



Thermal biology of the lesser grain borer *Rhyzopertha dominica* (F.) (Bostrichidae) and the warehouse pirate bug *Xylocoris flavipes* (Reuter) (Anthocoridae)  
by Tracy Lynn Mumm Campbell

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Entomology  
Montana State University  
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**Abstract:**

Cereal grains are stored in nearly every country around the world for personal consumption or economic reasons. The stored grain environment is thermally dynamic. Insect behavior and damage are highly influenced by these changing temperatures. Temperature-dependent behavior should be taken into consideration to successfully store grain. Many of the chemicals traditionally used to control pests are being banned worldwide. Thus, it is necessary to explore alternatives such as biological control.

I studied temperature-dependent walking speed, activity and mortality of two stored grain insects, *Rhyzopertha dominica* (F.) (Bostrichidae) and *Xylocoris flavipes* (Reuter) (Anthocoridae). I also investigated temperature-dependent adult position and oviposition preferences of these two species, and temperature-dependent predation rates of *A. flavipes*. The experiments were conducted on a laboratory thermal gradient which consisted of four parallel 15 x 100 x 1.0 cm thick aluminum bars. The ends of the bars rested upon hollow aluminum rods. Water from a hot water bath was pumped in a loop through rods beneath one end of the apparatus, while another pump passed cold water through rods at the other end. This resulted in a stable linear temperature gradient across the bars.

Walking speed increased linearly with temperature for both species. *Xylocoris flavipes* was faster than *R. dominica* at nearly all temperatures tested. *Rhyzopertha dominica* was 100% active at temperatures  $\geq 14.6 \pm 0.1^\circ\text{C}$  while *X. flavipes* was 100% active at temperatures  $\geq 17.3 \pm 0.01^\circ\text{C}$ . After 24 hours, *R. dominica* and *X. flavipes* were incapable of surviving temperatures  $\geq 46.5^\circ\text{C} \pm 0.1$  and  $\geq 39.1^\circ\text{C} \pm 0.2$ , respectively. The preferred temperature for *R. dominica* was  $29.4 \pm 0.1^\circ\text{C}$  and for *A. flavipes* it was  $26.2 \pm 0.1^\circ\text{C}$ . Both insects oviposited in the preferred range of the adults.

The results will improve managers' ability to accurately analyze sampling data when temperature-dependent biological characteristics are taken into consideration. Managers of stored grain ecosystems around the world can use these data to assist them in maintaining the integrity of grain from harvest to consumption or sale.

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BUG *XYLOCORIS FLAVIPES* (REUTER) (ANTHOCORIDAE)

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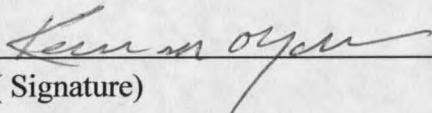
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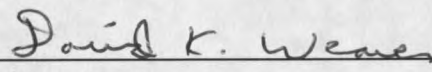
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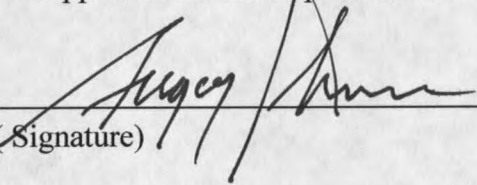
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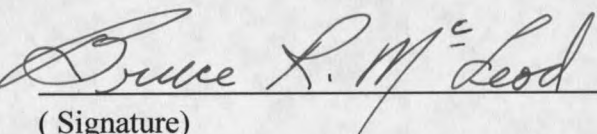
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## ABSTRACT

Cereal grains are stored in nearly every country around the world for personal consumption or economic reasons. The stored grain environment is thermally dynamic. Insect behavior and damage are highly influenced by these changing temperatures. Temperature-dependent behavior should be taken into consideration to successfully store grain. Many of the chemicals traditionally used to control pests are being banned worldwide. Thus, it is necessary to explore alternatives such as biological control.

I studied temperature-dependent walking speed, activity and mortality of two stored grain insects, *Rhyzopertha dominica* (F.) (Bostrichidae) and *Xylocoris flavipes* (Reuter) (Anthicoridae). I also investigated temperature-dependent adult position and oviposition preferences of these two species, and temperature-dependent predation rates of *X. flavipes*. The experiments were conducted on a laboratory thermal gradient which consisted of four parallel 15 x 100 x 1.0 cm thick aluminum bars. The ends of the bars rested upon hollow aluminum rods. Water from a hot water bath was pumped in a loop through rods beneath one end of the apparatus, while another pump passed cold water through rods at the other end. This resulted in a stable linear temperature gradient across the bars.

Walking speed increased linearly with temperature for both species. *Xylocoris flavipes* was faster than *R. dominica* at nearly all temperatures tested. *Rhyzopertha dominica* was 100% active at temperatures  $\geq 14.6 \pm 0.1^\circ\text{C}$  while *X. flavipes* was 100% active at temperatures  $\geq 17.3 \pm 0.01^\circ\text{C}$ . After 24 hours, *R. dominica* and *X. flavipes* were incapable of surviving temperatures  $\geq 46.5^\circ\text{C} \pm 0.1$  and  $\geq 39.1^\circ\text{C} \pm 0.2$ , respectively. The preferred temperature for *R. dominica* was  $29.4 \pm 0.1^\circ\text{C}$  and for *X. flavipes* it was  $26.2 \pm 0.1^\circ\text{C}$ . Both insects oviposited in the preferred range of the adults.

The results will improve managers' ability to accurately analyze sampling data when temperature-dependent biological characteristics are taken into consideration. Managers of stored grain ecosystems around the world can use these data to assist them in maintaining the integrity of grain from harvest to consumption or sale.

## CHAPTER 1

## INTRODUCTION

On-farm post-harvest storage of wheat and other cereal grains is a common practice worldwide (Sinha, 1995). Many farmers around the world store grain for personal consumption or seed (Scholler et al., 1997). However, the fundamental reason wheat is stored in the U.S., including Montana, is to increase its net value and sell it when prices are more advantageous (Anderson et al., 1995). Farmers in a broad region harvest their crops at the same time, thereby flooding the market and driving down prices. Producers that store their wheat and sell it at a time when the market is not inundated can reap a higher profit from the same yield.

Unfortunately, the practice of storing grain does not guarantee a higher profit. Positive economic returns can be offset because the overall quality of the grain is more likely to decrease the longer it is stored (Toews, 2000). Successful storage is achieved when the gain in revenue from postponing sale exceeds the costs associated with storage, costs that include diminished wheat quality due to insect damage and the expense of pest control. Therefore, much research has been aimed at improving the management of stored-grain ecosystems in ways that reduce these costs.

The combined attributes of stored grain insects have given rise to the serious global dilemma of preserving grain quality throughout the storage period. As grain was transported from region to region all over the world, so were the insects that depend on it for survival. Insects found in stored grain are among the most genetically diverse insects

in the world (Sinha, 1993; Guedes et al., 1997). Most stored grain insects have the ability to withstand extreme hot and cold temperatures even though they are believed to have originated from the tropics (Evans et al., 1983; Fields, 1992; Dermott and Evans, 1998). Many species are considered native to Africa and are naturally found under bark and detritus, as well as in rodent and bird nests (Linsley, 1944). Their wide-ranging thermal tolerances likely contributed to their global exploitation of stored grains. In addition to their complex genotype and cosmopolitan distribution, they have high reproductive rates and are remarkably resilient to environmental disturbances (Sinha, 1993). These characteristics, particularly genotypic complexity and high rates of reproduction, have enabled numerous populations to develop resistance to a wide range of insecticides (Champ and Dyte, 1976; Zettler and Cuperus, 1990).

### Justification

#### Management Decisions

Two of the key components of integrated pest management (IPM) are economic injury level (EIL) economic threshold (ET). The EIL is the point at which the loss of crop value due to damage is equal to the cost of control. Once an insect population surpasses the EIL, it is often too late for control measures to be effective. The ET is the point at which control measures should be taken to avoid reaching the EIL. The ET is often referred to as an action threshold, and it is always lower than the EIL in order to give producers, or managers, enough time for control measures to be effective. Both EIL and ET are a function of the value of the commodity and the cost of control. EIL and ET

are often expressed as insects per unit area, volume, or weight of commodity. Since these components require information about the insect populations present in the commodity, monitoring and sampling are integral to IPM programs, including those in stored grain (Ruesink and Kogan, 1982).

The probability that an insect population will reach the economic threshold may be directly linked to many factors. Some of the most influential factors in the stored grain ecosystem are grain moisture, temperature, and storage time (Hagstrum, 1987; Hagstrum and Throne, 1988). Sampling to determine pest density in stored grain is often done with probe traps. The first probe trap was developed and tested by Loschiavo and Atkinson (1967; 1973). A typical contemporary U.S. model is a cylindrical plastic probe trap, 37 cm long with 2.0 cm internal diameter and 192 2.8 mm downward-sloped round holes in the upper half (15.8 cm) of the trap (Burkholder, 1984). Insects are trapped and collected from the removable bottom portion. Probe traps are especially useful because they can be left in the grain over time and tend to capture more insects than an instantaneous grain sample. However, probe traps collect insects passively and the number collected relies not only on insect density and insect species but also on their activity levels. In this case, activity level refers to the proportion of time insects spend moving and the speed of their movement, both of which should be affected by temperature. Temperature gradients within a grain bin in the fall may reach 7 to 10°C/m (Hagstrum, 1987). In the fall and winter, grain temperatures around the periphery may be very cold, however temperatures near the center may remain warm enough to allow population growth through the winter (Flinn and Hagstrum, 1998). Hagstrum et al. (1998)

developed equations to account for thermal influence on trap catch. However, there is still the need to further investigate variables that will grant us an improved understanding of stored grain insect behavior (Sinha, 1993). When grain temperatures are warm,  $>25^{\circ}\text{C}$ , more insects are likely to be caught in probe traps. A manager may decide that control measures are necessary, when they might not be, due to the high numbers of insects caught; in this instance he is likely overestimating insect density when he does not consider the thermal influences on insect behavior. On the other hand, when grain temperatures are cooler,  $<20^{\circ}\text{C}$ , less insects may be caught in the traps. In this scenario a manager may decide that control measures are not necessary, when they might be, due to the low numbers of insects caught; in this case he may be underestimating insect density when temperature is not factored into the trap catch. This study examines one variable, temperature, which Hagstrum et al. (1998) used in their model. In summary, trap catch data may lead to inaccurate IPM decisions if the effects of temperature on distribution, movement, and general insect behavior are not considered (White et al., 1990).

### Biological Control

Many of the insecticides traditionally used to treat pest infestations in wheat bins are being phased out and will no longer be available for application (United Nations Environment Programme, 1998). In addition to the worldwide phase-out of many insecticides, there are increasing instances of target organism resistance (White and Bell, 1988; Guedes et al., 1996). As a result, we must investigate and implement viable, economical alternatives. One such alternative is the use of biological control. Natural

enemies may be applied against a diverse array of pests with negligible risk (Scholler et al., 1997). Furthermore, parasitoids and predators have an advantage over conventional methods because they are able to coevolve with their hosts (Scholler and Flinn, 2000). As a result, pests are unlikely to develop significant long-term resistance to biological control agents (Brower et al., 1995; Hokkanen et al., 1995). Furthermore, there has been little focus on using biological control agents in the stored-product ecosystem (Brower et al., 1995; Flinn et al., 1996), although this system is favorable for supporting natural enemy populations (Scholler and Flinn, 2000).

#### Overall Objectives

The overall objective of my research was to investigate the thermal biology of a stored-grain pest and a biological control agent. The pest species was *Rhyzopertha dominica* (F.), lesser grain borer (Coleoptera: Bostrichidae). The biological control agent was *Xylocoris flavipes* (Reuter), the warehouse pirate bug (Hemiptera: Anthocoridae). The major question addressed was whether the thermal preferences of predator and potential prey species overlapped. Thus, the results provide data concerning the efficacy of using *X. flavipes* to control *R. dominica* in a storage environment.

In addition to increasing our knowledge of the basic biology of these insects, the results of this project should provide useful information for assisting managers in implementing the appropriate IPM decisions. Grain is an excellent insulator (Jayas et al., 1994), which heats and cools relatively slowly in an unaerated grain bin. Temperatures

around the periphery of the bin, and on the grain surface, can be significantly different than the temperatures of the grain near the center and bottom (Arbogast et al., 1998). Since IPM decisions for stored-grain ecosystems are sometimes based on probe trap catch, and because probe trap catch is likely to be temperature sensitive, the results from my study should help improve the interpretation of trap catch data.

### Specific Objectives

To accomplish the overall objective I conducted a series of experiments on a laboratory thermal gradient to determine:

- 1) temperature-dependent movement rates, activity levels, and short-term mortality of adults of both species,
- 2) position and oviposition preferences of adults of both species across temperatures, and
- 3) predation rates of *X. flavipes* adults across temperatures.

## CHAPTER 2

TEMPERATURE-DEPENDENT MOVEMENT RATES AND MORTALITY OF  
*RHYZOPERTHA DOMINICA* (F.) (BOSTRICHIDAE) AND  
*XYLOCORIS FLAVIPES* (REUTER) (ANTHOCORIDAE)Abstract

Because temperature influences the movement, or change in spatial position, and behavior of stored grain insects, it may affect trap catch. Thus, integrated pest management decisions may be compromised if these attributes are not taken into consideration. I studied temperature dependent walking speed, activity, and mortality of two stored grain insects, *Rhyzopertha dominica* (F.) (Bostrichidae) and *Xylocoris flavipes* (Reuter) (Anthocoridae). Walking speed increased linearly with temperature for both species. At  $39.8 \pm 0.1^\circ\text{C}$ , *R. dominica* moved at rates from 0.25 to 1.0 cm/sec, with a mean of  $0.55 \pm 0.03$  cm/sec. At the same temperature, *X. flavipes* were capable of moving between 1.0 and 3.6 cm/sec, with a mean of  $2.0 \pm 0.1$  cm/sec. Activity levels, which were measured as the proportion of time the insects were active out of 60 seconds, did not vary linearly with temperature. In these experiments, the minimum temperature required for movement was  $9.0 \pm 0.02^\circ\text{C}$  and  $9.6 \pm 0.02^\circ\text{C}$ , for *R. dominica* and *X. flavipes*. At temperatures greater than, or equal to,  $14.6 \pm 0.1^\circ\text{C}$  and  $17.3 \pm 0.01^\circ\text{C}$ , all of the *R. dominica* and *X. flavipes* were 100% active. My data indicate that *R. dominica* and *X. flavipes* are incapable of surviving temperatures greater than, or equal to,  $46.5 \pm 0.1^\circ\text{C}$  and  $39.1 \pm 0.2^\circ\text{C}$  for 24 hours.

