user to explore “what if” scenarios (alter ambient and threshold temperatures, select a fuel type) of a heat treatment. The calculator was validated during heat treatment of a large pasta facility in the USA and the calculator heat energy estimates were compared with company heat energy values based on amount of natural gas consumed [Bhadriraju

**Figure 3.** A linear regression model describing mean  $\pm$  SE ( $n = 2$ ) egg mortality (A) and an exponential decay model describing adult mortality (B) of *T. castaneum* as a function of flour depth in rings placed on the first floor of Kansas State University pilot mill subjected to heat treatment with forced air gas heaters [58].



Subramanyam, unpublished data]. The calculator estimates were within 4% of the actual energy values and cost.

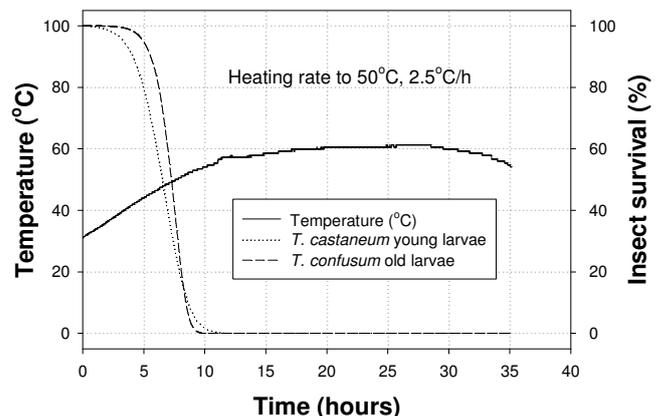
As briefly mentioned above, a novel thermal death kinetic model was developed and validated using the heat-tolerant stages of *T. confusum* (old larvae) [52] and *T. castaneum* (young larvae) [Bhadriraju Subramanyam and Rizana M Mahroof, unpublished data]. The model was based on a logarithmic decrease in insect numbers as a function of time at constant elevated temperatures, and a logarithmic decrease in insect numbers as a function of temperature. The model accurately predicted insect survival as a function of time, and the decrease in survival during a heat treatment was faster at higher heating rates. The model predicts insect survival based only on time-dependent temperature data (Figure 4). This model has been used to show survival curves for data collected from numerous facilities subjected to heat treatments. Initially, the limitation was that these curves were generated using the Microsoft Excel program after data collection, and this exercise took several weeks. In 2010, another software program (Super Nova) written in C-Sharp analyses time-dependent tempera-

ture data in Microsoft Excel (xls) format to provide the following estimates in less than 10 seconds: starting ambient temperature at each location, hours to 50°C, hours above 50°C, heating rate from ambient to 50°C, the maximum temperature, predicted hours required to kill 90 (LT<sub>90</sub>), 95 (LT<sub>95</sub>), or 99% (LT<sub>99</sub>) of young larvae of *T. castaneum* and old larvae of *T. confusum*. The program also gave the predicted insect survival data, so graphs such as those shown in Figure 4 can be easily generated using any graphing program.

