PREY UTILIZATION AND LABORATORY REARING OF *XYLOCORIS FLAVIPES* (REUTER) (HEMIPTERA: ANTHOCORIDAE)

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REARING OF *XYLOCORIS FLAVIPES*

(REUTER) (HEMIPTERA:

ANTHOCORIDAE)

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PREFACE

The first chapter of this thesis is a literature review focusing on biological control in stored products ecosystems and the biology of the predatory insect *Xylocoris flavipes*. Also included is the biology of the stored products pests examined during this study as well as basic information about functional response analysis and artificial diets. Subsequent chapters are formal manuscripts of the research I conducted during my M.S. program and are written in compliance with the publication policies and guidelines for manuscript preparation with the Entomological Society of America.

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Biological Control

Examples of natural enemies being used to control pests have existed for centuries, but biological control emerged as a scientific method only late in the nineteenth century (Huffaker and Messenger 1976). The term "biological control," first used by Smith (1919), signifies the use of predator, parasitoid, pathogen, antagonist, or competitor to suppress a pest population, making it less abundant and thus less damaging than it would otherwise be. Biological control may be the result of purposeful actions by people or may result from the unassisted action of natural forces (Van Driesche and Bellows 1996). There are many benefits associated with the use of biological control. These include reduction of pest numbers and a decrease in the harmful effects of pest damage, reductions in pesticide use and environmental contamination, increased productivity of agricultural or recreational areas, and recovery or protection of natural environments (Clausen 1978). Worldwide, over 543 pests have been the targets of more than 1200 introduction programs (Greathead and Greathead 1992). However, the ease and benefits associated with pesticide usage have caused a twelvefold worldwide increase in their use since the early 1950s (Eichers 1981). The increase in pesticide usage has caused numerous environmental problems, which in conjunction with legislation such as the Food Quality Protection Act (FQPA) may force managers to turn to other non-chemical options such as biological control in the near future.

Stored-Products

The focus of this study is on insects in the stored-products ecosystem. Approximately 900 million tons of grain are in storage throughout the world at any given time, about one

half of annual world production. Storage of these products needs to be properly engineered and managed to preserve harvested grain and to provide wholesome food, free of insect damage, live insects and mites, insect fragments, microflora, mycotoxins, and pesticides (Jayas et al. 1995). Managing stored grain wisely with minimal loss while maintaining its nutritional quality is a major concern at all stages. Post harvest food losses are estimated to range from 9% in the United States up to 50% in some developing nations (Pimentel 1991). According to a 1990 survey of extension specialists throughout the US, stored grain losses exceeded $500 million for that year alone (Harein and Meronuck 1995).

Stored-product managers had few pest control options other than pesticides until legislation, passed in 1992, exempted certain beneficial insects from the tolerance standards. The EPA exempted from previous standards all genera of parasitoids and predators known commonly to attack stored-food pests. It is now acceptable to use beneficial insects in stored raw whole grains and packaged food in warehouses so long as the insects do not become a component of the final food product (Anonymous 1992). The following are genera included on that list: the parasitic Hymenoptera *Trichogramma, Bracon (Habrobracon), Venturia, Mesostenus, Anisopteromalus, Choetospila, Lariophagus, Dibrachys, Habrocytus, Pteromalus, Cephalonomia, Holepyris,* and *Laelius*; and the predatory Hemiptera *Lyctocoris, Dufouriellus,* and *Xylocoris* (Subramanyam and Hagstrum 1996). The focus of this study will be to determine the functional response of the predator *Xylocoris flavipes* (Reuter) in order to evaluate its potential as a biological control agent for stored-products ecosystems.

Xylocoris flavipes

Xylocoris flavipes (Rueter) (Hemiptera: Anthocoridae) and several other insects in the subfamily Lyctocorinae frequently occur as predators in storage ecosystems. *Xylocoris flavipes* is a cosmopolitan predator found in stored commodities. Although Carayon (1972) found it in other localities, he was not convinced it occurred outside the stored product habitat naturally. *Xylocoris flavipes* may play an important role in biological control programs for storage ecosystems, as is indicated by facets of its biology and its success in regulating populations of storage pests (Arbogast 1978).

Adult *X. flavipes* are shiny brownish black with dark brown compound eyes and red ocelli. The sexes can be easily distinguished from the shape of their abdomens. Females are bilaterally symmetrical while males have a notch on the left side of segments 8 and 9 (Arbogast 1978). Although Awadallah and Tawfik (1972) observed mating on the day of adult emergence, adult females are not sexually mature for 1-2 d and there is a 3-4 d preoviposition period. Their ellipsoidal eggs are laid randomly throughout the habitat and take 3-4 d to hatch. When nymphs emerge they are pale tannish-orange darkening to reddish-orange as they mature. Instars one to four have a mean duration of 2 d each at 30° C, while the mean duration of the fifth instar is 3 d. There are usually five nymphal stages, which differ in overall size, size of developing wing pads, and color (Arbogast 1978). The size of the developing wing pads provides the most reliable method of distinguishing instars two through five (Awadallah and Tawfik 1972). In studies testing the effects of temperature and relative humidity on *Xylocoris flavipes,* it was found that both considerably affected the duration of the nymphal stage as well as the longevity of adult stage. A temperature rise from 15° to 35°C enhances the development of the egg

and the nymphal stages but shortens the life span of adults. Optimal conditions for egg laying are 25°C with 50% rh, while 25°C and 10% RH are optimal for egg hatch and nymph survival. No development will occur at 35°C with less than 10% RH or under 15° at any rh (Abdel-Rahman 1977).