## Introduction

Many environmental factors influence the likelihood of an insect population reaching a level that requires control. Some of the factors that are most influential in the stored grain ecosystem are grain moisture, temperature, and storage time (Hagstrum, 1987; Hagstrum and Throne, 1988). In addition to affecting population growth, these factors may bias sampling results when their effect on insect movement and behavior are not considered, thus potentially compromising IPM decisions. Flinn and Hagstrum (1998) showed that *Cryptolestes ferrugineus* (Stephens) were able to congregate at the warmest temperature even when provided with as little as a 1°C temperature gradient, from 20 to 21°C. Sampling is an essential element of integrated pest management. IPM decisions are based upon the interpretation of sampling results (Ruesink and Kogan, 1982) and managers of stored-grain ecosystems generally base their decisions on trap catches and grain samples. One of the more common monitoring devices for stored grain insects is the probe trap. Unlike an instantaneous grain sample, probe traps sample over a prolonged period of time; as a result, they tend to be more sensitive and are liable to capture more insects. Trap catch depends on both insect density and activity levels since the traps collect insects passively. Consequently, if the effects of temperature on distribution and movement are not considered, trap catch data may lead to imprecise IPM decisions (White et al., 1990).

The lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) may be one of the most economically devastating stored grain insects in the United States, if

not in the world (Storey et al., 1984; Longstaff, 1999). It is also one of the most heat-tolerant species in the stored grain ecosystems (Dermott and Evans, 1978). More research is needed to reliably predict population size from sampling results.

Insecticides have routinely been applied to stored grain in order to control insect infestations. The alarming number of target organisms that have become resistant to these chemicals may be a direct result of this routine practice (White and Bell, 1988; Guedes et al., 1996). Furthermore, the public has become increasingly dissatisfied with the overuse and misuse of these chemicals, and there is now a worldwide phase-out underway for many of the insecticides commonly used in stored grain. Accordingly, we must explore and put into practice alternatives that are economically and environmentally sustainable. Biological control organisms are one such alternative. Distinct from chemicals, parasitoids and predators have the advantage of coevolving with their hosts (Scholler and Flinn, 2000). This coevolution results in negligible long-term resistance to biological control agents (Brower et al., 1995; Hokkanen et al., 1995). Up until now there has been little focus on the application of biological control agents in stored-product ecosystems (Brower et al., 1995; Flinn et al., 1996), although it is possible for natural enemies to flourish in this system (Scholler and Flinn, 2000).

The warehouse pirate bug, *Xylocoris flavipes* (Reuter) (Hemiptera: Anthocoridae) was first identified as a potential biological control agent in the early 20<sup>th</sup> century (Willcocks, 1922). The post WWII advent of agricultural chemicals led to a near disappearance of the demand for biological control. Biological control did not regain popularity until the late 1960s. Jay et al. (1968) were the first to officially suggest using

the warehouse pirate bug as a biological control agent against stored grain pests. Their work proved that the population growth rate, of numerous stored grain pests, would be severely diminished in the presence of *X. flavipes*. Further research authenticated the previous study, and confirmed the insect's ability to prey on a diversity of species (LeCato and Davis, 1973) and life stages (LeCato, 1976). The most susceptible life stage of *R. dominica* is the egg and populations are significantly reduced in the presence of *X. flavipes* (Jay et al., 1968).

The objectives of this study were to determine temperature-dependent walking speed, activity levels, and mortality of *Rhyzopertha dominica* and *Xylocoris flavipes*. Since IPM decisions for stored-grain ecosystems are often based on probe trap catch, and because probe trap catch is likely to be temperature sensitive, the results should help improve the interpretation of trap catch data. In addition, they should expand our knowledge of the predator's capacity to control pests in the thermally dynamic stored grain ecosystem.

#### Materials and Methods

The research was conducted on a laboratory thermal gradient which consisted of four parallel 15×100×1 cm thick aluminum bars. The ends of the bars rested upon hollow aluminum rods oriented perpendicular to the long axis of the bars. Water from a hot water bath was pumped in a loop through two rods beneath one end of the apparatus, while another pump passed a cooled propylene glycol and water mixture through the rods

at the other end (Fig. 2.1). As a result, a stable linear temperature gradient was maintained along the length of the bars. Thermocouples were embedded within each of the bars at 10 cm intervals from 0 to 100 cm, for a total of 11 temperature measurements per bar. A 1.2 cm high wood frame surrounded each of the bars, each frame was covered with a sheet of plate glass to stabilize temperature and confine insects. The four bars also sat within a single large opaque enclosure. Thus, the insects remained in darkness to minimize outside disturbance and to ensure that they did not orient to an external light source that would confound their response to temperature. The bars were cleansed after each trial with a 70% ethanol solution and then washed with distilled water.

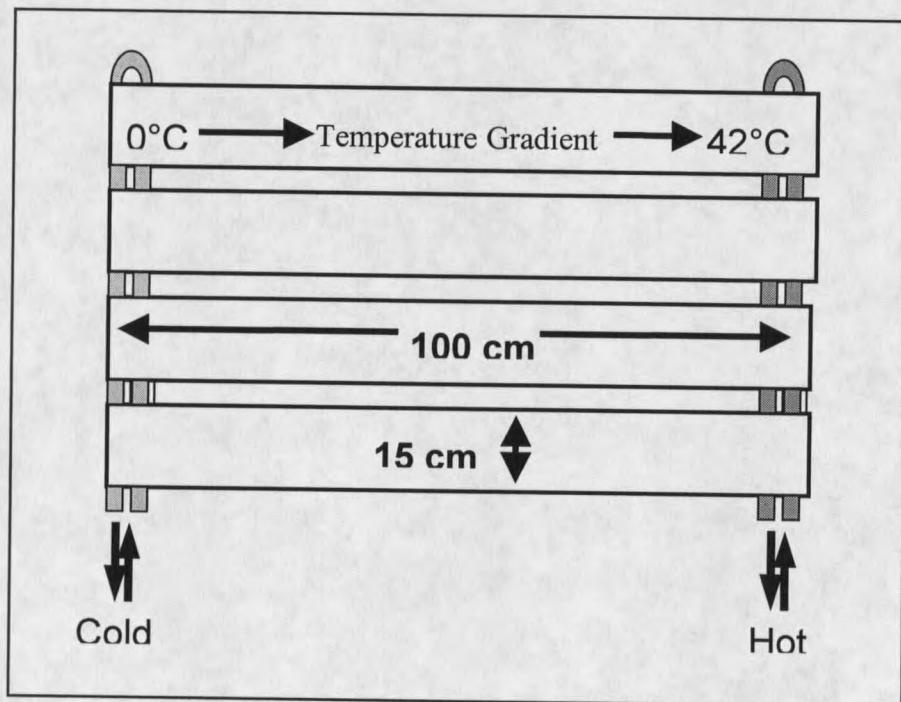


Figure 2.1. Diagram of the laboratory thermal gradient apparatus. The ends of the four aluminum bars rested upon hollow aluminum rods through which water was pumped at a constant temperature. One end was in contact with cold water while the other was in contact with hot water, this resulted in a stable linear temperature gradient across each of the bars.

The lesser grain borers (*R. dominica*) used in these experiments were collected from Miles City, Montana and reared on organic soft white wheat in a 12L/12D, 20/25°C incubator. The cultures were maintained in quart size glass jars with screen lids covering #2 filter paper. Humidity was provided by a pan of water at the bottom of the incubator. Adults were periodically sifted from the grain; this grain was placed into a clean jar, dated, and returned to the incubator. The approximate age of the beetles that emerged from this grain could be determined. The *R. dominica* used in these experiments were 0 to 30 days old.

The warehouse pirate bugs (*X. flavipes*) used in these experiments were obtained from the USDA ARS Center for Medical, Agricultural and Veterinary Entomology in Gainesville, Florida and were originally collected from Savannah, Georgia. The insects in my laboratory were reared on a population of *Oryzaephilus surinamensis* obtained from the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) Grain Marketing and Production Research Center (GMPRC) in Manhattan, Kansas. The cultures were maintained in quart size glass jars with screen lids covering #2 filter paper. The jars contained old-fashioned rolled oats and were kept in a 12L/12D, 20/25°C incubator. Humidity was provided by a pan of water at the bottom of the incubator. The *X. flavipes* used in these experiments were of unknown ages.

### Walking Speed

The linear walking speeds of individual insects were recorded after they were placed on filter paper set on the surface of the gradient. Two glass tubes, 15 cm long,

were placed 0.5 cm apart on top of the paper, providing a channel for the insects to walk forward. The tubes were placed perpendicular to the long axis of the gradient, this allowed for an even temperature across the walking channel. The filter paper was necessary for the insects to grip; otherwise they were unable to gain traction on the smooth gradient surface. The gradient water temperatures were set at  $-5.5$  and  $48.0^{\circ}\text{C}$ . The walking channels were spaced 10 cm apart along one of the gradient bars, so there were a total of 9 channels. This experiment was replicated 25 times for each temperature. Three separate thermocouple measurements were taken on the surface of the filter paper in the middle of each of the nine walking channels and a mean was calculated. Walking speed was transformed using a square root transformation ( $\sqrt{3/8 + Y}$ ). This transformation was used because it stabilized the variance while maximizing the mean to variance ratio. A regression line was fitted to transformed speed and back-transformed to be plotted for raw, or untransformed, speed. Temperatures at which no movement occurred were excluded to eliminate biased results.

### Activity Index

The activity indices were determined with individual insects placed in petri dishes on the surface of the gradient, with the pumps set at  $-7.0$  and  $43.0^{\circ}\text{C}$ . Each individual was observed for 60 seconds. Activity level is defined by the number of seconds they are active out of sixty. Eighteen 3.5 cm petri dishes were placed along one bar. This experiment was replicated 20 times. Two separate thermocouple measurements were taken on the surface of each petri dish to account for any difference between the bar

temperature and the actual temperature that the insect experienced. Data were analyzed with logistic dose response equation.

### Mortality

Survivorship at extreme temperatures was examined by placing individual insects in 15 cm long glass tubes, placed perpendicular to the long axis of the gradient. This allowed for even temperature across the tube and a total of 19 tubes per bar. Each tube contained one broken kernel of wheat for the pest and one late instar *Oryzaephilus surinamensis* larva for the predator. The gradient water temperatures were set at 25 and 60°C. Five separate thermocouple measurements were taken inside each of the tubes to account for any difference between the bar temperature and the actual temperature that the insect experienced. Insects were left on the gradient for 24 h, after which they were rated according to their condition. Insects that were healthy and active immediately upon removal from the tube were given a rating of 1; dead insects were given a rating of zero. Moribund individuals were given ratings of 0.25, 0.5, or 0.75, depending on their condition. Insects that were capable of moving only their antennae were given a rating of 0.25. Those that exhibited leg and antennal movement were given a rating of 0.5. The insects that were given a rating of 0.75 recovered at a slower rate than the healthy individuals. This experiment was replicated 10 times. Data were analyzed with a logistic dose response equation.

## Results

### Walking Speed

Walking speed increased linearly with temperature for both *R. dominica* (Fig. 2.2) and *X. flavipes* (Fig. 2.3). The regression equation predicts that *R. dominica* will not move at 7.1°C, and, in fact, all of the 25 *R. dominica* placed on the gradient where the mean of the three thermocouple measurements was  $6.6 \pm 0.1^\circ\text{C}$  remained immobile. Although the minimum temperature for movement by *X. flavipes* was  $10.0^\circ\text{C}$  (from the regression) and none moved at positions corresponding to  $6.9 \pm 0.1^\circ\text{C}$  or  $12.1 \pm 0.1^\circ\text{C}$ , they moved at a higher speed than *R. dominica* at nearly all temperatures tested.

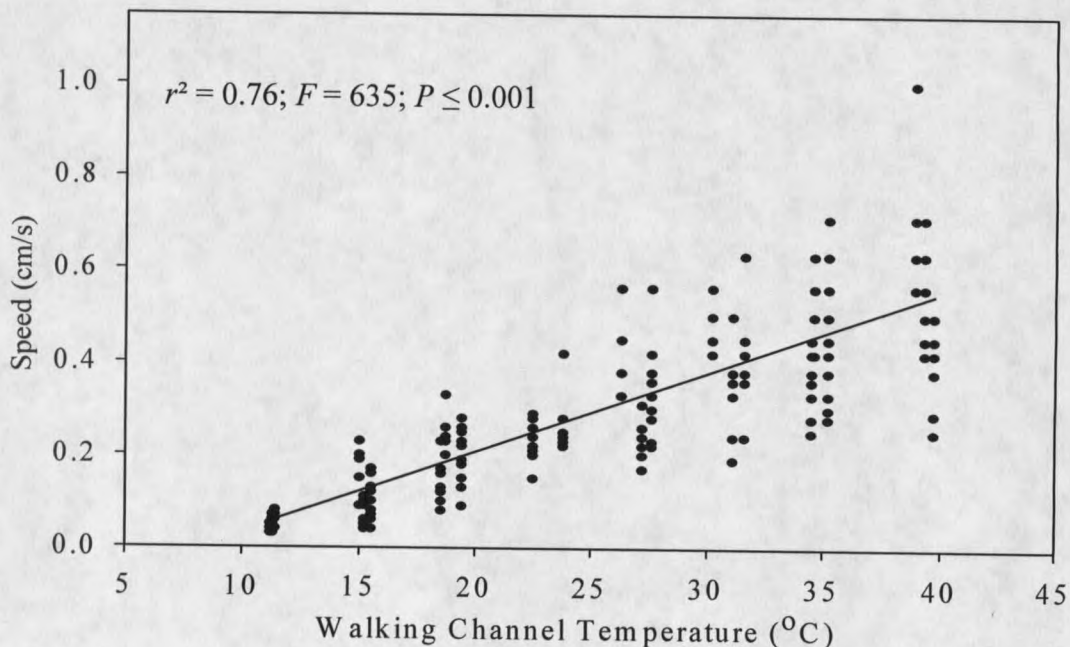


Figure 2.2. *Rhyzopertha dominica* walking speed as a function of temperature. Twenty-five adults were observed at each of nine temperatures; speed was measured over a 5 cm distance and expressed in cm/s. Back-transformed regression line plotted against untransformed speed. [ $\sqrt{3/8 + \text{Speed}} = .5372 + 0.0107 (\text{Temperature})$ ]

