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# Temperature and relative humidity profiles during heat treatment of mills and its efficacy against *Tribolium castaneum* (Herbst) life stages[☆]

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## Abstract

Heat treatment involves raising the temperature of a food-processing facility to 50–60°C and maintaining these elevated temperatures for 24–36 h to kill stored-product insects. The pilot feed and flour mills at Kansas State University, Manhattan, KS, were subjected to heat treatment using gas and steam heaters, respectively, during 4–8 August 2001 to characterize temperature and relative humidity profiles and to determine efficacy against developmental stages of the red flour beetle, *Tribolium castaneum* (Herbst). Temperature and relative humidity profiles in each mill were electronically monitored in 10 different locations. Susceptibility of eggs, young instars, old instars, pupae, and adults of *T. castaneum* to heat was determined at the same 10 locations within each mill. The number of hours required to reach the target temperature of 50°C, number of hours above 50°C, and maximum temperatures varied between the mills and among mill locations. Relative humidity decreased predictably with an increase in temperature, and was $\leqslant 21\%$ in most locations at 50°C or above. Two separate three-parameter non-linear regression models best described temperature and relative humidity profiles observed in each mill. Despite non-uniform heating at the sample locations, mortality of *T. castaneum* life stages was 100% in most locations, except in areas where temperatures were $<50°C$. Old instars and pupae appeared to be relatively heat tolerant when compared with other life stages, especially in the flour mill where lethal temperatures were attained. Possible reasons for the non-uniform heating observed at mill locations and survival of certain *T. castaneum* developmental stages are discussed.

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*Keywords:* Heat treatment; Methyl bromide alternative; Red flour beetle

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## 1. Introduction

Managing stored-product insect pests by heating the ambient air of a food-processing facility to lethal temperatures is an old and effective technology (Dean, 1911, 1913). There is renewed interest in utilizing heat treatments because of the impending phase-out of methyl bromide, an atmospheric ozone-depleting space fumigant currently used for disinfesting food-processing facilities in North America and Europe (Makhijani and Gurney, 1995; Menon et al., 2000). During heat treatment, an entire facility or a portion of it is heated to 50–60°C, and these high temperatures are maintained for 24–36 h to kill stored-product insects (Fields, 1992; Dowdy, 1999; Dowdy and Fields, 2002; Wright et al., 2002). The target temperature for effective disinfestation should be at least 50°C (Imholte and Imholte-Tauscher, 1999; Roesli et al., 2002; Wright et al., 2002).

The effectiveness of heat treatment depends on treated areas reaching and maintaining lethal temperatures (50–60°C) for an adequate amount of time. Horizontal and vertical stratification of temperatures within a facility during heat treatment result in non-uniform distribution of heat (Fields et al., 1997; Dowdy, 1999; Dowdy and Fields, 2002). Thus, some portions of a facility may be over- or under-heated (Dowdy, 1999). Overheating may result in damage to heat-sensitive equipment. Underheating may result in insects surviving the heat treatment. Generally, it is difficult to attain lethal temperatures (50–60°C) near floors, room corners, and floor-wall junctions without proper heating capacity and air circulation. Fans can help redistribute air from hotter areas of the facility to cooler areas, producing a more uniform heat treatment.

In this paper, we report temperature and relative humidity profiles monitored during a heat treatment at 10 locations each in the pilot feed and flour mills at Kansas State University. Eggs, young instars, old instars, pupae, and adults of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), a common pest associated with food-processing facilities worldwide (Mills and Pedersen, 1990), were exposed in each mill at the same 10 locations to characterize variation in mortality due to location and insect life stage.

## 2. Materials and methods

### 2.1. Insects

*Tribolium castaneum* cultures were reared on 95% whole-wheat flour and brewer's yeast (5% by wt) at 28°C and 65% r.h. Eggs and newly hatched larvae (first instars), collected periodically from cultures, were reared on bleached wheat flour and powdered brewer's yeast in the same ratio as above, for convenience in handling. Eggs (2-d-old), 6-d-old larvae (first or young instars), 22-d-old larvae (old instars), unsexed pupae (26-d-old), and unsexed adults (2-wk-old) were used in tests. The mean ± SE ($n = 15$) weight of young instars was 0.12 ± 0.01 mg and that of old instars was 3.59 ± 0.11 mg. Old instars, pupae, and adults were separated from the diet using a 0.83-mm aperture sieve. Eggs and young instars were separated from the diet using a 250-µm-aperture sieve, and counted under a stereomicroscope.

### 2.2. Layout of feed and flour mills

The pilot feed and flour mills are located in the Department of Grain Science and Industry, Kansas State University, Manhattan, KS, USA. The feed mill is vertically separated into a basement, and first, second, third, and fourth floors. Each floor, including the basement, measures 17 × 12 × 3.3 m. The basement has a small extension (5.8 × 2.9 × 1.1 m) on the northeast side. The first floor is attached on the north side to a warehouse (12.8 × 7.8 × 4.7 m). A concrete floor separates the basement and first floor. Metal grating separates first and second floors and second and third floors. Metal plates separate the third and fourth floors.

The pilot flour mill is vertically separated into four floors. Each floor is horizontally separated into a cleaning house that has equipment for cleaning wheat and a milling house that has equipment for milling wheat. Each cleaning house floor is 12.1 × 8.5 × 3.7 m, while each milling house floor is 11.6 × 9.9 × 3.7 m. Concrete walls separate both houses and vertical floors of the flour mill. The feed mill is located on the west side of the flour mill, and is separated from it by a concrete wall.

