

EFFECT OF ELEVATED TEMPERATURES ON *TROGODERMA VARIABILE* BALLION
LIFE STAGES

by

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Abstract

Heat treatment of grain-processing facilities involves using elevated temperatures of 50-60°C for 24 h or less to manage stored-product insects. Heat is an alternative to a non-ozone depleting fumigant sulfuryl fluoride, which was registered in the United States in 2004 for disinfestation of grain-processing facilities. In this study, life history traits of the warehouse beetle, *Trogoderma variabile* Ballion, were characterized on ground cat food at 28°C and 65% RH to facilitate harvesting life stages of a specific age for bioassays with heat. Eggs laid by females were observed for daily eclosion. Eggs hatched on days 6 through 10, and the mean proportion for egg hatching was 87%. Larvae hatching from eggs (first instars) were reared on ground cat food and their head capsule widths were measured every 2 d until all larvae became pupae. Head capsule widths indicated six instars and the total larval duration ranged from 28-40 d. Pupae became adults in 3-9 d. Newly eclosed unmated female adults lived 7 d longer than unmated males (16 d), whereas mated males lived 2 d longer than mated females (8 d). Eggs were not observed when food was not provided to male and female pairs. Females started laying eggs 2 d after pairing until the fifth day. The total number of eggs laid by mating pairs in the presence of food ranged from 30 to 135.

Exposure of eggs, young larvae, old larvae, pupae, and adults of *T. variabile* at 46, 50, and 54°C and 15-20% RH for four fixed time periods showed pupae to be generally more heat tolerant than other life stages. At 46, 50, and 54°C, complete mortality of all stages occurred at 1440, 120, and 30 min, respectively. Pupae also were generally more heat tolerant than other life stages during tests in pilot flour and feed mills at Kansas State University and in a commercial grain-processing facility. However, results from pilot and commercial mills were not as conclusive as the results at fixed temperatures in the laboratory.

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Dedication

This thesis is lovingly dedicated to my parents, Shri Ganpati Lal Bunkar and Smt. Keshri Devi, for their encouragement, support, and unconditional love throughout my life.

**Chapter 1 - WAREHOUSE BEETLE BIOLOGY AND ECOLOGY,
HEAT TREATMENTS, AND RATIONALE FOR THE STUDY**

Introduction

The warehouse beetle, *Trogoderma variabile* Ballion (Coleoptera: Dermestidae), is a major pest of packaged and processed stored products. The common name “warehouse beetle” was given by Okumura (1972), who regarded it as the next most serious dermestid pest after the khapra beetle, *Trogoderma granarium* Everts (Cross et al. 1977). Originating in central Asia, this species was first described in the United States by Beal in 1954 (Partida and Strong 1975; Loschiavo 1960). This species was found to be most prominent in western areas infesting a wide variety of seeds and stored products of both animal and vegetable origin (Vincent and Lindgren 1975). This pest is distributed throughout the tropical and subtropical parts of the world, and has been reported infesting 119 different commodities (Hagstrum et al. 2013). Although adults have been reported as occasional feeders, most of the damage to stored products is caused by larvae (Vincent and Lindgren 1975).

Food materials attacked

Commodities infested by *T. variabile* include barley, oats, maize, rice, rye, shelled maize, sorghum, wheat, barley cereal, instant oatmeal, maize cereal, maize meal, noodle, oatmeal, polished rice, rolled barley, rolled oats, wheat feed, wheat flour, wheat germ, white rice, safflower, copra meal, safflower meal, cowpea, kidney bean, lima bean, pinto bean, black eye bean, polished rice, whole wheat flour, alfalfa, Austrian peas, beet, brome, burnet, carrot, clover, corn, cotton, cucumber, dallis grass, dandelion, eggplant, fescue, garbanzos, lettuce, millet, muskmelon, onion, pepper, pumpkin, ryegrass, soybeans, spinach, squash, sudan, sunflower, sweet corn, tomato, vetch, watermelon, pollen and wheat grass. Animal products infested by this species include dead moths, fish meal and animal feeds (cat food, dog food, poultry laying mash,

cattle feed, rabbit feed). Mud dauber nests, spider egg masses and spider webs are the natural habitats for this species (Strong et al. 1959; Hagstrum et al. 2013; Partida and Strong 1975).

Damage caused by the insect

Damage to stored products caused by *T. variabile* usually results in loss of weight and decrease in quality. An infestation of this insect is characterized by the presence of larvae, masses of cast skins, live or dead insects, and fine dust (Vincent and Lindgren 1975). Although dermestid larvae are responsible for damage caused to commodities by feeding, generally all stages are present in an established infestation (Vingent and Lindgren 1975). A single insect only causes a few milligrams of weight loss, whereas populations measured by millions of *T. variabile* individuals can bring considerable weight loss. Emergence holes may be found in whole grains (<http://museumpests.net/wp-content/uploads/2014/03/Warehouse-Beetle.pdf>). Infestation of cereal grains and of seeds of beans and other plants could adversely affect germination as the germ is attacked (Partida and Strong 1975). In grains, it feeds on broken kernels (<http://www.eco2.nl/en/pest/commodity-feeders/warehouse-beetle-trogoderma-variabile>).

Description of life stages

The body of *T. variabile* is typically brownish black in color, oval, about 3.2 mm long (1/8th inch) and covered with fine pubescence. The elytra have distinct reddish-brown maculae which vary considerably in pattern within the species (Loschiavo 1960). The head bears three-segmented antennae with simple setae occurring on the mesal side of the basal segment only (Beal 1954). Six ocelli are present on each side of the head, five forming a crescent-like configuration. The prothorax is the longest of the body segments. The head is very small in relation to the rest of the body and is almost entirely covered by the pronotum. The antennal club

has three to eight segments, joined symmetrically. Males are distinctly smaller than females. The ratio of width to length is 1:1.8 in females and 1:1.7 in males (Loschiavo 1960). Unlike many *Trogoderma* species, the adult beetles are capable of flight (Wright and Morton 1995). Loschiavo (1960) also observed that adults normally crawl but if handled roughly or upon reaching the edge of a plane surface, adults spread their wings and fly in an almost vertical ascent. The adults are short-lived, and display concealment/exposure behavior, remaining hidden and inaccessible most of the time but emerging for purposes of mating, oviposition and dispersal for a certain period of the day, termed the exposure period (Shapas and Burkholder 1978; Wright and Morton 1995). The flight behavior of females is very different to that of male. *T. variabile* females fly most of the daylight hours, while males fly for several hours after sunrise (Wright and Morton 1995). Adults are inactive at temperatures below 15°C. Loschaivo (1960) reported that adults do feed but did not require food to lay eggs (Loschiavo 1967). The overall sex ratio of adults (male:female) is about 1.2:1 (Partida and Strong 1975).

The eggs of *T. variabile* are pearly-white, translucent, extremely fragile and covered with a sticky secretion. After two or three days it is less fragile and can be handled safely with a soft-haired brush. A typical egg is cylindrically elongate and bears a number of hairs at one end; a few eggs are banana-shaped. The length of the egg ranges from 0.54 to 0.71 mm and width ranges from 0.23 to 0.30 mm. The developing larva can be clearly visible through the transparent egg membrane about a day before hatching and gives the egg a reddish-brown appearance. Loschiavo (1960) observed five distinct brown spots or ocelli on each side at the anterior end of the egg. The posterior end was dark brown owing to the coiled, long, simple hairs of the last abdominal segment. The segments and erectile hairs on the dorsal surface were clearly visible in the developing larva. Shortly before hatching bulging movements occurred at different locations

on the surface of the egg. The egg ruptures at the anterior end. When inspected microscopically, the chorion appeared sculptured like a peanut shell (Loschiavo 1960).

Loschiavo (1960) stated that first instars emerge their head from eggs first and gradually pushing themselves from the egg case with legs and stopping periodically to rest. As the larva emerges, the long brush-like “tail” slowly unwinds. After emergence the larva crawls about actively in search of food. The larva has, on the last abdominal segment, a number of long simple hairs directed posteriorly to form a long “tail” increasing in length with each succeeding instar. Loschiavo (1960) reported the mean length of the “tail” in the first-, second-, third-, fourth-, fifth-, and sixth-instar larva to be 1.6, 0.9, 0.6, 0.5, and 0.4 times the mean body length respectively. Simple hairs projecting laterally and dorsally occur over the entire surface of the body; barbed hairs (hastisetæ) occur in paired tufts on the body segments and in fourth, fifth, and sixth instars are particularly dense on the fifth, sixth, seventh and eighth abdominal tergites. These features also occur in *Trogoderma granarium* Everts and *Trogoderma versicolor* Cruetzer (Hadaway 1956). The first-instar larva is yellowish-white but later instars are reddish-brown and usually dark brown just before molting. During molting the skin splits along the mid-dorsal line from the head to about the sixth abdominal segment and the larva crawls out leaving behind the cast skin. Larvae are active feeders and have strong mouth parts capable of chewing holes through acrylic plastic 1.7 mm thick. From the first- to the fourth- instar, larvae cast their skins on the surface of the food and penetrate not more than four millimeters into it; subsequent instars tunnel through the food and cast one out of three skins beneath the surface. Loschiavo (1960) observed that the greatest increase in size occurred between the third and fourth instars. The duration of each larval instar was progressively longer. The larval period was normally completed in six instars but many fully-developed larvae moulted 28 times in 11 months and did

not become pupae. These larvae were considered to be in diapause. At this stage, larval development was complete but larvae continued to molt at irregular periods. Daily disturbance and handling is an important factor in diapause. Burges (1961) found that the amount of space was an important factor affecting the incidence of diapause and larvae were in diapause stage as long as 2 years. Partida and Strong (1975) observed diapause in larvae when insects were reared singly and in groups, although a higher percentage of larvae reared individually failed to pupate. It appears likely that diapause in larvae of *T. variabile* has a complex genetic basis, and the phenomenon may be induced by several variables. Development of larvae stops when the temperature falls below 21.1°C or above 35°C (Partida and Strong 1975).

The pupal stage is passed in the last larval skin. At the last molt the larval skin splits dorsally but is not cast off. The pupa, being wider than the larva, forces the skin part, leaving the dorsal surface visible through the gap. It has a thin transparent skin which, at adult emergence, is passed to the posterior end of larval skin. More than 90% pupae are found at or near the surface of the food. The male pupa ranges from 4.31 to 4.62 mm in length and from 1.53 to 1.80 mm in width respectively. Female pupa ranges from 6.24 to 6.69 mm in length and 2.42 to 2.69 in width respectively. Males are distinctly smaller than females (Loschiavo 1960).

Effect of temperature and relative humidity on immature development and survival

Temperature is the single most important factor affecting development and survival of *T. variabile*. Partida and Strong (1975) reported that temperature of 35°C approached the upper limit for physiological development. Low temperatures slowed development and reproductive activity, and humidity effects varied with temperature. Loschaivo (1960) stated that development and hatching of eggs occur between 17.5 to 37.5°C. Partida and Strong (1975) indicated that the most severe temperature and humidity effects were observed at 37.8°C and 30% RH, where 20%

of the larvae were dead on the fourth day and 100% mortality was observed after the 35th day. No larvae died at 37.8°C and 50% RH. Temperatures of 35 and 21.1°C approached the upper and lower limits for larvae to develop. Pupation was earliest at 32.2°C and 70% RH, and larval development from the time of hatch to pupation took progressively longer at temp below 32.2°C. Development was fastest at 37.8°C and 50% RH, but a low number of insects pupated and reached adult stage at this temperature and humidity. The optimum humidity for larval development and pupation is 50%. Higher humidity prolonged the egg duration and lower humidity caused higher mortalities in larval development. These two environmental variables had more influence on the length of the pupal stage than on adult maturation.

Sex pheromone, mating, and reproduction

Pheromones are chemical messengers that influence the behavior or physiology of *T. variabile*. In general, pheromones produced by the female are attractive to male adults of the same species, while male pheromones are aphrodisiacs and are not attractive to females. Female sex pheromones are active over longer distances than those of males. Cross et al. (1977) observed that the calling activity was largely restricted to 7-h interval with a maximum at 2-4 h after light onset. Abdominal elevation by *T. variabile* females was considered part of a pattern of postural activities accompanying sex pheromone release. Like other *Trogoderma* pheromones, that of *T. variabile* has several components, of which the main attractant is (Z)-14-methyl-8-hexadecenal. Secretory epithelium on the inner surface of 7th abdominal sternites, is considered the site of sex pheromone production in *T. variabile* (Hammack et al. 1973).

Loschiavo (1960) indicated that copulation occurs shortly after emergence. Mating in *T. variabile* may be mediated by contact chemoreception of chemicals dissolved in the cuticular wax of the females, but which are not major components of the airborne pheromone, or by non

chemical means (Cross et al. 1977). Partida and Strong (1975) stated that males never fertilized more than one female during a 24-h period and more than 6 females per male can cause masking effect. Females do not require multiple matings to lay a full complement of eggs, but fertile eggs are laid by mated females only.

