Association of Operative Millers BULLETIN

Management of red flour beetles using elevated temperatures

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ABSTRACT

The use of elevated temperatures or heat treatment is a viable alternative to methyl bromide fumigation of flour and feed mills. However, very little is known about the stage-specific susceptibility of stored-product insects to high temperatures used during heat treatments, as well as effectiveness of heat treatment. We determined the susceptibility of eggs, young larvae, older larvaeold larvae, pupae, and adults of the red flour beetle, an economically important insect pest in mills worldwide, to temperatures used during heat treatments. Young larvae were the most heat-tolerant stage. Laboratory and field tests provided conflicting results on stage-specific susceptibility to elevated temperatures. We trapped insects before and after heat treatment inside and outside a feed mill to determine the degree and duration of insect suppression. Monitoring insects before and after heat treatment inside and outside the mill is important for determining heat treatment efficacy and for identifying control failures. The importance of these findings is discussed.

INTRODUCTION

Managing stored-product insect pests by heating ambient air of food-processing facilities to lethal temperatures is an old, but still effective, technology. During heat treatment, all portions of a facility are heated to 50 - 60°C, and these high temperatures are maintained for 24 - 36 h to kill stored-product insects. There is renewed interest in utilizing heat treatments because of the impending phase out of methyl bromide, an ozone-depleting space fumigant currently used for managing insects in food-processing facilities in North America and Europe.

The effectiveness of heat treatment depends on maintaining uniform heating of all portions of a facility. Horizontal and vertical stratification of temperatures during heat treatment can result in under- or over-heating some portions of the some portions of a facility. Over-heating may result in damage to heat-sensitive equipment. Under-heating may result in insects surviving the heat treatment. Very little quantitative information is available on time-mortality relationships of stored-product insect pests exposed to elevated temperatures typically used during heat treatments.

At Kansas State University we have been involved since 1999 in generating quantitative data on the stage-specific susceptibility of stored-product insects, primarily red flour beetles, exposed to elevated temperatures. Red flour beetles were chosen as test insect because this species is commonly associated with food-processing facilities worldwide. In this article, we report results from our investigations on the red flour beetle exposed to elevated temperatures. Our purpose in sharing these results is to (1) show variation among red flour beetle life stages to elevated temperatures under laboratory conditions; (2) determine susceptibility of red flour beetle life stages during an actual facility heat treatment; and (3) comment on the use of traps as a tool for evaluating effectiveness of heat treatments. Detailed information about the experimental protocols and results mentioned only briefly here are available in the papers listed at the end of this article.

EXPERIMENTAL METHODS

Insect stages used

Red flour beetle cultures were reared at 28°C and 65% relative humidity on whole-wheat flour fortified with 5% (by wt) of brewer's yeast. Eggs (2-d-old), 6-d-old larvae (young larvae or 1st instars), 22-d old larvae (old larvae or 6-7th instars), unsexed pupae (26-d-old), and unsexed adults (2-wk-old) were used for tests during mill heat treatments or in the laboratory growth chambers. The mean ± SE ($n = 15$) weight of young larvae was 0.12 ± 0.01 mg and that of old larvae was 3.59 ± 0.11 mg.

Susceptibility of red flour beetle life stages at fixed constant temperatures under laboratory conditions

Small square plastic boxes (4.5 x 4.5 cm) with insects (20 individuals) were exposed in growth chambers (Model I-36 VL, Percival Scientific, Perry, IA) set at constant temperatures of 42, 46, 50, 54, 58 and 60°C and 20-22% RH for establishing time-mortality relationships for each life stage of the red flour beetle. Boxes kept in a chamber set at 28.2 ± 0.2°C and 44.5 ± 0.3% served as the control treatment to measure natural mortality of insects. There were five boxes for each life stage at each temperature-time combination. At the end of both heat treatments boxes containing adults were held at 28°C and 42% RH for an additional 24 h before assessing mortality. Mortality of adults was based on number dead out of the total exposed. Pupae were held in the same boxes until emergence of adults. Boxes containing eggs, young larvae, and old larvae were transferred into 150-ml plastic containers holding 40 g of whole-wheat flour plus yeast (5% by wt). Mortality of immature was based on number out of the total that failed to emerge into adults.

Time-mortality data for life stages to elevated temperatures were not corrected for control mortality (<5%). Time-mortality data for each life stage at 42 to 60°C were fit to the complementary log-log (CLL) regression model to estimate the time required to kill 99% (LT_{99}) of the exposed insects. The change in LT_{99} with temperature for each stage was described using equation 1 (Anonymous, 1994):

$$\ln y = a + bx \quad (1)$$

where, y is the LT_{99} in min, x is the temperature in °C, and a and b are constants estimated from the LT_{99}-temperature data.

Susceptibility of red flour beetle life stages during an actual facility heat treatment

The pilot feed and flour mills in the Department of Grain Science and Industry, Kansas State University, Manhattan, KS, were heated during 6 - 8 and 4 - 8 August 2001, respectively. The feed mill was heated using natural gas heaters while the flour mill was heated using in-house steam heaters. In the pilot feed and flour mill, 10 different locations were selected to measure temperature and relative humidity changes, and to record insect mortality. These locations typically included floor-wall junctions, room corners, or "difficult-to-heat" areas, recognized from our experience during past heat treatments. HOBO data-logging units were used to measure temperature and relative humidity at these 10 locations. The rise in temperature during heat treatment was described as:

$$y = a + b(1-1/(1 + bcx)) \qquad (1)$$

where, y is the predicted temperature in °C, x is the time in hours, and a, b, and c, are constants estimated from the temperature-time data. The decrease in humidity during heat treatment was described by equation (2):

$$y = a + b\,exp(-x/c) \qquad (2)$$

where, y is the predicted relative humidity (%), x is time in hours, and a, b, and c are constants estimated from the humidity-temperature data.