### 2.3. Heat treatment

The pilot feed and flour mills were heated during 6–8 and 4–8 August 2001, respectively. Heat treatment started early in the flour mill because previous experience has shown that the built-in steam heater and two portable heaters used (described below) were inadequate to rapidly increase the mill temperature to 50°C or above. In both mills, windows and doors were not sealed to prevent heat loss. In each mill, 10 locations were selected (Table 1) to measure temperature and relative humidity changes and to record insect mortality.

Five natural gas heaters from Temp-Air® (Burnsville, MN, USA) were used to heat the feed mill. Each of the four heaters (THP-550) produced 38,598 kcal (550,000 BTUs) and one heater

Table 1
Sample locations in feed and flour mills where temperature, relative humidity, and mortality of *T. castaneum* life stages were measured during heat treatment

| Location | Pilot feed mill | | Pilot flour mill | |
|---|---|---|---|---|
| | Floor | Description | Floor | Description |
| 1 | B[a] | Floor, northwest corner | 2 | Milling house floor, southwest corner |
| 2 | B[a] | Floor, southwest corner | 2 | Cleaning house, inside Aeroglide dryer |
| 3 | 1 | Top of hopper bin with divider | 3 | Cleaning house floor, near entrance door |
| 4 | 1 | Floor, southwest corner | 3 | Milling house floor, southwest corner |
| 5 | 1 | Floor, center of room | 3 | Milling house, inside purifier |
| 6 | 2 | Floor, northwest corner | 3 | Cleaning house floor, northwest corner |
| 7 | 2 | Floor, southwest corner | 3 | Milling house floor, man lift area |
| 8 | 3 | Floor, north wall | 4 | Cleaning house floor, southwest corner |
| 9 | 4 | Floor, south wall | 4 | Milling house floor, northwest corner |
| 10 | 4 | Floor, center of room | 4 | Milling house floor, dust collection area |

[a] Basement.

(THP-1400) produced 352,794 kcal (1,400,000 BTUs). The airflow rate of a THP-550 heater was $20.3\,\mathrm{m}^3\,\mathrm{min}^{-1}$ ($3000\,\mathrm{ft}^3\,\mathrm{min}^{-1}$) and that of a THP-1400 heater was $54.2\,\mathrm{m}^3\,\mathrm{min}^{-1}$ ($8000\,\mathrm{ft}^3\,\mathrm{min}^{-1}$). All heaters were placed outside the mill. These heaters bring in air through the burners and heat it to 60–82°C. Heat generated by the units was discharged into the mill floors by 50.8-cm diameter nylon ductwork with 10-cm diameter openings at regular intervals. Ducts from the THP-550 units (one duct per heater) were placed in the basement and first floor, while ducts from the THP-1400 unit (two ducts per heater) were placed in the third floor. Theoretical engineering calculations, based on airflow rate of heaters and the volume of the facility, estimated that the air inside the feed mill was exchanged 2–5 times $\mathrm{h}^{-1}$ with hot air during heat treatment. Heaters were turned on at 8:00 p.m. (local time) on 6 August and turned off at 7:00 a.m. on 8 August. Ten fans distributed heat in the first, second, and third floors. Of these, three were Bayley® fans with a 50-cm blade diameter, producing an airflow rate of $48.1\,\mathrm{m}^3\,\mathrm{min}^{-1}$ ($7100\,\mathrm{ft}^3\,\mathrm{min}^{-1}$), and seven were Schaefer® fans with a 90-cm blade diameter and an air flow rate of $311.3\,\mathrm{m}^3\,\mathrm{min}^{-1}$ ($11{,}000\,\mathrm{ft}^3\,\mathrm{min}^{-1}$).

The milling house floors were heated using a built-in steam generator that vented hot air into each floor. The steam pressure was $6.3\,\mathrm{kg\,cm}^{-2}$, steam temperature was 100°C, and the outlet air temperature measured near the vents was 68°C. The cleaning house floors were heated using two portable steam heaters (Armstrong-Hunt, Cat. No. 3507, Milton, FL, USA). One portable heater was in the first floor and the other was in the third floor. These heaters operated at $17.6\,\mathrm{kg\,cm}^{-2}$ steam pressure (steam temperature = 100°C) and emitted a maximum temperature of 99°C. Each portable heater had a built-in fan (Baldor® Electric Corporation, Fort Smith, AR, USA) that operated at 1725 rpm. Besides these fans, no additional fans were used to circulate heat among the flour mill floors. The steam generator and portable heaters were turned on at 6:30 p.m. on 4 August. They were temporarily turned off on 6 August, between 7:30 a.m. and 4:30 p.m., to avoid overheating especially in rooms with the portable heaters, and to maintain temperatures near 50°C. In the past, failing to turn off the portable heaters resulted in activating the overhead sprinkler system. Heaters were turned off at the end of heat treatment (7:30 a.m.) on 8 August.

### 2.4. *Insect exposure and temperature and humidity profile characterization*

Different life stages of *T. castaneum* were transferred to square plastic boxes ($4.5 \times 4.5 \times 1.5$ cm) covered with perforated lids. Lid perforations were sealed with 600-μm aperture wire mesh screens to permit ventilation and prevent insect escape. Each box held a mean ± SE ($n = 20$ replicates) of $305 \pm 3$ mg of bleached wheat flour and 20 individuals of a *T. castaneum* life stage. Two boxes of each life stage (10 boxes total) were placed on the floor or a horizontal surface of equipment at each mill location. The control treatment consisted of a similar number of boxes placed in a laboratory growth chamber set at 28°C and 42% r.h. To verify if eggs hatched into neonate larvae or pupae transformed into adults during heat treatment, 20 eggs or 20 pupae were placed in separate 9-cm glass Petri dishes without any flour. Dishes with eggs or pupae were placed at locations 2, 7, and 10 in the feed mill and locations 2, 8, and 9 in the flour mill. Three dishes were placed at each of these locations.