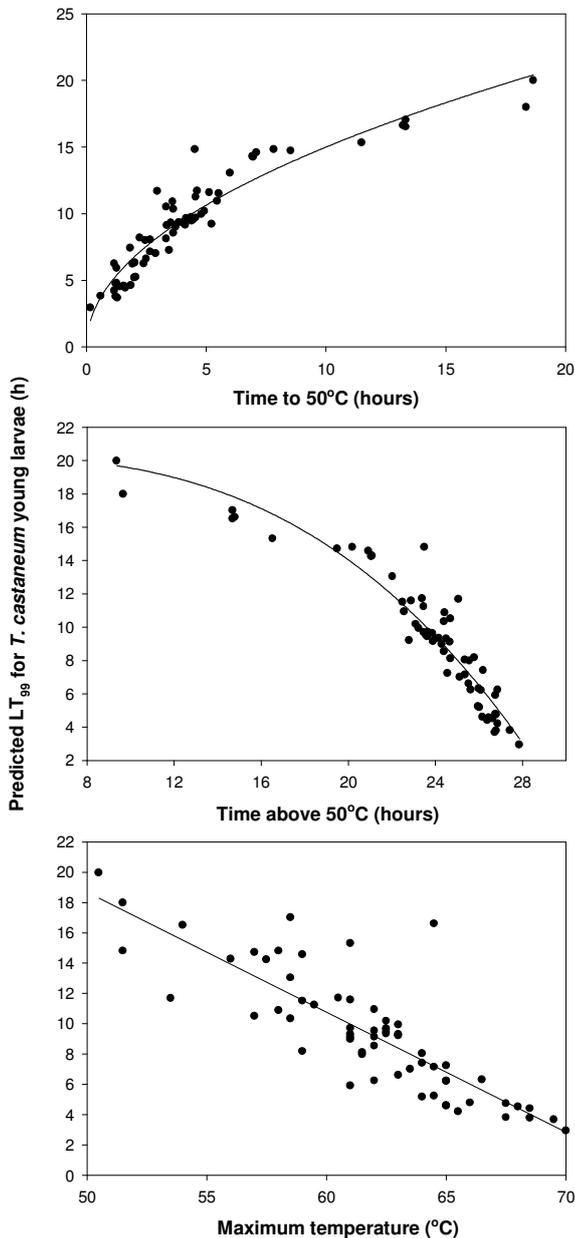
In nine commercial facilities, the predicted hours to 99% mortality of *T. castaneum* young larvae from temperature data collected from multiple locations during heat treatment were plotted as a function of time to 50°C, time above 50°C, and the maximum temperature. All of these curves showed a consistent pattern. The hours required for 99% mortality of *T. castaneum* young larvae was positively related to time to 50°C, but negatively related to time above 50°C and the maximum temperature. This relationship is illustrated using data from a grain-processing facility that was subjected to forced air gas heaters (Figure 5).

In the above examples, understanding how quickly insects die as a function of temperature is valuable only when conducting another heat treatment in the same facility, because temperature data analysis and insect mortality data that was observed or predicted occurred after the heat treatment. In order to provide “real time” insect survival estimates based on temperature data during a heat treatment, another software called E.A.R.T.H. (Efficacy Assessment in Real-Time during Heat treatment) was built using Java, Nest C and Postgre technologies. This powerful program provides the user with an interface to input and acquire heat treatment data to predict real-

**Figure 4.** Predicted survival of young larvae of *T. castaneum* and old larvae of *T. confusum* using time-dependent temperature data and thermal death kinetic models [52, Bhadriraju Subramanyam and Rizana Mahroof, unpublished data]. The temperature data were collected from one location in a ready-to-eat breakfast cereal company in the USA that was subjected to heat treatment using steam heaters.



**Figure 5.** Relationship between predicted time in hours for 99% mortality (LT<sub>99</sub>) of *T. castaneum* young larvae and time in hours to 50°C ( $y = 4.80x^{0.49}$ ;  $r^2 = 0.894$ ), time in hours above 50°C ( $y = 20.34 - 0.0008x^3$ ;  $r^2 = 0.902$ ), and the maximum temperature ( $y = 58.30 - 0.79x$ ;  $r^2 = 0.706$ ). These graphs were based on temperature data from 64 locations from a brewery in the USA subjected to heat treatments with forced air gas heaters.



time insect survival during heat treatments. There are two modules of the software: a wireless sensor network module and a real-time insect survival graph generation module.

The wireless sensor network module uses the MICAZ /MIB/ MTS technology from Crossbow Technology, Inc. (San Jose,

California, USA) to acquire data (temperature in our case) remotely from different locations on a real-time basis. The MICAZ mote is a radio board for enabling low power wireless sensor networks. The MIB520 serial interface and programming board allows the aggregation of sensor network data onto a PC. MTS310CB sensor boards are weather sensor boards that offer five basic environmental sensing parameters. For example, to get temperature readings during heat treatment of a facility, a MICAZ radio board along with a weather sensor board is placed at different locations. The weather sensor board acquires temperature and transmits this data to the base station using the radio board. The range of wireless sensor networks can be increased by placing intermediate radio boards for effective communication with the base station.