Loschiavo (1967) stated that a suitable oviposition site, which provided crevices for insertion of the ovipositor, was more important than food in stimulating egg production. Partida and Strong (1975) also noted that females extended their ovipositor and seemed to prefer placing eggs in crevices under the surface of loose food. Loschiavo (1960, 1967) reported that females laid eggs after a pre-oviposition period of one to two days and maximum egg production occurred in the first three days after emergence, followed by a rapid decline thereafter. They also reported that maximum oviposition occurred among females that were three to five days old.

Lethal effects of low and high temperatures

Larvae of *T. variabile* are very resistant to low temperatures. (Loschiavo 1960) reported that a six day exposure at -1, 10 and 20°C to mature larvae did not kill them. A one-week exposure at 10°C prevented oviposition, but did not affect the survival of young mated females. At 20°C many eggs were laid but none of these hatched. Brower and Tilton (1972) stated that all adults were sterilized when irradiated at 30 krad or above. Adult females were more sensitive to the sterilizing effects of gamma radiation than were males. Development of adults from treated eggs and larvae was prevented by 5 krad but some adults emerged from pupae at all treatment levels, with no reproduction after exposure at 30 krad or above. In a previous study in Australia, large larvae of *T. variabile* were found to be the most heat tolerant stage, based on experiments in a growth chamber at 56°C and 0% RH (Wright et al. 2002). A 4-min exposure at this temperature resulted in 79% mortality of large larvae, whereas mortality of eggs, diapausing larvae, pupae,

and female adults was 93-100%. They did not report the susceptibility of young larvae of *T. variabile* to elevated temperatures. Furthermore, Wright et al. (2002) developed a degree minute model using constant temperature data at 50, 52, 54 or 56°C for predicting mortality of large larval instars of *T. variabile*. The base temperature for accumulating degree minutes, and the intercept and slope values of the linear regression of mortality (expressed as the inverse of the standard normal deviate) against degree minutes were different at each of the four temperatures. Despite these differences, Wright et al. combined data across 52, 54 and 56°C to describe the relationship between mortality and degree minutes without giving any statistical or biological basis, even though the intercepts and slopes of the linear regressions at the three temperatures were different.

There is limited information on the effects of high (elevated) temperatures on the survival of life stages of *T. variabile*. Therefore, I conducted field and laboratory experiments, reported in this thesis, to determine the impact of elevated temperatures on the survival of *T. variabile* life stages. The first part of my research involved a careful study of the biology of *T. variabile* on ground, cat food at 28°C and 65% relative humidity to enable me to harvest specific stages for exposure to elevated temperatures. The second part of my research involved exposure of eggs, young larvae, old larvae, pupae, and adults of *T. variabile* to elevated temperatures to identify a heat tolerant stage. The work reported on this thesis forms a valid basis for use of elevated temperatures for management of *T. variabile* life stages in grain-processing facilities.

Chapter 2 - BIOLOGY OF THE WAREHOUSE BEETLE
***TROGODERMA VARIABILE* BALLION ON GROUND CAT**
FOOD

Introduction

Stored-product insects associated with grain-processing facilities are best managed with fumigants, aerosols, residual products and heat. The concept of heating the ambient air of the whole, or a portion of the facility, to 50 to 60°C and maintaining these temperatures for up to 24 h or less is termed as “heat treatment”. Heat treatments have been used effectively against stored-product insects for hundred years (Dean 1911). The phase out of methyl bromide in the USA in 2005, because of its adverse effects on stratospheric layer, made heat treatment a viable alternative to fumigation with methyl bromide. Since 1999, the research group in the Department of Grain Science and Industry, Kansas State University, Manhattan, KS, has been generating data on utilizing heat treatments as a non-chemical alternative to methyl bromide fumigation for management of stored-product insects associated with food and feed processing facilities (Mahroof et al. 2003a, b; 2004, 2005a, b; Roesli et al. 2003, Boina and Subramanyam 2004, Mahroof and Subramanyam 2006, Boina et al. 2008, Yu et al. 2011). Previous research at Kansas State University focused on evaluating susceptibility of various life stages of the red flour beetle, *Tribolium castaneum* (Herbst) (Mahroof et al. 2003a,b), confused flour beetle, *Tribolium confusum* (Jacquelin du Val) (Boina and Subramanyam 2004), Indian meal moth, *Plodia interpunctella* (Hübner) (Mahroof and Subramanyam 2006), and cigarette beetle, *Lasioderma serricorne* (F.) (Yu et al. 2011) to elevated temperatures. Quantitative data are limited (Wright et al. 2002) on susceptibility of various life stages of the warehouse beetle, *Trogoderma variabile* Ballion (Coleoptera: Dermestidae), exposed to elevated temperatures used for structural disinfestations.

In order to extract large numbers of insects of a specific life stage for exposure to elevated temperatures, it was very important to first conduct a detailed biological study of *T. variabile* on a suitable diet. The development and reproduction of *T. variabile* is influenced by

the food substrate (Partida and Strong 1975), and detailed information on the development and reproduction of this species on processed animal feed is lacking. Therefore, laboratory tests were conducted at 28°C and 65% RH to determine certain life history parameters, such as egg hatchability, duration and survival of immature stages, number of instars, adult longevity, and number of eggs laid by females. The processed animal feed (cat food) was finely ground and sifted for easy separation of life stages from the food substrate.

Materials and Methods

Insect diet

The diet used for rearing *T. variabile* at controlled environmental conditions and for all experiments was processed animal feed, Brand Meow Mix, manufactured by Big Heart Pet Brands, Topeka, KS, was obtained from local grocery store. This pet food contained crude protein (31.0% min.), crude fat (11.0% min.), crude fiber (4.0% max.), moisture (12.0% max.), calcium (1.0% min.), phosphorous (0.8% min.), selenium (0.125 mg/kg min.) and vitamin E (50 IU/kg min.). The major ingredients of the diet include: ground yellow corn, corn gluten meal, chicken by-product meal, soybean meal, beef tallow, turkey by-product meal, salmon meal, ocean fish meal, minerals (ferrous sulfate, zinc oxide, manganese oxide, copper sulfate, calcium iodate) and vitamins (B1, B2, B6, K, D3, B12). The cat food was stored in a freezer (-13°C) until use in experiments. For use in experiments, the cat food was thawed for 3 hours on a laboratory bench, and milled using table top Roller Mills (Serial No. 906, 907; Size 9X6; Ross Machine and Mill Supply Incorporation, Oklahoma City, OK.) in the Milling Laboratory, Department of Grain Science and Industry, Kansas State University, Manhattan, KS. For milling, two pair of rolls was used, the first pair of rolls was kept as apart as possible (> 0.457 mm) and the second rolls were at a distance of 0.125 mm. The ground cat food was sifted with 250 µm sieve for easy separation

of life stages from the diet required for further experiments. About 350 g of diet was placed in 0.94-liter glass jars, with wire-mesh and filter paper lid, and seeded with 200 *T. variabile* adults to start the cultures. Starting cultures of *T. variabile* reared on rolled oat, were obtained from United States Department of Agriculture, Center for Grain and Animal Health Research, to obtain large cultures on ground cat food. . All cultures were reared at 28°C and 65% RH in growth chambers (Model I-36 VL, Percival Scientific, Perry, IA).

Collection and rearing of eggs

To collect eggs, 60 male and female adults of *T. variabile* were placed in 150-ml plastic containers containing 10 g of the wheat flour sifted through a U.S. Standard Sieve No. 80 (Hogentogler & Co., Inc., Columbia, MD), with 180 µm openings. After 3 days adults were separated from the flour using sieves of two different mesh sizes and a bottom pan. The top sieve had 600 µm openings (U.S. Standard Sieve No. 30, Seedburo Equipment Company, Chicago, IL.) and the bottom sieve had 180 µm openings. Adults were collected on the top sieve, and the eggs were retained on the 180 µm sieve, while the wheat flour passed through the second sieve into the bottom pan. Eggs were gently removed from the sieve using a camel's hair brush into 9-cm glass petri dishes. Eggs (20) were placed in 9-cm glass petri dishes using camel's hair brush and kept in a growth chamber set at 28°C and 65% RH. Eggs were examined daily until the emergence of first instars to record duration and egg hatchability.

Determining number of instars

In a related experiment, newly hatched larvae (0-1d old) were removed from petri dishes using a very fine hair brush and placed in 30-ml plastic condiment cups similar to that used by Subramanyam et al. (1985) with 3 g of the diet per cup. Total 120 cups were used in the study. After placing 10 larvae in each cup, the cups were covered with a parafilm (American National

Can, Menasha, WI) and then holes were punched with a pin for air diffusion. All infested cups were incubated at 28°C and 65% RH. The cat food used in this experiment was pre-sifted using a 250 µm sieve (Fisher Scientific, Pittsburg, PA), for easy separation of first instars from the diet. Every 2 d, 3 cups were chosen and one larva from each cup was kept in 95% ethanol for further observations. The measurements for head capsule width were made under ethanol by pinning abdomens of larvae such that the dorsal region of the head capsule was clearly visible. The widest portion of the head capsule was measured using a stereomicroscope (Model SMZ 1000, Nikon Corporation, Japan), fitted with an ocular micrometer, at 8X magnification.

Determining adult longevity and female oviposition

When the larvae from the above experiment started to pupate, the food was sifted using a sieve with 600 µm openings, for collection of pupae from the diet. Individual pupae were kept in glass vials (1 cm dia., 4.4cm length and 4 ml. cap.) and covered with a parafilm. Holes were made with a pin in the parafilm for air diffusion. Pupae were examined daily until the emergence of adults to record duration of pupal stage and determine pupae to adult survivorship.

When adults emerged from pupae, they were sexed using differences in their antennae shown in Fig.2.1 (International Standards for Phytosanitary Measures, ISPM 27 Diagnostic Protocols, DP: 3 *Trogoderma granarium* Everts, 2012). A total of 27 unmated male adults and 120 unmated female adults were placed individually in the glass vials and checked daily for longevity.

Additionally, one male and one female were paired and placed in 30 ml plastic condiment cups containing 1 g of the diet sifted using 180 µm sieve, and covered with parafilm with pin holes (total 11 pairs). In another experiment, one male and one female were paired and placed in 30 ml plastic condiment cups without diet and covered with parafilm (total 13 pairs). Cups were

examined daily to record whether adults were alive or dead and for counting number of eggs laid by the females. To count number of eggs laid, the diet in each cup was sifted through a sieve with 180 μm openings to separate eggs from the diet as explained above. Fresh diet (1 g) was placed in cups and checked daily until all the adults were dead. A new cup was also made and checked daily for the mating pair without diet. The objective here was to determine the difference in egg production with diet and without diet and also the effect of diet, sex and mating on adult longevity.

Data analysis

Means and standard errors (SE) for the duration of each immature stage and for the egg-to-adult development were calculated using the Statistical Analysis System (SAS Institute 2003). The number of individuals surviving at each stage was expressed as a percentage. The egg hatchability and frequency distribution of head capsule widths by instar was plotted using Sigma Plot 12.5 (Systat Software, Inc., San Jose, CA). Differences in head capsule widths among instars were determined by subjecting data to DISCRIM procedure of SAS (SAS Institute 2003). One-way analysis of variance (ANOVA) and Ryan-Einot-Gabriel-Welsch multiple range test at $\alpha = 0.05$ level was used to determine significant differences among instars using the GLM procedure of SAS (SAS Institute 2003). This test was also performed on the longevity data to determine significant differences among various treatments on longevity.

Results

Development and survival of immature stages

Out of the 180 eggs, 87.2 % hatched, and the mean duration of this stage at 28°C and 65% RH was 7.6 days. Eggs started hatching on the sixth day and continued hatching till the

tenth day with a maximum percentage of hatching (53.3 %) on seventh day (Fig. 2.3). Measurement of head capsule width and length indicated six discrete instars (Table 2.2). There were significant differences in head capsule widths among the instars ($F= 8709.28$; $df= 5,410$; $P < 0.0001$) and the widths among the instars were significantly different from one another (Table 2.2). The first four instars took approximately 6 d each, whereas the fifth instar took 8 d and also there was overlap of time in fifth and sixth instar (Table 2.1). The number of misclassified observations was very small (4 out of 418 = 0.9%). In the fourth instar range only one observation (1.3% of 76) was misclassified as third instar and in the fifth instar range three observations (3.9% of 77) were misclassified as sixth instars. Pupae became adults in 5.8 days with a survivorship of 98% and there was no significant difference of time to become adult from pupa, between male and female since the confidence interval for male and female overlapped. Pupation occurs mostly on the surface of food. The total egg-to-adult development on ground, cat food took 40 to 58 days (Table 2.1).