Two HOBO® data-logging units (Onset Computer Corporation, Bourne, MA, USA) were placed in each feed and flour mill location to measure temperature and relative humidity during heat treatment. At each location, thermocouples from the HOBO® units were placed inside two of the 10 boxes. HOBO® units were launched by a computer to record temperature and relative humidity at 15 min intervals. Temperature and relative humidity outdoors were measured by two HOBO® units placed at ground level on the south side of the feed mill.

At the end of heat treatment, all plastic boxes containing insects were brought to the laboratory and incubated in the laboratory growth chamber at 28°C and 42% r.h. Boxes containing adults were placed in the chamber for an additional 24 h before assessing mortality. Mortality of adults was based on the number dead out of the total exposed. Pupae were held in the same boxes until emergence of adults. Boxes containing eggs, young instars, or old instars were transferred into 150-ml plastic containers with 40 g of whole-wheat flour plus yeast (5% by wt). These containers were incubated until emergence of adults. Mortality of immature stages was based on the number of individuals that developed into adults out of the total exposed to heat treatment.

Temperature and relative humidity data from HOBO® units were transferred to a desktop computer for statistical analyses. Temperature or humidity measurements taken at 15-min intervals from the two HOBO® units inside boxes at each mill location were averaged using the PROC MEANS procedure (SAS Institute, 1990). From the mean temperature data, the number of hours required to reach the target temperature of 50°C from the starting temperature at each location, number of hours above 50°C, and maximum temperature were determined. Rate of decrease in humidity from the beginning of heat treatment until the target temperature of 50°C and mean relative humidity from 50°C until the end of heat treatment were determined for each mill location.

Temperature and relative humidity data at each mill location were described by two separate three-parameter non-linear equations using TableCurve 2D® software (Anonymous, 1994). The rise in temperature during heat treatment was described as

$$y = a + b(1 - 1/(1 + bcx)) \tag{1}$$

where $y$ is the predicted temperature in °C, $x$ is the time in h, and $a$, $b$, and $c$ are constants estimated from the temperature–time data. The decrease in relative humidity during heat treatment was described as

$$y = a + b\exp(-x/c) \tag{2}$$

where $y$ is the predicted humidity, $x$ is the time in h, and $a$, $b$, and $c$ are parameters estimated from the humidity–time data.

### 2.5. *Analysis of insect mortality data*

Feed mill and flour mill data were analyzed separately. Percentage mortality data of insects exposed to heat treatment were not corrected for control mortality, because mortality of all stages in the control treatment was $<5\%$. Percentage mortality data ($x$) at each mill location were transformed to $\arcsin(x)^{0.5}$ (Zar, 1984) to stabilize heteroscedastic treatment variances. Data were subjected to two-way analysis of variance (ANOVA) using the PROC GLM procedure (SAS Institute, 1990), to determine mortality differences among mill locations and insect stages. For each stage, mortality differences among mill locations were determined by linear contrasts (SAS Institute, 1990). Treatments were considered significantly different at the $\alpha = 0.05$ level.

## 3. Results and discussion

### 3.1. Temperature and relative humidity profiles

Starting temperatures at all feed mill locations ranged from 32°C to 36°C (Table 2). At all flour mill locations they ranged from 34°C to 42°C (Table 3). The temperature outdoors during 4–8 August 2001 was 23–28°C.

Temperature in the southwest corner of the feed mill basement (location 2) never reached 50°C, and the maximum temperature attained was 46°C (Table 2). Puddles of standing water near the southwest corner could have contributed to the slow heating of this area. In the same basement, temperatures in the northwest corner (location 1) reached 50°C in the shortest time (6 h). This occurred because location 1 was close to a heating duct. The longest time (19 h) taken to reach 50°C occurred in the southwest corner of the second floor (location 7). Heating ducts were not placed in the second floor, and heat distribution was primarily due to chimney effect and air movement facilitated by fans. Temperatures above 50°C among mill locations were maintained for 18–31 h, and the maximum temperatures attained ranged from 46°C to 63°C. Temperatures exceeded 60°C in three of the 10 locations (1, 5, and 8).

In the flour mill, temperature reached 50°C quickly (4.5 h) in the third floor of the cleaning house (location 3) and slowly (47 h) in the third floor of the milling house (location 7) (Table 3). Location 3 had the highest starting temperature (42°C). In addition, the presence of a portable steam heater near location 3 contributed to the faster heating observed. In locations 2, 3, 5, and 6, which were in the second and third floors of the cleaning house, temperatures reached 50°C relatively fast compared with most other locations, because portable heaters were located in the first and third floors of the cleaning house. Temperatures above 50°C were held for variable lengths of time among the 10 flour mill locations. Maximum temperatures among locations

Table 2
Temperature changes at feed mill locations during heat treatment

| Location | Starting temperature (°C) | Time to 50°C (h) | Rate of increase (°C $h^{-1}$)[a] | Time above 50°C (h) | Maximum temperature (°C) |
|---|---|---|---|---|---|
| 1 | 34.9 | 6.0 | 2.5 | 31.3 | 62.7 |
| 2 | 31.5 | —[b] | —[b] | —[b] | 45.9 |
| 3 | 33.6 | 14.3 | 1.1 | 22.5 | 53.5 |
| 4 | 35.3 | 15.0 | 1.0 | 21.8 | 56.0 |
| 5 | 34.6 | 10.3 | 1.5 | 26.8 | 61.7 |
| 6 | 35.1 | 11.3 | 1.3 | 26.3 | 59.2 |
| 7 | 35.3 | 19.3 | 0.8 | 18.3 | 56.0 |
| 8 | 35.3 | 10.3 | 1.4 | 27.3 | 60.6 |
| 9 | 36.1 | 11.0 | 1.3 | 24.0 | 59.2 |
| 10 | 36.1 | 14.2 | 1.0 | 20.8 | 55.7 |

[a] (50°C−starting temperature (°C))/time to 50°C (h).