The efficacy of *X. flavipes* as a biological control agent can be ascertained by the results of several studies (Jay et al. 1968, Press et al. 1975, LeCato and Collins 1976, Arbogast 1976, LeCato et al. 1977, Keever et al. 1986, Brower and Press 1992). *Xylocoris flavipes* does not show a high degree of prey specificity and LeCato and Davis (1973) speculated that it would probably kill any prey it can subdue and penetrate with its stylet. It is presently known to prey on at least 13 species of insects belonging to three orders. It does prefer certain species and stages over others however, but these preferences probably reflect the predator's variable success in attacking prey of different sizes, degrees of sclerotization, defensive behavior, and/or other factors (LeCato and Davis 1973). *Xylocoris flavipes* has a high capacity to increase its numbers relative to its prey and a high level of prey consumption. In addition, it destroys large quantities of prey when prey are abundant (LeCato and Collins 1976). Suppression studies, carried out by adding *X. flavipes* to infested commodities, determined that the predator has a high searching capacity and is able to find prey even when scarce. A predator's ability to survive between contacts when prey densities are low is an important factor to consider, when determining the efficacy of a potential biological control agent (LeCato and Collins 1976). *Xylocoris flavipes* is capable of surviving at low prey densities and can develop with relatively little food, but is not considered highly resistant to starvation (Arbogast 1978). This is partially compensated for by their cannibalistic nature. Nymphs and

adults will both prey on conspecifics, and a small percentage of nymphs can complete development when no other prey are available (Arbogast 1979).

The use of Hemipteran predators as stored-product biological control agents has been criticized because it is thought that are unable to gain access to certain species or stages of storage pests. Their piercing sucking mouthparts are not adapted for penetrating seeds or very hard substances, and hence it is thought to be ineffective against most species that develop within seeds (LeCato and Press, personal communication, cited in Arbogast 1978). Sing (1997) tested the functional response of *X. flavipes* to several different bruchid species and determined that it could successfully subdue the adults of this family as well as the eggs and neonate larvae. Bruchids feed internally as larvae after boring into their food source as neonate larvae. However, Sing's (1997) research did not test the predator's efficacy on the internally feeding stages of this pest.

Studies have been done to determine what role *X. flavipes* could play in an IPM program, including tests to determine its sensitivity to several pesticides (Press et al. 1978, Baker and Arbogast 1995). These studies showed *X. flavipes* to have a higher tolerance to insecticides than the two tested parasitoids and several stored-products pest species (Arbogast 1978). *Xylocoris flavipes* possesses the attributes of a very effective predator and it could play a significant role as a biological control agent in stored product ecosystems.

Stored Product Pests

Many insect species thrive in the stored-products ecosystem, including the pests that consume the stored products and molds growing on them and those that occur as predators (Arbogast 1991). Stored products pests fall into two simple categories: internal

feeders that breed in seeds, fruits, and other fleshy parts of plants, and external feeders or scavengers (Linsley 1944). Internal feeders include some beetle species and a few species of moths. However, most stored-products pests are external feeders (Howe 1991). This study will involve four stored-products pests: the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae); the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Cucujidae); the Indianmeal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae); and the lesser grain borer, *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae).

Oryzaephilus surinamensis, a cosmopolitan pest, commonly feeds on cereal products and stored grain but is known to feed on a wide variety of products (Howe 1956a, Loschiavo and Smith 1970). It is an external feeder, unable to attack perfectly sound grain but known to attack grain with small lesions in the bran layer over the germ (Fraenkel and Blewett 1943). *Oryzaephilus surinamensis* lays its eggs singly in finely ground material, such as flour or whole grains. It typically goes through three instars before constructing a crude pupal cell of cemented food particles (Howe 1956a). Their optimal temperature range for development is approximately 30°-35°C. Their upper temperature limit is between 37.5° and 40°C; the lower is 17.5°C, making the species fairly cold hearty (Back and Cotton 1926). Adult females begin oviposition during their first week and reach maximum oviposition during the second or third week. Egg laying continues for about 10 wk and then declines steadily. The average total fecundity is approximately 280 eggs/female, but some can lay as many as 432 (Arbogast 1991).

Tribolium castaneum, a cosmopolitan species, feeds on a wide variety of plant and animal products but favors flour and other milled grain products (Good 1936). The

beetles are external feeders and do not attack sound grain, but feed on broken kernels (Arbogast 1991). Females lay eggs loosely in the flour or attach them to the surface of the storage container by coating eggshells with a glue-like substance. The number of instars varies from 5 to 11 but 7 is most common. Variation can be caused by individual characteristics, but is mostly the result of external conditions such as food, temperature, and relative humidity. The negatively phototactic larvae are fairly active but remain below the grain surface with final instar larvae moving to the surface for pupation (Arbogast 1991). Development cannot occur below 20°C or above 40°C; however, at intermediate relative humidities (50-70%) development can take place at slightly higher temperatures (Howe 1956b, Howe 1960). The average duration of the oviposition period is 148 d at 27°C or 174 d at room temperature with the maximum being 308 d. Average fecundity is approximately 327 eggs/female, with a possible maximum of 956 eggs/female (Good 1936).

Plodia interpunctella, a cosmopolitan species, feeds on dried fruits, cereals, and many other products, such as nuts and oilseeds (Cox and Bell 1991). The developmental temperature limits are 18-35° (Bell 1975, Tsuji 1963). Egg hatch will not occur below 15°C (Bell 1975, Tzanakakis 1959), but early instars can survive at temperatures down to 10°C (Stratil and Reichmuth 1984). Females lay an average of 150-200 eggs the first few days after mating but the numbers then drop dramatically there after (Cotton 1956, Lum and Flaherty 1969, Tzanakakis 1959). *Plodia interpunctella* usually have only one or two generations per year, but in warmer climates there may be up to eight per year (Tsuji 1963).