Adult longevity

In general, the longevity of both mated male and female adults was lower than unmated males and females (Table 2.3). Both sex and mating had significant effect on adult longevity ($F = 87.86$; $df = 5,189$; $P < 0.0001$). Unmated females lived approximately three times longer (23.4 d) than mated females (8.7 d), and this difference was significant ($P < 0.0001$). When no diet was provided to the mating pair, female longevity was significantly higher than male longevity ($P < 0.0001$). However, when diet was provided to the mating pair, males lived for a longer period of time, but this difference is not statistically significant. Unmated females always lived longer than mated females or unmated males.

Female oviposition

Among the 26 mated pairs with diet, females started laying eggs on the second day after pairing with males and continued to lay eggs for five days. The maximum number of eggs was laid on the second day after mating. Table 2.4 shows that egg production was significantly higher with diet when compared to egg production without diet ($F= 76.67$; $df = 1, 42$; $P < 0.0001$). Females, on average, laid 62.7 eggs with a mean daily egg production of 20.9 eggs. When laying eggs females extended their ovipositor and seemed to prefer placing eggs under the surface of loose food. Eggs were usually deposited singly, but occasionally they were found in short chains of 3 to 4 eggs also.

Discussion

Loschiavo (1960) reared *T. variabile* on mixture of equal parts of finely-ground wheat and bran at 32°C and 70 % RH, and observed the egg, larval, and pupal periods to be about 6.9, 34, and 4.1d, respectively. On ground cat food, the egg, larval and pupal periods were 7.6, 33 and 5.8 d, respectively, at 28°C and 65% RH. Given that the rearing conditions used by Loschiavo (1960) and in this study were different, the similarity in immature developmental times for *T. variabile* on mixture of finely-ground wheat and bran and ground, cat food suggests that the latter is also an optimal diet for rearing this species in the laboratory.

Development of *T. variabile*, especially the larval stage, is influenced by the food substrate. Partida and Strong (1975) studied the influence of different kinds of food on populations of *T. variabile* (see Table 2.5). Different kinds of food were infested with 10 pairs of young adults or 30 large larvae and populations were counted after 6, 12 and 18 weeks of infestation. Larval development was slow on raisins and shelled nuts, and the original stock of large larvae tended to remain in the larval stage on these foods. Rolled barley and oats were more

suitable hosts than the corresponding whole cereal grains. As a group, processed animal feeds were better host materials than foods found in any other category. Based on this finding, cat food was chosen as a diet for mass rearing of *T. variabile* cultures.

In our study, six discrete instars were identified based on the head capsule widths. Loschaivo (1960) also reported six instars in his study based on the measurements of head capsule. Partida and Strong (1975) found that males usually completed 5 molts and females 6 before pupation. Based on our study two instars: third instars (young larvae) and fifth instars (old larvae), were selected for exposure to elevated temperatures (see Chapter 3). Young larvae and old larvae that were 12-14 d and 20-26 d old after eclosing from the eggs were used in experiments with elevated temperatures.

Loschiavo (1960) reported that adults live from 8 to 20 days, the mean life span being 14.3 days at 32°C and 70 % RH. When young adults were exposed to 10°C for seven days, no mortality was observed but oviposition was prevented. However, when adults were exposed to 20°C, they laid 399 eggs but none of these hatched. Partida and Strong (1975) reported that adult longevity increased with decreases in temperature explaining that exposures at low temperatures slows the rate of metabolism and thereby increases longevity. Loschiavo (1967) reported that at temperatures below 12.8°C longevity decreased rapidly. Adult longevity was found to be affected by sex and mating in our study. Unmated male and female adults lived for 7-33 d and mated adults lived for 6-25 d. Unmated females usually lived longer than unmated males and mated females, but when diet was provided to the mating pair, males lived for a longer period of time indicating that females laid eggs in the presence of food. In the temperature range associated with high egg production, productive females probably required considerable energy for synthesis (Loschiavo 1967). This view is supported by the fact that ovipositing females had

much shorter life spans than non-productive females. Mated females had a shorter life span than males at all temp except mean temperature of 15.6°C (Partida and Strong 1975). Loschiavo (1960) found that at 32°C, high egg production was associated with high adult mortality, particularly among females.

In a study by Loschiavo (1967) the lengths of the preoviposition and oviposition period were found to be inversely related with temperature. At 17.5 and 27.5°C, the preoviposition and oviposition periods were 16.5, 10.2, 0.7 and 6.3 days respectively. The lengths of preoviposition and oviposition periods in our study at 28°C and 65% RH were, 2 and 3 d respectively which were close to 2.5 and 3.9 d for preoviposition and oviposition period at 26.7°C reported by Partida and Strong (1975). On ground cat food, 26 females of *T. variabile* laid 30 to 135 eggs during their life time with mean daily egg production of 20.9 eggs which is very close to 20.2 eggs at 27.5°C reported by Loschiavo (1967). Partida and Strong (1975) stated that egg production by mated females was extremely variable among individuals, even under the most favorable conditions. Loschiavo (1968) in a study found that a suitable oviposition site, which provided crevices for insertion of the ovipositor, was more important than food in stimulating egg production. As similar to Partida and Strong (1975) study, virgin females were found to lay eggs, but none of these eggs hatched.

In summary, our results suggest that ground cat food to be an optimal laboratory diet for mass rearing *T. variabile* at 28°C and 65% RH based on the speed of development of immature stages and number of eggs laid by mated females. Characterizing stage-specific development and adult longevity of *T. variabile* on ground cat food in the laboratory enabled us to harvest eggs, young larvae, old larvae, pupae and adults of specific age in large numbers for detailed studies at elevated temperatures.

Figure 2.1Antennae of *Trogoderma variabile* adults used for sexing male and female in the adult stage.



Male



Female

Table 2.1 Development and survival of *T. variabile* immature stages on ground cat food.

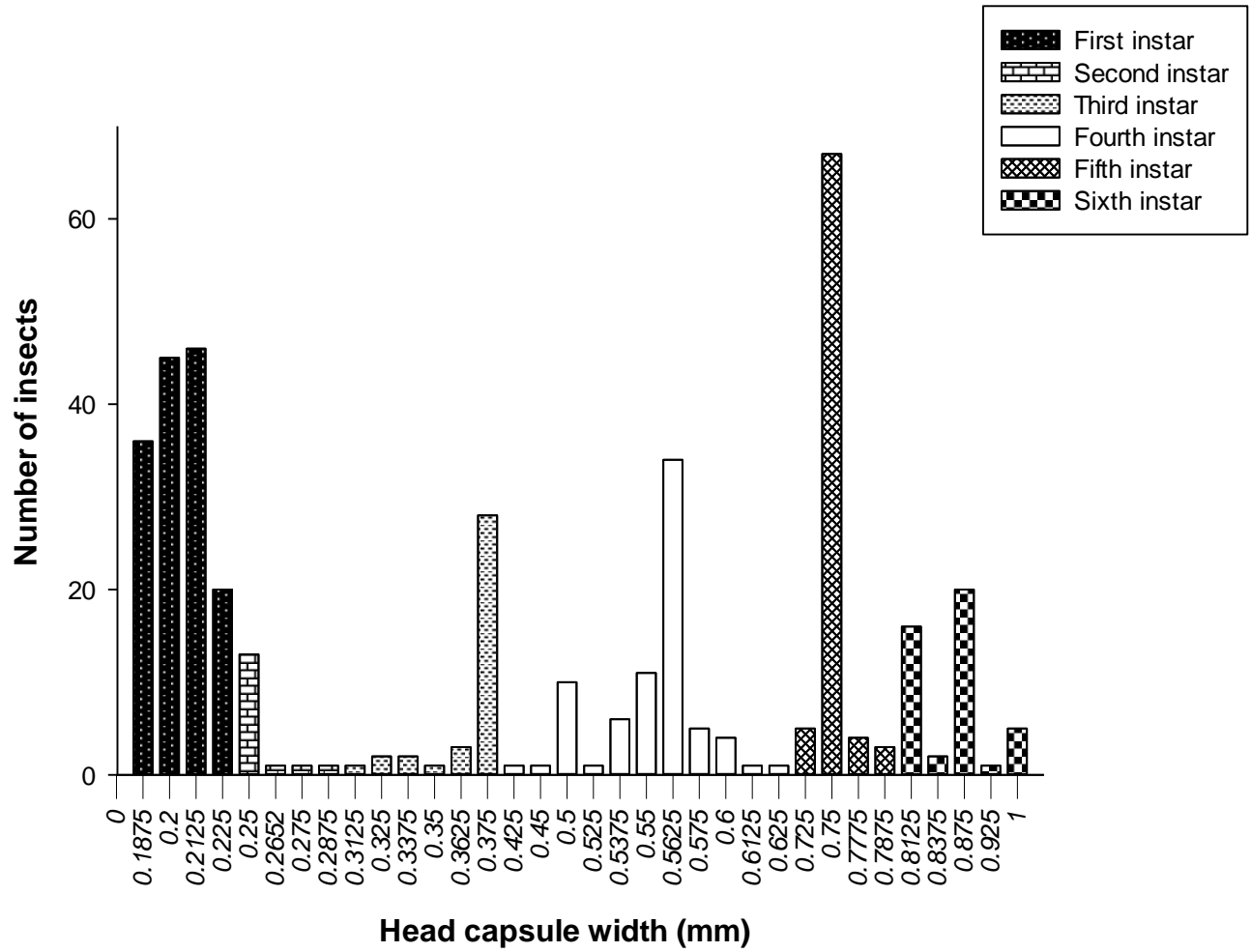
Life Stage	Number of insects	Median development time (range) in days
Egg	180	8 (6 – 10)
First instar	83	4.5 (4 – 5)
Second instar	83	5.5 (5 – 6)
Third instar	55	4 (3 – 5)
Fourth instar	76	5.5 (5 – 6)
Fifth instar	77	7.5 (7 – 8)
Sixth instar	44	8.5 (5 – 12)
Pupae	147	5.5 (5 – 6)
Egg-to-adult	745	49 (40 – 58)

Table 2.2 Head capsule width and length measurements of *T. variabile* instars.

Instar	Number of insects	Head capsule width (mm) ^a	Range (mm)	Length (mm)	Range (mm)
		Mean \pm SE		Mean \pm SE	
First	83	0.19 \pm 0.01a	0.19 – 0.22	0.92 \pm 0.35	0.72 – 3.90
Second	83	0.26 \pm 0.01b	0.25 – 0.29	1.47 \pm 0.32	0.95 – 2.50
Third	55	0.37 \pm 0.01c	0.31 – 0.37	2.26 \pm 0.38	1.5 – 2.85
Fourth	76	0.55 \pm 0.03d	0.42 – 0.62	3.50 \pm 0.68	1.5 – 4.50
Fifth	77	0.75 \pm 0.01e	0.72 – 0.79	3.90 \pm 0.44	2.8 – 4.75
Sixth	44	0.86 \pm 0.05f	0.81 – 1.00	4.34 \pm 0.43	3.4 – 5.50

^aMeans followed by different letters are significantly different ($P < 0.05$, by REGWQ test).

Figure 2.2 Frequency distribution of head capsule widths of six instars of *T. variabile* reared on ground cat food.



Based on 415 larvae.

Table 2.3 Longevity of unmated and mated *T. variabile* adults.

Treatment	Number of individuals/pairs	Mean \pm SE Longevity (d) ^a
Unmated female	120	23.5 \pm 0.3a
Unmated male	27	16.7 \pm 0.9b
Mated female with diet	11	8.7 \pm 0.4c
Mated male with diet	11	10.7 \pm 0.6c
Mated female without diet	13	18.6 \pm 1.5b
Mated male without diet	13	8.5 \pm 0.7c

^a Means followed by different letters are significantly different ($P < 0.05$; by REGWQ test).

Table 2.4 Egg production of mated *T. variabile* females with diet and without diet.