[b] Time to 50°C and time above 50°C could not be computed because temperature did not reach 50°C.

Table 3
Temperature changes at flour mill locations during heat treatment

| Location | Starting temperature (°C) | Time to 50°C (h) | Rate of increase (°C h$^{-1}$)[a] | Time above 50°C (h) | Maximum temperature (°C) |
|---|---|---|---|---|---|
| 1 | 33.8 | 22.0 | 0.7 | 17.8 | 59.9 |
| 2 | 35.1 | 18.3 | 0.8 | 71.3 | 61.3 |
| 3 | 41.5 | 4.5 | 1.9 | 63.0 | 69.1 |
| 4 | 34.9 | 20.8 | 0.7 | 68.5 | 59.2 |
| 5 | 35.3 | 15.5 | 0.9 | 74.0 | 62.7 |
| 6 | 37.7 | 14.3 | 0.9 | 27.3 | 62.0 |
| 7 | 33.6 | 47.0 | 0.3 | 42.3 | 56.3 |
| 8 | 35.3 | 32.8 | 0.4 | 9.0 | 54.4 |
| 9 | 37.9 | 22.0 | 0.6 | 65.0 | 58.2 |
| 10 | 37.9 | 20.5 | 0.6 | 68.8 | 58.6 |

[a] (50°C−starting temperature (°C))/time to 50°C (h).

ranged from 54°C to 69°C. Differences among locations in the time taken to reach 50°C, time above 50°C, and maximum temperature are likely due to non-uniform distribution of heat.

Temperatures increased faster in feed mill locations than in flour mill locations (Figs. 1 and 2). The number, efficiency, and capacity of heaters used in the feed mill were greater than those used in the flour mill. The built-in steam generator in the flour mill, installed 40 years ago, was inefficient as indicated by low outlet temperature which resulted in the slow heating observed at the mill locations. The two portable steam heaters were also insufficient to rapidly increase the temperature at all mill locations to 50°C. The absence of strategically placed fans in the flour mill also contributed to the slow heating observed. Placing additional portable heaters and fans in the flour mill may have helped in attaining lethal temperatures within a short time ($<$24 h).

Although the capacity of heaters and number of fans used for feed mill heat treatment were adequate, the rate of heating at the 10 sample locations was not uniform. Non-uniform heating was also observed at the flour mill sample locations. Horizontal and vertical stratification of temperatures, poor air movement, less than optimum placement of heaters or ducts carrying hot air, and loss of heat from various surfaces (windows, doors, floor, and roof vents) may have contributed to non-uniform heating observed in sample locations in both mills. Therefore, it is important to monitor temperatures regularly at several locations during heat treatment and take corrective action to redistribute heat from hotter to cooler areas of the mill by using additional heaters and/or fans. Non-uniform distribution of heat within and among floors of a pet food-processing facility, flour mills, and feed mill during heat treatments were reported by Dean (1911), Heaps and Black (1994), Dowdy (1999), Dowdy and Fields (2002), and Roesli et al. (2002). Dean (1911) observed significant differences in the rate of heating of several flour mill floors and locations within the mill, such as elevator boots and roll stands. Temperature rise was faster at $>$1.5 m above the mill floor when compared with temperatures close to the floor. Dowdy and Fields (2002) reported differences within north and south corners of the second and third floors of the Kansas State University pilot flour mill subjected to a steam heat treatment during March 1998. In their study, the maximum temperatures attained ranged from 48°C to 57°C, and the time