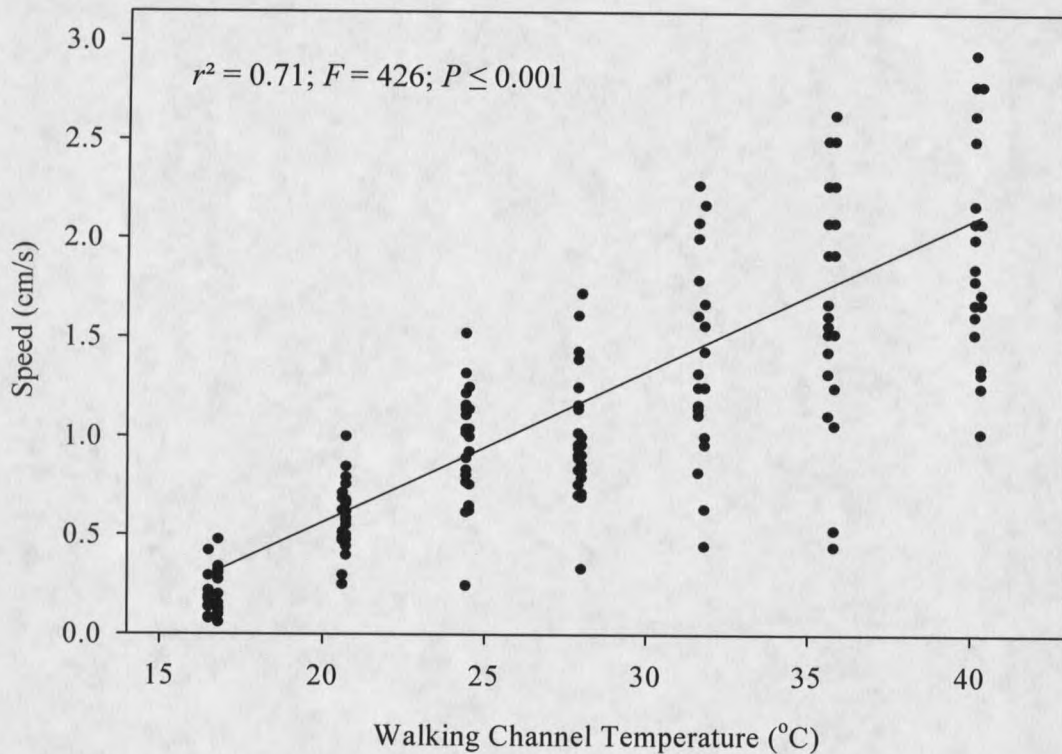


Figure 2.3. *Xylocoris flavipes* walking speed as a function of temperature. Twenty-five adults were observed at each of nine temperatures; speed was measured over a 5 cm distance and expressed in cm/s. Back-transformed regression line plotted against untransformed speed. [ $\sqrt{3/8 + Speed} = 0.2939 + 0.03189 (\text{Temperature})$ ]

### Activity Index

The proportions of time that *R. dominica* (Fig. 2.4) and *X. flavipes* (Fig. 2.5) were active were non-linear functions of temperature. On the gradient, none of the *R. dominica* moved at  $6.7 \pm 0.1^\circ\text{C}$ , whereas they moved constantly at temperatures  $\geq 14.6 \pm 0.1^\circ\text{C}$ . No *X. flavipes* moved at  $5.4 \pm 0.01^\circ\text{C}$  or  $7.4 \pm 0.01^\circ\text{C}$ , whereas they moved constantly at temperatures  $\geq 17.3 \pm 0.01^\circ\text{C}$ . *Xylocoris flavipes* achieved levels of activity similar to those of *R. dominica* only at higher temperatures. The logistic dose response equation predicts that *R. dominica* will be active half of the time at a

temperature of 10.5°C (Fig. 2.4), whereas *X. flavipes* exhibit a similar level at 12.6°C (Fig. 2.5). The difference of ~2.1°C is similar to the ~2.9°C difference in thresholds for activity predicted from the regression equations from the walking speed observations.

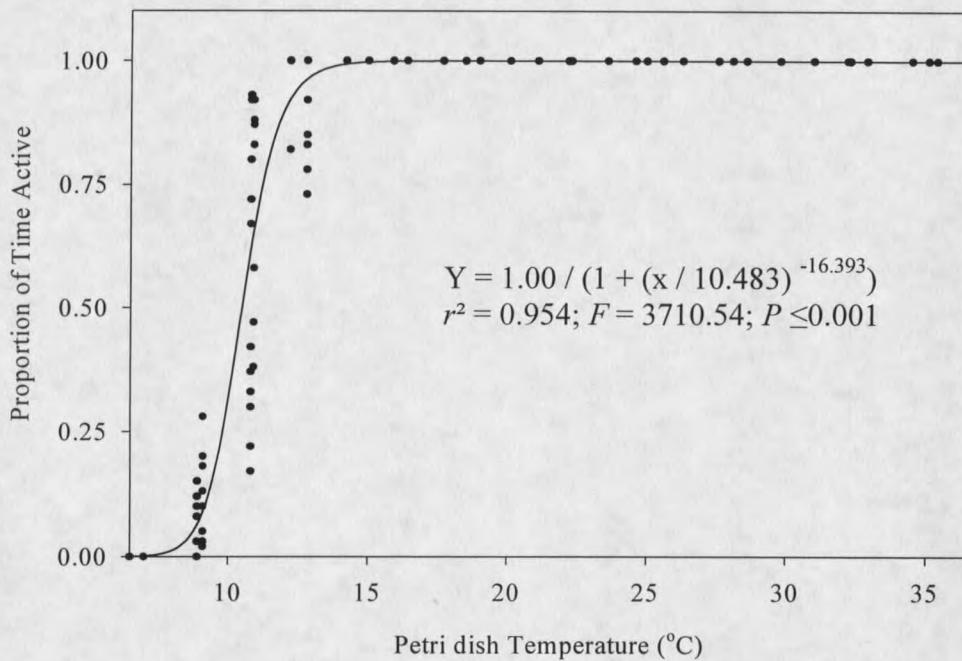


Figure 2.4. Logistic dose response of activity indices of *R. dominica* as a function of temperature. Twenty adults were observed for 60 s at each of 18 temperatures; activity index was calculated as the proportion of time active out of 60 s.

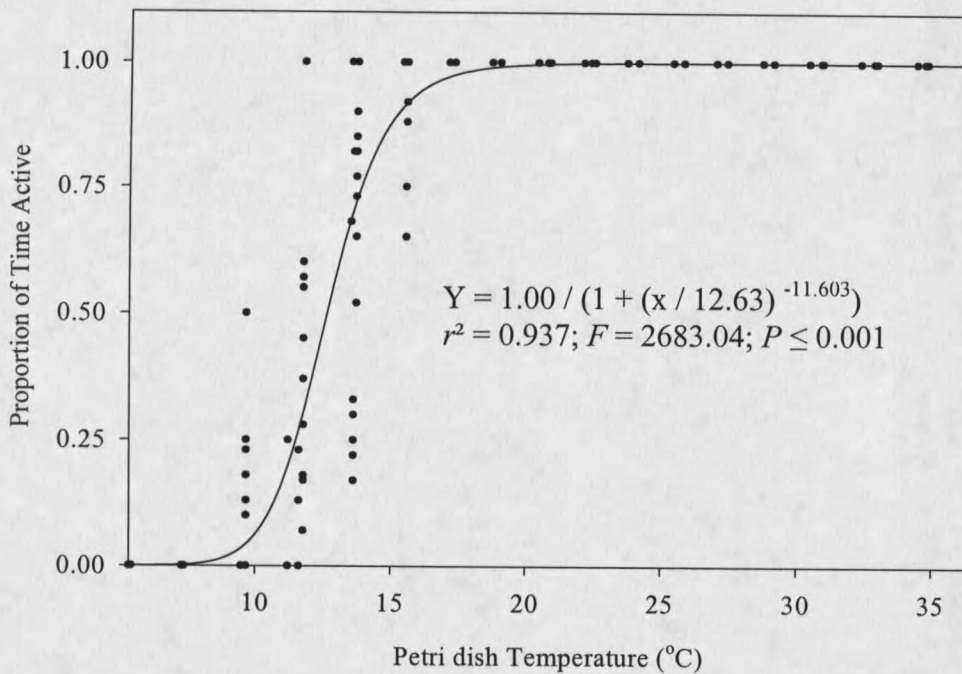


Figure 2.5. Logistic dose response of activity indices of *X. flavipes* as a function of temperature. Twenty adults were observed for 60 s at each of 18 temperatures; activity index was calculated as the proportion of time active out of 60 s.

### Mortality

Survivorship also varied non-linearly with temperature for both species (Figs. 2.6 and 2.7). Although *R. dominica* survived for 24 hours at temperatures from  $25.9 \pm 0.1^\circ\text{C}$  to  $44.4 \pm 0.1^\circ\text{C}$ , all died at temperatures  $\geq 46.6 \pm 0.1^\circ\text{C}$ . *Xylocoris flavipes* survived for 24 hours at temperatures from  $25.9 \pm 0.1^\circ\text{C}$  to  $37.5 \pm 0.2^\circ\text{C}$ , but all died at temperatures  $\geq 39.1 \pm 0.2^\circ\text{C}$ . The logistic dose response equations predict a 0.5 moribundity value, or  $LD_{50}$ , for *X. flavipes* at  $35.5^\circ\text{C}$ , but that condition is not predicted for *R. dominica* until  $42.5^\circ\text{C}$ .

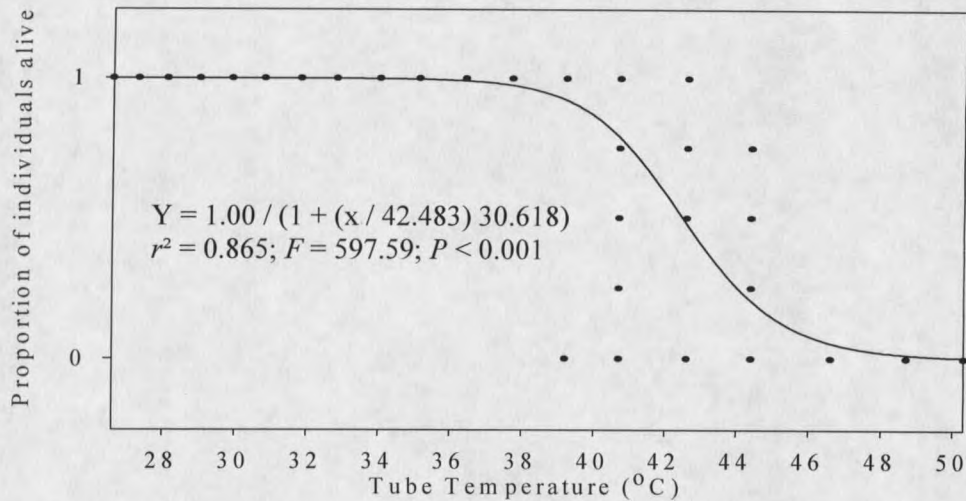


Figure 2.6. Logistic dose response for *R. dominica* mortality, expressed as proportion of individuals alive as a function of temperature. Ten adults were placed individually in each of 19 glass tubes and rated according to condition after 24 h. Dead insects were rated as 0, moribund individuals were rated as 0.25, 0.5, and 0.75 depending on the level of moribundity and apparently healthy insects were rated as 1.

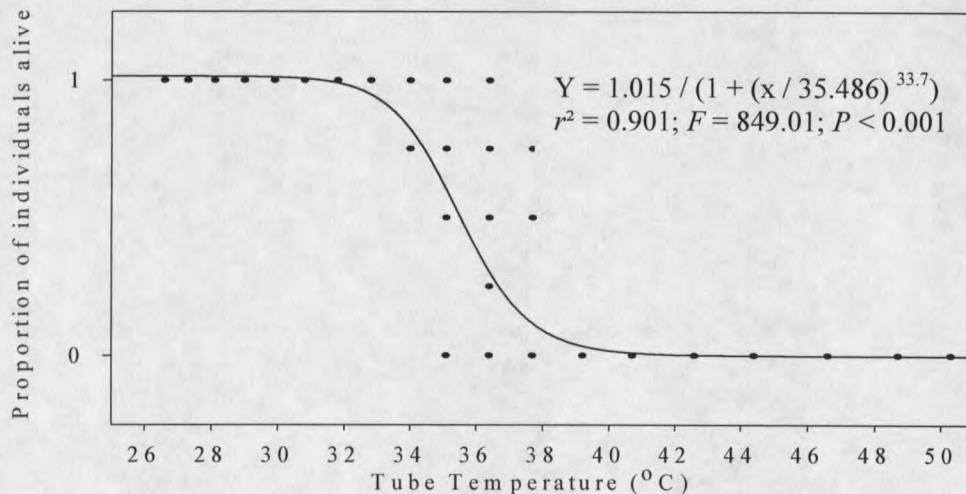


Figure 2.7. Logistic dose response for *X. flavipes* mortality, expressed as proportion of individuals alive as a function of temperature. Ten adults were placed individually in each of 19 glass tubes and rated according to condition after 24 h. Dead insects were rated as 0, moribund individuals were rated as 0.25, 0.5, and 0.75 depending on the level of moribundity and apparently healthy insects were rated as 1.

Discussion

My results indicate that, compared to *X. flavipes*, *R. dominica* move more slowly when active, but that they remain active at lower temperatures and survive higher temperatures. The mortality data generally concur with previous studies which demonstrated that *R. dominica* can survive temperatures of 39°C for 24 hours (Birch, 1945; Longstaff, 1999).