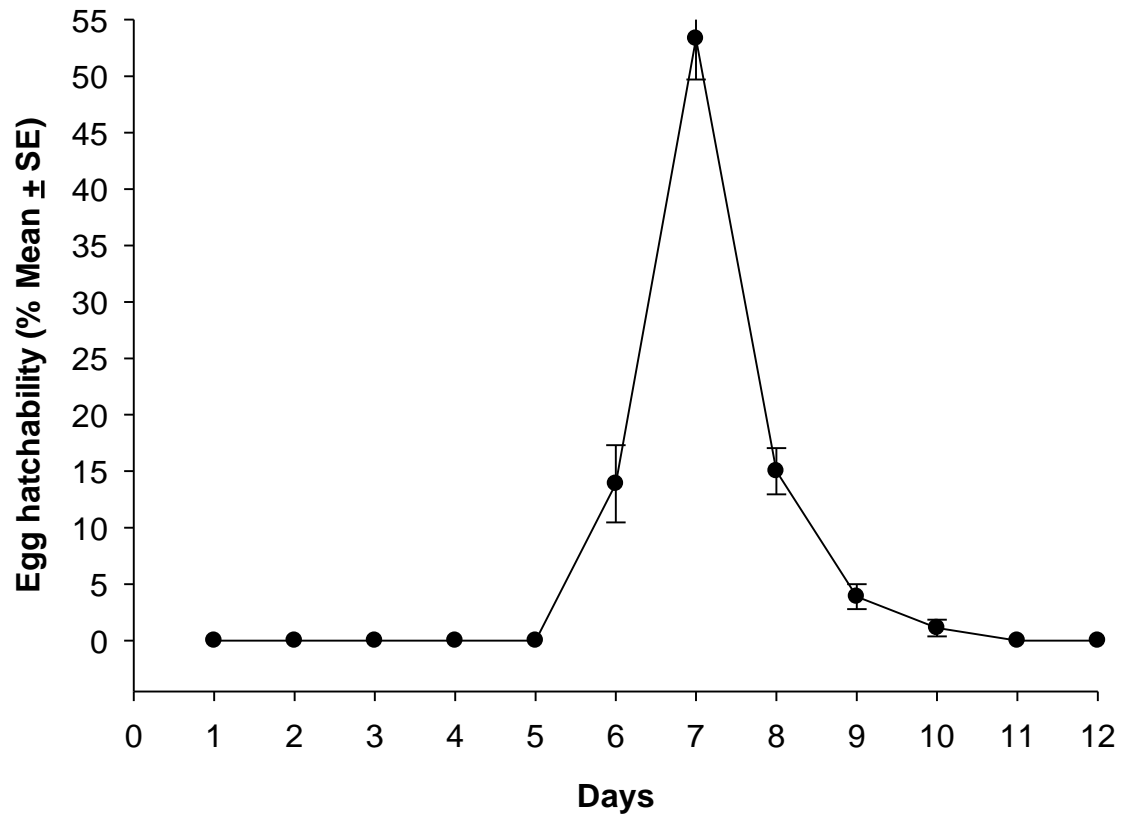
Treatment	Total number of eggs	Number of pairs	Mean \pm SE Eggs/female ^a	Mean \pm SE oviposition period (d)	Eggs/female per day
Without diet	23	18	1.3 \pm 2.6	1 \pm 0.0	1.2
With diet	1632	26	62.8 \pm 29.6	3 \pm 0.8	20.9

^a $F = 76.67$; $df = 1, 42$; $P < 0.0001$ (ANOVA Type III SS)

Table 2.5 Influence of different types of food on populations of *T. variabile*. (Source: Partida and Strong1975)

Food	Multiple of original number of weeks after infestation with					
	10 Adults			30 Large larvae		
	6	12	18	6	12	18
Barley	30	29	141	10	28	40
Milo	28	11	14	24	--	16
Oats	31	32	18	11	--	16
Rye	32	26	15	9	25	--
Wheat (western)	35	32	80	16	23	36
Cat food (friskies)	38	20	262	26	--	41
Dog food (kibbled)	38	270	228	29	23	125
Poultry laying mash	45	35	150	12	125	104
Rolled barley	39	116	394	27	35	107
Rolled oats	38	47	244	21	21	65

Figure 2.3 Egg hatchability of *T. variabile* eggs over days at 28°C and 65 % RH.



Total number of eggs is 180.

**Chapter 3 - EFFECT OF ELEVATED TEMPERATURES ON
TROGODERMA VARIABLE BALLION LIFE STAGES**

Introduction

The use of elevated temperatures, also termed heat treatments, has long been documented as an effective approach for managing stored-product insects infesting food-processing facilities (Mahroof et al. 2003a). It is becoming popular as a methyl bromide alternative because of the phase out of methyl bromide in 2005 in the United States (Boina and Subramanyam 2004, Dosland et al. 2006). During heat treatment the ambient temperature of the entire facility, or a portion of it, is raised to 50-60°C, and these elevated temperatures are held for 24 h or less to facilitate heat penetration throughout the entire space for effective disinfestation.

Time-mortality relationships at constant elevated temperatures were described for eggs, young larvae, old larvae, pupae and adults of the red flour beetle, *Tribolium castaneum* (Herbst) (Mahroof et al. 2003a); confused flour beetle, *Tribolium confusum* (Jacqueline du Val) (Boina and Subramanyam 2004); Indian meal moth, *Plodia interpunctella* (Hübner) (Mahroof and Subramanyam 2006); cigarette beetle, *Lasioderma serricorne* (Fabricius) (Yu et al. 2011), and drug store beetle *Stegobium paniceum* (Abdelghany et al. 2010). The most heat tolerant stage based on these studies identified the following: young larvae (first instars) for *T. castaneum*, *S. paniceum*; old larvae for *T. confusum* and *P. interpunctella*; and eggs for *L. serricorne*. Among all the insect species and stages tested, the young larvae of *T. castaneum* were found to require longer exposure times at temperatures of 50-60°C (Abdelghany et al. 2010 and Subramanyam et.al 2011). However, during actual facility heat treatments, where temperatures are dynamically changing over time, the young larvae were not found to be heat tolerant (Mahroof et al. 2003b). In a more recent study (Brijwani et al. 2012) reported adults of *T. castaneum* to be the most heat tolerant stage during heat treatment of pilot flour mill. Yu et al. (2011) also reported eggs to be heat tolerant at constant elevated temperatures, but this finding

was difficult to confirm during commercial facility. Heat tolerance among life stages within a species may be related to heating rates during commercial heat treatments (Subramanyam et al. 2011). Therefore, it is important to conduct experiments at constant elevated temperatures and during commercial heat treatments to definitively identify a heat tolerant stage. It is also essential to confirm which stage is heat tolerant at different heating rates, which can occur in different locations during commercial heat treatments (Subramanyam et al. 2011).

Limited quantitative data are available on relative susceptibility of life stages and time-mortality relationships for the warehouse beetle, *Trogoderma variabile* Ballion, an important pest associated with food-processing facilities (Campbell and Mullen 2004; Arthur et al. 2014), exposed to elevated temperatures. In a previous study in Australia, large larvae of *T. variabile* were found to be the most heat tolerant stage, based on experiments in a growth chamber at 56°C and 0% RH (Wright et al. 2002). A 4-min exposure at this temperature resulted in 79% mortality of large larvae, whereas mortality of eggs, diapausing larvae, pupae, and female adults was 93-100%. They did not report the susceptibility of young larvae of *T. variabile* to elevated temperatures. Furthermore, Wright et al. (2002) developed a degree minute model using constant temperature data at 50, 52, 54 or 56°C for predicting mortality of large larval instars of *T. variabile*. The base temperature for accumulating degree minutes, and the intercept and slope values of the linear regression of mortality (expressed as the inverse of the standard normal deviate) against degree minutes were different at each of the four temperatures. Despite these differences, Wright et al. combined data across 52, 54 and 56°C to describe the relationship between mortality and degree minutes without giving any statistical or biological basis, even though the intercepts and slopes of the linear regressions at the three temperatures were different. The studies described in this paper were conducted using eggs, young larvae, old larvae, pupae,

and adults of *T. variabile* exposed to three constant elevated temperatures at fixed times, and in pilot scale and commercial facilities subjected to heat treatments. The three constant temperatures selected were 46, 50 and 54°C. The elevated temperatures tested ($\geq 46^\circ\text{C}$) were well above the range (21.1- 35°C) for development and survival of *T. variabile* (Partida and Strong 1975). Although 50°C is the minimum temperature required for effective disinfestation (Wright et al. 2002, Roesli et al. 2003, Boina et al. 2008), vertical and horizontal stratification of temperatures during heat treatment may result in temperatures below or above 50°C in some portions of the facility (Dosland et al. 2006). Therefore, temperatures between 46 and 54°C were selected for this study. Our objective was to determine the relative susceptibility of *T. variabile* life stages at constant temperatures in the laboratory and during facility heat treatments, where temperatures are dynamically changing over time (Subramanyam et al. 2011).

Materials and Methods

Insect cultures and collection of life stages

Cultures of *T. variabile* were reared on ground, cat food (Brand Meow Mix, Big Heart Pet Brands, Decatur, AL, USA) obtained from a local store. After thawing, cat food was milled and sifted using table top Roller Mills (Serial No. 906, 907; Size 9X6; Ross Machine and Mill Supply Incorporation, Oklahoma City, OK) in the Milling Laboratory, Department of Grain Science and Industry, Kansas State University, Manhattan, KS. For milling, two pair of rolls was used, the first pair of rolls was kept as apart as possible (> 0.475 mm) and the second pair of rolls was at a distance of 0.125 mm. This ground diet was sifted for 1 minute with 250 μm sieve. About 350 g of diet was placed in 0.94-liter glass jars, with wire-mesh and filter paper lid, and seeded with 200 adults to start the cultures. All cultures were reared at 28°C and 65% RH in growth chambers (Model I-36 VL, Percival Scientific, Perry, IA).

Field and laboratory experiments were conducted using various life stages of *T. variabile*. To collect eggs, 60 male and female adults of *T. variabile* were placed in 150-ml plastic containers containing 10 g of the wheat flour sifted through a U.S. Standard Sieve No. 80 sieve (Hogentogler & Co., Inc., Columbia, MD), with 180 μm openings. After 3 d adults were separated from the flour using sieves of two different mesh sizes; the top sieve had 600 μm opening and the bottom sieve had 180 μm openings, with a bottom pan. Adults were collected on the top sieve, and the eggs were retained on the 180 μm sieve, while the wheat flour passed through the second sieve into the bottom pan. Eggs were gently removed from the sieve using a camel's hair brush into 9-cm glass petri dishes. Twenty eggs were placed in plastic test boxes (4.5 x 4.5 x 1.5 cm), each containing 1 ± 0.05 g of the rearing medium, using camel's hair brush. All other life stages were separated from the rearing media with a U.S. Standard Sieve No.60 (Fisher Scientific, Pittsburg, PA) with 250 μm openings. For both field and laboratory experiments, 20 individuals of each life stage were transferred to separate plastic test boxes (4.5 x 4.5 x 1.5 cm) each containing 1 ± 0.05 g of the rearing medium. Test boxes had perforated lids (3-cm diameter perforation) covered with mesh (600 μm openings) for ventilation.

Laboratory heating conditions

A growth chamber (Model I-36 L, Percival Scientific, Perry, IA) was used for exposing life stages of *T. variabile* to elevated constant temperatures of 46, 50 and 54°C and 15-20% RH. A humidity of 15-20% was used because during heat treatment, the humidity inside the facility is around 22-25% (Roesli et al. 2003, Mahroof et al. 2003a). Another growth chamber set at 28°C and 65% RH, served as the control treatment. The internal volume of growth chambers was 0.84 m³ (29.5 ft³). Air velocity, measured with an electronic wind speed indicator (Davis Instruments, San Leandro, CA), inside the growth chambers at 46-54°C ranged from ~0.6-1.2 m/s. In order to

verify that the insects in test boxes are exposed to the set chamber temperature (46, 50, or 54°C) and humidity (22%), the air temperature and relative humidity inside growth chambers were measured using HOBO® data-logging units (Onset Computer Corporation, Bourne, MA). At each temperature, a HOBO® unit was placed in each of the four corners and the center of the top shelf inside growth chambers in a similar manner as the test boxes were kept inside the chamber. The accuracy of each HOBO® unit was verified with a mercury thermometer before use and was within 0.1°C of the reading from the mercury thermometer.

Lab experiments at elevated temperatures

In order to determine the most heat tolerant stage, square test boxes each with 20 specific life stages of *T. variabile* were exposed to 46, 50 and 54°C and 15-20 % RH. Eggs (1-3 d old), young larvae (12-14 d old from the time of eclosion from eggs), old larvae (20-26 d old from the time of eclosion from eggs), pupae (2-4 d old from the time of pupation), and adults (3-6 d old from the time of eclosion from pupae) were used. At each elevated temperature four different exposure times were used, because time to death is inversely related to elevated temperatures (Mahroof et al. 2003a, Boina et al. 2004, Yu et al. 2011). At 46°C, the exposure times were 120, 240, 480 and 1440 min; at 50°C the exposure times were 20, 30, 60 and 120 min; and at 54°C the exposure times were 5, 10, 15 and 30 min. After the desired exposure time, three test boxes of each stage were taken out of the chamber and the contents of the test boxes were transferred to 150 ml round plastic containers with perforated lids each holding 19 ± 1 g of *T. variabile* diet. The plastic containers were placed in a growth chamber set at 28°C and 65% RH. After 24 h, the diet was sifted using a 600 µm sieve to separate adults from the diet. Adult mortality, expressed as a percentage, was determined based on number of dead adults out of the total exposed. Immature stages were reared to adulthood in the plastic containers as described above, and the

mortality of immature stages was based on number of adults that emerged out of the total exposed. Each of these experiments was replicated three times by conducting tests on different dates, along with the control treatment consisting of life stages placed separately in test boxes with diet at 28°C and 65% RH, and sampled at the same fixed times corresponding to each elevated temperature.