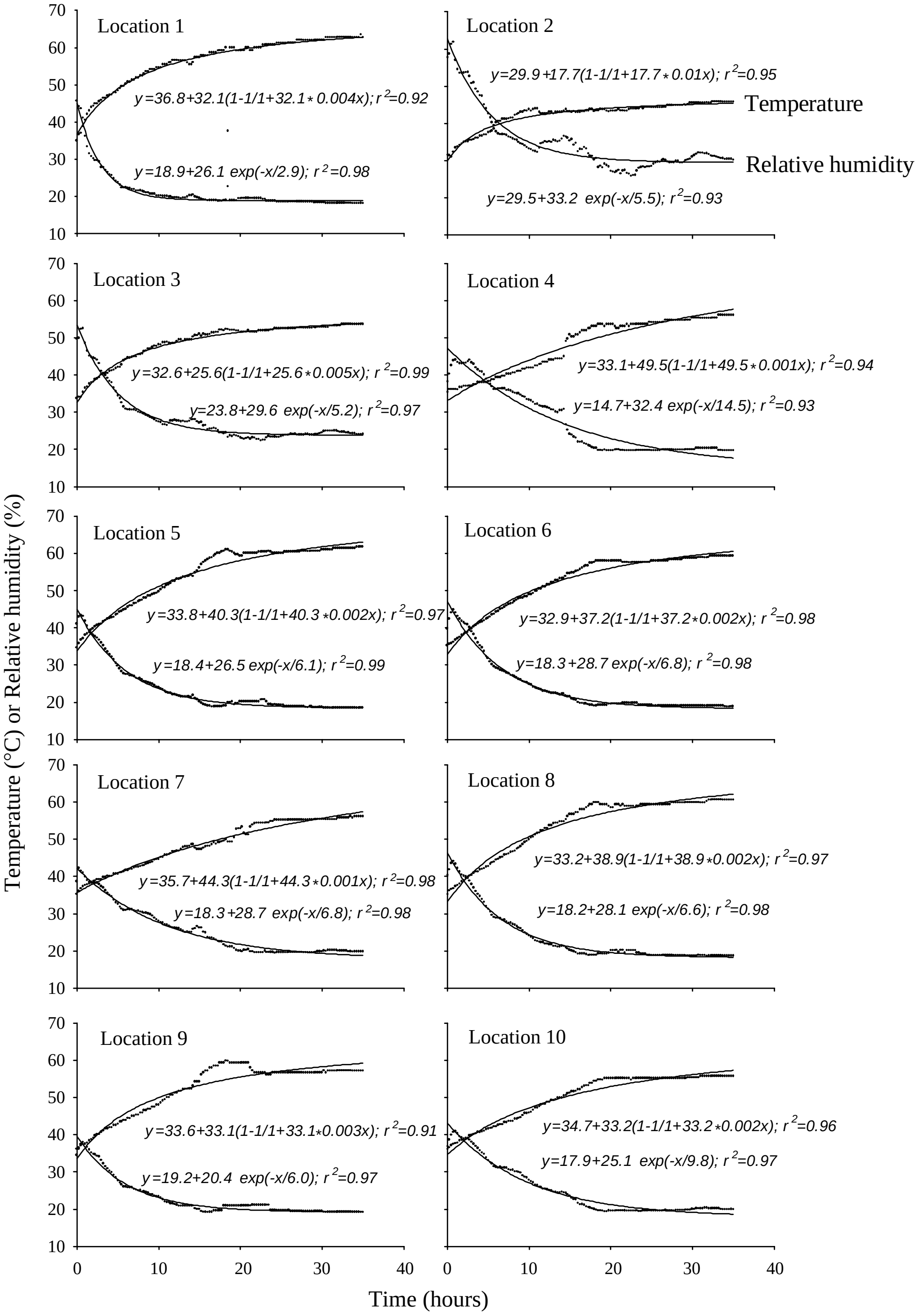

Fig. 1. Temperature and relative humidity profiles at 10 feed mill locations during heat treatment. Temperature and relative humidity profiles were described by fitting two separate three-parameter non-linear regression models to data. Each fitted line (solid line) was based on $n = 141$ temperature or relative humidity observations. The adjusted $r^2$ values are presented in each graph.