The same five stages of red flour beetles mentioned above were placed in square boxes with flour and exposed at the 10 locations of each mill. There were two boxes for each stage at each mill locations. Mortality of life stages was assessed after heat treatment using procedures outlined above.

Traps as tools for gauging heat treatment effectiveness

Food-baited and pheromone-baited traps were used in the Kansas State University pilot feed mill four weeks before and four weeks after a heat treatment to show the immediate effect of heat treatment on stored-product insects and the duration of treatment effectiveness. Each food-baited trap had 15 drops of food oil attractant and lures for red/confused flour beetles, cigarette beetles, and warehouse beetles. The use of food oil and pheromones is known to enhance trap catch. These food-baited traps were placed on the mill floor, whereas the sticky traps were hung generally at eye level to any suitable mill structure. The feed mill was heated during August 14-16, 2000 using natural gas heaters. There were five food-baited and five pheromone-baited sticky traps on each of the four floors of the feed mill. In addition, five traps were placed on the south side of the feed mill and on the mill roof. Adults of stored-product insects captured in traps were identified and expressed as numbers captured per trap per week.

RESULTS AND CONCLUSIONS

Susceptibility of red flour beetle life stages at fixed constant temperatures under laboratory conditions

The mortality of the five red flour beetle life stages generally increased with an increase in temperature and exposure time. The differences among stages in susceptibility were not consistent among the six constant temperatures. Eggs and young larvae were more susceptible to heat than other stages at 42°C, and eggs were more susceptible than young larvae. At 42°C, older old larvae, pupae, and adults were relatively more heat tolerant than eggs and young larvae. However, the susceptibility of these stages greatly increased at or above 46°C. In general, young larvae were the most heat tolerant stage at 50°C, because it had the highest LT_{99} value (432.8 min, Fig. 1). Young larvae also had the highest LT_{99} value at 54°C (81.9 min). Young larvae had significantly higher LT_{99} value at 58°C when compared with that for eggs and adults. Similarly, young larvae had significantly higher LT_{99} value at 60°C when compared with that for eggs, old larvae, pupae, and adults. The decreased susceptibility of young larvae at elevated temperatures could be due to higher metabolism or production of heat shock proteins. Our laboratory is currently rearing red flour life stages at different (hot and cold optimum) temperatures to study expression of the heat shock proteins. A review of published literature has shown that newly hatched larvae (young larvae) of red flour beetles had greater oxygen consumption per unit body weight compared with eggs, old larvae, and pupae. , and t The higher respiration rates may be attributable to higher metabolic rates often connected with a reaction to stress, and may enhance survival under unfavorable environmental conditions.

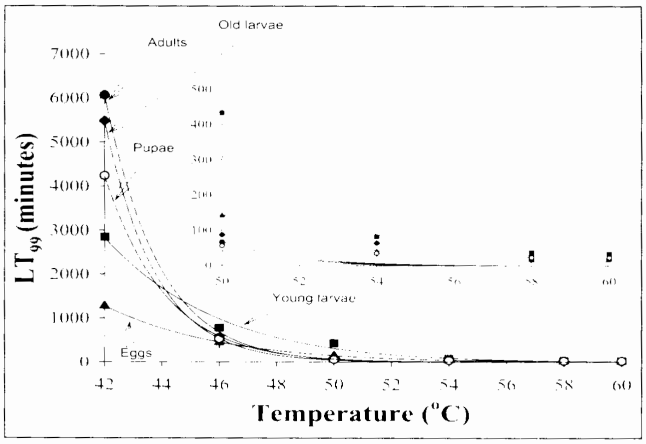

Fig. 1. Time required to kill 99% of eggs (2-d old), young larvae (6-d old; wt. 0.12 mg), old larvae (22-d old; wt. 3.59 mg), pupae (26-d old), and adults (≤2-wk old) of the red flour beetle at 42 to 60 C. A nonlinear regression equation ($\ln y = a + bx$) was fit to LT_{99}–temperature data of each stage to describe heat tolerance. Regression equations for each stage were: eggs, $\ln y = 12.346 + (-0.002)x$; young larvae, $\ln y = 13.244 + (0.003)x$; old larvae, $\ln y = 19.682 + (-0.006)x$; pupae, $\ln y = 20.280 + (-0.006)x$; and adults, $\ln y = 18.267 + (-0.005)x$.

The longer time periods (21 to 83 h) required to kill 99% of all exposed stages at 42°C suggest that it is preferable to use temperatures ≥50°C during heat treatment, because of the reduced time it takes to kill insects. At temperatures of 50°C or more eggs, old larvae, pupae, and adults become highly susceptible to heat. At 50°C, to kill 99% of the exposed young larvae required 7.2 h, as opposed to 1.8 h for eggs, 1.1 h for old larvae, 1.5 h for pupae, and 0.9 h for adults. Similarly , at 60°C, a 42-min exposure was necessary to kill 99% of the exposed young larvae, whereas the remaining stages required less than 20 min. Therefore, during heat treatments, temperatures should be monitored to ensure that a minimum of 50°C is achieved throughout the area being heated for at least 7.2 h to kill the most heat tolerant young larvae. Our data suggest that heat treatments aimed at controlling young larvae should be able to control all other stages. In addition, young larvae should be used as test insects to gauge the effectiveness of heat treatment against *T. castaneum* red flour beetles in a food-processing facility. The information reported here provides baseline data for successful use of elevated temperatures for management of red flour beetle life stages associated with food-processing facilities.