Exposure of life stages during commercial facility heat treatments

The Kansas State University pilot-scale O. H. Kruse Feed Technology Innovation Center was heated during 2–4 April 2014. Only Cargill Feed Safety Research Center of the O. H. Kruse Feed Technology Innovation Center was heated during the heat treatment. The total volume of the heated facility is 1017.44 m³ and it has three built-in steam heaters, out of which two are on the first and fourth floor (FAQ-202, Armstrong-Hunt Inc., Granby, QC, Canada) and one is on the second floor (AQ-122, Armstrong-Hunt Inc., Granby, QC, Canada). Each of these heaters produced 55 Kw/h. Heat treatment started at 3 pm of 2nd April and continued until 3 pm of 4th April. Four locations were selected to place test boxes: three locations were the three corners (North-west, West-south and North-east) of the room and the fourth location was the center of the room (right below the heater). At each of the four locations, a HOBO® data-logging unit was placed to record temperature at one minute intervals during the entire heat treatment period (41 h).

All life stages of *T. variabile*: eggs, young larvae, old larvae, pupae, and adults were used in the pilot scale heat treatment. All test boxes were placed on the ground floor of the Cargill Feed Safety Research Center, subjected to heat treatment. Test boxes were collected at 6, 17, 24 and 41 h into the heat treatment. For young larvae and old larvae, test boxes were collected at 20 h instead of 17 h. The number of test boxes collected at the specific time intervals varied from 1

to 3 as per the availability of life stages. Test boxes were brought back to the laboratory after 15 min of collection and the contents transferred to 150-ml plastic containers, as described above. Control treatment consisted of life stages placed separately in test boxes with diet at 28°C and 65% RH, and sampled at the same fixed times corresponding to each elevated temperature. After 48 h, adult mortality, expressed as a percentage, was determined based on number of dead adults out of the total exposed. Immature stages were reared to adulthood in the plastic containers as described above, and the mortality of immature stages was based on number of adults that emerged out of the total exposed.

Another experiment with *T. variabile* life stages was conducted at a grain- processing facility subjected to heat treatment during 19-20 August 2014. This particular facility conducts heat treatments in several rooms and processing areas twice a year using propane heaters from a heat treatment service provider. The total volume of the facility is 26051.49 cubic meters. Four propane gas heaters from Temp-Air® (Burnsville, MN, USA) were used to heat the facility. Each of the four heaters (THP-4500) produced 1318.87 kW/h. All heaters were placed outside of the mill because of an open flame. Heat generated by the units was discharged into the mill floor by nylon ductwork of 80 cm diameter with round holes. Heaters were turned on at 5:00 p.m. (local time) on 19 August and turned off at 5:30 p.m. on 20 August. Forty five fans were used to distribute the heat evenly throughout the processing area. Of these, 19 were Schaefer® fans with a 90-cm blade diameter, 18 were Box® fans with a 122-cm blade diameter and 8 were Bayley® fans with a 50-cm blade diameter. Two locations were selected to place test boxes based on the distance from the heating source: the first location was at a distance of 4.3 m from the heating source (duct) and the second location was at a distance of 7.8 m from the heating source. At each of the two locations and at a distance starting from 3 to 11.6 m from the heating source, a

HOBO® data-logging unit was placed to record temperature and relative humidity at one minute intervals during the entire heat treatment period (27 h).

Eggs, young larvae, old larvae, pupae, and adults were used. All the test boxes were placed on the ground floor of the processing area. Test boxes were collected when the temperature reached 46, 48, 50, 52 and 54°C at each of the location. At the end of the heat treatment (after 27 h), last set of test boxes was collected. Three test boxes for each stage were collected at the required temperatures. The contents were transferred to 150-ml plastic containers with perforated lids each holding 19 ± 1 g of *T. variabile* diet and placed in a growth chamber set at 28°C and 65% RH. Control samples were kept in a separate room in the processing area and sampled at same intervals as the treatment. After 48 h, adult mortality, expressed as a percentage, was determined based on number of dead adults out of the total exposed, as described in the above field experiment. Immature stages were reared to adulthood in the plastic containers at 28°C and 65% RH, and the mortality of immature stages was based on number of adults that emerged out of the total exposed.

The last heat treatment was conducted at the Kansas State University pilot flour mill occurred during 10-11 September 2014. 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× 3.7m, while each milling floor is 11.6× 9.9× 3.7m. Concrete walls separate both houses and vertical floors of the flour mill. Heaters were started at 10:00 a.m. in the morning because previous experience has shown that the built –in steam heater were inadequate to rapidly increase the mill temperature to 50°C or above. Doors of first and third floor were not sealed to prevent heat loss. Four locations were chosen to place test boxes, based on the distance from the

heaters: Two locations were on the first floor (location 1 at a distance of 0.7 m from the heater and location 2 at a distance of 3.2 m from the heater) and two locations were on the third floor (location 3 at a distance of 0.6 m from the heater and location 4, 3.3 m away from the heater).

All life stages similar to the above described heat treatments were used. Test boxes were kept at the decided locations and two test boxes of each stage were collected at 5.25, 18.75, 24 and 27 h into the heat treatment. Test boxes were brought back to the laboratory after 5 minutes of collection and the contents transferred to 150-ml plastic containers, as described above. Control treatment consisted of life stages placed separately in test boxes with diet at 28°C and 65% RH, and sampled at the same fixed times corresponding to each elevated temperature. After 48 h, adult mortality, expressed as a percentage, was determined based on number of dead adults out of the total exposed. Immature stages were reared to adulthood in the plastic containers as described above, and the mortality of immature stages was based on number of adults that emerged out of the total exposed.

Data analysis

Mortality data for *T. variabile* life stages was corrected for natural mortality by using Abbott's (1925) formula in both laboratory and field experiments. Corrected mortality data at each elevated temperature for fixed time responses was subjected to two-way and one-way analysis of variance (ANOVA) and Ryan-Gabriel-Welsch multiple range test at $\alpha = 0.05$ level to determine significant differences for mortality among stages using the GLM procedure of SAS (SAS Institute of 2003).

The temperature data from HOBO® units at each of the locations from the commercial heat treatments was used to determine the starting temperature, time required to reach 50°C, time above 50°C, and the maximum temperature. Corrected mortality was expressed against degree-

hours by converting the exposure time into degree hours using the following formula:
(Temperature in °C at time of insect collection – ambient temperature in °C) × time in hours at sample collection. The corrected mortality of *T. variabile* life stages were summarized by each location, for each commercial treatment. Corrected mortality data were plotted as a function of degree-hours using Sigma Plot 12.5 (Systat Software, Inc., San Jose, CA).

Results

Temperature and humidity measurements in growth chambers

The temperatures recorded by HOBO® data-logging units on the top shelf of growth chambers and inside test boxes with 1 ± 0.05 g of *T. variabile* diet were similar to the set chamber temperature and humidity levels. This indicated that the insects were exposed to predetermined treatment and control temperatures and humidity levels.

Fixed time-mortality responses of life stages

The natural (control) mortality among the stages ranged from 0 to 24%. High mortalities observed for egg and larval stages is due to fact that in insects higher mortalities occur in initial stages of development. At 46°C, two-way ANOVA showed that there were significant differences in mortality among life stages ($F = 387.72$; $df = 4, 40$; $P < 0.0001$) and among the exposure times ($F = 1642.79$; $df = 3, 40$; $P < 0.001$). The stage and exposure time interaction was significant ($F = 48.84$; $df = 12, 40$; $P < 0.0001$). Generally, differences among stages were less apparent at longer exposure times because of near or complete mortality of all life stages. One-way ANOVA at each exposure time also showed significant differences among the life stages (F , range among exposure times = 99.08 – 1022.65; $df = 4, 10$; $P < 0.0001$). A 24 h exposure resulted in 100% mortality of all stages except the pupa stage (Table 3.2).

At 50°C, two-way ANOVA showed that there were significant differences in mortality among life stages ($F = 17.60$; $df = 4, 40$; $P < 0.0001$) and among the exposure times ($F = 138.09$; $df = 3, 40$; $P < 0.0001$). The stage and exposure time interaction was significant ($F = 5.66$; $df = 12, 40$; $P < 0.0001$). One-way ANOVA at 20 and 30 min exposure time showed that differences among life stages were significant (F , range among exposure times = 4.79-88.11; $df = 4, 10$; $P < 0.0203$).

At 54°C also, two-way ANOVA showed that there were significant differences in mortality among life stages ($F = 32.91$; $df = 4, 40$; $P < 0.0001$) and among the exposure times ($F = 272.54$; $df = 3, 40$; $P < 0.0001$). The stage and exposure time interaction was significant ($F = 8.11$; $df = 12, 40$; $P < 0.0001$). One-way ANOVA showed that at 5, 10 and 15 min of exposure time differences among life stages were significant (F , range among exposure times = 5.02 – 31.37; $df = 4, 10$; $P < 0.0176$). A 15-min exposure at 54°C resulted in 100% mortality of all stages except for the pupa stage (Table 3.6).

The data at 54°C and 46°C showed pupae to be the most heat-tolerant stage. However, at 50°C, differences among postembryonic life stages were not apparent, because of increased susceptibility at these high temperatures (Table 3.4). Also at a temperature of 50°C and an exposure time ≥ 60 min, no significant differences in susceptibility among the stages were observed ($P = 0.3$). Similarly, at 54°C and 30 min exposure, no significant differences in susceptibility among the stages were observed. Eggs and adults were found to be the most susceptible stages consistently respectively, followed by young larvae and old larvae (Table 3.2; 3.4 and 3.6).

In general, mortality of *T. variabile* developmental stages increased with an increase in temperature and also with an increase in exposure time at a particular temperature except for

pupae, when they were exposed to 240 min at 46°C. Fixed-time mortality responses of life stages suggest pupae to be the most heat tolerant stage.

Temperature profiles and responses of life stages during a facility heat treatments

The starting temperature at all four locations of the Feed Technology Innovation Center was different and ranged from 18 to 20°C. At locations L1, L2 and L4, the maximum temperature was less than 50°C while at location L3, the maximum temperature was 60°C. The time required reaching 50°C at location L3 was 25.3 h because of the slow heating rate (1.24°C/h), and temperatures above 50°C were maintained only for 15.5 h (Table 3.7). The mean control mortality of eggs, young larvae, pupae, and adults at the four observation times ranged from 16.6-50, 22.5-62.5, 10-45, 3.3-20, and 0-10, respectively (Table 3.8). High control mortality could be due to improper handling of stages and movement of test boxes to and from the feed facility to laboratory.

Among all the life stages, 100 % mortality was observed after 41 h into the heat treatment except for pupae, which concludes that pupa was the most heat tolerant stage. However, old larvae and young larvae had 100 % mortality only at location L3 (Table 3.8 and Fig. 3.1). The lack of consistent trends in stage-specific susceptibility made it difficult to discern a heat-tolerant stage based on results at locations 1 through 4. Pupae were the most heat-tolerant stage at all locations, except location 1 where young larvae were found to be heat tolerant. Eggs and adults were found to be the most heat susceptible stages as the mortality was 100 % at all locations after 41 h into the heat treatment.

Starting temperatures at all locations of the grain-processing facility were high as compared to the temperatures at the pilot feed facility. The initial temperatures at both the locations were 30°C. The time required reaching 50°C was less than an hour (48 minutes) at

location 2 while at location 1 it took 1 hour and 18 minutes to reach 50°C. This is evident from the high heating rates of 24.97 and 16.72°C/h at location 2 and location 1 respectively (Table 3.9). The mean control mortality of eggs, young larvae, pupae, and adults at the six observation times ranged from 10-18.3, 1.7-5, 1.7-5, 0-1.7, and 3.3-13.3, respectively. Control mortalities were less as compared to previous heat treatment.

At both locations of the grain-processing facility, 100% mortality was observed among all the life stages of *T. variabile* after 27 h into the heat treatment. As can be seen from figure 3.2, at all degree-hours, 0% mortality for old larvae and pupae was noted except at 12 degree-hours for location 1 and 20 degree-hours for location 2. For young larvae also, 0 % mortality was noted at 42 and 34 degree-hours, at location 1 and 2 respectively. As similar to the data from KSU pilot feed mill, the lack of consistent trends in stage-specific susceptibility made it difficult to discern a heat-tolerant stage based on results at locations 1 and 2. Eggs and adults were found to be heat susceptible consistently at both locations.

During heat treatment at the pilot flour mill, the initial temperatures were close to 24-25°C at all locations. Location 4 was the hottest and location 1 was the coolest followed by location 3 and 2 since the time to 50°C were 2.08, 23.53, 21.32 and 12.27 h, respectively at each of the location. Temperatures reached above 50°C at all locations and temperatures above 50°C were maintained for sufficiently longer period of time at each location (Table 3.11). High variability in heating rates at location 1 and 4 was observed. Heating rates ranged from 1.08 to 11.96°C/h at all locations. The mean control mortality of eggs, young larvae, pupae, and adults at the four observation times ranged from 10-15, 5-7.5, 2.5-7.5, 0, and 0-2.5, respectively.