Rhyzopertha dominica, a cosmopolitan pest, feeds internally on a wide variety of foods including most grains. The beetle will lay its eggs in clusters on kernels of grain or singly in their own frass. The first instars will burrow into the seed coat to feed. The following instars get progressively less active while feeding until the beetle pupates within the seed. The beetle may have as many as seven instars, but three to four is most common (Potter 1935). *Rhyzopertha dominica* reared on wheat at 34°C with 75% RH will complete development from egg hatch to adult emergence in approximately 28-33 d (Kapoor 1964). The developmental temperature limits are between 18.2° and 38.6°C at 70% RH. The highest average fecundity occurs between 26° and 34°C with the highest, 415 eggs/female, occurring at 34° with 14% RH. Females held under optimal conditions may have oviposition periods lasting up to 112 d (Birch 1945).

Functional Response

Understanding predator-prey interactions is crucial if a biological control program is to be successful. Basic theoretical population ecology emerged between 1920 and 1935. Pearl and Reed (1920) rediscovered Verhulst's (1838) "logistic" model for single-species population growth, and Lotka (1925) and Volterra (1926) developed a model for two-species competition. Thompson (1924), Nicholson (1933), and Nicholson and Bailey (1935) researched simple interactions between hosts and parasitoids. Their work is the foundation of mathematical descriptions of these interactions (Van Driesche and Bellows 1996). The classic works of Holling (1959a, b), Watt (1959), and Ivlev (1961) took theoretical studies to a much higher level. These researchers disagreed with the basic tenant of the Lotka-Volterra and Nicholson-Bailey models, which stated that the attack rate per predator is a linear function of prey density rising indefinitely as prey density

increases. Researchers have since determined that few insects, if any, have responses that increase linearly in such a manner. Holling (1959b) stated that an upper level is reached when predators become satiated or when parasites run out of eggs, and that even predators who continue to kill after being satiated reach an upper limit bounded by the time required to kill. It is thought by many that the equations of Lotka, Volterra, Nicholson and Bailey do not accurately describe predator responses (Hassell 1978).

When determining the effects of predation on prey populations, it is critical to distinguish between those factors affecting predator abundance and those affecting predator searching efficiency. This is most commonly done through the determination of functional and numerical responses. Solomon (1949) first defined these terms which were later used by Holling (1959a, b, 1961, 1965, 1966) in more depth. Functional response defines the relationship between the number of prey consumed per predator, and prey density (Hassell 1978). The number of prey consumed can then be used to help determine predator development, survival, and reproduction (Oaten and Murdoch 1975). Numerical response defines increases in predator populations as prey densities increase (Holling 1959a). Control is customarily achieved through the numerical response although it is usually considered a derivative of the functional response (Huffaker and Messenger 1976).

Original functional response research conducted by Holling (1959a) involved a blindfolded person searching for disks of sandpaper which had been glued to a tabletop. From this experimentation, Holling derived a mathematical model to estimate functional response, incorporating the time spent searching and handling the disks. The following is

the simplest expression of his functional response model known now as Holling's (1959a) disk equation for Type I functional responses:

$$N_A = aT_sN_o\ ,$$

where N_A is the number of disks removed, N_o is the initial density of disks, T_s is the time available for searching, and a is the instantaneous rate of discovery. This basic equation was designed as a first step toward explaining real functional responses. When Holling first generated this equation it accurately described all the functional responses of insects for which data had been published thus far (Holling 1959b).

Holling (1959a) went on to theorize that functional responses could conceivably have three basic forms which he called Types I, II, and III. The mathematically simplest, Type I, is exemplified by a predator with a constant search rate over all densities and a random search pattern. Thus, the number of prey killed per predator would be directly proportional to prey density, yielding a linear response until satiation is reached (Hassell 1978). DeBach and Smith (1941) determined that the parasitic fly *Muscidifurax raptor* exhibits this type of response (Holling 1959a). However, for most insect predators linear functional responses do not accurately estimate their true response (Hassell 1978).

DeBach and Smith (1941), Ullyett (1949) and Burnett (1951, 1956) demonstrated the more complex Type II functional responses for a number of insect parasites. However, it was Holling (1959b) who determined that handling time is the essential biological ingredient that distinguishes Type II from Type I responses. Handling time (T_h) collectively refers to the act of subduing, killing, and eating a prey, and then perhaps cleaning and resting. All of these behaviors reduce the time available for search. This

led Holling (1959b) to distinguish between the total time initially available for search T_t, and the actual searching time T_s, which could be represented by the formula:

$$T_s = T_t - T_h N_A.$$

This equation can then be factored into Holling's basic disk equation to yield the Type II functional response equation:

$$N_A = aT_t N / (1 + aT_h N).$$

Most arthropod predators have been shown, with some exceptions (Sandness and McMurtry 1970, Tostowaryk 1972, Hassell et al. 1977), to possess a Type II response (Holling 1961, Royama 1971, Murdoch and Oaten 1975, Hassell 1978, Luck 1985).

The third form of functional response, Type III, is sigmoidal with the search rate initially increasing as prey density does, then decreasing as satiation is reached. The sigmoidal shape is thought to be the result of predator learning (Holling 1959a). Type III responses were thought to describe vertebrate predators only. However, Murdoch and Oaten (1975), van Lenteren and Bakker (1976), and Hassell et al. (1977) suggest that sigmoidal responses are also widespread among arthropod predators and parasitoids. Hassell et al (1977) analyzed Type III functional responses using the following model:

$$N_a = N[1 - \exp(-a(T - T_h N_a))].$$

Xylocoris flavipes has previously been tested with bruchid species to determine its functional response by Sing (1997), who found the predator's response was best described by Holling's Type II model and not the Type III.