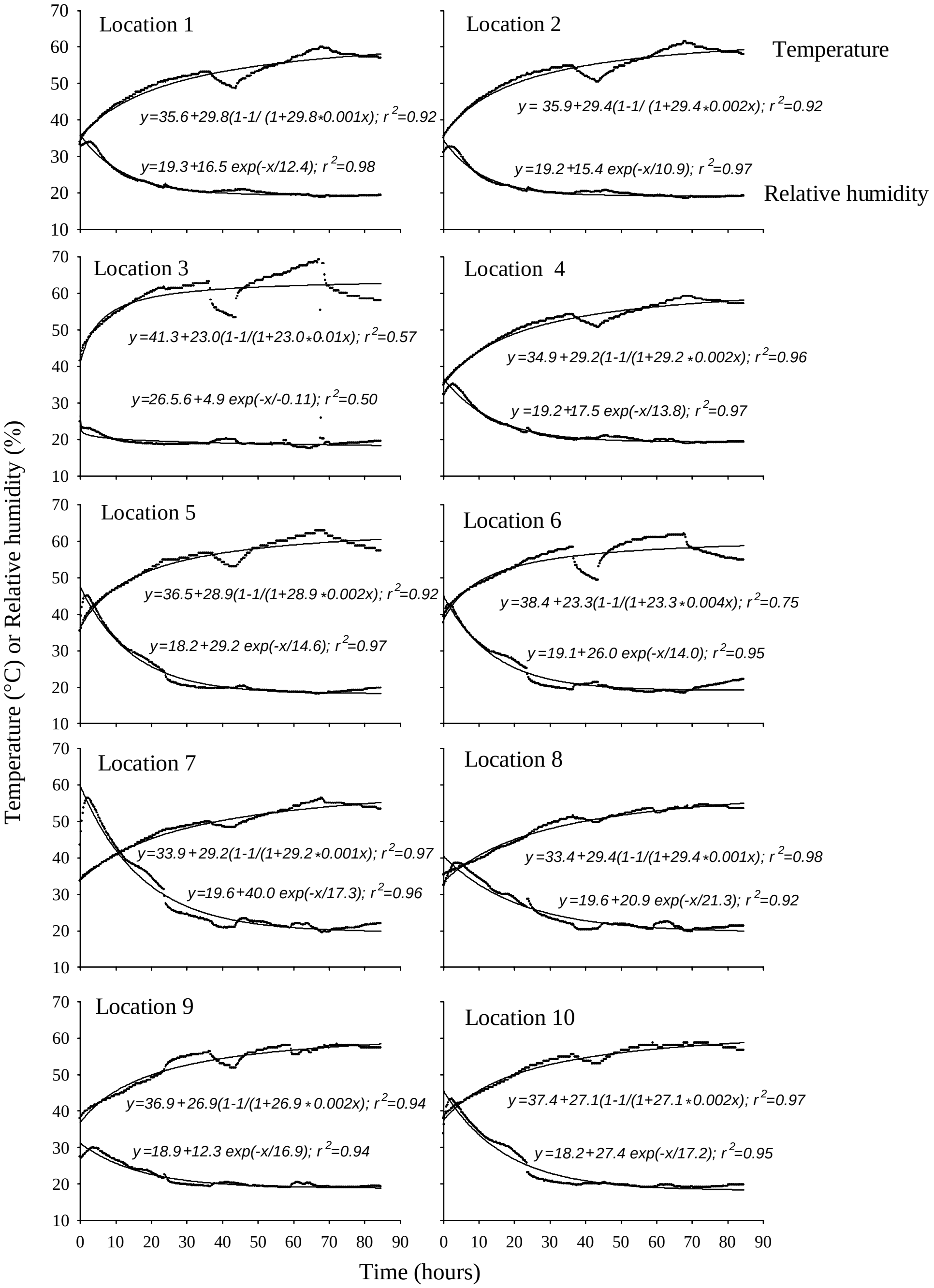

Fig. 2. Temperature and relative humidity profiles at 10 flour mill locations during heat treatment. Temperature and relative humidity profiles were described by fitting two separate three-parameter non-linear regression models to data. Each fitted line (solid line) was based on $n = 339$ temperature or relative humidity observations. The adjusted $r^2$ values are presented in each graph.

to reach 47°C took 30–51 h. Temperature profiles observed in our study in the flour mill were similar to those observed by Dowdy and Fields (2002). Although temperatures were above 60°C in three feed mill and four flour mill locations, no adverse effects on structural integrity of the mills or functioning of mill equipment were observed after the heat treatment.

Equation (1) satisfactorily described (adjusted $r^2 = 0.91-0.99$) temperature increases during heat treatment among feed mill locations (Fig. 1), despite differences in the starting temperature and rate of increase in temperature. In eight of the 10 flour mill locations, the model described temperature data well (adjusted $r^2 = 0.92-0.98$) (Fig. 2). In the third floor of the cleaning house (locations 3 and 6), the model explained 57–75% of the total variation in temperature data. In these locations, temperatures were highly variable as the steam heater directly discharged heat into this area. The heater had to be turned off on one occasion and the direction changed on other occasions to avoid overheating. Our results show that Eq. (1) is suitable for describing temperature profiles observed in mills subjected to gas and steam heat treatments and it is independent of the starting temperature and rate of increase in temperature during heat treatment. Eq. (1) also was found to be suitable for describing temperature increases during mill heat treatments conducted in the summer, fall, and winter months (Roesli et al., 2002; Bh. Subramanyam, unpublished data).

Outdoor relative humidity during heat treatment ranged from 37–78%. Relative humidity at the beginning of the heat treatment among the feed and flour mill locations was variable. Humidity across feed mill locations was 34–58% (Table 4) and across flour mill locations was 25–44% (Table 5). The humidity levels observed during heat treatment were inversely related to temperature (Figs. 1 and 2). In the feed mill, the rate of decrease in humidity as the temperature climbed to 50°C was faster (3.9% $h^{-1}$) in location 1 and slower (0.9% $h^{-1}$) in locations 4, 7,

Table 4
Relative humidity changes at feed mill locations during heat treatment

| Location | Starting humidity (%) | Rate of decrease in humidity until 50°C (% $h^{-1}$)[a] | Mean ± SE humidity (no. observations)[b] | Rate of decrease in humidity after 50°C (% $h^{-1}$)[c] |
|---|---|---|---|---|
| 1 | 45.7 | 3.9 | 19.3 ± 0.1 (116) | 0.1 |
| 2 | 57.7 | —[d] | 34.8 ± 1.4[e] (141) | —[d] |
| 3 | 49.6 | 1.5 | 30.2 ± 0.5 (84) | 0.6 |
| 4 | 38.1 | 0.9 | 20.2 ± 0.2 (80) | 0.2 |
| 5 | 41.0 | 1.7 | 19.6 ± 0.2 (99) | 0.2 |
| 6 | 39.6 | 1.4 | 19.7 ± 0.3 (96) | 0.2 |
| 7 | 38.5 | 0.9 | 19.2 ± 0.04 (63) | 0.1 |
| 8 | 40.3 | 1.6 | 19.6 ± 0.2 (99) | 0.2 |
| 9 | 34.4 | 1.1 | 19.9 ± 0.2 (96) | 0.1 |
| 10 | 37.0 | 0.9 | 20.1 ± 0.2 (83) | 0.2 |

[a] (Starting humidity−humidity at 50°C)/time to 50°C.
[b] Mean ± SE humidity values were calculated from observations starting at 50°C until the end of heat treatment.
[c] (Humidity at 50°C−humidity at the end of heat treatment)/time from 50°C until end of heat treatment.
[d] Values could not be calculated because temperature did not reach 50°C.
[e] The mean ± SE humidity value was calculated from observations collected throughout the heat treatment.

Table 5
Relative humidity changes at flour mill locations during heat treatment

| Location | Starting humidity (%) | Rate of decrease in humidity until 50°C (% $h^{-1}$)[a] | Mean ± SE humidity (no. Observations)[b] | Rate of decrease in humidity after 50°C (% $h^{-1}$)[c] |
|---|---|---|---|---|
| 1 | 33.1 | 0.5 | 19.9 ± 0.1 (251) | 0.04 |
| 2 | 31.0 | 0.5 | 19.6 ± 0.1 (265) | 0.04 |
| 3 | 24.8 | 0.6 | 19.0 ± 0.1 (321) | 0.03 |
| 4 | 32.3 | 0.5 | 20.0 ± 0.1 (255) | 0.05 |
| 5 | 35.4 | 0.4 | 20.3 ± 0.3 (277) | 0.14 |
| 6 | 39.4 | 0.7 | 20.9 ± 0.3 (281) | 0.11 |
| 7 | 43.5 | 0.4 | 21.2 ± 0.1 (150) | 0.02 |
| 8 | 32.4 | 0.3 | 21.1 ± 0.1 (207) | 0.03 |
| 9 | 27.4 | 0.3 | 19.7 ± 0.1 (250) | 0.04 |
| 10 | 33.3 | 0.2 | 20.1 ± 0.2 (256) | 0.14 |

[a] (Starting humidity−humidity at 50°C)/time to 50°C.