Difference in heating rates influences the life stages to become heat tolerant. At location 1, after 27 h into heat treatment, young larvae were found to be heat tolerant with only 5.4%

mortality, whereas at location 4 100% mortality for all life stages was achieved only after 18.75 h into heat treatment. At this location pupae were found to be heat tolerant, with only 2.5% mortality after 5.25 h into heat treatment. Similarly, at location 3, where the temperature profile was similar to location 1 (Table 3.11), young larvae and adults were found to be heat tolerant whereas at location 2, where the temperature profiles were close to location 4, pupae were found to be least susceptible to heat. As similar to above described commercial heat treatments, the lack of consistent trends in stage-specific susceptibility made it difficult to clearly identify a heat tolerant stage based on the results from all locations.

Discussion

The fixed-time mortality experiments showed pupae to be the most heat-tolerant, of all *T. variabile* life stages. Unlike *T. variabile*, the most heat-tolerant stage of *T. castaneum*, *T. confusum*, *P. interpunctella* and *L. serricornis* at elevated temperatures were observed to be young larvae, old larvae, old larvae (wandering stage) and eggs, respectively (Mahroof et al. 2003b, Boina and Subramanyam 2004, Mahroof and Subramanyam 2006, Yu et al. 2011). These studies show that stage-specific susceptibility to elevated temperatures varies among the species. Within a given species heat tolerance among life stages may vary based on heating rates or temperatures. The rapid drop in lethal time estimates at 50 and 54°C as compared to 46°C is due to rapid mortality of life stages at higher temperatures. The data from Wright et al. (2002) suggested old larvae to be heat tolerant however the method of insect exposure was different than the methods we used. In our experiments, stages were exposed to constant temperatures of 46, 50 and 54°C and 15-20% RH, while Wright et al. (2002) exposed stages at 0% RH.

The exposure of life stages of *T. variabile* during a facility heat treatment, to identify a heat tolerant stage was not very successful. Pupae were found to be generally more heat tolerant

then other life stages, in both the laboratory experiments and the field experiments. Mahroof et al. (2003a) exposed eggs, young larvae, old larvae, pupae, and adults of the red flour beetle, *Tribolium castaneum* (Herbst), during heat treatment of a flour mill and observed pupae to be the most heat-tolerant stage. However, laboratory test using the same life stages at six constant elevated temperatures between 42 and 60°C showed young larvae to be the most heat tolerant stage (Mahroof et al. 2003b). Yu et al. (2010) also reported eggs to be heat tolerant at constant elevated temperatures, but this finding was difficult to confirm during commercial facility. It is unclear why there is a discrepancy in definitively identifying a heat tolerant stage during facility heat treatments. It is plausible that heating rates may have an impact on which stages develop heat tolerance during facility heat treatments and this aspect warrants further study.

In summary, pupae of *T. variabile* were generally the most heat tolerant of all stages tested at 46-54°C. For example, at 54°C, and 15 min exposure, mortality for other life stages ranged from 96.7 to 100%, while for pupae it was 91% only. Also at 46°C, after 24 h exposure, mortality for all life stages was 100% except for pupae which was 94.5% only. Pupae also were generally more heat tolerant than other life stages during tests in pilot flour and feed mills at Kansas State University and in a commercial grain-processing facility. However, these results were not as conclusive as the results at fixed temperatures. Therefore, pupae should be used as test insects in evaluating heat treatment effectiveness because heat treatment designed to control pupae should be able to control all other *T. variabile* life stages. The information presented in this article provides a quantitative basis for successful use of elevated temperatures for managing *T. variabile* life stages associated with food-processing facilities.

Table 3.1 Control mortality of *T. variabile* life stages corresponding to 46°C.

Temp (°C)	Stage	Mean ± SE % mortality at:			
		120 min	240 min	480 min	1440 min
28	Egg	18.8 ± 3.4	13.3 ± 1.9	17.2 ± 3.0	19.4 ± 1.5
	Young larvae	14.4 ± 0.9	17.2 ± 2.2	21.1 ± 2.0	14.4 ± 2.2
	Old larvae	12.2 ± 1.7	10.0 ± 3.3	22.2 ± 2.8	19.4 ± 1.4
	Pupae	2.2 ± 1.5	6.1 ± 2.0	6.1 ± 1.1	8.3 ± 2.5
	Adults	0.8 ± 0.4	5.0 ± 2.5	2.2 ± 1.6	10.0 ± 0.9

Each mean in based on $n = 3$.

Table 3.2 Corrected mortality of *T. variable* life stages for fixed-time responses at 46°C.

Temp (°C)	Stage	Mean ± SE ^a % corrected mortality at:			
		120 min	240 min	480 min	1440 min
46	Egg	62.4 ± 1.4a	83.3 ± 1.3a	100 ± 0.0a	100 ± 0.0a
	Young larvae	15.0 ± 1.3b	37.6 ± 2.3b	92.2 ± 0.7b	100 ± 0.0a
	Old larvae	0.0 ± 0.0c	6.8 ± 2.2c	87.1 ± 1.2c	100 ± 0.0a
	Pupae	6.8 ± 1.1d	2.8 ± 2.5c	46.2 ± 0.6d	94.5 ± 1.0b
	Adult	28.3 ± 5.8e	69 ± 0.6a	100 ± 0.0a	100 ± 0.0a

^a Means followed by different letters are significantly different ($P < 0.05$, by REGWQ test).

The mean control mortality of eggs, young larvae, pupae, and adults at the four observation times ranged from 11.4-22.2, 13.5-23.1, 6.7-25, 0.7-10.8, and 0.4-10.9, respectively.

Table 3.3 Control mortality of *T. variabile* life stages corresponding to 50°C

Temp (°C)	Stage	Mean ± SE % mortality at:			
		20 min	30 min	60 min	120 min
28	Egg	21.7 ± 3.3	18.7 ± 1.6	22.2 ± 2.2	18.9 ± 3.4
	Young larvae	16.7 ± 0.9	12.0 ± 2.1	17.8 ± 0.9	14.4 ± 0.9
	Old larvae	12.8 ± 1.7	9.2 ± 2.0	11.7 ± 1.9	12.2 ± 1.7
	Pupae	2.7 ± 1.0	2.7 ± 1.0	0.5 ± 0.5	2.2 ± 1.6
	Adults	1.1 ± 1.1	3.5 ± 2.3	1.3 ± 0.5	1.9 ± 1.0

Each mean is based on $n = 3$.

Table 3.4 Corrected mortality for *T. variabile* life stages for fixed-time responses at 50°C.

Temp (°C)	Stage	Mean ± SE ^a % corrected mortality at:			
		20 min	30 min	60 ^b min	120 ^c min
50	Egg	85.8 ± 1.9a	96.6 ± 1.8a	98.6 ± 1.4	100 ± 0.0
	Young larvae	36.7 ± 1.3b	55.2 ± 1.7b	94.0 ± 3.5	100 ± 0.0
	Old larvae	7.1 ± 1.7c	44.9 ± 1.8b	93.1 ± 5.1	100 ± 0.0
	Pupae	4.6 ± 1.5c	48.6 ± 9.4b	95.5 ± 1.5	98.3 ± 1.7
	Adult	34.8 ± 6.8b	40.8 ± 20.4b	100 ± 0.0	100 ± 0.0

^a Means followed by different letters are significantly different ($P < 0.0001$ by REGWQ test).

^b Susceptibility among life stages not significant ($F = 1.42$; $df = 4, 10$; $P = 0.2952$, one-way ANOVA).

^c Susceptibility among life stages not significant ($F = 1.00$; $df = 4, 10$; $P = 0.4516$, one-way ANOVA).

The mean control mortality of eggs, young larvae, pupae, and adults at the four observation times ranged from 15.5-25, 9.9-18.7, 7.2-14.5, 0-3.7, and 0-5.8, respectively.

Table 3.5 Control mortality of *T. variabile* life stages corresponding to 54°C.

Temp (°C)	Stage	Mean ± SE % mortality at:			
		5 min	10 min	15 min	30 min
28	Egg	23.8 ± 2.9	20.8 ± 2.8	21.6 ± 3.3	18.7 ± 1.6
	Young larvae	18.8 ± 2.4	16.1 ± 0.7	15.0 ± 2.8	12.0 ± 2.0
	Old larvae	16.6 ± 0.9	12.2 ± 1.0	16.1 ± 1.4	9.2 ± 2.0
	Pupae	0.0 ± 0.0	0.0 ± 0.0	1.1 ± 0.5	2.7 ± 1.0
	Adult	0.5 ± 0.5	0.8 ± 0.5	0.0 ± 0.0	3.5 ± 2.3

Each mean is based on $n = 3$.

Table 3.6 Corrected mortality for *T.variabile* life stages for fixed-time exposures at 54°C.

Temp (°C)	Stage	Mean ± SE ^a % corrected mortality at:			
		5 min	10 min	15 min	30 min
54	Egg	75.9 ± 0.0a	94.4 ± 1.8a	100 ± 0.0a	100 ± 0.0
	Young larvae	19.9 ± 1.2b	70.9 ± 1.7ab	100 ± 0.0a	100 ± 0.0
	Old larvae	1.4 ± 0.7c	68.4 ± 3.3ab	96.7 ± 1.7a	100 ± 0.0
	Pupae	1.1 ± 0.5c	48.9 ± 14.0b	91.0 ± 2.0b	100 ± 0.0
	Adult	49.7 ± 15.1a	86.0 ± 7.8a	100 ± 0.0a	100 ± 0.0

^aMeans followed by different letters are significantly different ($P < 0.0006$ by REGWQ test).

The mean control mortality of eggs, young larvae, pupae, and adults at the four observation times ranged from 17.1-26.7, 10-21.2, 7.2-17.5, 0-3.7, and 0-5.8, respectively.

Table 3.7 Temperature data at four locations where test boxes with *T. variable* life stages were placed during heat treatment at Feed Technology Innovation Center.

Location	Initial temp. (°C)	Time to 50°C (h)	Rate to 50°C (°C/h) ^a	Time above 50°C (h)	Max temp (°C)	Temp. at sample collection time in hours				
						6	17	20	24	41
1	19.0	--	--	--	47.5	31.8	41.9	44.2	47.1	47.5
2	18.9	--	--	--	45.5	28.8	38.3	41.1	44.3	45.5
3	19.9	25.3	1.2	15.5	60	31.8	41.8	44.8	47.7	60
4	17.9	--	--	--	43	27.1	36.1	38.4	41.9	42.5

^a Rate = (50°C – Initial temp.) / time to 50°C.

Location 1 North-west, Location 2 West-south, Location 3 North-east corner of the room and Location 4 is the center of the room.

Table 3.8 Mortality of *T. variabile* life stages in test boxes at four locations sampled at four different time intervals during heat treatment at Feed technology Innovation Center.

Location	Stage ^a	Sample collection time (h)	No. of test boxes	Treatment		Control		Corrected Mortality (%)
				No. dead insects/total	Mortality (%)	No. dead insects/total	Mortality (%)	
1	A	6	3	3/60	5	2/60	3.3	1.7
		17	3	2/60	3.3	0/60	0	3.3
		24	2	1/40	2.5	0/40	0	2.5
		41	1	20/20	100	1/20	5	100
	P	6	3	8/60	13.3	8/60	13.3	0
		17	3	3/60	5	7/60	11.6	0
		24	2	2/40	5	8/40	20	0
		41	1	14/20	70	2/20	10	66.6
	OL	6	2	2/40	37.5	17/40	42.5	0
		20	2	4/40	35	14/40	35	0
		24	1	7/20	50	9/20	45	0
		41	1	12/20	40	7/20	35	38.5
	YL	6	2	21/40	52.5	25/40	62.5	0
		20	2	23/40	57.5	9/40	22.5	38.7
		24	1	10/20	50	7/20	35	38.5
		41	1	6/20	30	12/20	60	12.5
E	6	3	52/60	86.6	10/60	16.6	84.0	
	17	3	54/60	90	12/60	20	87.5	
	24	2	26/40	65	11/40	27.5	51.7	
	41	1	20/20	100	5/20	25	100	
2	A	6	3	5/60	8.3	1/60	1.6	6.8
		17	3	10/60	16.6	0/60	0	16.7
		24	2	5/40	12.5	0/40	0	12.5
		41	1	20/20	100	2/20	10	100