Though many functional response studies are performed in laboratory settings, their results may bear little resemblance to those measured in the field (O'Neil 1989). Luck et al. (1988), O'Neil (1989), and Wiedenmenn and O'Neil (1991) have conducted studies to

better understand the important differences between laboratory and field environments, in an attempt to explain the difficulties encountered when trying to apply laboratory results to field situations. Both laboratory and field studies show predator search efficiency to be an inverse function of prey density. However, where the rates of predation and predator search in the laboratory are associated with predator consumptive behaviors, such as handling time, in the field predation is primarily associated with the predator's ability to find prey (O'Neil 1989). O'Neil (1989) found handling time to be the limiting factor in laboratory studies because the scale of prey density offered was so unnaturally high. Applying the results of laboratory studies to field settings is difficult because of the incompatible prey densities and corresponding attack rates. Unfortunately, the vast majority of predation studies done in the field have utilized prey numbers in excess of field-realistic conditions as laboratory studies tend to (Luck et al. 1988). Prey may be dispersed over a more natural and realistic searching arena but with unrealistic numbers the focus still remains on effects of handling time, which may be misleading. Prey densities need to reflect realistic field conditions if the roles of predator search and handling time, in predator-prey dynamics, are to be ascertained (Gilbert et al. 1976).

Artificial Diet for Rearing Predatory Bugs

It can be difficult and expensive to rear large colonies of natural enemies for use in mass release biological control programs. One of the major difficulties is mass propagation of prey insects to serve as food for the beneficials. A natural rearing system utilizes the predator's natural prey, which has also been reared on one of its natural food

plants. The labor costs for rearing plants and herbivores make some biological control programs too expensive to compete with chemical controls or at least too expensive to use on lower value crops. Thus, alternative food sources are often utilized at either the plant or herbivore trophic level to make these programs more economically competitive (Van Driesche and Bellows 1996). The development of nutritionally adequate artificial diets devoid of insect components has helped make biological control programs more feasible (Cohen 1985, Cohen and Urias 1986, Greenberg et al. 1994, Guerra and Martinez 1994, Guerra et al. 1994, Legaspi et al. 1994, Ogura and Hosoda 1995, Rojas et al. 1996, Xie et al. 1997).

Diet can influence the size, vigor, fecundity, sex ratio, and host recognition ability of biological control agents, which can in turn influence their effectiveness. The natural diet of an insect is generally assumed to be the best diet for the production of vigorous individuals with natural behaviors (Cohen 1985). The primary goal of artificial diet research is to determine if the diet adversely affects the natural enemy's performance. The nutritional quality of artificial diets is the first obstacle that must be overcome. Diets are evaluated for amino acid, vitamin, lipid, and protein content. Once a suitable match to natural food sources is found, presentation of the artificial diet is tested. The complexities of host finding and the behavioral mechanisms involved are not well understood, which makes artificial diet presentation challenging. Many factors may be involved in the natural enemy's searching strategy. Some insects use the physical characteristics of the host such as shape and texture (Weseloh 1971, Bragg 1974), others use size (Richerson and DeLoach 1972), developmental stage (Isenhour 1985), or movement and vibration (Monteith 1956, Bragg 1974). Many beneficial insects also use

chemical cues or semiochemicals to elicit their host seeking behavior. These chemicals may be found in host frass (Hendry et al. 1973, Jones 1971), host mandibular or labial gland secretions (Mudd and Corbet 1973, Weseloh 1977), hemolymph (McLain 1979), or chemicals found in the herbivore's host plants or from synomones produced by the plants when damaged by herbivore feeding (Guerra et al. 1994, Dicke et al. 1993, Nordlund et al. 1981, Vinson 1976, 1981). All of these factors need to be taken into consideration when presenting an artificial diet to a natural enemy. Another factor for consideration is the ability of arthropods to learn, which has been demonstrated in both parasitoids and predators (Arthur 1966, Murdoch 1969). This ability can cause predators reared on artificial diets to have reduced efficiency on the natural or target hosts. However, Herard et al. (1988) determined that this phenomenon could be overcome by exposing the predator to target or natural hosts prior to use as a biological control agent.

Objectives:

In the second chapter of this thesis, the functional response of *Xylocoris flavipes* was observed via two aspects, prey species and habitat. The objectives of this study were to determine differences in male and female functional response to four stored-products pests. Experiments were conducted in empty jars to ascertain their general capabilities and then in jars of wheat at lower prey densities in an attempt to simulate natural grain storage conditions.

The primary goal of the third chapter of this thesis was to determine if *X. flavipes* could be mass reared on a meat-based artificial diet. The objective of this study was to determine whether the diet adversely affected the natural enemy's performance. This was ascertained by comparing developmental time, survivorship and fecundity of insects reared on the diet compared to those reared on a natural food source.

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Chapter II: Functional Response of *Xylocoris flavipes* (Reuter) (Hemiptera: Anthocoridae): Effects of Prey Species and Habitat

Abstract

Xylocoris flavipes (Reuter), the warehouse pirate bug, is a well known predator of many stored-products pests. This study compared the functional response of *X. flavipes* to varied densities of the prey species *Tribolium castaneum* (Herbst), *Oryzaephilus surinamensis* (L.), *Plodia interpunctella* (Hübner) and *Rhyzopertha dominica* (F.) in two different habitats: empty glass jars and jars of wheat kernels designed to simulate grain bin conditions. Differences in the functional response of *X. flavipes* to all combinations of prey densities and grain conditions were compared to the predicted functional response curves from Holling's Type I and Type II models, and to Hassell's Type III model. Results indicate that the functional response of *X. flavipes* is best described by Holling's Type II model, but a Type III response may occur with prey that are more difficult to subdue, such as *T. castaneum* larvae. Male and female attack rates differed significantly (P=0.05) in both habitats for many prey life stages and species, but male attack rates were not so low as to rule out their use as biological control agents. The range of individual predatory attack rates for the different species were as follows: 23-27 eggs, 11-27 small larvae and 15-17 large larvae in empty jar assays over a 24 h period; and 23-27 eggs, 11-27 small larvae and 15-17 large larvae in wheat jar assays over a 48 h period. All results indicate that *X. flavipes* could be a viable biological control agent in stored-products ecosystems.