The results have several implications for pest population sampling and biological control in stored grain systems, which are temporally dynamic and spatially diverse (Arbogast, 1998). First, although *R. dominica* can apparently deal with a wider range of temperatures, the range at which *X. flavipes* is active overlaps broadly with that of *R. dominica* and it can move (at least in two dimensions) much more rapidly than its prey. Assuming that bin temperatures do not change too rapidly and that non-lethal temperatures are available somewhere in the grain bulk, *X. flavipes* should be able to find safe refuges when necessary. Thus, although temperature may restrict the distribution of *X. flavipes* within stored grain more than it does *R. dominica*, it may not preclude the use of this predator in the biological control of this pest. Although *X. flavipes* predation may be curtailed at lower temperatures, damage caused by *R. dominica* is also likely to be limited under those conditions. On the other hand, the greater short-term survival of *R. dominica* at higher temperatures may provide the pest with refuges against predation by *X. flavipes*. Additionally, *R. dominica* may escape predation by moving deeper into the

grain bulk since *X. flavipes* are more common near the grain surface (Arbogast, et al. 1998). The most susceptible life stage of *R. dominica* is the egg and populations are significantly reduced in the presence of *X. flavipes* (Jay et al., 1968).

Second, pest management decisions for stored grain ecosystems rely primarily on accurate estimations of insect density within bulk-stored grain (Cuperus et al., 1990; Hagstrum and Flinn, 1992). Probe traps are one of the best choices for monitoring because they can be left in the grain and tend to be more sensitive than other tools (Loschiavo and Atkinson, 1967; Lippert and Hagstrum, 1987). However, trap catch is contingent upon various factors, such as insect species, grain temperature, and trapping duration (Cuperus et al., 1990; Hagstrum et al., 1998). Managers need to be able to convert trap catch into an absolute sampling estimate like insects/kg grain. Thus, the results of this study will assist managers in accurately analyzing trap catches when biological characteristics, such as temperature-dependent movement and activity, are taken into consideration.

## CHAPTER 3

TEMPERATURE PREFERENCES OF *RHYZOPERTHA DOMINICA* (F.)  
BOSTRICHIDAEAbstract

The stored grain environment is thermally dynamic and pest population densities and distributions are highly dependent on the presence, and spatial distribution of favorable temperatures. I studied the temperature-dependent adult position and oviposition preferences of *Rhyzopertha dominica* (F.). After 7 days, most of the beetles introduced to the gradient were found resting, mating, or feeding on grain where the temperature range was  $29.4 \pm 0.1^{\circ}\text{C}$ . Emergence data indicate that the beetles oviposited in these areas. I also studied temperature-dependent adult position preferences of *R. dominica* in the absence of food. Results from these trials were inconclusive due to thigmotaxis rather than thermotaxis.

Introduction

The bostrichid beetle, *R. dominica*, is one of the most damaging cosmopolitan pests commonly found in the stored grain ecosystem. To effectively manage and monitor populations, we must first have a comprehensive understanding of their distribution in grain; several factors seem primary in influencing distribution: temperature, moisture and social interactions. Temperature is an important factor not only because of its potential effect on the beetles but because the stored grain environment is thermally dynamic.

Grain is an excellent insulator that heats and cools relatively slowly (Jayas et al., 1994). Therefore, temperatures can be significantly different depending upon position within the grain mass (Arbogast et al., 1998).

In addition to the effects of temperature on distribution, it is also important to note that temperature plays a key role in determining population densities and growth rates. The duration of the immature stages (egg, larva, and pupa) typically tends to decrease as temperature increases. *Rhyzopertha dominica* require 281 days to develop at 18°C, whereas it only takes 31 days at 33°C (Longstaff, 1999). Development from egg to adult ceases at temperatures  $\geq 39^\circ\text{C}$ , even though adults are capable of enduring these higher temperatures for a period of time (Birch, 1945; Longstaff, 1999).

The distribution and density of beetles in grain is not only influenced by temperature but also by social interactions. These interactions are chiefly due to the aggregation pheromone secreted by the males, and to which both males and females respond equally (Khorramshahi and Burkholder, 1980). This pheromone, containing the two major compounds dominicalure 1 and dominicalure 2, was first isolated by Williams et al. (1980). Population densities and the distribution of beetles in grain may be the result of chemical cues as well as thermal preferences.

This study focused on temperature-dependent distribution of *R. dominica*. Managers are able to make more informed decisions regarding pest management by monitoring temperatures within the grain mass and having knowledge of the insect's preferred temperatures. This study is aimed to give managers the information they need for accurate stored grain ecosystem management.

## Materials and Methods

The Materials and Methods section in Chapter 2 (pp. 10-12) provides a complete description of the laboratory thermal gradient apparatus and the source of the *R. dominica* that were used in this study.

### Adult Position and Oviposition Preferences

Twenty-four hours prior to introducing adult insects to the bars, the thermal gradient was established with the surface of the bars covered with a single-grain layer of intact wheat grains. Water bath fluid temperatures were set at -7 and 43°C. To begin each experiment, 3 adults were introduced to the surface of the grain at each of 15 5 cm intervals from 15 to 85 cm. The bars were then covered with glass and placed in darkness. The location of all visible adults was recorded after 7 days. I included only mobile beetles in the analyses; beetles placed on the cold end of the gradient never moved and were therefore excluded from the analyses. After 7 days, the grain was removed in 5 cm bands (i.e., ~2°C ranges), and placed in rearing containers. Adults were immediately removed and counted, and the containers were placed in a 12L/12D, 20/25°C incubator for rearing. After 30 days, each container was examined at two-day intervals to remove and count second-generation adults until emergence ceased. Distribution of insects along the gradient was analyzed with ANOVA general linear model. A square root transform,  $\sqrt{3/8 + Y}$  was chosen for each data set because it stabilized the variance while maximizing the mean to variance ratio. This experiment was replicated six times: four times with the temperature gradient established and twice

(controls) with it off. Temperature data from all trials were used to calculate mean positional temperatures.

#### Adult Positions without Food Present

The condition of the grain and eggs at different temperatures, and insect interaction may confound responses to temperature. To provide a check against these possibilities, I ran a second set of observations in which the insects were placed individually (without food) in 90 cm long glass tubes lying on the surface of the gradient bars. A single strand of dental floss was strung through each tube to provide traction, as they are unable to move forward on smooth glass. Each beetle was positioned in the middle of the tube on the gradient, from 45 to 55 cm, with each tube containing wooden plugs on either end to prevent escape. The water fluid bath temperatures were set at 15 and 43°C. There were a total of 40 tubes per replicate, with 10 tubes placed on each gradient bar. The tubes were rotated 180° after 2 hours and the final positions were recorded after 18 hours. In this situation, they should be able to make the same behavioral choices among temperatures without the possible confounding factors of food condition and social interactions. Food condition is important, because although they might not respond to variable food condition, they could be responding to lack of food and start wandering. This experiment was replicated 6 times, 4 with the thermal gradient established and 2 controls with the gradient off. Distribution of insects along the gradient was analyzed with an ANOVA general linear model. A Chi-Square goodness-of-fit test was used to determine whether the outcome of the controls differed from random.

ResultsAdult Positional Preferences

Seven days after the beetles were introduced to the gradient, most were found between 65 and 75 cm or  $29.4 \pm 0.1^\circ\text{C}$  (Fig. 3.1). Position or temperature (Table 3.1) was the only significant factor in trials with the gradient on (Table 3.2). The mean temperature for the control trials was  $24.2 \pm 0.1^\circ\text{C}$ . Position and bar by position interaction significantly influenced the response in the control trials (Table 3.3). The distribution of the adults differed between trials with the gradient established versus the controls as reflected in Figure 3.1 and Table 3.4.

Table 3.1. Gradient position, range on the gradient in cm, and mean temperature  $\pm$  SE $^\circ\text{C}$  for *R. dominica* adult positional and ovipositional preferences.

Position	Range (cm)	Mean Temperature $\pm$ SE $^\circ\text{C}$
1	5-15	$3.1 \pm 0.2$
2	15-25	$7.8 \pm 0.2$
3	25-35	$12.6 \pm 0.2$
4	35-45	$17.0 \pm 0.2$
5	45-55	$21.2 \pm 0.1$
6	55-65	$25.3 \pm 0.1$
7	65-75	$29.4 \pm 0.1$
8	75-85	$33.7 \pm 0.1$
9	85-95	$37.7 \pm 0.1$

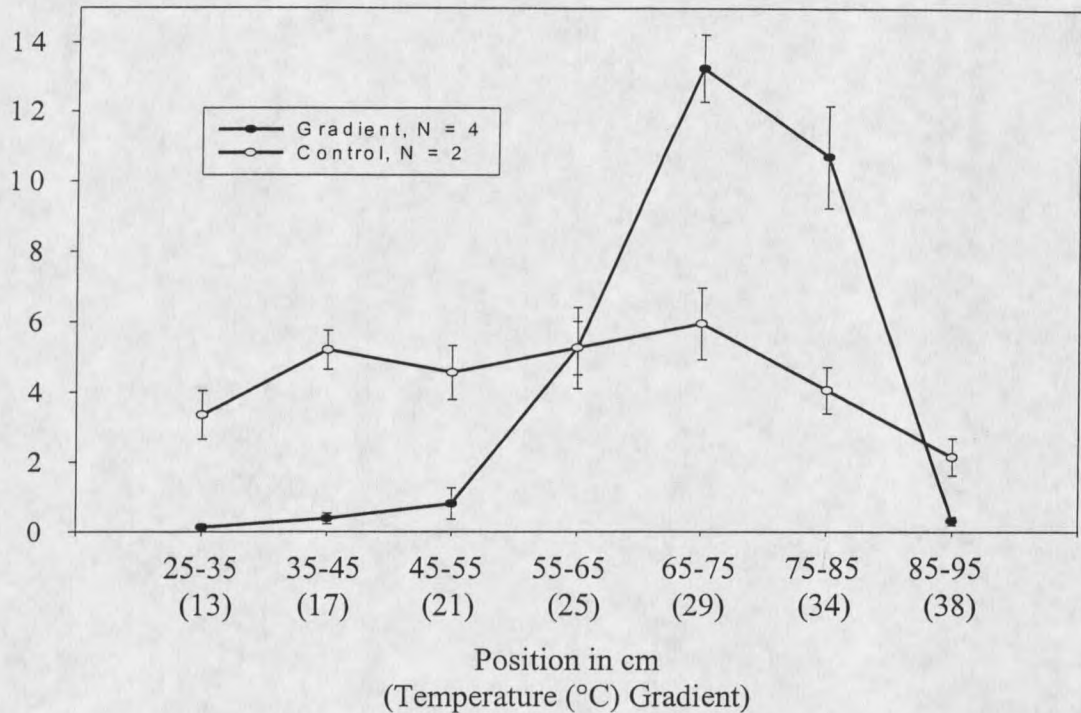


Figure 3.1. Mean ( $\pm$  SE) adult *R. dominica* per bar as a function of position in cm and temperature ( $^{\circ}$ C). Three adults were placed every 5 cm, from 15 to 85 cm, across each of the 4 laboratory thermal gradient bars, for a total of 45 adults per bar. Adult positions were recorded after 7 days on the apparatus. Mean temperature for the control was  $24.2 \pm 0.1^{\circ}$ C.

Table 3.2. ANOVA for gradient on, with transformed proportion of adult *R. dominica* as the response. Positions were recorded seven days after being introduced to the apparatus; 45 adults were evenly spaced across each of the four bars and allowed to self-disperse in each of the trials with the temperature gradient established.

Source	DF	Seq SS	Adj MS	F	P
Trial	3	0.00087	0.00029	0.08	0.971
Bar	3	0.00002	0.00001	0.00	1.000
Position	8	1.60415	0.20052	54.91	0.000
Trial*Bar	9	0.00146	0.00016	0.04	1.000
Trial*Position	24	0.11392	0.00475	1.30	0.196
Bar*Position	24	0.10907	0.00455	1.24	0.236
Error	72	0.26291	0.00365		
Total	143	2.09239			

Table 3.3. ANOVA for controls (gradient off), with transformed proportion of adult *R. dominica* as the response. Positions were recorded seven days after being introduced to the apparatus; 45 adults were evenly spaced across each of the four bars and allowed to self-disperse in each of the control trials.

Source	DF	Seq SS	Adj MS	F	P
Trial	4	0.00045	0.00005	0.01	1.000
Bar	3	0.00010	0.00000	0.00	1.000
Position	8	0.12481	0.01501	4.53	0.000
Trial*Bar	12	0.00142	0.00008	0.02	1.000
Trial*Position	32	0.09331	0.00283	0.85	0.688
Bar*Position	24	0.13974	0.00582	1.76	0.029
Error	96	0.31801	0.00331		
Total	179	0.67783			

Table 3.4. ANOVA for gradient on versus controls, with transformed proportion of adult *R. dominica* as the response. Positions were recorded seven days after being introduced to the apparatus; 45 adults were evenly spaced across each of the four bars and allowed to self-disperse in each of the treatments.

Source	DF	Seq SS	Adj MS	F	P
Treatment	1	0.00578	0.00578	1.76	0.186
Bar	3	0.00011	0.00003	0.01	0.999
Position	8	1.02536	0.14871	45.18	0.000
Treatment*Bar	3	0.00002	0.00001	0.00	1.000
Treatment*Position	8	0.70389	0.08799	26.73	0.000
Bar*Position	24	0.13247	0.00552	1.68	0.027
Error	276	0.90838	0.00329		
Total	323	2.77600			

### Ovipositional Preferences

Emergence data indicate that the beetles oviposited in the areas where the mean temperature was  $29.4 \pm 0.1^\circ\text{C}$  (Fig. 3.2). Position, or temperature, was significant in trials with the gradient established, while trial  $\times$  position was also significant (Table 3.5).

None of the factors in the control trials contributed significantly to the response (Table 3.6). The distribution of second-generation beetles differed significantly between treatments (Table 3.7). The significant factors in this analysis were position and treatment  $\times$  position.