Susceptibility of red flour beetle life stages during an actual facility heat treatment

Temperature during heat treatment in the southwest corner of the feed mill basement (location 2) never reached 50°C, and the maximum temperature attained was 46°C (Fig. 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 within the shortest time (6 h). This occurred because a heating duct was placed close to the HOBO data data-logging units and insect samples. In general, the time to reach 50°C among other mill locations varied between 6 and 19 h. Time above 50°C and maximum temperature varied among mill locations. For example, temperatures above 50°C among mill locations varied from 18 - 31 h, and maximum temperatures varied from 46 - 63°C. Temperatures exceeded 60°C in three of the 10 feed mill locations. Temperature reached 50°C quickly (4.5 h) in the third floor of cleaning house (location 3), and slowly (47 h) in the third floor of milling house (location 7) of the flour mill (Fig. 3). Improper air movement, placement of heaters or ducts, and loss of heat from various surfaces (windows, doors, floor, and roof vents) may have resulted in horizontal and vertical stratification of temperatures within each mill. Therefore, during heat treatment, temperatures in various locations of the facility should be monitored frequently and corrective action taken to

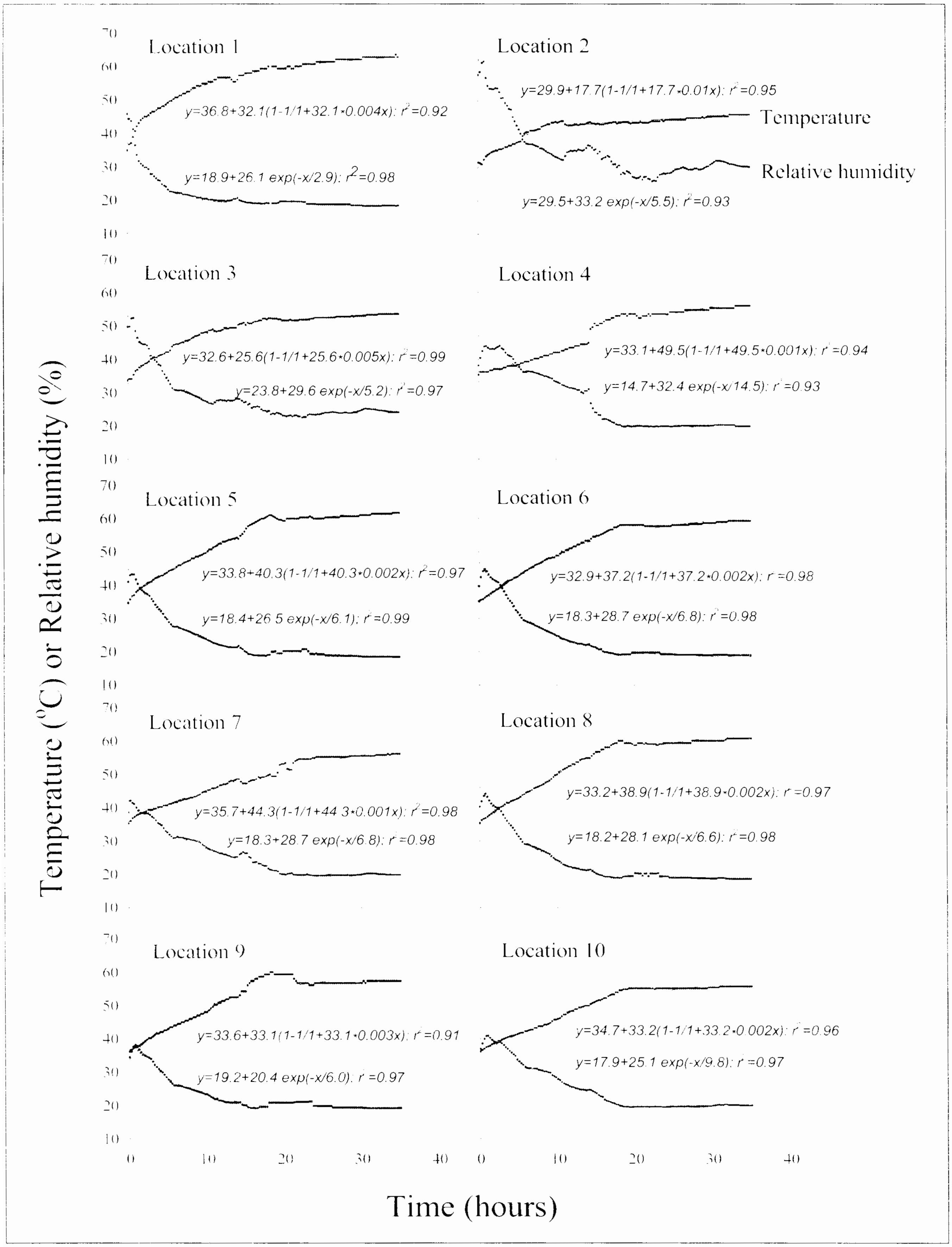

Fig. 2. Temperature and relative humidity profiles at 10 feed mill locations during heat treatment. Temperature and relative humidity profiles were described by fitting two separate three-parameter nonlinear regression models to data. Each fitted line (solid line) was based on n = 141 temperature or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