Introduction

Examples of natural enemies being used to control pests have existed for centuries, but biological control emerged as a scientific method only late in the nineteenth century (Huffaker and Messenger 1976). Increases in pesticide usage have caused numerous environmental problems, which in conjunction with legislation such as the Food Quality Protection Act (FQPA) may force stored products managers to turn to other non-chemical options such as biological control in the near future. Stored product managers had few pest control options other than pesticides until legislation, passed in 1992, exempted certain beneficial insects from the tolerance standards. The EPA exempted from previous standards all genera of parasitoids and predators known commonly to attack stored food pests. It is now acceptable to use beneficial insects in stored raw whole grains and packaged food in warehouses so long as the insects do not become a component of the final food product (Anonymous 1992). Included on that list of tolerated beneficial organisms is the stored products predator *Xylocoris flavipes* (Reuter) (Hemiptera: Anthocoridae) (Subramanyam and Hagstrum 1996).

Xylocoris flavipes and several other insects in the subfamily Lyctocorinae frequently occur as predators in storage ecosystems. It is presently known to prey on at least 13 species of insects belonging to three orders. *Xylocoris flavipes* can play an important role in biological control programs for storage ecosystems, as indicated by facets of its biology and its success in regulating populations of storage pests (Arbogast 1978). The efficiency of *X. flavipes* as a biological control agent can be ascertained by the results of several studies (Jay et al. 1968, Press et al. 1975, LeCato

and Collins 1976, Arbogast 1976, LeCato et al. 1977, Keever et al. 1986, Brower and Press 1992).

Understanding predator-prey interactions is crucial if a biological control program is to be successful. Determining the effects of predation on prey populations is most commonly done through the analysis of functional and numerical responses (Huffaker and Messenger 1976). Functional response defines the relationship between the number of prey attacked per predator, and prey density (Hassell 1978). The number of prey attacked can then be used to help predict predator development, survival, and reproduction (Oaten and Murdoch 1975). Original functional response research was conducted by Holling (1959a). This study utilized Holling's Type I and II models (Holling 1959a, b) and Hassell's Type III model (Hassell et al. 1977):

$$\text{Type I: } N_A = aTN,$$

$$\text{Type II: } N_A = aTN / (1 + aT_hN),$$

$$\text{Type III: } N_a = N[1 - \exp(- a(T - T_h N_a))]$$

In these models, N_A is the number of prey killed, N is the initial density of prey, T is the time available for searching during the experiment, a is the instantaneous rate of discovery, and T_h is the amount of time the predator handles each prey killed. Type I responses are the mathematically simplest and are exemplified by a predator with a constant search rate over all densities and a random search pattern. Thus, the number of prey killed per predator would be directly proportional to prey density, yielding a linear response until satiation is reached (Hassell 1978). Type II responses incorporate predator handling time, which refers to the act of subduing, killing, and eating a prey, and then perhaps cleaning and resting before moving on to search for more prey. Most arthropod

predators have been shown, with some exceptions (Sandness and McMurtry 1970, Tostowaryk 1972, Hassell et al. 1977), to possess a Type II response (Holling 1961, Royama 1971, Murdoch and Oaten 1975, Hassell 1978, Luck 1985). The third form of functional response, Type III, is sigmoidal with the searching rate first increasing with increased prey density and then decreasing. The sigmoidal shape is thought to be the result of predator learning (Holling 1959a).

Though many functional response studies are performed in laboratory settings, their results may bear little resemblance to those measured in the field (O'Neil 1989). Luck et al. (1988), O'Neil (1989), and Wiedenmenn and O'Neil (1991) have conducted studies to better understand the important differences between functional response studies conducted in laboratory and field environments, in an attempt to explain the difficulties encountered when trying to apply laboratory results to field situations. Both laboratory and field studies show predator search efficiency to be an inverse function of prey density. However, the rates of predation and predator search in the laboratory are associated with predator consumptive behaviors, such as handling time, but in the field predation is primarily associated with the predator's ability to find prey (O'Neil 1989). O'Neil (1989) found handling time to be the limiting factor in laboratory studies because the scale of prey density offered was so unnaturally high. Prey densities need to reflect realistic field conditions if the roles of predator search and handling time, in predator-prey dynamics, are to be ascertained (Gilbert et al. 1976). Sing (1997) has tested the functional response of *X. flavipes* to several different bruchid species and determined that it exhibited a Type II functional response.

The functional response of *Xylocoris flavipes* was observed in this study via two aspects, prey species and habitat. The objectives were to determine differences in male and female functional responses to four stored-products pests. Experiments were conducted in empty jars to ascertain their general capabilities and then in jars of wheat at lower prey densities in an attempt to simulate natural grain storage conditions.

Materials and Methods

Predator. *Xylocoris flavipes* were obtained from a commercial producer of beneficial insects and a preexisting colony from the University of Florida at Gainesville. The colonies were combined and kept at 30° C and 65% RH with a 16:8 (L:D) photoperiod. All experiments were conducted under the same conditions. The bugs in the colony were fed ad libitum eggs of the indianmeal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae). Three to five day old adult *X. flavipes* were gathered from the colony for this study and briefly chilled in order to separate them by sex.