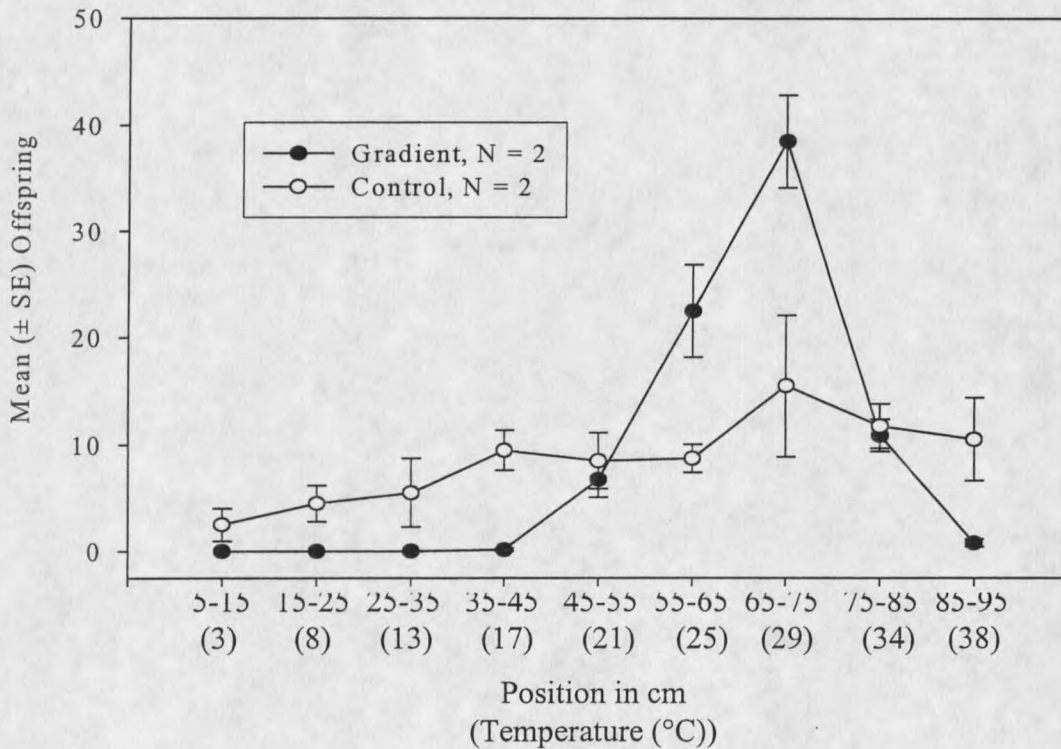


Figure 3.2. Mean ( $\pm$  SE) *R. dominica* offspring as a function of position in cm and temperature ( $^{\circ}$ C). Three adults were placed every 5 cm, from 15 to 85 cm, across each of the 4 laboratory thermal gradient bars, for a total of 45 adults per bar. After 7 days the grain was removed in 10 cm bands, the adults were removed, and the grain was placed in rearing containers in an incubator. The offspring were collected and counted upon emergence. Mean temperature for the control was  $24.2 \pm 0.1^{\circ}$ C.

Table 3.5. ANOVA for gradient on, with transformed proportion of *R. dominica* offspring as the response. Three adults were placed every 5 cm, from 15 to 85 cm, across each of the 4 laboratory thermal gradient bars, for a total of 45 adults per bar. After 7 days the grain was removed in 10 cm bands, the adults were removed, and the grain was placed in rearing containers in an incubator. The offspring were collected and counted upon emergence.

Source	DF	Seq SS	Adj MS	F	P
Trial	1	0.00002	0.00002	0.02	0.902
Bar	3	0.00014	0.00005	0.03	0.992
Position	8	0.79403	0.09925	68.79	0.000
Trial*Bar	3	0.00014	0.00005	0.03	0.992
Trial*Position	8	0.02770	0.00346	2.40	0.046
Bar*Position	24	0.02489	0.00104	0.72	0.788
Error	24	0.03463	0.00144		
Total	71	0.88155			

Table 3.6. ANOVA for controls (gradient off), with transformed proportion of *R. dominica* offspring as the response. Three adults were placed every 5 cm, from 15 to 85 cm, across each of the 4 laboratory thermal gradient bars, for a total of 45 adults per bar. After 7 days the grain was removed in 10 cm bands, the adults were removed, and the grain was placed in rearing containers in an incubator. The offspring were collected and counted upon emergence.

Source	DF	Seq SS	Adj MS	F	P
Trial	1	0.00061	0.00061	0.09	0.762
Bar	3	0.00015	0.00005	0.01	0.999
Position	8	0.11492	0.01437	2.20	0.065
Trial*Bar	3	0.00026	0.00009	0.01	0.998
Trial*Position	8	0.03003	0.00375	0.57	0.789
Bar*Position	24	0.21761	0.00907	1.39	0.215
Error	24	0.15695	0.00654		
Total	71	0.52053			

Table 3.7. ANOVA for gradient on versus controls, with transformed proportion of *R. dominica* offspring as the response. Three adults were placed every 5 cm, from 15 to 85 cm, across each of the 4 laboratory thermal gradient bars, for a total of 45 adults per bar. After 7 days the grain was removed in 10 cm bands, the adults were removed, and the grain was placed in rearing containers in an incubator. The offspring were collected and counted upon emergence.

Source	DF	Seq SS	Adj MS	F	P
Treatment	1	0.00058	0.00058	0.15	0.700
Bar	3	0.00027	0.00009	0.02	0.995
Position	8	0.69780	0.08723	22.35	0.000
Treatment*Bar	3	0.00001	0.00000	0.00	1.000
Treatment*Position	8	0.21115	0.02639	6.76	0.000
Bar*Position	24	0.11822	0.00493	1.26	0.212
Error	96	0.37463	0.00390		
Total	143	1.40267			

#### Adult Positions without Food Present

In both treatments, most of the insects rested against the plug in the end of the glass tube. Beetles in trials with the gradient established rested against the plug at the cooler end of the gradient, while beetles in the controls rested against either plug indiscriminately (Chi-Square = 0.1;  $P = 0.752$ ) (Fig. 3.3). Analyses showed a significant position effect in both treatments individually (Tables 3.8 and 3.9), and when compared against one another (Table 3.10). There was also a significant treatment  $\times$  position interaction suggesting that the position of the beetles differed between the treatments.

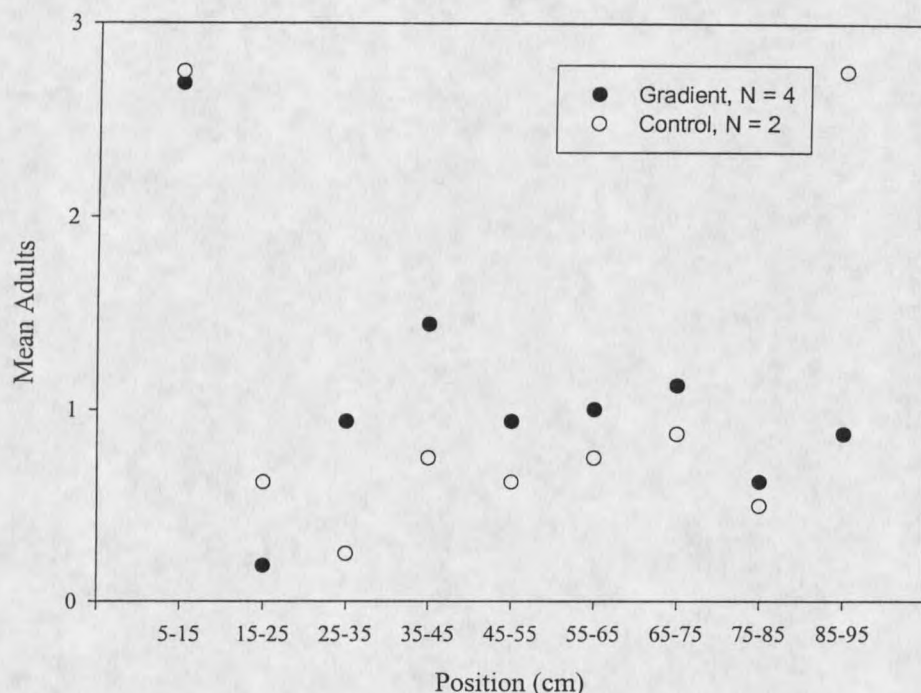


Figure 3.3. Mean number of adult *R. dominica* as a function of position. Insects were placed individually in 100 cm long glass tubes which ran parallel with the long axis of the apparatus; there were ten tubes per bar in each trial. All insects were placed in the middle of the tube at the start of the experiment, from 45 to 55 cm. The tubes were rotated 180° after 2 h and final positions were recorded after 18 h.

Table 3.8. ANOVA for gradient on (without food), with transformed proportion of adult *R. dominica* as the response. Insects were placed individually in 100 cm long glass tubes which ran parallel with the long axis of the apparatus; there were ten tubes per bar in each trial. All insects were placed in the middle of the tube at the start of the experiment, from 45 to 55 cm. The tubes were rotated 180° after 2 h and final positions were recorded after 18 h.

Source	DF	Seq SS	Adj MS	F	P
Trial	3	0.00125	0.00042	0.07	0.974
Bar	3	0.00030	0.00010	0.02	0.997
Position	8	0.27938	0.03492	6.21	0.000
Trial*Bar	9	0.00049	0.00005	0.01	1.000
Trial*Position	24	0.19630	0.00818	1.45	0.114
Bar*Position	24	0.13440	0.00560	1.00	0.483
Error	72	0.40486	0.00562		
Total	143	1.01698			

Table 3.9. ANOVA for controls (without food), with transformed proportion of adult *R. dominica* as the response. Insects were placed individually in 100 cm long glass tubes which ran parallel with the long axis of the apparatus; there were ten tubes per bar in each trial. All insects were placed in the middle of the tube at the start of the experiment, from 45 to 55 cm. The tubes were rotated 180° after 2 h and final positions were recorded after 18 h.

Source	DF	Seq SS	Adj MS	F	P
Trial	1	0.00017	0.00017	0.04	0.839
Bar	3	0.00119	0.00040	0.10	0.959
Position	8	0.25731	0.03216	8.10	0.000
Trial*Bar	3	0.00014	0.00005	0.01	0.998
Trial*Position	8	0.04459	0.00557	1.40	0.245
Bar*Position	24	0.18384	0.00766	1.93	0.057
Error	24	0.09525	0.00397		
Total	71	0.58250			

Table 3.10. ANOVA for gradient on versus control, with transformed proportion of adult *R. dominica* as the response. Insects were placed individually in 100 cm long glass tubes which ran parallel with the long axis of the apparatus; there were ten tubes per bar in each trial. All insects were placed in the middle of the tube at the start of the experiment, from 45 to 55 cm. The tubes were rotated 180° after 2 h and final positions were recorded after 18 h.

Source	DF	Seq SS	Adj MS	F	P
Treatment	1	0.00000	0.00000	0.00	0.979
Bar	3	0.00023	0.00018	0.03	0.992
Position	8	0.42047	0.05164	9.35	0.000
Treatment*Bar	3	0.00127	0.00042	0.08	0.973
Treatment*Position	8	0.11621	0.01453	2.63	0.010
Bar*Position	24	0.13376	0.00557	1.01	0.457
Error	168	0.92753	0.00552		
Total	215	1.59948			

### Discussion

The results of this study aid in furthering our knowledge of the basic thermal preferences of *Rhyzopertha dominica*. When given a choice, they nonrandomly aggregate toward a specific temperature. Adult positional and ovipositional data show that this species preferred temperature is  $29.4 \pm 0.1^{\circ}\text{C}$ . In control trials there was a significant position effect in both adult position and oviposition, although it was not as pronounced as it was in the trials with the gradient established. The effect of position in the controls is perhaps due to the aggregation pheromone produced by the males (Williams et al. 1980), because temperature remained relatively constant along each bar, as well as between the four bars. Bar  $\times$  position also influenced adult positional preference in the controls. The position of the beetles depended upon the bar. This is acceptable for the control trials because distribution was not the same on each bar, whereas the adults in trials with the gradient established exhibited a similar distributional pattern on all bars. The distribution of the adults and second-generation emergences differed between treatments, as indicated by the strong treatment  $\times$  position interaction. This interaction demonstrated that the distribution of the beetles within the bars depended on the treatment.

There was a strong trial  $\times$  position interaction for oviposition in trials with the gradient established. The distribution of second-generation beetles varied between trials, so trials within this treatment may not be considered exact replicates of one another. However, bar and trial  $\times$  bar were not significant. Therefore, the individual bars may be

considered independent replicates because the distribution of second-generation emergences from each bar did not vary within a trial or across trials.

In the trials without food present, treatment  $\times$  position was significant. This was probably because of thigmotaxis rather than thermotaxis. In trials with the gradient established, the beetles rested against the cool plug and avoided the hot end of the gradient. While beetles in the controls rested against either plug without preference for one end versus the other. The distribution of beetles differed between the treatments and that is why there is a significant treatment  $\times$  position interaction. This experiment was shown to be an insufficient test for temperature preference without food present. The beetles distributed themselves according to a preferred physical niche, against a plug, rather than a preferred temperature.

*Rhyzopertha dominica* is one of the most damaging cosmopolitan pests of stored grain. Consequently, this study provides valuable information to stored grain managers throughout the world. The experiments I conducted have shown that distribution of *R. dominica* is significantly influenced by temperature. Managers can use species-dependent thermal preferences to more reliably monitor and control insect populations in stored grain.

## CHAPTER 4

THERMAL PREFERENCES AND TEMPERATURE-DEPENDENT PREDATION RATES OF *XYLOCORIS FLAVIPES* (REUTER) ANTHOCORIDAEAbstract

I studied adult position and oviposition temperature preferences of *Xylocoris flavipes* (Reuter), as well as the temperature-dependent predation rates of this species. After 7 days, most of the predators were observed to rest, mate, and feed where the mean temperature was  $30.1 \pm 0.1^\circ\text{C}$ . Oviposition data indicate the predators oviposited in areas where the mean temperature was  $26.2 \pm 0.1^\circ\text{C}$ . I also studied temperature-dependent adult position preferences in the absence of food. Results from this study were inconclusive due to a thigmotactic response from the insects rather than a thermotactic response. Temperature-dependent predation rates were also examined. Predation rates were relatively constant over a wide range of temperatures, but declined at temperatures  $\geq 36.1 \pm 0.1^\circ\text{C}$ .

Introduction

Insect infestations are one of the major problems facing managers of stored grain ecosystems around the world. Many common agricultural chemicals, such as methyl bromide, are being eliminated worldwide (United Nations Environment Programme 1998). The global ban of these chemicals is necessitating the development of alternatives to these chemicals (Bell et al. 1996). One of the most promising alternatives is the use of

biological control organisms. Most are already present in the system, and may only need to be augmented for control to be successful. We must evaluate the efficacy of these organisms in the context of the thermally dynamic stored grain environment.

A beneficial characteristic that distinguishes biological control agents, like predators, from traditional chemical controls is their ability to coevolve with their prey (Scholler and Flinn, 2000). Coevolution typically prevents the pests from developing significant long-term resistance to their predators. Resistant traits are usually ephemeral because natural selection favors the predators with traits that overcome the prey's resistance (Brower et al., 1995; Hokkanen et al., 1995); chemicals are incapable of doing this without costly human intervention. To date, there has not been substantial effort applied to the exploration of utilizing beneficial insects in stored grain (Brower et al., 1995; Flinn et al., 1996). However, it has been shown that many biological control agents are naturally found in the stored grain ecosystem and that this system favors the reproductive success of beneficial insects (Scholler and Flinn, 2000).

