



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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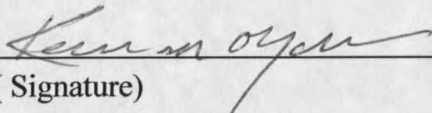
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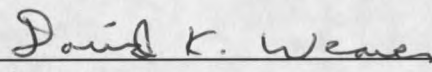
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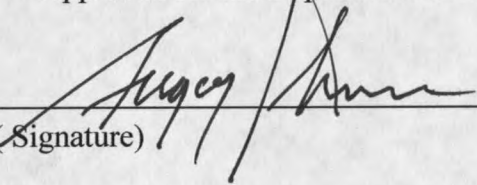
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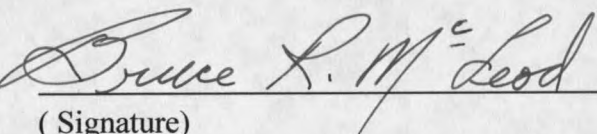
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ABSTRACT

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 ± 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 ± 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 20th 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.

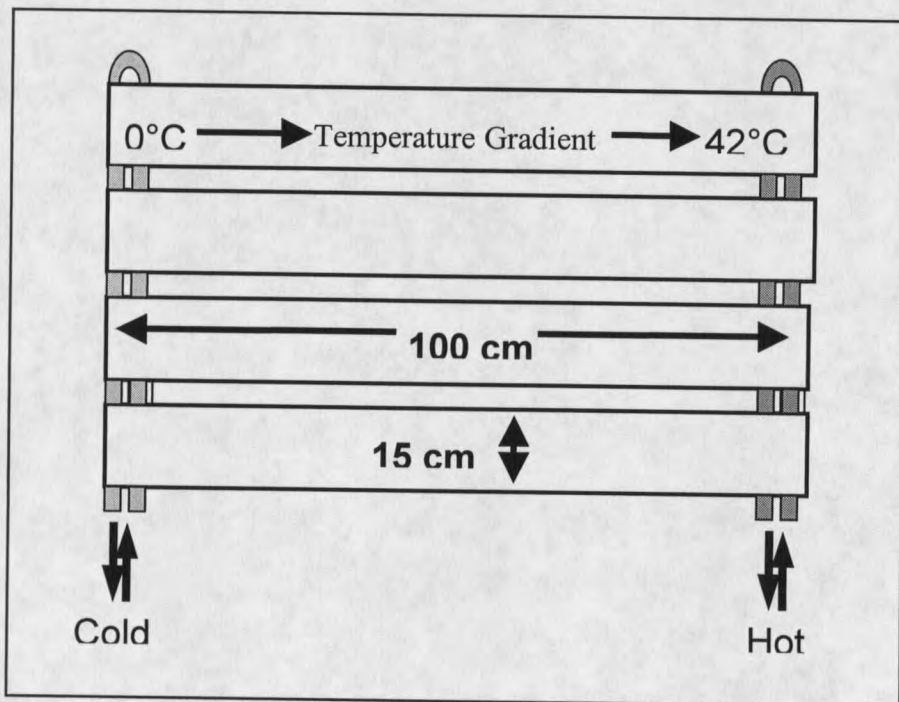


Figure 2.1. Diagram of the laboratory thermal gradient apparatus. The ends of the four aluminum bars rested upon hollow aluminum rods through which water was pumped at a constant temperature. One end was in contact with cold water while the other was in contact with hot water, this resulted in a stable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

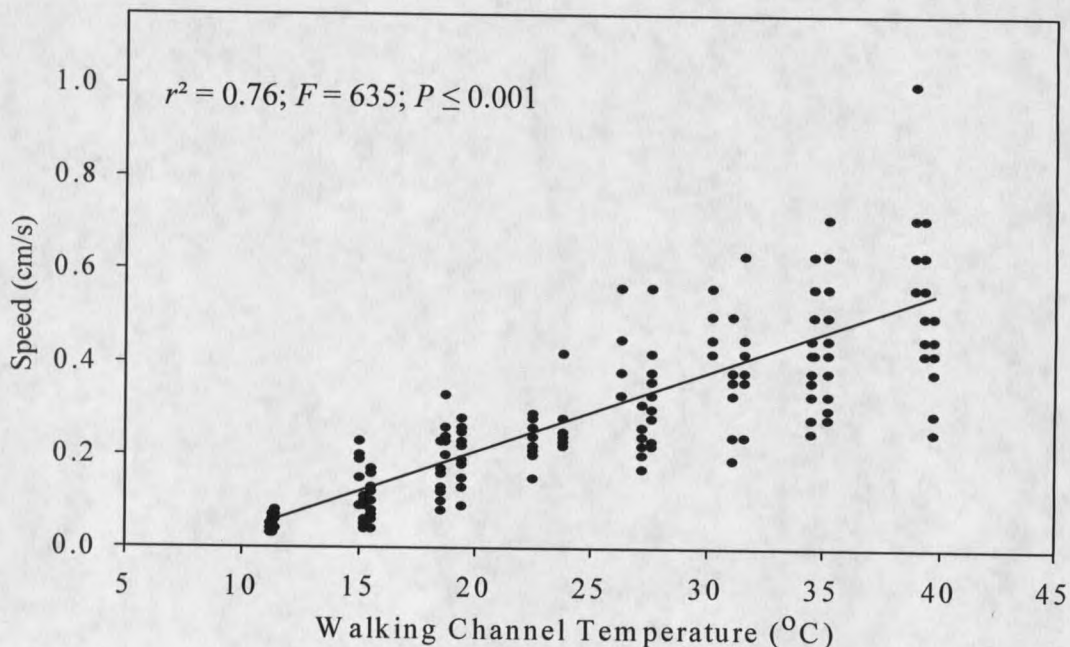


Figure 2.2. *Rhyzopertha dominica* walking speed as a function of temperature. Twenty-five adults were observed at each of nine temperatures; speed was measured over a 5 cm distance and expressed in cm/s. Back-transformed regression line plotted against untransformed speed. [$\sqrt{3/8 + \text{Speed}}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