Prey. Prey species used in this study were *Oryzaephilus surinamensis* (L.) (Coleoptera: Cucujidae), *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), *P. interpunctella* and *Rhyzopertha dominica*(F.) (Coleoptera: Bostrichidae). All experimental prey species were reared at Oklahoma State University following established procedures (e.g., Howe 1950, 1991). *Tribolium castaneum* used in experiments were small first and second instars (≤2.5mm) and large fourth and fifth instars (≥4.5mm) larvae. The small *Oryzaephilus surinamensis* used in these assays were first and second instars (≤2.5mm) and the large larvae used were fourth through sixth instars, (≥3.0mm). The first and second instar *P. interpunctella* used in the assays were ≤2.5mm in length. *Rhyzopertha dominica* used in these assays were first and second instars which were feeding internally on wheat kernels. *Tribolium castaneum* and *O. surinamensis* larvae were obtained from the laboratory colony jars using #14, #20 and #40 sized stacked sieves. *Plodia interpunctella* eggs were obtained by shaking eggs through screen covered 945ml glass jars that contained moth adults. First and second instar *P. interpunctella* larvae were obtained by hatching out their eggs in Petri dishes.

Rhyzopertha dominica eggs were obtained by placing 50 to 100 adults in containers of flour which is sifted daily through #20, #40 and #100 sized stacked sieves. To obtain wheat kernels containing first and second instar *Rhyzopertha dominica*, eggs were placed in 100-welled microtiter plates with each well containing a single kernel of wheat. Eggs were allowed to hatch (approximately 7-9 d at 30° C) and larvae were given 3-4 d to burrow into the kernels. Individual kernels were then examined under a microscope to identify those kernels with entrance holes packed with frass.

Empty Jar Assays. Laboratory functional response assays were conducted with the above pest species in 240ml glass jars with a bottom diameter of 5.4cm. These assays are designated as empty jar experiments. Other functional response experiments were conducted in 945ml glass jars containing 500g of a wheat flour mixture (5% whole wheat flour) in an attempt to simulate field conditions. The predator's functional response to immature stages of *O. surinamensis, T. castaneum, P. interpunctella* and *R. dominica* was determined. The empty jar assays conducted with external feeding larvae actually contained 0.10g of whole-wheat flour to eliminate prey cannibalism that was witnessed in preliminary assays. Predators separated by sex, starved for 24 h, then added singularly to the glass jars containing various densities of each prey species. The densities for the empty jar treatments were determined in preliminary assays to ensure maximum attacks rates would be reached. Densities used in the empty jar assays were as follows: *T. castaneum* large larvae at 5, 15, 20, and 30, and small larvae at 10, 20, 30, 40, and 50; *O. surinamensis* large and small larvae each at 10, 20, 30, 40, 50, and 60; *P. interpunctella* eggs at 10, 50, 100, 150, 200, and 300, and small larvae at 10, 20, 30, 40, 50, and 60; *R. dominica* eggs 10, 25, 50, 100, 150, and 200, and small larvae within kernels of wheat at

5, 10, 15, 20, 25, and 30. The predators fed for 24 h before being removed so the number of prey killed could be quantified. Each density was examined 30 times with 10 control replications to account for natural mortality and prey cannibalism, 10 replications with a male predator, and 10 with females.

Wheat Jar Assays. The wheat jar treatments done in 946ml glass jars evaluated predation on three species: *O. surinamensis*, *P. interpunctella*, and *R. dominica*. The 500g wheat-flour mixture contained approximately 18,400 kernels of wheat and 25g of flour for the predators to hunt through. The predetermined densities for the wheat jar treatments were as follows: *O. surinamensis* large and small larvae each at 5, 10, 15, 20, 25, and 30; *P. interpunctella* eggs at 50, 100, 150, 200, and 300, and small larvae at 5, 10, 15, 20, 25, and 30; *R. dominica* eggs at 10, 25, 50, 100, 150, and 200, and small larvae inside kernels of wheat at 5, 10, 15, 20, 25, and 30. Larval prey, added at the aforementioned densities, were allowed 1 h to disperse. Jars with eggs added were tapped on a hard surface five times in order to disperse eggs among the top layer of wheat kernels as they would be in stored grain. Wheat kernels with *R. dominica* larvae feeding internally were added to the jars, which were then turned on their sides, rotated five times, and then turned end over end five times to ensure even disbursement of infested kernels throughout the jar.

Male and female predators were starved for 24 h, introduced singularly to the jars for 48 h and then removed so the number of prey killed could be scored. Predators were given 48 h in the wheat jar treatments as opposed to the 24 h in the empty jars to let them better acclimate themselves to the more complex habitat. Jars containing eggs and small larvae were kept in a growth chamber until the pests were large enough to count. A

control treatment with no predator was used to determine the amount of natural mortality and prey cannibalism occurring during this time. Ten replications for each prey species and density were conducted for both male and female predators.

Data Analysis. The coefficients of determination (r^2 values) were calculated by SAS PROC NLIN (SAS Institute 1989) to determine which nonlinear model, Holling's disk equations for Types I and II or Hassell's Type III, best fits the experimental values. Parameters a and T_h from the functional response models were estimated using SAS PROC NLIN (SAS Institute 1989) also. All analysis of density and male to female comparisons were completed using SAS PROC MIXED (SAS Institute 1989).

Results

Tribolium castaneum. Predators assayed with large larvae of *T. castaneum* did not exhibit a typical functional response. The lack of functional response was indicated by the absence of substantial difference in the number of larvae killed over the varying densities. Since the average number killed did not exceed 1.60 larvae over the 24 h test period, it would be incorrect to insinuate that any functional response occurred. I observed that the predators had a difficult time subduing the large *T. castaneum* larvae. When attacked, large larvae would thrash around and often dislodge the predator.

The predators assayed with small *T. castaneum* larvae were far more successful at subduing the prey. The response of male and female predators to small beetle larvae was best fit by Hassell's Type III model (Table 2.1). The number of prey killed increased significantly as initial prey density increased. Female predators killed a significantly higher number of prey over the 24 h test period (Table 2.2). Females had a maximum attack rate of approximately 27 larvae, while males peaked at only 11 larvae.