[b] Mean ± SE humidity values were calculated from observations starting at 50°C until the end of heat treatment.

[c] (Humidity at 50°C−humidity at the end of heat treatment)/time from 50°C until end of heat treatment.

and 10. The rate of decrease in humidity among flour mill locations ranged from 0.2% to 0.7% $h^{-1}$, and the decrease was slower than in the feed mill. The slow drop in humidity was related to the slow increase in temperature in the flour mill as opposed to the feed mill. Once the temperature reached 50°C, humidity in both the feed and flour mills stabilized around 19–21%, and the rate of change in humidity above 50°C was generally very small (0.02–0.2% $h^{-1}$, Tables 4 and 5). Norstein (1996) and Dowdy and Fields (2002) reported a similar decrease in relative humidity in flour mills during heat treatment. In the flour mill locations, there was a slight increase in humidity soon after the heaters were turned on. Dowdy and Fields (2002) also observed a slight increase in humidity at the beginning of heat treatment. Moisture evaporating from flour in the boxes, grain dust, or hygroscopic surfaces in the mill may explain this small rise in humidity during the initial phase of heat treatment.

Irrespective of the starting relative humidity and rate of decrease of humidity during heat treatment, Eq. (2) adequately described humidity profiles in most feed and flour mill locations (adjusted $r^2 = 0.92-0.99$) (Figs. 1 and 2). Humidity data in location 3 of the cleaning house was not satisfactorily explained by Eq. (2) (adjusted $r^2 = 0.50$). Highly variable and fluctuating temperatures on this floor affected humidity levels, resulting in greater variation of data around the fitted line.

### 3.2. *Mortality of* T.castaneum *life stages*

During heat treatment, eggs and pupae in Petri dishes without food did not develop into larvae and adults, respectively. Therefore, mortality reported here corresponds to the specific life stage exposed to heat. Two-way ANOVA indicated that mortality of *T. castaneum* varied among the feed mill locations ($F = 192.02$; $df = 9, 50$; $P < 0.0001$) and life stages ($F = 35.9$; $df = 4, 50$; $P < 0.0001$). Mortality of all life stages in location 2 was significantly lower ($P < 0.05$) than

Table 6
Mortality of *T. castaneum* life stages among locations of the feed mill subjected to heat treatment

| Location | Mean ± SE mortality (%)[a,b] | | | | |
|---|---|---|---|---|---|
| | Eggs[c] | Young instars[c] | Old instars[c] | Pupae[c] | Adults[c] |
| 1 | 100.0 ± 0.0a | 97.5 ± 0.9a | 100.0 ± 0.0a | 100.0 ± 0.0a | 100.0 ± 0.0a |
| 2 | 82.5 ± 4.8b | 5.0 ± 1.9b | 7.5 ± 0.9b | 22.5 ± 0.9c | 0.0 ± 0.0b |
| 3 | 100.0 ± 0.0a | 100.0 ± 0.0a | 100.0 ± 0.0a | 100.0 ± 0.0a | 100.0 ± 0.0a |
| 4 | 100.0 ± 0.0a | 100.0 ± 0.0a | 100.0 ± 0.0a | 100.0 ± 0.0a | 100.0 ± 0.0a |
| 5 | 100.0 ± 0.0a | 100.0 ± 0.0a | 100.0 ± 0.0a | 100.0 ± 0.0a | 100.0 ± 0.0a |
| 6 | 100.0 ± 0.0a | 100.0 ± 0.0a | 100.0 ± 0.0a | 100.0 ± 0.0a | 100.0 ± 0.0a |
| 7 | 100.0 ± 0.0a | 100.0 ± 0.0a | 100.0 ± 0.0a | 100.0 ± 0.0a | 100.0 ± 0.0a |
| 8 | 100.0 ± 0.0a | 100.0 ± 0.0a | 100.0 ± 0.0a | 100.0 ± 0.0a | 100.0 ± 0.0a |
| 9 | 100.0 ± 0.0a | 100.0 ± 0.0a | 50.0 ± 1.8bc | 50.0 ± 7.8bc | 100.0 ± 0.0a |
| 10 | 100.0 ± 0.0a | 100.0 ± 0.0a | 30.0 ± 1.9bc | 30.0 ± 1.9bc | 100.0 ± 0.0a |

[a] Mortality of eggs, young instars, old instars, pupae, and adults in the control treatment (28°C and 42% r.h.) was 5%, 0%, 5%, 0%, and 0%, respectively.

[b] Each mean ± SE was based on $n = 2$ replications.

[c] For each stage, means followed by different letters are significantly different ($P < 0.05$, linear contrasts).

mortality in other locations (Table 6), because the target temperature of 50°C was not attained in this location. Significant differences among life stages were a result of less than 50% mortality of old instars and pupae in locations 2, 9, and 10 when compared with 100% mortality observed in remaining locations.