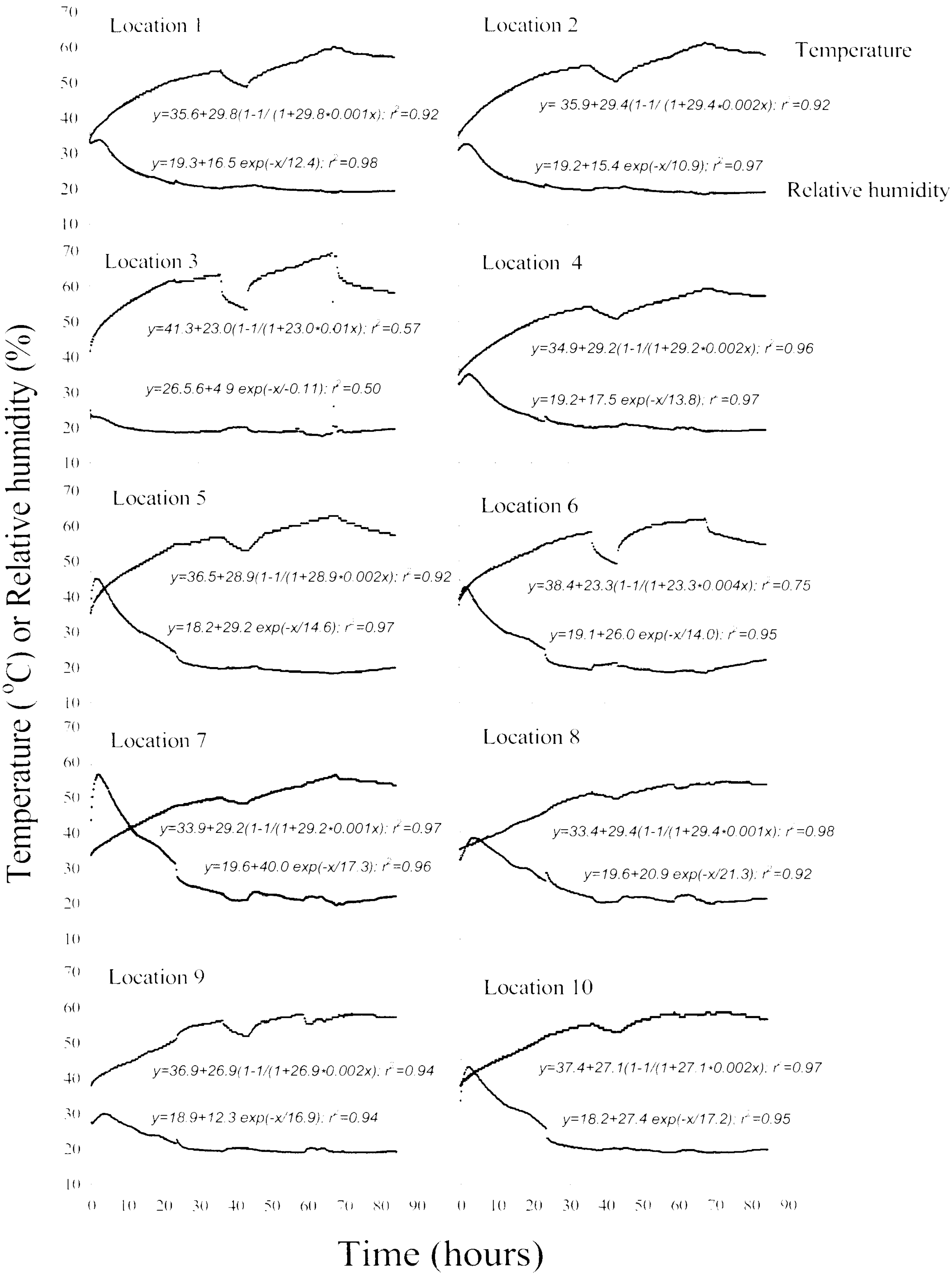

Fig. 3. Temperature and relative humidity profiles at 10 flour mill locations during heat treatment. Temperature and relative humidity profiles were described by fitting two separate three-parameter nonlinear regression models to data. Each fitted line (solid line) was based on n = 339 temperature or relative humidity observations. The adjusted r^2 values are presented in each graph.

redirect heat to areas that are under-heated.

Despite differences in the rate of temperature increases among mill locations, equation 1 satisfactorily described (r^2 = 0.91 - 0.99) temperature profiles among the feed mill locations (Fig. 2). In eight of the 10 flour mill locations, the model fit the temperature data well (r^2 = 0.92 - 0.98) (Fig. 3). In third floor of the cleaning house (locations 3 and 6), the model explained 60 - 80% of the total variation in the temperature data. In third floor of the cleaning house, temperatures were highly variable as the steam heater directly discharged heat into this area and the heater had to be turned off on one occasion, and the heat regulated on other occasions to avoid overheating. Our results suggest that equation 1 is independent of the initial ambient temperature observed in the mills and the rate of increase in temperature during heat treatment.

The humidity levels observed during heat treatment were inversely related to temperature (Figs. 2 and 3). In the feed mill, the rate of decrease in humidity as the temperature climbed to 50°C was faster (3.9%/h) in location 1 and slower (0.9%/h) in locations 4, 7, and 10. The rate of decrease in humidity among flour mill locations ranged from 0.2-0.7 %/h, 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). Irrespective of the starting relative humidity and rate of decrease of humidity during heat treatment, equation 2 adequately described humidity profiles in most feed and flour mill locations (adjusted r^2 = 0.92-0.99). Humidity data in location 3 of the cleaning house was not satisfactorily explained by equation 2 (adjusted r^2 = 0.50). Highly variable and fluctuating temperatures in this floor affected humidity levels, resulting in greater variation of data around the fitted line.