Oryzaephilus surinamensis. Both male and female predatory responses of *X. flavipes* to *O. surinamensis* larvae in empty jars were best described by Holling's Type II model (Table 2.3). Number of larvae attacked increased significantly as initial prey density increased for both male and female predators (Table 2.4). There was no significant difference between average male and female response. Males and females attained maximum attack rates of approximately 23 and 24 larvae respectively during the 24 h period.

Both male and female functional responses to small *O. surinamensis* larvae in jars of wheat were best described by the Type II model (Table 2.3). Number of larvae killed

increased significantly as initial prey density increased (Table 2.5). There was no significant difference in male and female responses with maximum attack rates being reached at approximately 13 and 14 prey for each sex respectively during the 48 h test period.

Male and female predators best fit a Type II response to large *O. surinamensis* larvae in empty jars (Table 2.3). There was a significant increase in the number of larvae killed as initial prey density increased (Table 2.4). Male and female attack rates were significantly different, consuming approximately 15 and 17 large larvae over 24 h.

Male and female responses to large *O. surinamensis* larvae in wheat jars were best described by the Type II model (Table 2.3). There was a significant increase in predation as the initial prey density increased (Table 2.5). There was no significant difference in male and female maximum attack rates with the males peaking at approximately 12 larvae and females at approximately 13 larvae during the 48 h testing period.

Plodia interpunctella. Male and female responses to *P. interpunctella* eggs in empty jar assays were best described by the Type II model (Table 2.6). In both male and female treatments, the average number of eggs attacked increased significantly as initial prey density increased (Table 2.7). There was no significant difference in male and female maximum attack rates. Both peaked at approximately 27 eggs over the 24 h test period.

Holling's Type II model best fit the male and female functional response to *P. interpunctella* eggs in the wheat jar assays (Table 2.6). In both treatments, the number of eggs attacked increased significantly as initial prey density increased (Table 2.8). Unlike the empty jar assays, there was a significant difference in male and female attack rates in

the wheat jar assays. Females had a higher peak attack rate of approximately 41 eggs over the 48 h testing period, while males reached only 37 eggs in 48 h.

Male and female functional responses to small larvae of *P. interpunctella* in empty jars was best described by the Type II (Table 2.6). Both treatments showed a significant increase in number of larvae killed as initial prey density increased (Table 2.7). There was no significant difference in male and female maximum attack rates, with both attacking approximately 23 larvae over the 24 h testing period.

Female functional response to *P. interpunctella* larvae in wheat jars was best described by the Type II model while the males were best described by a Type III response (Table 2.6). There was a significant increase in the number of larvae killed as initial prey density increased (Table 2.8). However, male predators did not have as sharp an initial increase in predation, causing their response to fit Hassell's Type III model better than Holling's Type II. Overall, there was no significant difference in maximum male and female attack rates; both reach peaked at approximately 14 larvae over the 48 h test period.

Rhyzopertha dominica. Both male and female functional responses to *R. dominica* eggs in empty jars were best described by the Type II model (Table 2.9). There was a significant increase in the number of eggs killed as initial prey density increased (Table 2.10). There was a significant difference in male and female maximum attack rates. Females were able to attack as many as 26 eggs over the 24 h period, while males reached peaked at only 23 eggs.

Predation by male and female *X. flavipes* on *R. dominica* eggs in wheat jars was best described by Holling's Type II model (Table 2.9). There was a significant increase in the

number of eggs killed as the initial egg density increased (Table 2.11). Male and female responses were only significantly different at the satiation point. Males reached satiation at approximately 19 eggs while females did not reach satiation until approximately 22 eggs over the 48h time period.

Both male and female predators were successful at killing *R. dominica* larvae that were feeding inside wheat kernels. The predators would search an infested wheat kernel until the beetle's entrance hole was found and then they would insert their stylet through that hole to feed. Both male and female functional responses to *R. dominica* larvae in empty jars were best described by the Type II model (Table 2.9). There was a significant increase in the number of larvae killed as the initial prey density increased (Table 2.10). There was also a significant difference in maximum male and female attack rates in the empty jar assays. Females were able to find and kill as many as 17 larvae over the 24 h testing period, while males peaked at only 12 larvae.

Both male and female predators successfully found and killed the internal feeding larvae within the 18,000 kernels of wheat contained in the jars. Type II and Type III r^2 values for both sexes were similar. The male Type II value was 0.9354 while the Type III value was only slightly lower at 0.9327. The female Type II value was 0.9462 while the Type III value was slightly higher at 0.9472 (Table 2.9). These figures show the sexes to have different functional responses, the female response appears to fit the Type III model best, while males seem to fit the Type II best. However, since the r^2values were so high for both models, it might be more correct to say both male and female functional responses were neither Type II nor III, but somewhere in between the two traditional models for this prey species. The number of larvae killed by both sexes

increased significantly as initial prey density increased (Table 2.11). Male and female maximum attack rates were not significantly different. Males reached peaked at approximately 11 larvae females at 12 larvae over the 48 h test period.

Discussion

Xylocoris flavipes apparent Type III functional response to *T. castaneum* larvae was not surprising. Even the small larvae of this pest have a higher degree of sclerotization than the other pests tested. The *T. castaneum* larvae also thrash around when the predator inserts its stylet to begin feeding, making them difficult to subdue. Type III responses have a slower rate of increase at the beginning of their curves which has often been ascribed to predator learning (Holling 1959a, b). If the predator had to learn how to subdue this pest, a Type III curve would best fit their functional response as was observed.