The data aggregated by the base station can be stored in PostgreSQL server (database). This data is used by the graph generation module to generate insect survival graphs in real time (Figure 6) as an instantaneous function of time-dependent temperature profile. This information is extremely valuable in understanding which areas are being under-heated (less than 50°C) and which areas are being over-heated (greater than 60°C), so corrective actions can be taken to improve heat treatment efficiency against insects during a heat treatment. Some corrective actions for example may include placing an extra heater or moving a fan to redistribute heat or to eliminate cool spots. The software generates and archives a comprehensive report before, during, and after a heat treatment based on checklists provided by Dosland *et al.* [41].

The new version of E.A.R.T.H., just completed in 2011, is equipped with state-of-the-art wireless technology that renders efficient performance in terms of battery life, multi-hopping, usability, maintenance and alleviates the problem of connection errors. The increase in scope and performance of multithreading in the graph generation module enables the product to scale up enormously in terms of number of motes supported so that a whole facility can be fully covered.

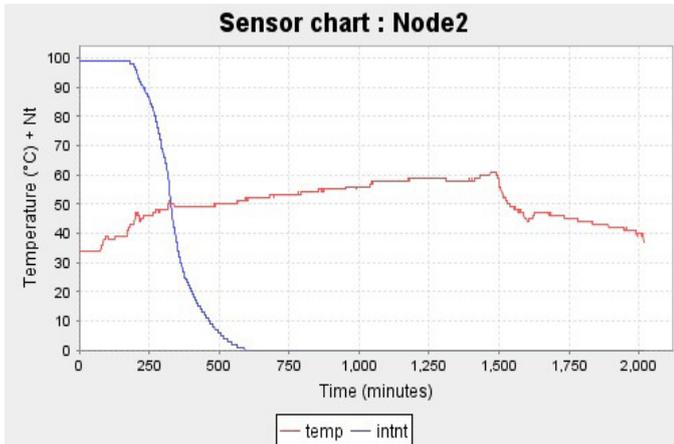
### A success story

A major ready-to-eat breakfast cereal company in the USA does heat treatments for 34 hours in their large facility at approximately monthly intervals using steam heaters. Our research at this facility using the tools described in this article showed that the young larvae and adults of *T. castaneum* were completely killed within 12 hours. As a result the company currently does heat treatments for only 24 hours with cost savings of \$25,000 per year. The use of the Heat Treatment Calculator software, thermal death kinetic models, and the E.A.R.T.H. software are recent developments, and should be used to improve and evaluate facility heat treatments. The use of these tools will improve heat treatment efficacy while at the same time reducing costs to the users.

### Research needs

The use of heat treatment for disinfecting facilities is not a

**Figure 6.** The E.A.R.T.H. software gives a graph illustrated below. This graph represents the end of heat treatment, but the graph can be viewed at any time during a heat treatment. The line that starts at 100 is the predicted survival curve for *T. castaneum* young larvae based on the thermal death kinetic model. The curve that starts around 34 represents temperature measured over time with a wireless sensor during heat treatment of a grain-processing company in the USA with steam heaters.



new idea. However, research in the last decade and half has shed new light on how to improve heat treatment effectiveness against insects. The susceptibility of other economically important insect species not reported here should be evaluated both at constant elevated temperatures in the laboratory and in commercial facilities subjected to heat treatments where temperatures are changing dynamically over time. There is still scope for lot of research to be done on improving heat treatment effectiveness, especially in commercial facilities. In large commercial facilities, it is difficult to maintain temperatures between 50 and 60°C in all locations. Methods for controlling insects in locations where temperatures are less than 50°C should be explored. The methods may include application of chemical or non-chemical insecticides [33] or thorough inspection and sanitation. The rate of heating and development of heat tolerance in specific life stages of stored-product insects needs to be explored further. The sub-lethal effects of commercial heat treatments on population rebounds of insects should be verified through carefully designed experiments. The impact of high temperatures or repeated heat treatments on the performance of equipment and on adverse effects to structural components should be scientifically documented. It is important to recognise that heat treatment may not be suitable for all facilities. However, where it is suitable, heat treatment can be a viable methyl bromide alternative.