One of the predators in the stored grain ecosystem is the anthocorid *Xylocoris flavipes* (Reuter) (Arbogast et al., 1971). Research with this potential biological control agent dates back to the early 20<sup>th</sup> century. Willcocks (1922) documented *X. flavipes* predation on the immature stages of nearly all stored grain pests. The demand for biological control grew quite steeply until WWII ended. After the war, the market was inundated with agricultural chemicals, which nearly ended the need for biological control. Insecticide resistance and the tragic environmental consequences of these new chemicals (Carson, 1962) facilitated the revival of biological control. In 1968, Jay et al.

were the first to professionally suggest application of the warehouse pirate bug, *X. flavipes*, as a biological control agent in stored grain. Their study showed dramatic reductions in a variety of stored grain insect populations by *X. flavipes*. Additional studies confirmed the insect's innate capacity to prey on numerous species (LeCato and Davis 1973; Arbogast 1979) and life stages (LeCato, 1976). Arbogast (1979) suggested that the predator would consume any insect that it could subdue, especially considering they are known cannibals. Abdel-Rahman et al. (1983) concluded that beetle larvae were the preferred prey of *X. flavipes*. The most susceptible life stage of *R. dominica* is the egg and populations are significantly reduced in the presence of *X. flavipes* (Jay et al., 1968).

After establishing the predator's ability to suppress pest populations, investigations began to further explore its versatility. Researchers began experiments that would demonstrate its exact impact on a pest population. LeCato and Collins (1976) looked at the maximum number of prey the predator could consume in a lifetime. They used *Tribolium castaneum* as the prey species. In a laboratory environment, one individual *X. flavipes* would be able to consume a mean number of 539 eggs, 34 larvae, or 14 pupae. Aside from the mean numbers of prey taken, it is also practical to examine the percent reduction in population due to the predator. When *X. flavipes* was added to an *Oryzaephilus surinamensis* culture, the prey population was reduced by 95% after 15 weeks (Arbogast, 1976). Likewise, fourteen weeks after being added to a *T. castaneum* culture, the pest population was suppressed by more than 90% (Press et al., 1975).

Research continued to confirm that *X. flavipes* was an effective biological control agent. Unfortunately, most, if not all, of the studies had been accomplished in a laboratory and control had only been demonstrated in small culture jars (Press et al., 1974; LeCato and Arbogast, 1979). The scientific community recognized the need for larger scaled experiments to verify the predator's practicality in the field. One such study followed, which clearly demonstrated the predator's ability to suppress pest populations in a large storage facility (Press et al., 1975). The researcher's concluded that statistically significant pest suppression could be achieved with less than 10 pairs of *X. flavipes* per bushel. Another large-scale study was conducted using two commercial warehouses containing farmers stock peanuts (Keever et al., 1986). A combination of parasitoids (*Bracon hebetor*) and predators (*X. flavipes*) reduced moth populations 54-83% more than the control warehouse, which was treated with malathion.

*Xylocoris flavipes* has the potential to significantly reduce pest populations in bulk stored grain; however its efficacy may be affected by temperature since population densities and growth rates are temperature-dependent. For example, developmental time from egg to adult increased from 19 d to 59 d as temperature decreased from 30 to 20°C (Arbogast, 1975). This study also showed that maximum oviposition rates occurred at 25 and 30°C. Arbogast (1975) concluded that the optimum conditions for population increase are temperatures between 29 and 31°C.

This study aimed to determine the thermal preferences of the anthocorid predator *Xylocoris flavipes*. The thermal preferences of the adults were observed, as well as the

preferred oviposition temperatures and subsequent nymphal emergences. I also examined temperature-dependent predation rates. The results have expanded our understanding of the predator's role in stored grain ecosystems and assessed their potential applicability for biological control in this thermally dynamic environment.

### Materials and Methods

The Materials and Methods section in Chapter 2 (pp. 10-12) provides a complete description of the laboratory thermal gradient apparatus and the source of the *X. flavipes* that were used in this study.

#### Adult Position and Oviposition Preferences

Twenty-four hours prior to introducing adult insects to the bars, the thermal gradient was established with the surface of the bars covered with a single-grain layer of intact wheat grains. Water fluid bath temperatures were set at -7 and 43°C. To begin each experiment, 2 adults were introduced to each gradient bar at each of 15 5 cm intervals from 15 and 85 cm. At each of these intervals ~250 *Plodia interpunctella* eggs were deposited daily for 7 days using a small aluminum scoop 0.5 cm wide × 0.25 cm deep. *Plodia interpunctella* eggs were used because they provided an immobile food source; this experiment investigated temperature preferences of *X. flavipes*, not its prey. The bars were covered with glass and placed in darkness. The location of all visible adults was recorded after 7 days. I included only the mobile predators for the analyses; insects placed on the cold end of the gradient never moved and were therefore excluded

from the analyses. After 7 days, the grain was removed in 5 cm bands (i.e.,  $\sim 2^\circ\text{C}$  ranges), and placed in rearing containers. Adults were immediately removed and counted; *X. flavipes* eggs were also counted at this time. Then the containers, with the eggs, were placed in a 12L/12D, 20/25°C incubator for rearing. After 24 h, each container was examined at one-day intervals to remove and count nymphs. Distribution of insects along the gradient was analyzed with an ANOVA general linear model (MINITAB 13.1). A square root transform,  $\sqrt{3/8 + Y}$  was chosen for each data set because it stabilized the variance while maximizing the mean to variance ratio. The experiment was replicated six times: four times with the temperature gradient established and twice (controls) with it off. Temperature data from all trials were used to calculate mean positional temperature.

#### Adult Positions without Food Present

The condition of the grain and eggs at different temperatures, rather than direct responses to temperature itself, may possibly influence the observed positions of the insects on the gradient. Another confounding factor may be insect interaction. Insect interaction may influence not only their choice of temperature but also even their ability to choose a temperature. To provide a check against these possibilities, I ran a second set of observations in which the insects were placed individually (without food) in 90 cm long glass tubes lying on the surface of the gradient bars. A single strand of floss was strung through each tube to give the predators traction, as they are unable to move forward on smooth glass. Each insect was positioned in the middle of the tube on the gradient, with each tube containing wooden plugs on either end to prevent escape. The water fluid

bath temperatures were set at 15 and 43°C. There were a total of 40 tubes per replicate, with 10 tubes placed on each gradient bar. The tubes were rotated 180° after 2 hours and the final positions were recorded after 18 hours. In this situation, they were able to make the same behavioral choices among temperatures without the possible confounding factors of food condition and social interactions. Food condition is important, because although they might not respond to variable food condition, they could be responding to lack of food and start wandering. This experiment was replicated 6 times, 4 with the thermal gradient established and 2 controls with the gradient off. Distribution of predators along the gradient was analyzed with an ANOVA general linear model.

#### Temperature-dependent Predation Rates

The effectiveness of the predators at different temperatures was determined by placing 1 female predator, along with 50 *P. interpunctella* eggs, within covered petri dishes placed along the thermal gradient. *Plodia interpunctella* eggs were used because they were readily available and more precisely scored than *R. dominica* eggs. The 2.5 cm diameter dishes were placed every 5 cm, from 5 to 95 cm, for a total of 19 dishes. Control dishes were placed next to the treatment dishes on the same bar at the same temperature. The control dishes were identical, except for the absence of a predator. After 24 hours, the condition of the eggs in both dishes was evaluated and the number of collapsed or eaten eggs was counted. The gradient temperature was set at -7 and 43°C. I conducted 4 replicates at each temperature. This experiment, conducted over 24 hours, allowed me to determine predation rates at different temperatures. Data were analyzed

using simple linear regression excluding the results associated with the two highest temperatures where predation rates declined.

## Results

### Adult Positional Preferences

Seven days after the predators were placed on the gradient, most were found between 55 and 65 cm or  $26.2 \pm 0.1^\circ\text{C}$  (Fig. 4.1). Position or temperature (Table 4.1) was the only significant factor in trials with the gradient on (Table 4.2). The mean temperature for the control trials was  $24.2 \pm 0.1^\circ\text{C}$ . None of the factors in the control trials were significant (Table 4.3). The distribution of the adults differed between trials with the gradient established versus the controls as reflected in Figure 4.1 and Table 4.4.

Table 4.1. Gradient position, range on the gradient in cm, and mean temperature  $\pm$  SE $^\circ\text{C}$  for *X. flavipes* adult positional and ovipositional preferences.

Position	Range (cm)	Mean Temperature $\pm$ SE $^\circ\text{C}$
1	5-15	$4.5 \pm 0.1$
2	15-25	$9.1 \pm 0.1$
3	25-35	$13.8 \pm 0.1$
4	35-45	$18.2 \pm 0.2$
5	45-55	$22.2 \pm 0.2$
6	55-65	$26.2 \pm 0.1$
7	65-75	$30.1 \pm 0.1$
8	75-85	$34.3 \pm 0.1$
9	85-95	$38.1 \pm 0.1$

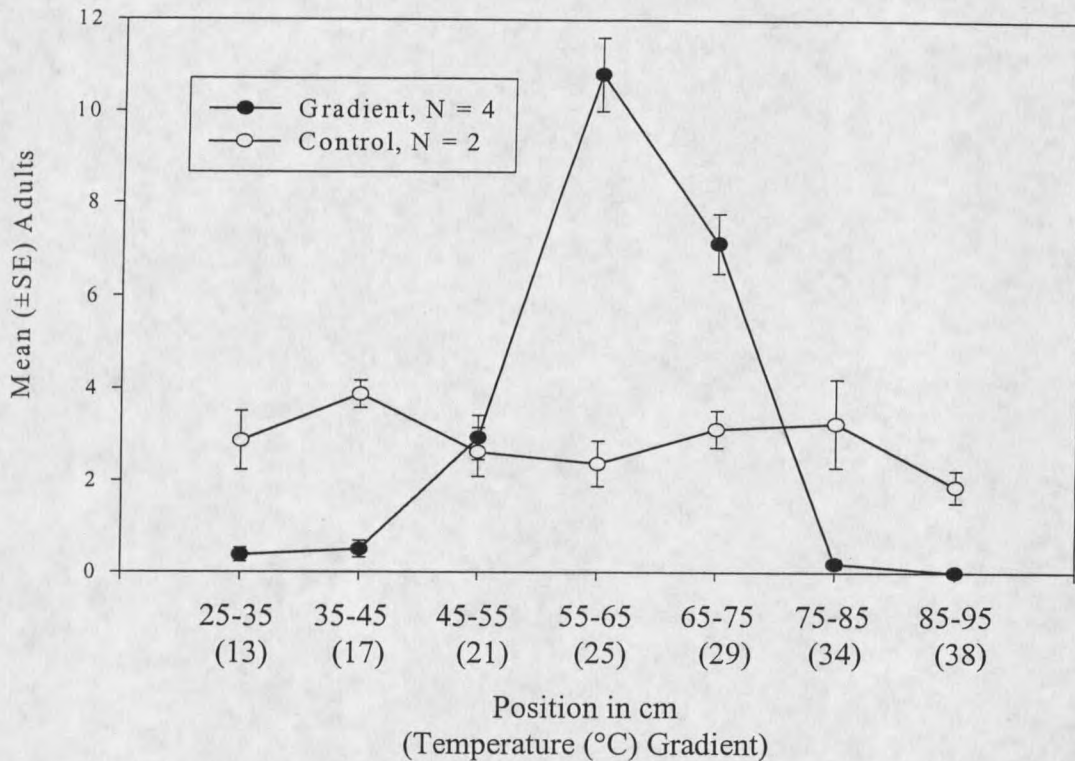


Figure 4.1. Mean ( $\pm$  SE) adult *X. flavipes* per bar as a function of position in cm and temperature ( $^{\circ}$ C). Two adults were placed every 5 cm, from 15 to 85 cm, across each of the 4 laboratory thermal gradient bars, for a total of 30 adults per bar. Adult positions were recorded after 7 days on the apparatus. Mean temperature for the control was  $26.2 \pm 0.1^{\circ}$ C.

Table 4.2. ANOVA for gradient on, with transformed proportion of adult *X. flavipes* as the response. Positions were recorded seven days after being introduced to the apparatus; 30 adults were evenly spaced across each of the four bars and allowed to self-disperse in each of the trials with the temperature gradient established.

Source	DF	Seq SS	Adj MS	F	P
Trial	3	0.00006	0.00002	0.01	0.999
Bar	3	0.00004	0.00001	0.01	0.999
Position	8	1.87256	0.23407	111.22	0.000
Trial $\times$ Bar	9	0.00017	0.00002	0.01	1.000
Trial $\times$ Position	24	0.01829	0.00076	0.36	0.997
Bar $\times$ Position	24	0.03251	0.00136	0.64	0.887
Error	72	0.15153	0.00211		
Total	143	2.07516			

Table 4.3. ANOVA for controls (gradient off), with transformed proportion of adult *X. flavipes* as the response. Positions were recorded seven days after being introduced to the apparatus; 30 adults were evenly spaced across each of the four bars and allowed to self-disperse in each of the control trials.

Source	DF	Seq SS	Adj MS	F	P
Trial	1	0.00001	0.00001	0.00	0.946
Bar	3	0.00005	0.00002	0.01	0.999
Position	8	0.02490	0.00311	1.18	0.349
Trial×Bar	3	0.00010	0.00004	0.01	0.998
Trial×Position	8	0.01803	0.00225	0.86	0.564
Bar×Position	24	0.04076	0.00170	0.65	0.854
Error	24	0.06311	0.00263		
Total	71	0.14697			

Table 4.4. ANOVA for gradient on versus controls, with transformed proportion of adult *X. flavipes* as the response. Positions were recorded seven days after being introduced to the apparatus; 30 adults were evenly spaced across each of the four bars and allowed to self-disperse in each of the treatments.