The mortality of red flour beetle life stages varied among the feed mill locations (Table 1). Also, mortality varied among the life stages. Mortality of all life stages in location 2 was lower than mortality in nine other locations. In the flour mill, red flour beetle mortality among locations was similar but it was different among the life stages (Table 2). Generally, immature stages of stored-product insects are more tolerant to high temperatures than adults., and our Our results show that pupae appeared to be more heat tolerant than young larvae, especially in the flour mill where the temperature increase was much slower than in the feed mill.

Our results show that equations 1 and 2 are suitable for describing temperature and relative humidity profiles profiles observed in mills subjected to gas and steam heat treatments and it is independent of the starting temperature and the rate of increase in temperature during heat treatment. These equations were also was found to be suitable for describing temperature increases during mill heat treatments conducted in the summer, fall, and winter months (data not shown).

Heat-related mortality can be caused by changes in carbohydrates, lipids, proteins, DNA, and RNA; cellular changes; and perturbation of ionic activities, and these changes could be different in the various developmental stages. Susceptibility of an insect species to heat varies within a developmental stage and among stages. Generally, immature stages of stored-product insects are more heat-tolerant of high temperatures than adults. Mortality of red flour beetle life stages was 100% in most mill locations, except in areas where the temperature was lower than 50°C. Survival of old larvae and pupae in two feed mill locations and pupae in nine flour mill locations is unclear, because lethal temperatures were attained in these locations. This finding is contrary to work done on red flour beetle life stages at fixed constant temperatures, where young larvae were found to be the most heat-tolerant stage. Additional research is needed to study the importance of exposure to gradually increasing temperatures and or heat shock protein synthesis onheating rates on the thermotolerance of development in red flour beetle life stages.

Relative humidity does not play a significant role in insect mortality, although some researchers have suggested that rapid desiccation at high temperatures could contribute toenhance heat-related mortality. Low relative humidity was maintained for twice as many hours in the flour mill than 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 mortality of red flour beetle life stages. Further work is needed to determine the interaction of temperature and relative humidity on insect mortality at elevated temperatures.

Traps as tools for gauging heat treatment effectiveness

Temperatures among the four feed mill floors prior to heat treatment ranged from 26.3 to 35.7°C. Temperatures on the mill roof during heat treatment ranged from 23.1 to 35.8°C, and on the south side of the feed mill (at ground level) ranged from 25.4 to 36.7°C. Temperatures reached 50°C within three to four hours in the basement and first, second, and third floors of the mill (Table 3). The fourth floor was the slowest to heat, and

Table 1. Mean mortality (%) of red flour beetle life stages among locations of the feed mill subjected to heat treatment.

Location	Eggs	Young larvae	Old larvae	Pupae	Adults
1	100.0	97.5	100.0	100.0	100.0
2	82.5	5.0	7.5	22.5	0.0
3	100.0	100.0	100.0	100.0	100.0
4	100.0	100.0	100.0	100.0	100.0
5	100.0	100.0	100.0	100.0	100.0
6	100.0	100.0	100.0	100.0	100.0
7	100.0	100.0	100.0	100.0	100.0
8	100.0	100.0	100.0	100.0	100.0
9	100.0	100.0	50.0	50.0	100.0
10	100.0	100.0	30.0	30.0	100.0

Table 2. Mean mortality (%) of red flour beetle life stages among locations of the flour mill subjected to heat treatment.

Location	Eggs	Young larvae	Old larvae	Pupae	Adults
1	100.0	100.0	100.0	78.0	100.0
2	100.0	100.0	100.0	93.0	100.0
3	100.0	97.5	100.0	98.0	100.0
4	100.0	100.0	100.0	90.0	100.0
5	100.0	100.0	100.0	95.0	100.0
6	100.0	100.0	100.0	85.0	100.0
7	100.0	100.0	100.0	90.0	100.0
8	100.0	100.0	100.0	80.0	100.0
9	100.0	100.0	100.0	88.0	100.0
10	100.0	100.0	100.0	100.0	100.0

Table 3. Temperature profiles among feed mill floors during 14 - 16 Aug 2000 heat treatment of the feed mill.

Mill floor	Staring temp (°C)	Time to 50°C (h)[a]	Rate (°C/h)[b]	Time above 50°C (h)	Max temp (°C)
Basement	33.6	4.0	4.1	30.0	62.7
First	34.4	3.0	5.2	31.0	62.0
Second	35.3	3.6	4.1	30.4	60.6
Third	34.9	4.0	3.8	30.0	59.9
Fourth	34.0	7.3	2.2	26.7	55.4

[a]From starting temp to 50°C.
[b](50°C - starting temp)/time to 50°C.