### Acknowledgements

Research reported here was supported by grants from the USDA-NIFA (CSREES); Propane Education and Research Council, Washington, D.C., USA; Region VII US-EPA; Temp

-Air, Inc., Burnsville, Minnesota, USA; and Kansas State University Agricultural Experiment Station. We are grateful to the grain and food industry cooperators for permitting us to conduct research in their facilities subjected to heat treatments.

### References

- 1 Wagner GB and Cotton RT. Factors affecting insect abundance in flour mills. Northwestern Miller, November, 1935: 522–523.
- 2 Smallman BN and Loschiavo SR. Mill sanitation studies. I. Relative susceptibilities of mill stocks to infestation by the confused flour beetle. Cereal Chemistry 1952: 29: 431–440.
- 3 Good NE. Insects found in the milling streams of flour mills in the southwestern milling area. Journal of Kansas Entomological Society 1937: 10: 135–148.
- 4 Dyte CE. Studies on insect infestation in the machinery of three English flour mills in relation to seasonal temperature changes. Journal of Stored Products Research 1965: 1: 129–144.
- 5 Dyte CE. Studies on the abundance of *Cryptolestes turcicus* (Grouv.) (Coleoptera, Cucujidae) in different machines of an English flour mill. Journal of Stored Products Research 1966: 1: 341–352.
- 6 Hosny MM, Hassanein MH and Kamel AH. Ecological studies on *Anagasta kuehniella* and *Corcyra cephalonica* infesting flour mills in Cairo. Bulletin of the Entomological Society of Egypt 1968: 52: 445–456.
- 7 Rilett RO and Weigel RD. A winter survey of Coleoptera in feed and flour mills. Journal of Economic Entomology 1956: 49: 154–156.
- 8 Salmond KF. The insect and mite fauna of a Scottish flour mill. Bulletin of Entomological Research 1956: 47: 621–630.
- 9 Salama HS and Salem SA. Seasonal fluctuations in the populations of insect fauna in flour mills. Zeitschrift für Angewandte Entomologie 1973: 74: 160–169.
- 10 Buchelos CT. Moth populations at a typical flour mill. Annals of the Institute of Phytopathology Benaki, (N.S.) 1980: 12: 188–197.
- 11 Buchelos CT. Coleoptera populations at flour mills and related areas. Annals of the Institute of Phytopathology Benaki, (N.S.) 1981: 13: 6–29.
- 12 Campbell JF and Arbogast RT. Stored-product insects in a flour mill: population dynamics and response to fumigation treatments. Entomologia Experimentalis et Applicata 2004: 112: 217–225.
- 13 Williams GC. Infestation of a disused flour mill. Milling, January, 1961: 92.
- 14 Banks HJ. Impact, physical removal and exclusion for insect control in stored products. In: Proceedings of 4<sup>th</sup> International Working Conference on Stored-Product Protection. Edited by Donahayae E and Navarro S., Tel Aviv, Israel, 1986: 165–184.
- 15 Chowanec T. Sanitation costs—the bottom line. Association of Operative Millers Bulletin, December, 1986: 4867–4871.
- 16 Foulk JD. Pest-related aspects of sanitation audits. Pest Control Technology 1990: 18: 32.
- 17 Stratil VH and Wohlgemuth R. *Untersuchungen sum wirkungsmechanismus vonPrallmaschinen auf vorratsschadliche insekten*. Anzeiger für Schadlingskunde Pflanzenschutz Umweltschutz 1989: 62: 41–47.
- 18 Watters FL. Physical methods to manage stored-food pests. In: Ecology and management of food-industry pests. Edited by Gorham JE, Association of Official Analytical Chemists, Arlington, Virginia, USA, 1991: 399–414.
- 19 Subramanyam Bh. Heaps J and Harein P. Insecticide recommendations for food-processing plants. University of Minnesota Extension Service Fact Sheet 3921-A, 1993.
- 20 Makhijani A and Gurney KR. Mending the ozone hole: science, technology and policy. MIT Press, Cambridge, Massachusetts, USA, 1995.
- 21 Dean GA. Heat as a means of controlling mill insects. Journal of Economic Entomology 1911: 4: 142–158.
- 22 Fields PG and White NDG. Alternatives to methyl bromide treatments