In the flour mill, *T. castaneum* mortality among locations was not significantly different ($F = 1.5$; $df = 9, 50$; $P = 0.18$), but it did differ among life stages ($F = 52.9$; $df = 4, 50$; $P < 0.0001$) (Table 7). Except for pupae, mortality of the remaining life stages was 100%. The reduced mortality of pupae resulted in the significant differences observed among the stages.

Heat-related mortality can be caused by changes in carbohydrates, lipids, proteins, DNA, and RNA; cellular changes; and perturbation of ionic activities (Denlinger and Yocum, 1999), and these changes could be different in the various developmental stages. Susceptibility of an insect species to heat varies within a developmental stage (Davison, 1969; Bursell, 1973) and among stages (Oosthuizen, 1935; Evans, 1981). Generally, immature stages of stored-product insects are more tolerant of high temperatures than adults (Beckett et al., 1998; Lewthwaite et al., 1998). In this study, old instars and pupae were found to be less susceptible to heat than eggs, young instars, and adults. However, in laboratory experiments at constant temperatures between 50°C and 60°C, young (first) instars of *T. castaneum* were more tolerant than eggs, old instars, pupae, and adults (R. Mahroof, Bh. Subramanyam, and A. Menon, unpublished data). For example, a 60 min exposure at 60°C was required to kill all exposed young instars of *T. castaneum*, while only 20 min was necessary to kill the remaining stages. In the laboratory *T. castaneum* life stages reared at 28°C and 65% r.h. were exposed to high temperatures without acclimation, and death could be due to the sudden heat shock. During heat treatment, *T. castaneum* life stages are exposed to gradually increasing temperatures. Differences in acclimation of the various stages to gradually increasing temperatures could affect subsequent susceptibility at high temperatures.

Table 7
Mortality of *T. castaneum* life stages among locations of the flour mill subjected to heat treatment

| Location | Mean ± SE mortality (%)[a,b] | | | | |
|---|---|---|---|---|---|
| | Eggs[c] | Young instars[c] | Old instars[c] | Pupae[c] | Adults[c] |
| 1 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 78.0 ± 0.9 | 100.0 ± 0.0 |
| 2 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 93.0 ± 0.9 | 100.0 ± 0.0 |
| 3 | 100.0 ± 0.0 | 97.5 ± 0.9 | 100.0 ± 0.0 | 98.0 ± 0.9 | 100.0 ± 0.0 |
| 4 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 90.0 ± 1.9 | 100.0 ± 0.0 |
| 5 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 95.0 ± 1.9 | 100.0 ± 0.0 |
| 6 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 85.0 ± 3.9 | 100.0 ± 0.0 |
| 7 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 90.0 ± 3.9 | 100.0 ± 0.0 |
| 8 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 80.0 ± 1.9 | 100.0 ± 0.0 |
| 9 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 88.0 ± 0.9 | 100.0 ± 0.0 |
| 10 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 | 100.0 ± 0.0 |

[a] Mortality of eggs, young instars, old instars, pupae, and adults in the control treatment (28°C and 42% r.h.) was 2.5%, 0%, 0%, 2.5%, and 0%, respectively.

[b] Each mean ± SE was based on $n = 2$ replications.

[c] Mortality of *T. castaneum* life stages among locations was not significant ($F = 1.49$, $df = 9, 50$, $P = 0.18$, two-way ANOVA).

Bijok (1996) and Emekci et al. (2002) reported differences in the metabolic (respiration) rates of *T. castaneum* life stages. Changes in metabolic rates or production of metabolites that enhance survival under adverse environmental conditions, such as those occurring during heat treatment, could differentially affect the survival of various developmental stages. Currie and Tufts (1997) and Lewis et al. (1999) stated that high temperature tolerance could be enhanced by the synthesis of heat shock proteins that prevent cell damage and death. We are presently studying the production of heat shock proteins in various life stages of *T. castaneum* exposed to high (constant) temperatures in the laboratory and during heat treatment to better understand the stage-specific susceptibility to heat.

Relative humidity does not play a significant role in insect mortality, although Denlinger and Yocum (1999) have suggested that rapid desiccation at high temperatures could contribute to heat-related mortality. Low relative humidity was maintained for twice as many hours in the flour mill as in the feed mill. However, mortality of all stages was greater in the feed mill than in the flour mill. These data indirectly indicate that temperature and rate of increase of temperature during heat treatment contributed to *T. castaneum* mortality. The mortality of *T. castaneum* adults during heat treatment was unaffected at 32% and 63% r.h., especially if a temperature of 50°C was maintained for 50 min (R. Roesli and Bh. Subramanyam, unpublished data). The responses may be similar for the remaining *T. castaneum* life stages.

In summary, we have provided two separate regression models for quantitatively describing temperature and relative humidity changes during an actual heat treatment of pilot feed and flour mills. The starting temperature and relative humidity, and the rate of temperature increase or relative humidity decrease, varied among locations within each mill. Despite these differences, the models satisfactorily described the data. This indicated that the models are fairly general and can be used to make quantitative comparisons of temperature or humidity changes among mill locations or floors. Parameters of the temperature–time model (Eq. (1)) can be used to predict the rate of temperature increase for any given starting temperature. Insect mortality data collected at various temperature–time combinations can be integrated with Eq. (1) for developing and validating heat accumulation models (degree-minute models; Wright et al., 2002) to predict insect mortality during heat treatment.

Mortality of *T. castaneum* life stages was 100% in most mill locations, except in areas where the temperature was below 50°C. The survival of old instars and pupae of *T. castaneum* in two feed mill locations and pupae in nine flour mill locations is unclear, because lethal temperatures were attained in these locations. Additional research is needed to study the importance of exposure to gradually increasing temperatures and heat shock protein synthesis on thermotolerance of *T. castaneum* life stages.

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