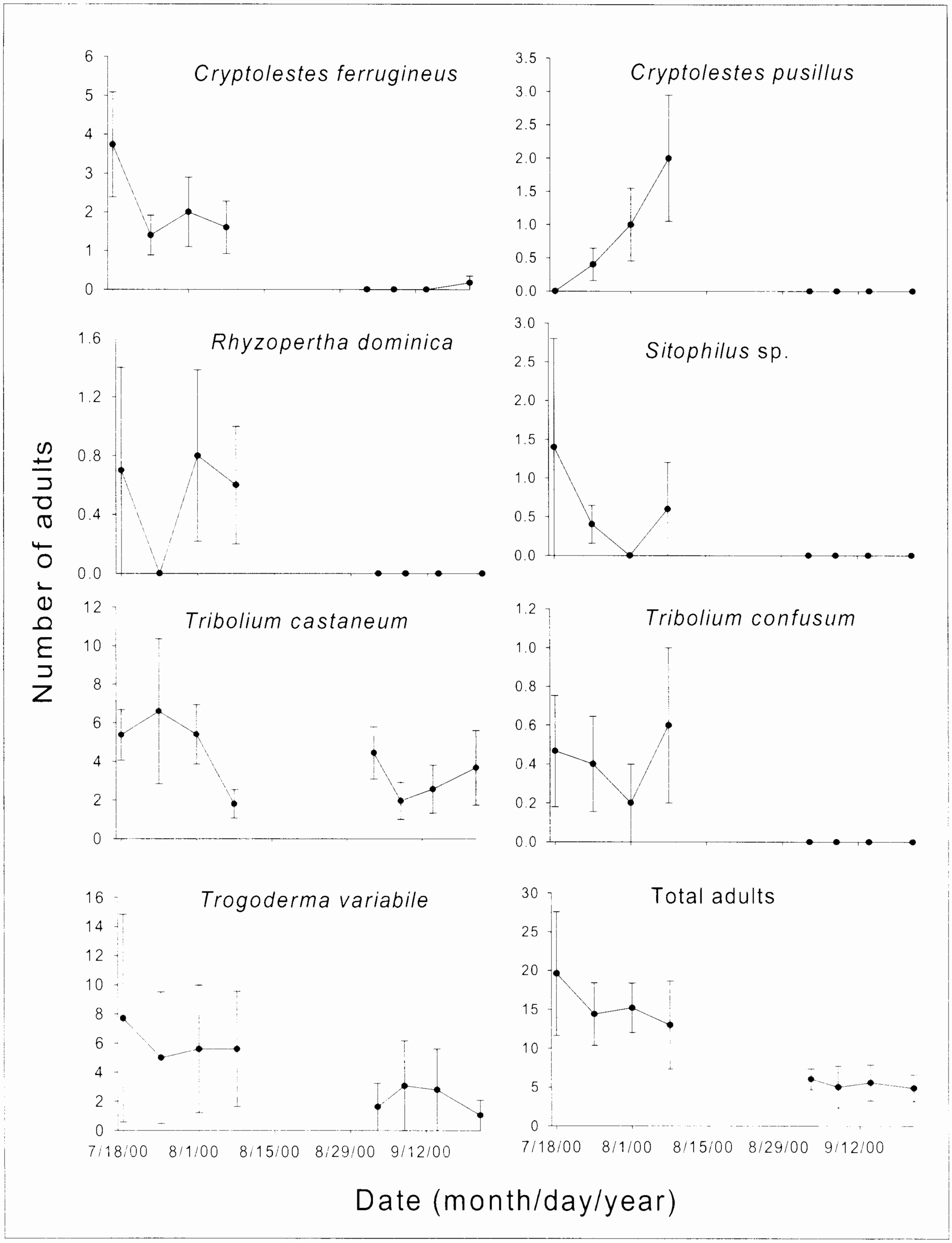

Fig. 4. Number of beetle adults (mean ± SE trap week) captured in food-baited traps in the first floor of the feed mill. The *y*-axis scale is different for different species. *Cryptolestes ferrugineus* is rusty grain beetle: *Cryptolestes pusillus* is flat grain beetle: *Rhyzopertha dominica* is lesser grain borer; *Sitophilus* sp. (weevils); *Tribolium castaneum* is red flour beetle: *Tribolium confusum* is confused flour beetle: *Trogoderma variabile* is warehouse beetle.