- for stored-product and quarantine insects. *Annual Review of Entomology* 2002; 47: 331–359.
- 23 Monceau DHL and Tillet M. Histoire d'un insect qui devore les grains de l'Angoumois, avec les moyens que l'on peut employer pour le detruire. Paris, 1762: 314.
- 24 Oosthuizen MJ. The effect of high temperature on the confused flour beetle. University of Minnesota Agricultural Experiment Station Bulletin 1935: 107: 1–45.
- 25 Webster FM. Observations on the Angoumois grain moth and its parasites. 12<sup>th</sup> Report of the State Entomologist on Noxious and Beneficial Insects of Illinois, 1883: 144–154.
- 26 Lintner, J.A. *Tribolium ferrugineum* (Fabr.). 2<sup>nd</sup> Reprint of injurious and other insects of New York, 1885: 136–139.
- 27 Goodwin WH. Some factors affecting results in the use of high temperature for the control of insects injuring cereal products. *Journal of Economic Entomology* 1914; 7: 313–322.
- 28 de Ong ER. Effect of excessive sterilization measures on the germination of seeds. *Journal of Economic Entomology* 1919; 12: 343–345.
- 29 Dean GA. Milled and stored grain insects. *Kansas State Agricultural College Technical Bulletin* 1913: 189: 139–176.
- 30 Goodwin WH. Heat for control of cereal insects. *Ohio Agricultural Experiment Station Bulletin* 1922: 354: 1–18.
- 31 Pepper JH and Strand AL. Superheating as a control for cereal-mill insects. *Montana State College Agricultural Experiment Station Bulletin* 1935: 297:1–26.
- 32 Imholte TJ and Imholte-Tauscher T. Engineering for food safety and sanitation. Technical Institute of Food Safety, Woodinville, Washington D.C., USA, 1999.
- 33 Dowdy AK and Fields PG. Heat combined with diatomaceous earth to control the confused flour beetle (Coleoptera: Tenebrionidae) in a flour mill. *Journal of Stored Products Research* 2002; 38: 413–425.
- 34 Wright EJ, Sinclair EA and Annis PC. Laboratory determination of the requirements for control of *Trogoderma variabile* (Coleoptera: Dermestidae) by heat. *Journal of Stored Products Research* 2002; 38: 147–155.
- 35 Tilley DR, Casada ME and Arthur FH. Heat treatment for disinfestations of empty grain storage bins. *Journal of Stored Products Research* 2007; 43: 221–228.
- 36 Mahroof R, Subramanyam Bh and Eustace D. Temperature and relative humidity profiles during heat treatment of mills and its efficacy against *Tribolium castaneum* (Herbst) life stages. *Journal of Stored Products Research* 2003a; 39: 555–569.
- 37 Roesli R, Subramanyam Bh, Fairchild FJ and Behnke KC. Trap catches of stored-product insects before and after heat treatment in a pilot feed mill. *Journal of Stored Products Research* 2003; 39: 521–540.
- 38 Beckett SJ, Fields PG and Subramanyam Bh. Disinfestation of stored products and associated structures using heat. In: Heat treatments for postharvest pest control: theory and practice. Edited by Tang J, Mitcham E, Wang S and Lurie S. CAB International, Oxon, UK, 2007: 182–236
- 39 Hansen JD and Sharp JL. Thermal death studies of third instar Caribbean fruit fly (Diptera: Tephritidae). *Journal of Economic Entomology* 1998; 91: 968–973.
- 40 Beckett SJ and Morton R. Mortality of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) at grain temperatures ranging from 50°C to 60°C obtained at different heating rates in a spouted bed. *Journal of Stored Products Research* 2003; 39: 313–332.
- 41 Dosland O, Subramanyam Bh, Sheppard K and Mahroof R. Temperature modification for insect control. In: *Insect management for food storage and processing*, Second Edition. Edited by Heaps J. American Association of Cereal Chemists International, St. Paul, Minnesota, USA, 2006: 89–103.
- 42 Subramanyam Bh. Heat treatment calculator. *Milling Journal*, Third Quarter 2003: 46–48.
- 43 Denlinger DL and Yocum GD. Physiology of heat sensitivity. In: *Temperature sensitivity in insects and application in integrated pest management*. Edited by Hallman GJ and Denlinger DL. Westview Press, Boulder, Colorado, USA, 1999: 6–53.