Source	DF	Seq SS	Adj MS	F	P
Treatment	1	0.00368	0.00368	2.16	0.144
Bar	3	0.00005	0.00002	0.08	0.970
Position	8	1.25544	0.07995	46.9	0.000
Treatment×Bar	3	0.00004	0.00001	0.01	0.999
Treatment×Position	8	0.64202	0.08025	47.08	0.000
Bar×Position	24	0.03819	0.00159	0.93	0.557
Error	168	0.28638	0.00171		
Total	215	2.22580			

#### Ovipositional Preferences - Eggs

Oviposition data indicate that the predators introduced to the gradient oviposited in areas where the mean temperature was  $26.2 \pm 0.1^\circ\text{C}$  (Fig. 4.2). Position, or temperature, was significant in trials with the gradient established, while trial×position

was also significant (Table 4.5). The mean temperature for the control trials was  $24.2 \pm 0.1^\circ\text{C}$ . Position and bar $\times$ position significantly influenced the response in the control trials (Table 4.6). The distribution of eggs differed between trials with the gradient established versus the controls, as reflected in Figure 4.2 and Table 4.7.

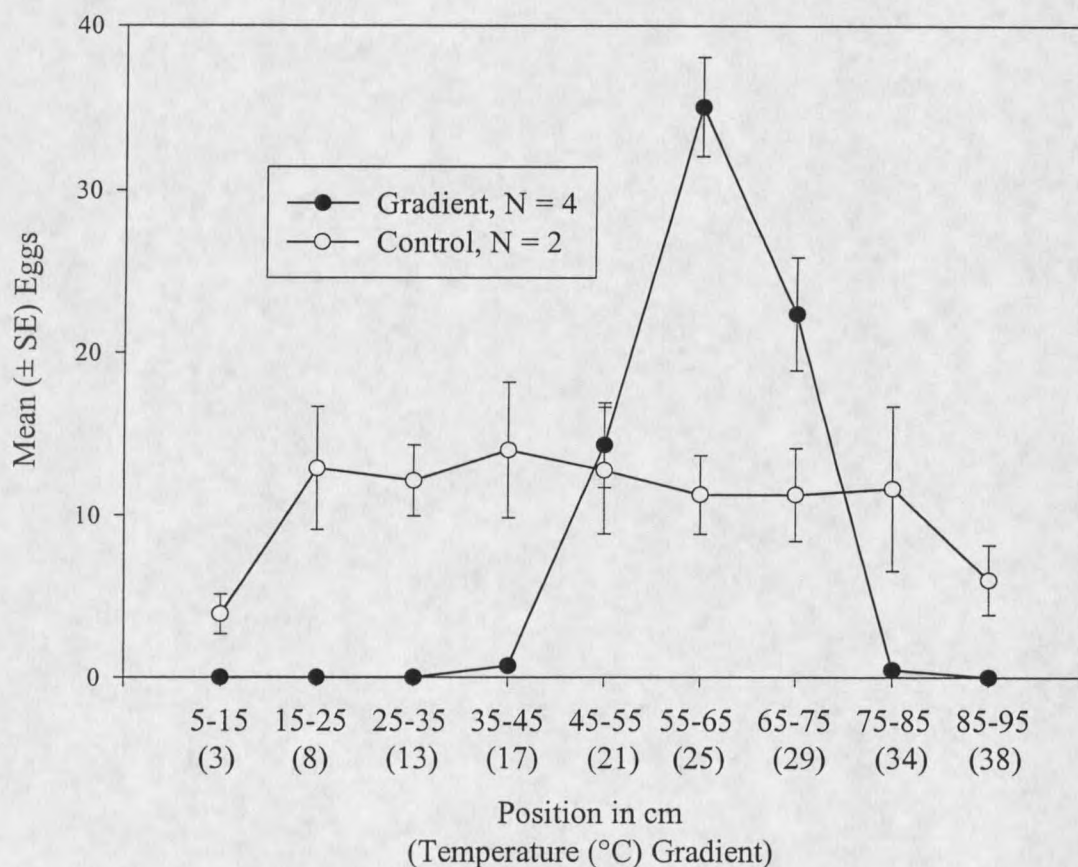


Figure 4.2. Mean ( $\pm$  SE) *X. flavipes* eggs per bar as a function of position in cm and temperature ( $^\circ\text{C}$ ). Two adults were placed every 5 cm, from 15 to 85 cm, across each of the 4 laboratory thermal gradient bars, for a total of 30 adults per bar. After 7 days the grain was removed in 10 cm bands, the adults were removed, and the *X. flavipes* eggs were counted. The grain, along with the eggs, was placed in rearing containers in an incubator. Mean temperature for the control was  $26.2 \pm 0.1^\circ\text{C}$ .

Table 4.5. ANOVA for gradient on, with transformed proportion of *X. flavipes* eggs as the response. Two adults were placed every 5 cm, from 15 to 85 cm, across each of the 4 laboratory thermal gradient bars, for a total of 30 adults per bar. After 7 days the grain was removed in 10 cm bands, the adults were removed, and the *X. flavipes* eggs were counted.

Source	DF	Seq SS	Adj MS	F	P
Trial	3	0.00015	0.00005	0.03	0.993
Bar	3	0.00002	0.00001	0.00	1.000
Position	8	1.80225	0.22528	133.43	0.000
Trial×Bar	9	0.00036	0.00004	0.02	1.000
Trial×Position	24	0.08816	0.00367	2.18	0.006
Bar×Position	24	0.02498	0.00104	0.62	0.908
Error	72	0.12156	0.00169		
Total	143	2.03748			

Table 4.6. ANOVA for controls (gradient off), with transformed proportion of *X. flavipes* eggs as the response. Two adults were placed every 5 cm, from 15 to 85 cm, across each of the 4 laboratory thermal gradient bars, for a total of 30 adults per bar. After 7 days the grain was removed in 10 cm bands, the adults were removed, and the *X. flavipes* eggs were counted.

Source	DF	Seq SS	Adj MS	F	P
Trial	3	0.00004	0.00004	0.02	0.882
Bar	3	0.00010	0.00004	0.02	0.995
Position	8	0.04703	0.00588	3.79	0.005
Trial×Bar	3	0.00005	0.00002	0.01	0.998
Trial×Position	8	0.01473	0.00184	1.19	0.347
Bar×Position	24	0.07898	0.00329	2.12	0.036
Error	24	0.03724	0.00155		
Total	71	0.17817			

Table 4.7. ANOVA for gradient on versus controls, with transformed proportion of *X. flavipes* eggs as the response. Two adults were placed every 5 cm, from 15 to 85 cm, across each of the 4 laboratory thermal gradient bars, for a total of 30 adults per bar. After 7 days the grain was removed in 10 cm bands, the adults were removed, and the *X. flavipes* eggs were counted.

Source	DF	Seq SS	Adj MS	F	P
Treatment	1	0.00460	0.00460	2.45	0.119
Bar	3	0.00001	0.00001	0.01	0.999
Position	8	1.30954	0.09056	48.27	0.000
Treatment×Bar	3	0.00012	0.00004	0.02	0.996
Treatment×Position	8	0.53974	0.06747	35.96	0.000
Bar×Position	24	0.05107	0.00213	1.13	0.312
Error	168	0.31517	0.00188		
Total	215	2.22025			

#### Ovipositional Preferences – Nymphal Emergences

Emergence data indicate that the predators introduced to the gradient oviposited in areas where the mean temperature was  $26.2 \pm 0.1^\circ\text{C}$  (Fig. 4.3). Position, or temperature, was the only significant factor in trials with the gradient on (Table 4.8). The mean temperature for the control trials was  $24.2 \pm 0.1^\circ\text{C}$ . Position was the only significant factor in the control trials (Table 4.9). The distribution of nymphs differed between trials with the gradient established versus the controls, as reflected in Figure 4.3 and Table 4.10.

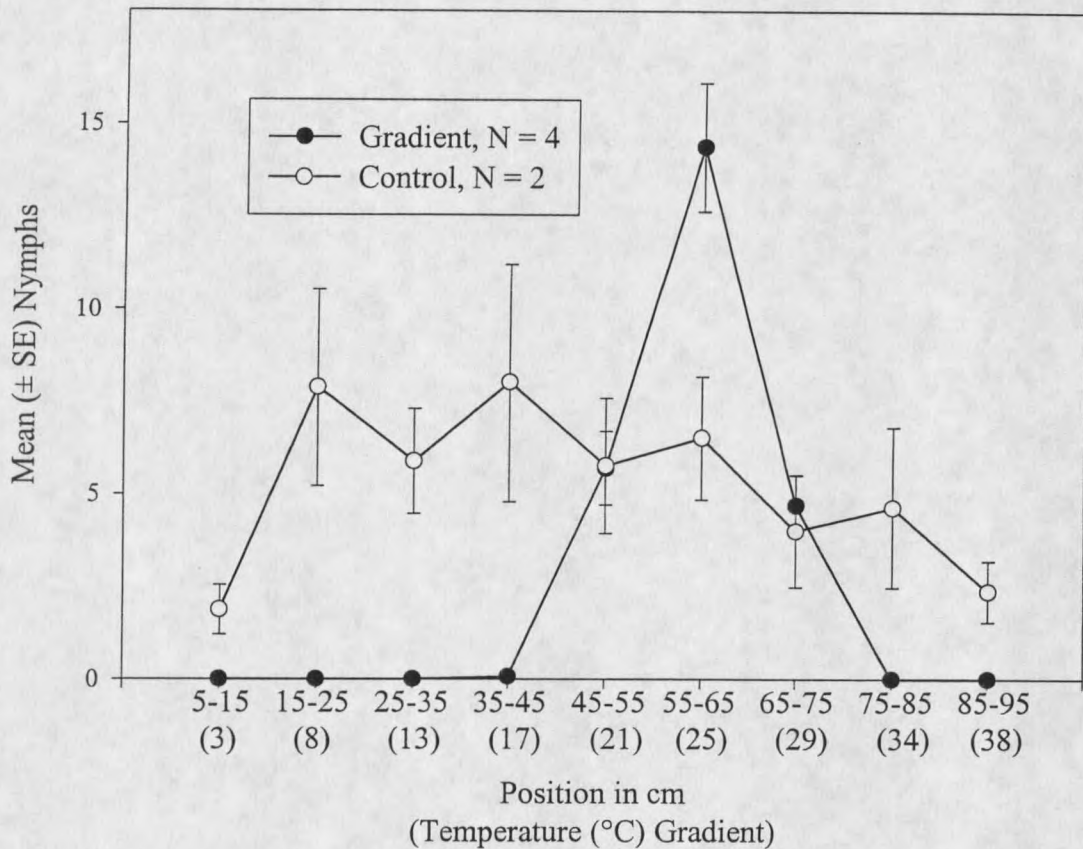


Figure 4.3. Mean ( $\pm$  SE) *X. flavipes* nymphs per bar as a function of position in cm and temperature ( $^{\circ}$ C). Two adults were placed every 5 cm, from 15 to 85 cm, across each of the 4 laboratory thermal gradient bars, for a total of 30 adults per bar. After 7 days the grain was removed in 10 cm bands, the adults were removed, and the grain was placed in rearing containers in an incubator. The offspring were collected and counted upon emergence. Mean temperature for the control was  $26.2 \pm 0.1^{\circ}$ C.

Table 4.8. ANOVA for gradient on, with transformed proportion of *X. flavipes* nymphs as the response. Two adults were placed every 5 cm, from 15 to 85 cm, across each of the 4 laboratory thermal gradient bars, for a total of 30 adults per bar. After 7 days the grain was removed in 10 cm bands, the adults were removed, and the grain was placed in rearing containers in an incubator. The offspring were collected and counted upon emergence.

Source	DF	Seq SS	Adj MS	F	P
Trial	3	0.00024	0.00008	0.03	0.994
Bar	3	0.00014	0.00005	0.02	0.997
Position	8	2.04230	0.25529	86.63	0.000
Trial×Bar	9	0.00031	0.00004	0.01	1.000
Trial×Position	24	0.01012	0.00042	0.14	1.000
Bar×Position	24	0.03098	0.00129	0.44	0.987
Error	72	0.21217	0.00295		
Total	143	2.29626			

Table 4.9. ANOVA for controls (gradient off), with transformed proportion of *X. flavipes* nymphs as the response. Two adults were placed every 5 cm, from 15 to 85 cm, across each of the 4 laboratory thermal gradient bars, for a total of 30 adults per bar. After 7 days the grain was removed in 10 cm bands, the adults were removed, and the grain was placed in rearing containers in an incubator. The offspring were collected and counted upon emergence.

Source	DF	Seq SS	Adj MS	F	P
Trial	1	0.00000	0.00000	0.00	0.982
Bar	3	0.00012	0.00004	0.01	0.998
Position	8	0.06627	0.00828	3.02	0.017
Trial×Bar	3	0.00004	0.00001	0.00	1.000
Trial×Position	8	0.01884	0.00236	0.86	0.563
Bar×Position	24	0.11450	0.00477	1.74	0.091
Error	24	0.06578	0.00274		
Total	71	0.26553			

Table 4.10. ANOVA for gradient on versus controls, with transformed proportion of *X. flavipes* nymphs as the response. Two adults were placed every 5 cm, from 15 to 85 cm, across each of the 4 laboratory thermal gradient bars, for a total of 30 adults per bar. After 7 days the grain was removed in 10 cm bands, the adults were removed, and the grain was placed in rearing containers in an incubator. The offspring were collected and counted upon emergence.

Source	DF	Seq SS	Adj MS	F	P
Treatment	1	0.00480	0.00480	2.03	0.156
Bar	3	0.00022	0.00007	0.03	0.993
Position	8	1.48058	0.10274	43.41	0.000
Treatment×Bar	3	0.00004	0.00001	0.00	1.000
Treatment×Position	8	0.62799	0.07850	33.17	0.000
Bar×Position	24	0.05538	0.00231	0.98	0.502
Error	203	0.39759	0.00237		
Total	215	2.56660			

#### Adult Positions without Food Present

In both treatments, most of the insects rested against the plug in the end of the glass tube (Fig. 4.4). Analyses showed a significant position effect in both treatments individually (Tables 4.11 and 4.12). There was also a significant trial×position effect in trials with the gradient established. When comparing between the treatments, the results suggest that the distribution of predators differed between trials with the gradient established and the control trials (Table 4.13).