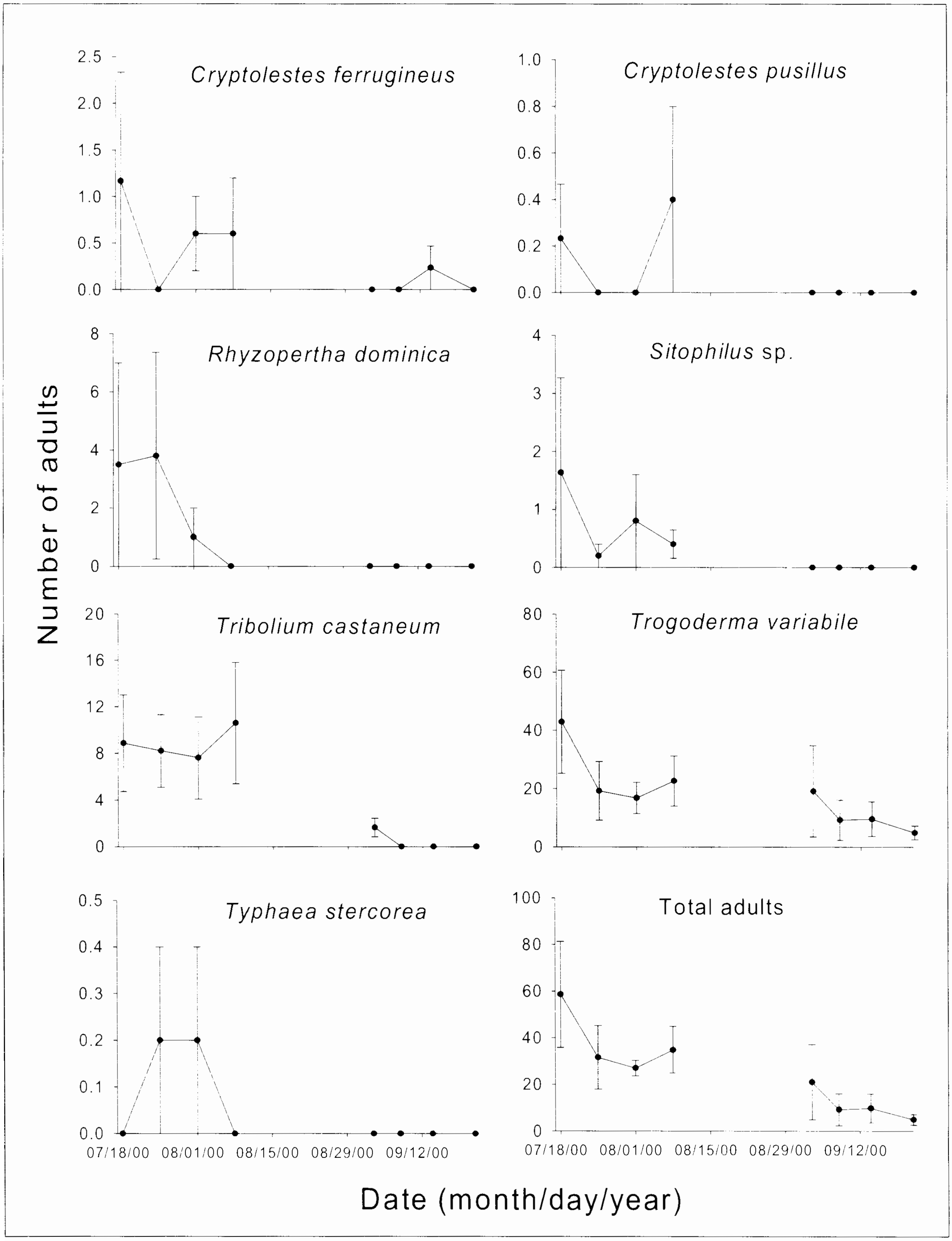

Fig. 5. Number of beetle adults (mean ± SE/trap/week) captured in food-baited traps on the south side of the feed mill. The *y*-axis scale is different for different species. For common names of insect species, see Fig. 4; *Typhaea stercorea* is hairy fungus beetle.

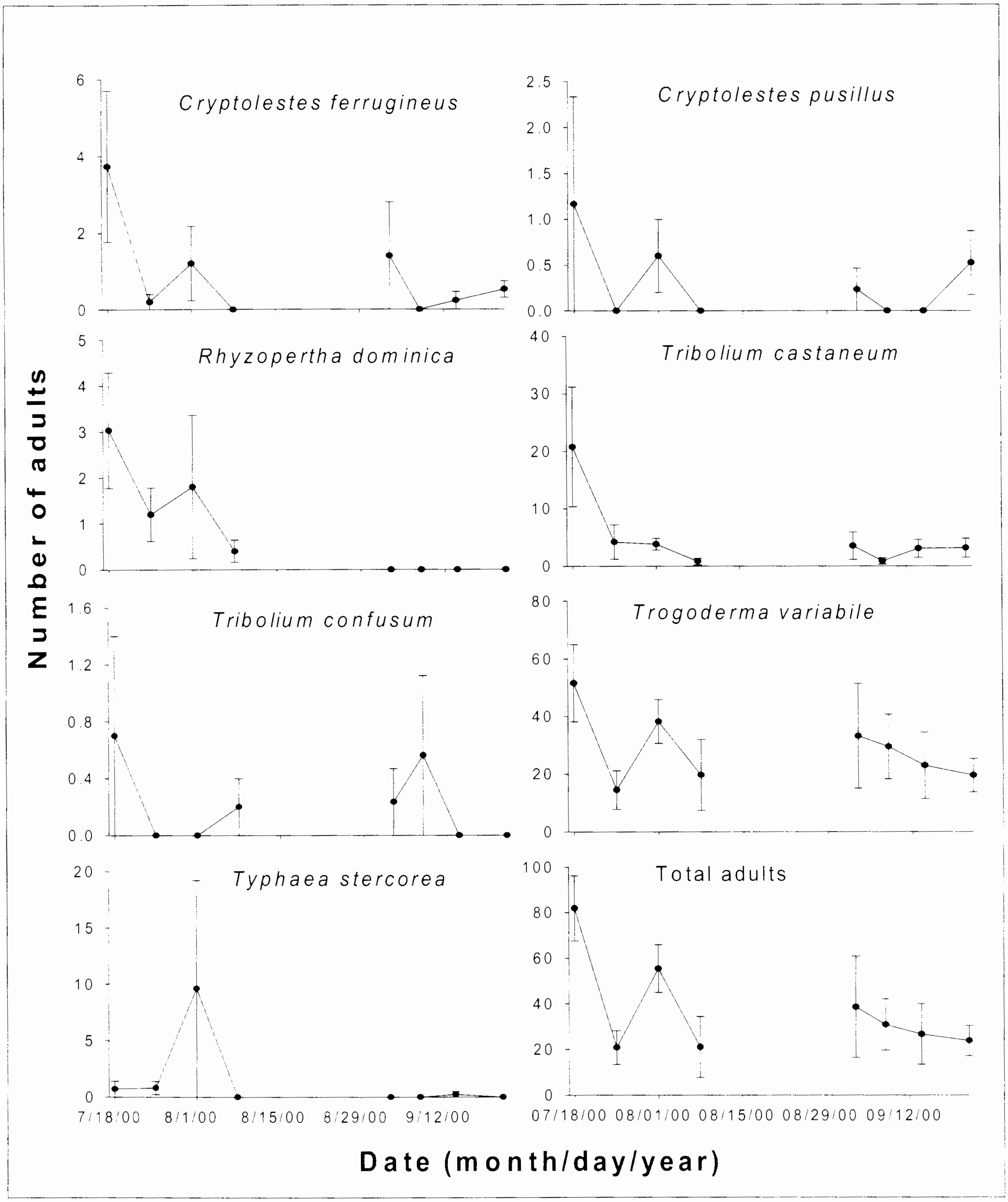

Fig. 6. Number of beetle adults (mean ± SE/trap/week) captured in food-baited traps on the feed mill roof. The *y*-axis scale is different for different species. See Figs. 4 and 5 for common names of insect species.