3	P	6	3	1/60	1.6	7/60	11.7	0
		17	3	1/60	1.6	3/60	5	0
		24	2	1/40	2.5	4/40	10	0
		41	1	0/20	0	2/20	10	0
	OL	6	2	5/40	45	9/40	22.5	0
		20	2	8/40	20	16/40	40	0
		24	1	8/20	25	8/20	40	0
		41	1	11/20	55	6/20	30	35.7
	YL	6	2	24/40	60	17/40	42.5	8.7
		20	2	18/40	45	13/40	32.5	7.4
		24	1	10/20	50	11/20	55	0
		41	1	11/20	55	9/20	45	18.2
	E	6	3	52/60	86.7	19/60	31.7	80.5
		17	3	51/60	85	15/60	25	80
		24	2	25/40	62.5	13/40	32.5	44.4
		41	1	20/20	100	10/20	50	100
	A	6	3	5/60	8.3	0/60	0	8.3
		17	3	13/60	21.7	1/60	1.7	20.4
		24	2	22/40	55	2/40	5	52.6
		41	1	20/20	100	1/20	5	100
	P	6	3	2/60	3.3	3/60	5	0
		17	3	0/60	0	5/60	8.3	0
		24	2	2/40	5	2/40	5	0
		41	1	4/20	20	3/20	15	5.9
	OL	6	2	5/40	35	13/40	32.5	0
		20	2	9/40	40	10/40	25	0
		24	1	11/20	55	5/20	25	40
		41	1	20/20	100	2/20	10	100
YL	6	2	21/40	52.5	19/40	47.5	14.3	
	20	2	27/40	67.5	18/40	45	22.7	
	24	1	14/20	70	9/20	45	54.5	
	41	1	20/20	100	8/20	40	100	
E	6	3	47/60	78.3	11/60	18.3	73.5	

		17	3	55/60	91.7	22/60	36.7	86.8
		24	2	37/40	92.5	8/40	20	90.6
		41	1	20/20	100	0/20	0	100
4	A	6	3	4/60	6.7	0/60	0	6.7
		17	3	6/60	10	1/60	1.7	8.5
		24	2	6/40	15	0/40	0	15
		41	1	20/20	100	2/20	10	100
	P	6	3	1/60	1.7	6/60	10	0
		17	3	2/60	3.3	2/60	3.3	0.0
		24	2	20/40	50	3/40	7.5	45.9
		41	1	1/20	5	2/20	10	0
	OL	6	2	1/40	55	8/40	20	0
		20	2	2/40	42.5	11/40	27.5	0
		24	1	5/20	75	4/20	20	6.2
		41	1	10/20	35	7/20	35	23.1
	YL	6	2	21/40	52.5	13/40	32.5	0
		20	2	13/40	32.5	20/40	50	0
		24	1	2/20	10	8/20	40	0
		41	1	12/20	60	10/20	50	20
	E	6	3	55/60	91.7	17/60	28.3	88.7
		17	3	48/60	80	18/60	30.0	71.4
		24	2	32/40	80	10/40	25	73.3
		41	1	20/20	100	4/20	20	100

^a A, adults, P, pupae, YL, young larvae, OL, old larvae, E, eggs. The mean control mortality of eggs, young larvae, pupae, and adults at the four observation times ranged from 16.6-50, 22.5-62.5, 10-45, 3.3-20, and 0-10, respectively.

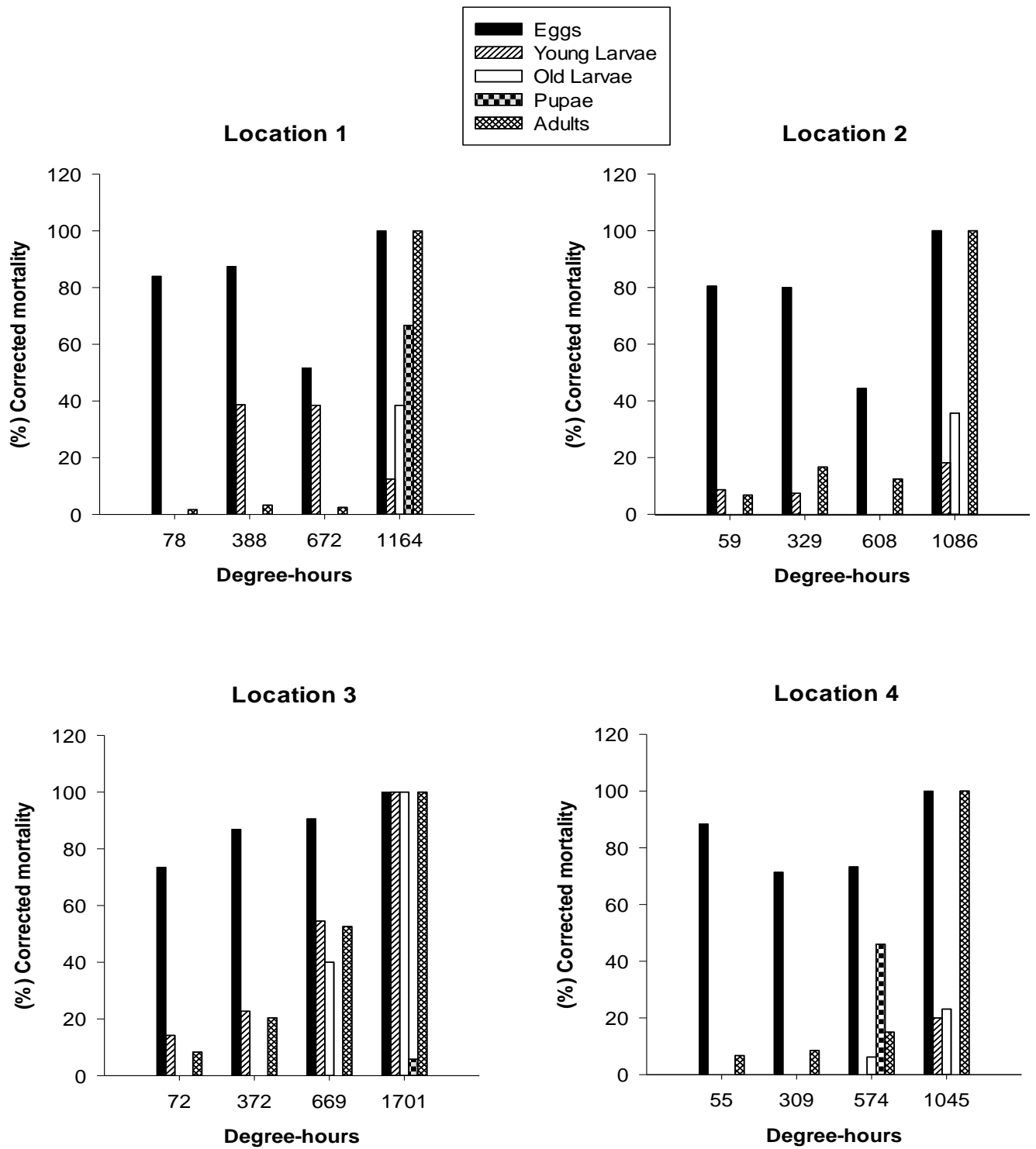


Figure3.1 Corrected mortality for *T. variabile* life stages against degree-hours at four locations during heat treatment at KSU Feed Technology Innovation Center.

Table 3.9 Temperature data at two locations where test boxes with *T. variable* life stages were placed during heat treatment at a grain-processing facility.

Location	Initial Temp. (°C)	Time to 50°C (h)	Rate to 50°C (°C/h) ^a	Time above 50°C (h)	Max Temp(°C)
1	30.9	1.2	16.0	25.8	60.3
2	30.1	0.8	25.0	26.2	61.2

^a Rate = (50°C – Initial temp.) / time to 50°C.

Table 3.10 Mortality of *T. variabile* life stages in test boxes at two locations sampled at six different time intervals during heat treatment at a grain-processing facility.

Location	Stage ^a	Sample collection time (h)	Temp at sample collection	No. of test boxes	Treatment		Control		Corrected Mortality (%)
					No. dead insects/total	Mortality (%)	No. dead insects/total	Mortality (%)	
1	A	0.92	44.391	3	32/60	53.3	5/60	8.3	49.1
		1.03	45.257	3	38/60	63.3	7/60	11.7	58.52
		1.42	48.328	3	28/60	46.7	3/60	5.0	43.86
		1.65	49.643	3	24/60	40.0	8/60	13.3	30.8
		2.05	51.465	3	14/60	23.3	2/60	3.3	20.72
		27	51.364	3	60/60	100	3/60	5.0	100
	P	0.91	44.391	3	1/60	1.7	0/60	0	1.7
		1.03	45.257	3	0/60	0	0/60	0	0
		1.41	48.328	3	0/60	0	0/60	0	0
		1.65	49.643	3	0/60	0	1/60	1.7	0
		2.05	51.465	3	0/60	1.7	0/60	0	0
		27	51.364	3	60/60	100	1/60	1.7	100
	OL	0.91	44.391	3	2/60	3.3	3/60	5.0	0
		1.03	45.257	3	2/60	3.3	2/60	3.3	0.03
		1.41	48.328	3	0/60	0	2/60	3.3	0
		1.65	49.643	3	0/60	0	1/60	1.7	0
		2.05	51.465	3	1/60	1.7	1/60	1.7	0

		27	51.364	3	60/60	100	2/60	3.3	100
	YL	0.91	44.391	3	15/60	25.0	3/60	5.0	21
		1.03	45.257	3	2/60	3.3	1/60	1.7	1.7
		1.41	48.328	3	18/60	30.0	2/60	3.3	27.61
		1.65	49.643	3	11/60	18.3	2/60	3.3	15.54
		2.05	51.465	3	2/60	3.3	2/60	3.3	0.03
		27	51.364	3	60/60	100	3/60	5.0	100
	E	0.91	44.391	3	57/60	95.0	8/60	13.3	94.2
		1.03	45.257	3	54/60	90.0	10/60	16.7	88
		1.41	48.328	3	56/60	93.3	7/60	11.7	92.4
		1.65	49.643	3	55/60	91.7	8/60	13.3	90.4
		2.05	51.465	3	58/60	96.7	6/60	10.0	96.3
		27	51.364	3	60/60	100	11/60	18.3	100
2	A	0.91	51.495	3	16/60	26.7	5/60	8.3	20.0
		1.03	52.022	3	18/60	30.0	7/60	11.7	0
		1.41	53.811	3	22/60	36.7	3/60	5.0	33
		1.65	55.13	3	26/60	43.3	8/60	13.3	34.7
		2.05	56.59	3	25/60	41.7	2/60	3.3	39.7
		27	50.653	3	60/60	100	3/60	5.0	100
	P	0.91	51.495	3	7/60	11.7	0/60	0	11.7
		1.03	52.022	3	0/60	0	0/60	0	0
		1.41	53.811	3	0/60	0	0/60	0	0

	1.65	55.13	3	0/60	0	1/60	1.7	0
	2.05	56.59	3	0/60	0	0/60	0	0
	27	50.653	3	60/60	100	1/60	1.7	100
OL	0.91	51.495	3	0/60	0	3/60	5.0	0
	1.03	52.022	3	1/60	1.7	2/60	3.3	0
	1.41	53.811	3	2/60	3.3	2/60	3.3	0.03
	1.65	55.13	3	2/60	3.3	1/60	1.7	1.7
	2.05	56.59	3	0/60	0	1/60	1.7	0
	27	50.653	3	60/60	100	2/60	3.3	100
YL	0.91	51.495	3	4/60	6.7	3/60	5.0	1.7
	1.03	52.022	3	4/60	6.7	1/60	1.7	0
	1.41	53.811	3	2/60	3.3	2/60	3.3	0.03
	1.65	55.13	3	0/60	0	2/60	3.3	0
	2.05	56.59	3	3/60	5	2/60	3.3	3.4
	27	50.653	3	60/60	100	3/60	5.0	100
E	0.91	51.495	3	58/60	96.7	8/60	13.3	96.1
	1.03	52.022	3	54/60	90.0	10/60	16.7	88
	1.41	53.811	3	60/60	100	7/60	11.7	100
	1.65	55.13	3	53/60	88.3	8/60	13.3	86.5
	2.05	56.59	3	52/60	86.7	6/60	10.0	85.2
	27	50.653	3	60/60	100	11/60	18.3	100

^a A, adults, P, pupae, YL, young larvae, OL, old larvae, E, eggs. The mean control mortality of eggs, young larvae, pupae, and adults at the six observation times ranged from 10-18.3, 1.7-5, 1.7-5, 0-1.7, and 3.3-13.3, respectively.