- 44 Evans DE and Dermott T. Dosage-mortality relationships for *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) exposed to heat in a fluidized bed. *Journal of Stored Products Research* 1981; 17: 53–64.
- 45 Fields PG. The control of stored product insects and mites with extreme temperatures. *Journal of Stored Products Research* 1992; 28: 89–118.
- 46 Mahroof R, Subramanyam Bh, Throne JE and Menon A. Time-mortality relationships for *Tribolium castaneum* (Coleoptera: Tenebrionidae) life stages exposed to elevated temperatures. *Journal of Economic Entomology* 2003b; 96: 1345–1351.
- 47 Hepburn HR. Structure of the integument. In: *Comprehensive insect physiology, biochemistry and pharmacology*, Volume 3. Edited by Kerkut GA and Gilbert LI. Pergamon Press, London, UK, 1985: 1–58.
- 48 Neven LG. Physiological responses of insects to heat. *Postharvest Biology and Technology* 2000; 21: 103–111.
- 49 Mahroof R, Subramanyam Bh and Flinn PW. Reproductive performance of *Tribolium castaneum* (Coleoptera: Tenebrionidae) exposed to the minimum heat treatment temperature as pupae and adults. *Journal of Economic Entomology* 2005a; 98: 626–633.
- 50 Fields PG. 2007. Efficacy assessment report. In: *Comparative evaluation of intensive pest management, heat treatments and fumigants as alternatives to methyl bromide for control of stored product pests in Canadian flour mills*. Edited by Harrison G. Canadian National Millers Association, Ottawa, Canada, Agriculture and Agri-Food Canada 2007: 1–67.
- 51 Boina DR and Subramanyam Bh. 2004. Relative susceptibility of *Tribolium confusum* life stages exposed to elevated temperatures. *Journal of Economic Entomology* 2004; 97: 2168–2173.
- 52 Boina DR, Subramanyam Bh and Alavi S. Dynamic model for predicting survival of mature larvae of *Tribolium confusum* during facility heat treatments. *Journal of Economic Entomology* 2008; 101: 989–997.
- 53 Mahroof R and Subramanyam Bh. Susceptibility of *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) to high temperatures used during structural heat treatments. *Bulletin of Entomological Research* 2006; 97: 539–545.
- 54 Yu C, Subramanyam Bh, Flinn PW and Gwirtz JA. Susceptibility of *Lasioderma serricorne* (F.) life stages to elevated temperatures used during structural heat treatments. *Journal of Economic Entomology* 2011; 104: 317–324.
- 55 Hulasare R, Subramanyam Bh, Fields PG and Abdelghany AY. 2010. Heat treatment: a viable methyl bromide alternative for managing stored-product insects in food-processing facilities. In: *Proceedings of the 10<sup>th</sup> International Working Conference on Stored-Product Protection*, 27 June - 2 July, 2010, Estoril, Portugal. Edited by Carvalho MO, Fields PG, Adler CS, Arthur FH, Athanassiou CG, Campbell JF, Fleurat-Lessard F, Flinn PW, Hodges RJ, İşikber AA, Navarro S, Noyes RT, Riudavets J, Sinha KK, Thorpe GR, Timlick BH, Trematerra P and White NDG. *Julius-Kühn-Archiv*, 425, 2010: 661–667 (<http://pub.jki.bund.de/index.php/JKA/issue/view/719>).
- 56 Mahroof R, Zhu KY and Subramanyam Bh. Changes in expression of heat shock proteins in *Tribolium castaneum* (Coleoptera: Tenebrionidae) in relation to developmental stage, exposure time, and temperature. *Annals of the Entomological Society of America* 2004; 98: 100–107.
- 57 Mahroof R, Zhu KY, Neven L, Subramanyam Bh and Bai J. Expression patterns of three heat shock protein 70 genes among the developmental stages of the red flour beetle, *Tribolium castaneum*. *Comparative Biochemistry and Physiology, Part A* 2005b; 141: 247–256.
- 58 Brijwani M. Effect of sanitation on responses of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) life stages to structural heat treatments. M.S. thesis, Kansas State University, Manhattan, Kansas, USA, 2011.