50 C was attained after more than seven hours. The rate of heating was 2.2 C/h in the fourth floor and 5.2 C/h in the first floor of the feed mill. Temperatures above 50 C among mill floors were maintained for 27 to 31 h. Temperatures slightly exceeded 60 C in the basement and first floor.

Several species of stored-product insects beetles were captured in food-baited traps and Indianmeal moth and almond moth males were captured in pheromone-baited traps. To illustrate the utility of traps for gauging heat treatment effectiveness we will present only the beetle trap capture data from the first floor of the feed mill, along with data of captures from the south side of the feed mill, and the feed mill roof. Interested readers are requested to contact the senior author for the full data set or the paper.

More beetles were captured in food-baited traps placed inside the feed mill than those placed on the south side of the feed mill or the roof before the heat treatment. However, after heat treatment, there were more insects on the roof than the other locations. Heat treatment had a major impact on captures at all locations. For example, captures of beetles in traps on the south side of the feed mill decreased by 73%, whereas captures inside the mill decreased by 71% and on the roof by 37%. Adult lesser grain borers and hairy fungus beetles were not captured in traps inside the mill after heat treatment. Lesser grain borers also were not captured outdoors after the heat treatment. Weevils and hairy fungus beetles were not captured on the south side of the feed mill after heat treatment. Species such as *Oryzaephilus* (sawtoothed or merchant grain beetles) were captured on the south side of the feed mill only after heat treatment, but in very small numbers.

There were differences in beetles captured in food-baited traps among the feed mill floors, including the basement. Therefore, impact of heat on trap captures was examined by floor. In the first floor of the feed mill, traps failed to capture adults of flat and rusty grain beetles, lesser grain borers, maize weevils, and confused flour beetles after the heat treatment (Fig. 4). The warehouse beetle captures were lower after the heat treatment when compared with captures before the heat treatment, whereas red flour beetle captures were unaffected. The impact of heat on other species was difficult to evaluate, because trap captures following heat treatment did not follow any consistent trend among the mill floors. There was a consistent decrease in the trap catchescaptures of adults of seven insect species in traps placed at ground level on the south side the feed mill following heat treatment (Fig. 5), whereas trap catchescaptures on the feed mill roof were not severely affected (Fig. 6).

The capture of stored-product insect species in traps, especially before the heat treatment period, both inside and outside the mill, was highly variable as indicated by the large standard errors associated with the mean captures. The observed differences in trap captures of insect species among mill floors could be due to differences in the absolute density of insect species and their spatial distribution, which is may be strongly influenced by the distribution of patchy resources in the feed mill. Environmental (temperature, food availability, moisture, trap location, trap design) and biological factors (age, mating status, sex, mobility, feeding status) that affect insects could impact trap captures. In addition, in feed mill environments, traps and lures are competing with food odors. Despite these limitations, traps are valuable tools for insect monitoring and for gauging treatment effectiveness. The alternative approach would involve sampling product within the mill to estimate the absolute density of insects and use these estimates to gauge heat treatment effectiveness.

The effectiveness of heat treatment against red flour beetles varied among mill floors, and between indoor and outdoor habitats. Red flour beetle numbers were reduced in the basement, second floor, fourth floor, and on the south side of the feed mill, but not in the first and third floors of the feed mill and the roof. It is likely that red flour beetle life stages may have escaped the effects of heat by insulating themselves within feed product residues or reinfested the mill from new products brought into the mill. The efficacy of phosphine fumigation was not monitored in the feed products removed from the mill and brought inside after the heat treatment. It is possible that red flour beetles surviving this fumigation reinfested the mill.

The decreased capture of beetles in traps placed outdoors following heat treatment of the mill suggested movement of insects between these two habitats. Heat treatment did not affect insect numbers on the mill roof, and the source of these insects is unclear. In conclusion, the heat treatment reduced the overall numbers of insect species found inside the feed mill. Despite temperatures reaching >60 C or more on some floors, there was no indication of damage to the electronic equipment and structural features of the mill. The mill was fully operational after temperatures cooled to ambient conditions.

Traps can be used to gauge heat treatment effectiveness. However, our present and past research has shown that insects are captured at levels similar to levels prior to heat treatment very quickly, usually 2-4 weeks. It is unclear whether the captures are from new insects entering the mills or from insects that were not killed during the heat treatment. We suspect that the captures following heat treatment predominantly represents insects coming in from the outside, either on infested raw ingredients or from insects outdoors. The benefits of heat treatment in suppressing insects can be extended by preventing reinfestation from insect species that are active outdoors. Simple practices such as closing mill doors and windows or using positive pressure air flow systems near entrances will help in excluding pest insects from entering the feed mill. Regular sanitation of dump-pit and all mill equipment, followed by a continuous monitoring program, inspection of raw ingredients and processed products, stock rotation, application of residual pesticides to nonfood surfaces, cracks, and crevices, could complement and extend the degree and duration of insect suppression obtained by heat treatment.

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