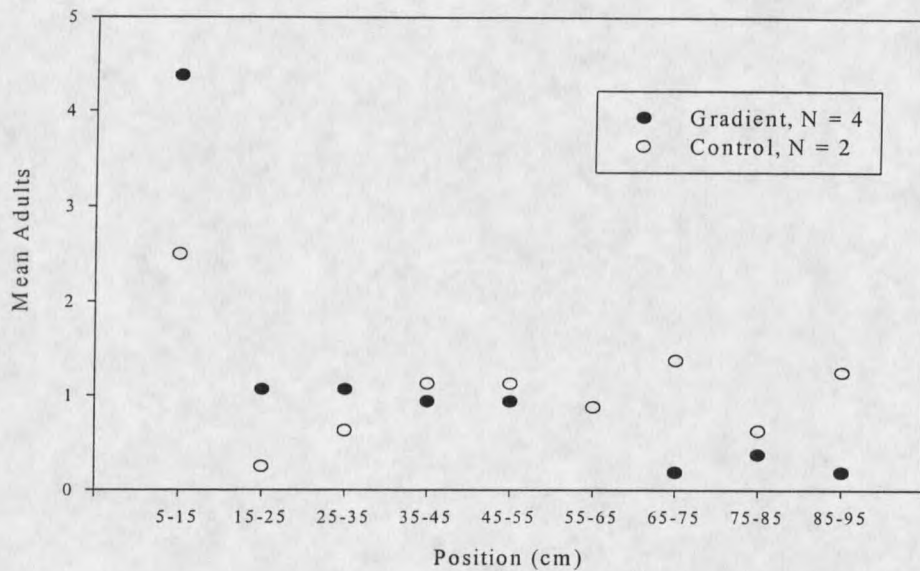


Figure 4.4. Mean number of adult *X. flavipes* as a function of position. Insects were placed individually in 100 cm long glass tubes which ran parallel with the long axis of the apparatus; there were ten tubes per bar in each trial. All insects were placed in the middle of the tube at the start of the experiment, from 45 to 55 cm. The tubes were rotated 180° after 2 h and final positions were recorded after 18 h.

Table 4.11. ANOVA for gradient on (without food), with transformed proportion of adult *X. flavipes* as the response. Insects were placed individually in 100 cm long glass tubes which ran parallel with the long axis of the apparatus; there were ten tubes per bar in each trial. All insects were placed in the middle of the tube at the start of the experiment, from 45 to 55 cm. The tubes were rotated 180° after 2 h and final positions were recorded after 18 h.

Source	DF	Seq SS	Adj MS	F	P
Trial	3	0.00105	0.00035	0.10	0.959
Bar	3	0.00033	0.00011	0.03	0.992
Position	8	0.86386	0.10798	31.33	0.000
Trial × Bar	9	0.00133	0.00015	0.04	1.000
Trial × Position	24	0.23442	0.00977	2.83	0.000
Bar × Position	24	0.11455	0.00477	1.38	0.147
Error	72	0.24819	0.00345		
Total	143	1.46373			

Table 4.12. ANOVA for controls (without food), with transformed proportion of adult *X. flavipes* as the response. Insects were placed individually in 100 cm long glass tubes which ran parallel with the long axis of the apparatus; there were ten tubes per bar in each trial. All insects were placed in the middle of the tube at the start of the experiment, from 45 to 55 cm. The tubes were rotated 180° after 2 h and final positions were recorded after 18 h.

Source	DF	Seq SS	Adj MS	F	P
Trial	1	0.00004	0.00004	0.01	0.917
Bar	3	0.00015	0.00005	0.02	0.997
Position	8	0.12991	0.01624	5.15	0.001
Trial×Bar	3	0.00015	0.00005	0.02	0.997
Trial×Position	8	0.05003	0.00625	1.98	0.093
Bar×Position	24	0.09769	0.00407	1.29	0.268
Error	24	0.07564	0.00315		
Total	71	0.35360			

Table 4.13. ANOVA for gradient on versus controls, with transformed proportion of adult *X. flavipes* as the response. Insects were placed individually in 100 cm long glass tubes which ran parallel with the long axis of the apparatus; there were ten tubes per bar in each trial. All insects were placed in the middle of the tube at the start of the experiment, from 45 to 55 cm. The tubes were rotated 180° after 2 h and final positions were recorded after 18 h.

Source	DF	Seq SS	Adj MS	F	P
Treatment	1	0.00053	0.00053	0.13	0.715
Bar	3	0.00040	0.00011	0.03	0.994
Position	8	0.89163	0.08087	20.33	0.000
Treatment×Bar	3	0.00008	0.00003	0.01	0.999
Treatment×Position	8	0.10213	0.01277	3.21	0.002
Bar×Position	24	0.15482	0.00645	1.62	0.042
Error	168	0.66827	0.00398		
Total	215	1.81786			

### Temperature-dependent Predation Rates

Predation rates were a nonlinear function of temperature (Fig. 4.5). The highest proportions of eggs were consumed between  $31.7 \pm 0.1$  and  $33.8 \pm 0.1^\circ\text{C}$ .

Consumption decreased at temperatures  $\geq 36.1 \pm 0.1^\circ\text{C}$ . No eggs were consumed at temperatures  $\leq 8.8 \pm 0.1^\circ\text{C}$ .

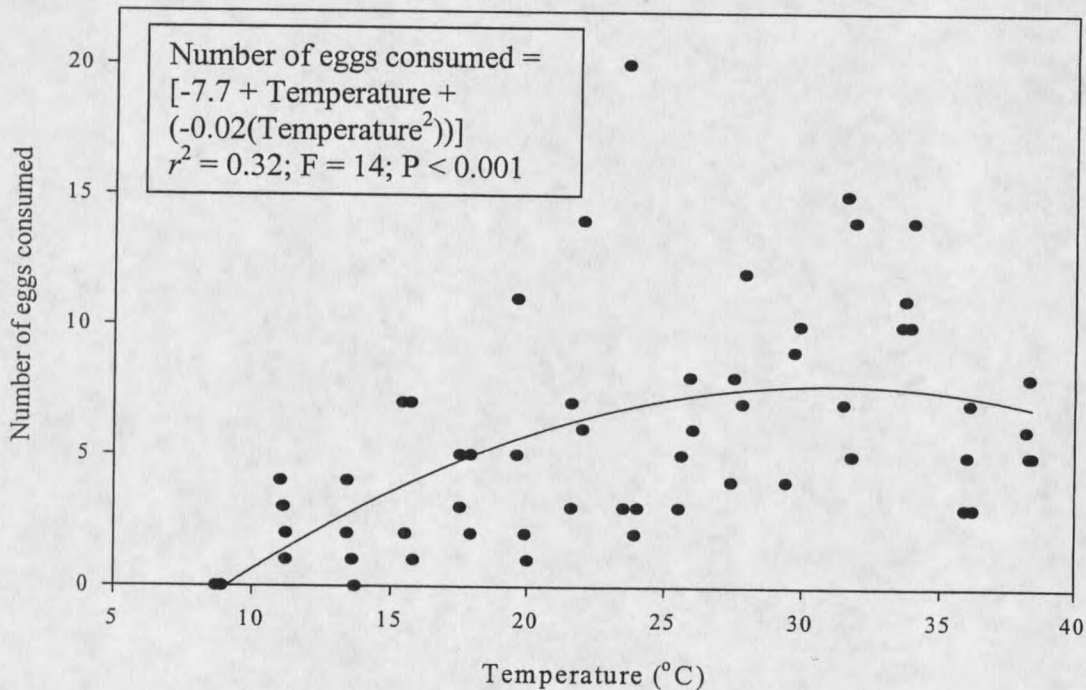


Figure 4.5. The number of eggs consumed by one female *X. flavipes* expressed as a function of temperature. One female *X. flavipes* was placed in each of 18 petri dishes along one bar of the apparatus, in addition to 50 *P. interpunctella* eggs. The number of eggs consumed was recorded after 24 h. This trial was conducted 4 times.

### Discussion

The results of this study aid in our understanding of the thermal preferences of the anthocorid predator *Xylocoris flavipes*. Most of the adults were found in areas where the

mean temperature was  $26.2 \pm 0.1^{\circ}\text{C}$ . Eggs, and subsequent nymphal emergences, were most common in areas where the mean temperature was also  $26.2 \pm 0.1^{\circ}\text{C}$ . My results indicate that the preferred temperature for *X. flavipes* is  $26.2 \pm 0.1^{\circ}\text{C}$ , rather than between 29 and  $31^{\circ}\text{C}$ , as previously suggested (Arbogast, 1975).

#### Adult Positional Preferences

The only factor that affected adult distribution in trials with the gradient on was temperature, indicated by position. The insects were distributed randomly across the bars in the control trials. The distribution differed between the trials with the gradient on versus the control trials, as indicated by a strong treatment  $\times$  position interaction. The distribution of the beetles on the bars depended on the treatment. Position, or temperature, is significant when comparing between treatments because it is the most influential factor affecting distribution in trials with the gradient established. Since all trials are being considered in this analysis, position is significant here as well.

#### Ovipositional Preferences - Eggs

In addition to temperature, trial  $\times$  position was also significant in trials with the gradient established. The distribution of eggs varied between trials, so trials within this treatment may not be exact replicates on one another. However, bar, trial  $\times$  bar, and bar  $\times$  position were not significant. Therefore the individual bars may be considered independent replicates because the distribution of adults across each bar did not vary between bars within a trial or across trials. In the controls, position and bar  $\times$  position were significant. The eggs were not distributed randomly in this treatment. However,

the distribution varied between bars. This is acceptable for the control trials because distribution was not the same on each bar, whereas the eggs in the trials with the gradient established exhibited a similar distributional pattern on all bars. The distribution differed between the treatments, as indicated by a significant treatment  $\times$  position interaction.

The distribution of the beetles on the bars depended on the treatment. Position, or temperature, is significant when comparing between treatments because it influences the distribution in trials with the gradient established.

#### Ovipositional Preferences -- Nymphal Emergences

The only factor that affected distribution of nymphal emergences in trials with the gradient on as well as in the controls was temperature, or position. This effect was not as pronounced in the controls as it was in trials with the gradient established. In both cases, nymphs were non-randomly distributed across the bars in each of the treatments.

However, the distribution of nymphs differed between treatments as indicated by a significant treatment  $\times$  position interaction. The distribution of nymphs depended on the treatment. Position, or temperature, was also significant when comparing treatments since it influences the distribution in trials with the gradient established.

#### Adult Positions without Food Present

Most of the insects rested against the plug at one end of the glass tube. The analysis showed a significant position, or temperature, effect in the individual treatments, as well as a significant treatment  $\times$  position effect when comparing treatments.

However, most of the insects responded thigmotactically rather than thermally. For the purpose of this study, this was an insufficient test for temperature preference in the absence of food. The predators distributed themselves according to a preferred physical niche, against a plug, rather than a preferred temperature.

#### Temperature-dependent Predation Rates

The predators are capable of consuming prey at a relatively constant rate over a wide range of temperatures and did not have a significant linear increase. Predation rates declined at temperatures  $\geq 36.1 \pm 0.1$  °C. The minimum temperature required for predation is  $\geq 8.8 \pm 0.1$  °C. The relatively constant rate of predation across temperatures by *X. flavipes* is very promising for the use of the predator as a biological control agent in the stored grain ecosystem.

## CHAPTER 5

SUMMARY

The results of my research provide information to managers regarding temperature-dependent behavior, distribution, and mortality of two cosmopolitan stored grain insects, *Rhyzopertha dominica* and *Xylocoris flavipes*. The dynamic daily and seasonal temperature fluctuations within stored grain (Hagstrum 1987; Arbogast et al 1998) make managing this system both unique and challenging. An integrated pest management (IPM) decision relies on accurate interpretation of sampling and trapping data. In stored grain the knowledge of the basic thermal biology of the insects is integral to the success of these programs (White et al. 1990). Because many bin sampling programs use probe traps that capture insects passively, the interpretation of trap catches depends upon knowledge of the movement and activity of the insects present (Fargo et al. 1989; Cuperus et al. 1990). Managers need to be able to convert trap catch into an absolute sampling estimate like insects/kg grain. Therefore, temperature must be taken into consideration to accurately analyze the data and make informed decisions regarding pest management (White et al. 1990). Without taking temperature into account, population estimates are liable to be erroneous; and subsequent decisions will likely result in grain damage due to inadequate control, or monetary loss due to unnecessary control.

The worldwide phase-out of many of the chemicals traditionally used to treat stored grain infestations has necessitated the exploration of economically and

environmentally sustainable alternatives (Bell et al., 1996). Biological control agents may provide a solution to this problem (Haines, 1994; Brower et al., 1995; Hokkanen et al., 1995) because the efficacy and desirability of many chemicals has rapidly faded since their inception since World War II. We have put extreme pressure on insect populations to develop resistance so we need to look for alternatives to insecticides.

A biological control agent in stored grain must be thermally compatible with its prey species. My results indicate that, when compared to *X. flavipes* *R. dominica* move more slowly when active, but that they remain active at lower temperatures and survive higher temperatures. The mortality data concur with previous studies that demonstrated that *R. dominica* can survive temperatures of 39°C for 24 hours (Birch 1945; Longstaff 1999). Although *X. flavipes* predation may be curtailed at lower temperatures, damage caused by *R. dominica* is also likely to be limited under those conditions. On the other hand, the greater short-term survival of *R. dominica* at higher temperatures may provide the pest with refuges against predation by *X. flavipes*. In addition, *R. dominica* may escape by moving deeper into the grain mass since *X. flavipes* are more common near the grain surface (Arbogast, et al. 1998).

The preferred temperatures for the adults of both species were not significantly different; most *R. dominica* adults were observed in areas where the mean temperature was  $29.4 \pm 0.1^\circ\text{C}$  and most *X. flavipes* adults were observed in areas where the mean temperature was  $26.2 \pm 0.1^\circ\text{C}$ . *Rhyzopertha dominica* and *X. flavipes* oviposited in these warm areas as well. With the exception of *X. flavipes* ovipositional preferences, the results corroborate previous work (Arbogast 1975; Longstaff 1999). Temperature-

dependent predation rates of *X. flavipes* indicate that it will consume a relatively consistent amount prey over a wide range of temperatures.

Although initial consumption may occur at the most preferred temperature, these data support the applicability of using the warehouse pirate bug, *X. flavipes*, as a biological control agent. Jay et al. (1968) demonstrated a significant reduction in *R. dominica* populations when *X. flavipes* was added to laboratory cultures. My study indicated that *X. flavipes* was capable of withstanding relatively high grain temperatures for a short period of time and would actively consume prey over a wide range of temperatures. More importantly, its thermal preferences overlapped with those of *R. dominica*, thus indicating that their optimal environments are similar enough for successful control.

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