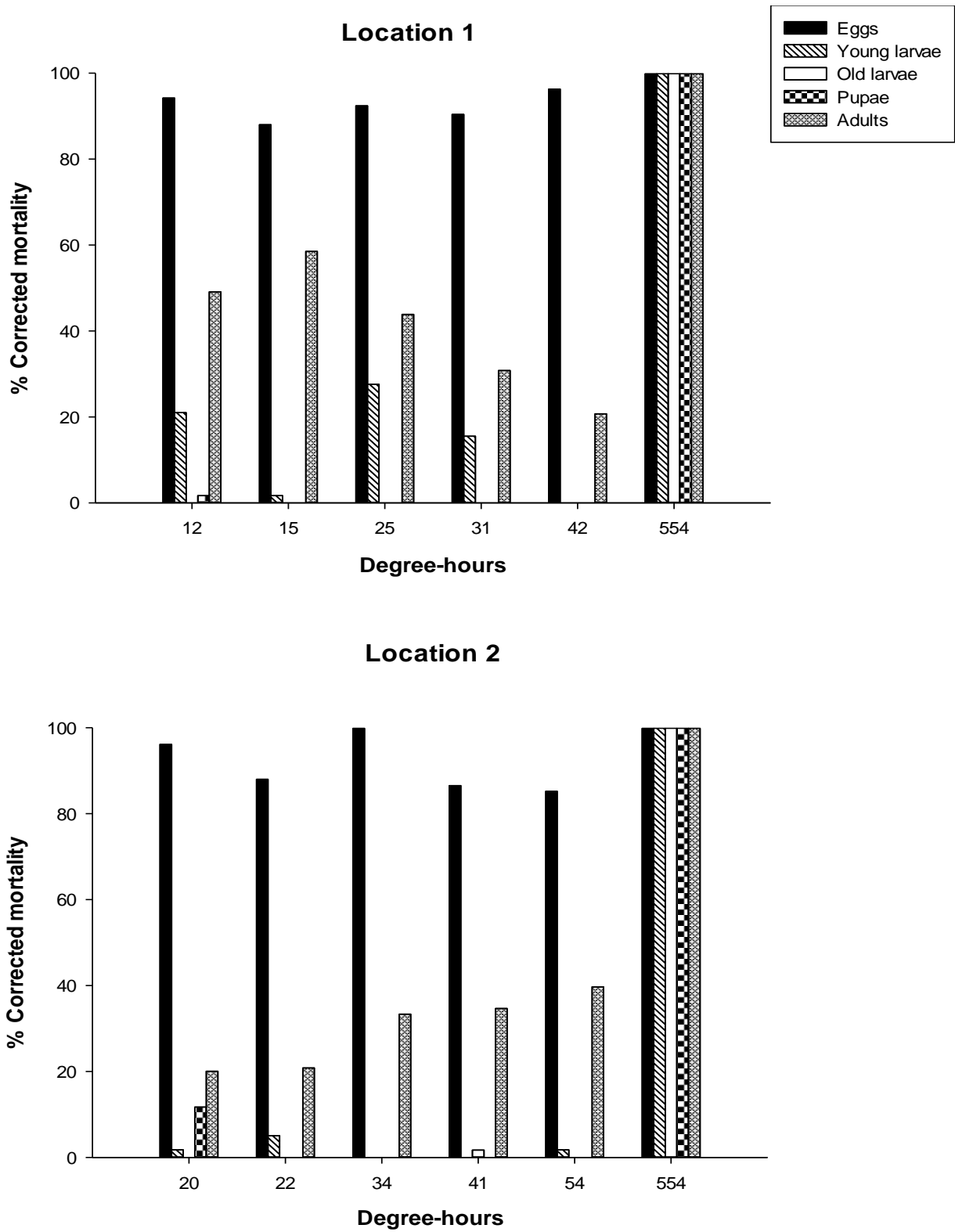


Figure 3.2 Corrected mortality for *T. variabile* life stages against degree-hours at two locations during heat treatment at a grain-processing facility.

Table 3.11 Temperature data at four locations where test boxes with *T. variabile* life stages were placed during heat treatment at KSU pilot flour mill.

Location	Initial temp. (°C)	Time to 50°C (h)	Rate to 50°C (°C/h) ^a	Time above 50°C (h)	Max temp (°C)	Temp at sample collection time in hours			
						5.25	18.75	24	27
1	24.6	23.5	1.1	3.5	54.6	42.8	47.5	52.6	54.0
2	24.8	12.3	2.0	14.8	61.5	46.3	51.8	59.5	60.8
3	25.0	21.3	1.2	5.7	55.6	39.0	47.9	52.4	54.8
4	25.1	2.1	12.0	24.9	66.6	55.30	61	65.1	65.6

^a Rate = (50°C – Initial temp.) / time to 50°C.

Table 3.12 Mortality of *T. variabile* life stages in test boxes at four locations sampled at four different time intervals during heat treatment at KSU pilot flour mill.

Location	Stage ^a	Sample collection time (h)	No. of test boxes	Treatment		Control		Corrected Mortality (%)
				No. dead insects/total	Mortality (%)	No. dead insects/total	Mortality (%)	
1	A	5.25	2	5/40	12.5	0/40	0	12.5
		18.75	2	18/40	45	0/40	0	45
		24	2	38/40	95	1/40	2.5	94.9
		27	2	40/40	100	0/40	0	100
	P	5.25	2	0/40	0	0/40	0	0
		18.75	2	0/40	0	0/40	0	0
		24	2	12/40	30	0/40	0	30
		27	2	39/40	97.5	0/40	0	97.5
	OL	5.25	2	0/40	0	3/40	7.5	0
		18.75	2	0/40	0	2/40	5.0	0
		24	2	3/40	7.5	1/40	2.5	5.1
		27	2	13/40	32.5	2/40	5.0	28.9
	YL	5.25	2	0/40	0	2/40	5.0	0
		18.75	2	2/40	5	2/40	5.0	0
		24	2	8/40	20	4/40	10.0	11.1
		27	2	5/40	12.5	3/40	7.5	5.4
E	5.25	2	37/40	92.5	5/40	12.5	91.4	
	18.75	2	38/40	95	3/40	7.5	94.5	

		24	2	40/40	100	6/40	15	100
		27	2	40/40	100	4/40	10	100
2	A	5.25	2	2/40	5	0/40	0	5
		18.75	2	40/40	100	0/40	0	100
		24	2	40/40	100	1/40	2.5	100
		27	2	40/40	100	0/40	0	100
	P	5.25	2	0/40	0	0/40	0	0
		18.75	2	4/40	10	0/40	0	10
		24	2	38/40	95	0/40	0	95
		27	2	40/40	100	0/40	0	100
	OL	5.25	2	0/40	0	3/40	7.5	0
		18.75	2	20/40	50	2/40	5.0	47.4
		24	2	40/40	100	1/40	2.5	100
		27	2	40/40	100	2/40	5.0	100
	YL	5.25	2	1/40	2.5	2/40	5.0	0
		18.75	2	2/40	5	2/40	5.0	0
		24	2	40/40	100	4/40	10.0	100
		27	2	40/40	100	3/40	7.5	100
	E	5.25	2	38/40	95	5/40	12.5	94.3
		18.75	2	38/40	95	3/40	7.5	94.5
		24	2	40/40	100	6/40	15	100
		27	2	40/40	100	4/40	10	100

3	A	5.25	2	3/40	7.5	0/40	0	7.5
		18.75	2	0/40	0	0/40	0	0
		24	2	39/40	97.5	1/40	2.5	97.4
		27	2	40/40	100	0/40	0	100
	P	5.25	2	0/40	0	0/40	0	0
		18.75	2	40/40	100	0/40	0	100
		24	2	40/40	100	0/40	0	100
		27	2	40/40	100	0/40	0	100
	OL	5.25	2	0/40	0	3/40	7.5	0
		18.75	2	31/40	77.5	2/40	5.0	76.3
		24	2	40/40	100	1/40	2.5	100
		27	2	40/40	100	2/40	5.0	100
	YL	5.25	2	0/40	0	2/40	5.0	0
		18.75	2	33/40	82.5	2/40	5.0	81.6
		24	2	39/40	97.5	4/40	10.0	97.2
		27	2	40/40	100	3/40	7.5	100
	E	5.25	2	39/40	97.5	5/40	12.5	97.1
		18.75	2	38/40	95	3/40	7.5	94.5
		24	2	40/40	100	6/40	15	100
		27	2	40/40	100	4/40	10	100
4	A	5.25	2	38/40	95	0/40	0	95
		18.75	2	40/40	100	0/40	0	100

	24	2	40/40	100	1/40	2.5	100
	27	2	40/40	100	0/40	0	100
P	5.25	2	1/40	2.5	0/40	0	2.5
	18.75	2	40/40	100	0/40	0	100
	24	2	40/40	100	0/40	0	100
	27	2	40/40	100	0/40	0	100
OL	5.25	2	40/40	100	3/40	7.5	100
	18.75	2	40/40	100	2/40	5.0	100
	24	2	40/40	100	1/40	2.5	100
	27	2	40/40	100	2/40	5.0	100
YL	5.25	2	17/40	42.5	2/40	5.0	39.5
	18.75	2	40/40	100	2/40	5.0	100
	24	2	40/40	100	4/40	10.0	100
	27	2	40/40	100	3/40	7.5	100
E	5.25	2	37/40	92.5	5/40	12.5	91.4
	18.75	2	39/40	97.5	3/40	7.5	97.3
	24	2	40/40	100	6/40	15	100
	27	2	40/40	100	4/40	10	100

^a A, adults, P, pupae, YL, young larvae, OL, old larvae, E, eggs. The mean control mortality of eggs, young larvae, pupae, and adults at the four observation times ranged from 10-15, 5-7.5, 2.5-7.5, 0, and 0-2.5, respectively.

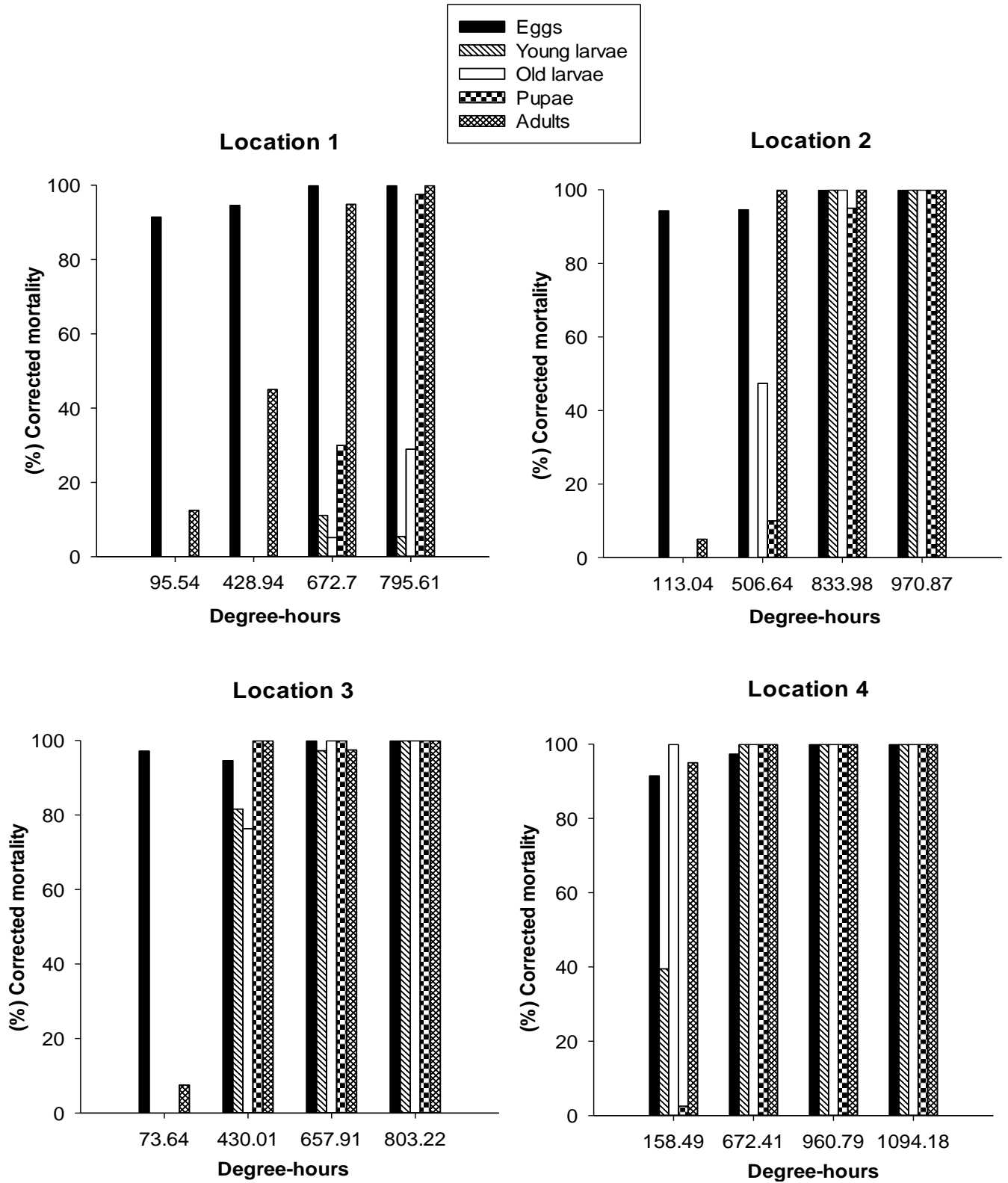


Figure 3.3 Corrected mortality for *T. variable* life stages against degree-hours at four locations during heat treatment at KSU pilot flour mill.

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