The other two instances of *X. flavipes* exhibiting Type III responses are not as easy to explain nor were they predicted. Functional responses have often been criticized because of the inherent problems associated with their statistical analysis. Several papers have been published on the statistical analysis of functional responses (Livdahl 1983, Houck and Strauss 1985, Williams and Juliano 1985). The papers offer many methods of analysis, criticizing some while recommending others. This research was analyzed using nonlinear regression (SAS PROC NLIN) to estimate the missing parameters of attack rate (a) and handling time (T_h) from Holling's and Hassell's models (SAS Institute 1989). Methods for estimating parameters by nonlinear regression have been described by many authors (Chambers 1973, Silvert 1979, Houck and Strauss 1985, Williams and Juliano 1985). The two unexpected instances of *X. flavipes* exhibiting a Type III response might be the result of the parameter estimation and overall analysis. The male predators that exhibited the Type III response to *P. interpunctella* larvae in wheat jars also had a very high coefficient of determination (r^2 value) for the Type II model (0.9598 Type III and

0.9207 Type II). A similar pattern was seen for female predators on *R. dominica* larvae in wheat jars. The Type III response had the highest coefficient of determination (0.9472) but the Type II was scarcely lower (0.9462). Since the two anomalous Type III r^2 values are so similar to the Type II values, it would not be incorrect to say that the predator's functional response was best described by Holling's Type II model for all pests assayed with the exception of the more robust *T. castaneum*.

As previously mentioned the wheat jar assays were conducted in 945ml glass jars containing 500g of a wheat flour mixture (5% whole wheat flour) in an attempt to simulate field conditions. Jay et al. (1968), who first suggested using *X. flavipes* as a possible stored products biological control agent, tested it in 945ml glass jars containing 150g of rolled oats. In their study the predator achieved suppression after 30 d of three of the pest species tested in this study. Arbogast (1976) assayed *X. flavipes* in fiber drums 80cm high x 39.5cm internal diameter containing shelled corn to a depth of 26cm. The predator was able to achieve suppression of *O. surinamensis* in this microenvironment after the 15 wk experiment also. There was some concern in the present study that the predators would not be able to penetrate the small interstitial spaces of the wheat and flour mixture to reach the crawling larvae of *O. surinamensis* and *P. interpunctella*. When the predators were removed at the end of the assay period, some were found alive on the very bottom of the 945ml jars, which indicated that the small spaces were large enough for them to maneuver and hunt effectively.

If *X. flavipes* is ever going to be prescribed as a biological control agent in grain storage facilities, it will be important to know how many prey each individual predator can attack in a given period of time. In empty jar arenas when feeding on eggs of both

pests assayed (*R. dominica* and *P. interpunctella*), the predators were able to find and consume approximately 22-27 eggs in 24 h. There was more variation in attack rates in the simulated natural habitat but their efficacy in these jars containing 500g of wheat was still impressive. *Plodia interpunctella* eggs are the natural diet of the *X. flavipes* used in these experiments, so these predators are well adapted to their natural chemical cues. The predators should have had no trouble hunting down the *P. interpunctella* eggs within the wheat jars, but *X. flavipes* had no previous exposure to *R. dominica*, so the high attack rates were unexpected.

The predator's ability to find and attack the internally feeding *R. dominica* larvae was much better than predicted. The wheat jar assays demonstrated that the predator could not only gain access to and kill the internal feeder but that it could search out and find as few as 5 infested kernels within a jar containing over 18,000 kernels of wheat. This could have far reaching implications for this predator. Biological control for stored products has always been hampered by the inaccessibility of the internally feeding pests to certain natural enemies (Arbogast 1978).

The performance of female predators often surpasses that of their male counterparts (DeBach and Smith 1941). This was not the case with *X. flavipes* in simulated grain bin (wheat jar) assays. The male and female maximum predatory attack rates only differed significantly on the *P. interpunctella* eggs that they are accustomed to eating in their colony. There was more variation in the empty jar assays where consumptive capabilities are the major limiting factor since searching skills would not be a key factor in the 5.4cm diameter arena (O'Neil 1989). The male attack rates that did differ from the females (*X. flavipes* on *R. dominica* eggs and larvae and large larvae of *O. surinamensis*) did so at the

highest prey densities where satiation was probably reached. These differences, if merely a result of satiation could be caused by the differences in size between male and female predators. Since rates of predation in natural habitats are more closely associated with searching efficacy and not consumptive capabilities, the male *X. flavipes* should be just as effective biological control agents as their female counterparts (O'Neil 1989).

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Table 2. 1. Coefficients of determination (r^2 values) for *Xylocoris flavipes* functional response to *Tribolium castaneum*

Arena	Life stage	Type	Sex	r^2
Empty jars	**Small larvae**	I	Male	0.8118
		I	Female	0.9156
		II	Male	0.8495
		II	Female	0.9213
		III	Male	0.9063*
		III	Female	0.9730*

* Model that best fits the observed data.

Table 2.2. Predation by *X. flavipes* on *Tribolium castaneum* at varying densities in empty jars

Density	Mean No. killed (SE) [1]		Sex difference [2]
	Male	Female	p - value
Small larvae:			
10	5.90a (0.38)	4.00a (0.97)	0.3514
20	7.60b (0.40)	9.20b (1.00)	0.4323
30	7.70bc (1.51)	13.50b (1.82)	0.0053
40	11.20c (1.37)	19.40c (1.45)	0.0001
50	11.00bc (1.69)	27.30d (2.42)	0.0001
	$F = 6.46$ df = 4, 16 $P = 0.0027$	$F = 33.84$ df = 4, 14.5 $P = 0.0001$	
Large larvae:			
5	0.78a (0.15)	1.20a (0.13)	0.2267
15	0.75a (0.25)	2.00a (0.26)	0.0008
20	1.10ab (0.22)	1.56a (0.29)	0.1925
30	1.60b (0.23)	1.40a (0.34)	0.5547
	$F = 3.37$ df = 3, 33 $P = 0.0299$	$F = 1.67$ df = 3, 35 $P = 0.1909$	

[1] Means in a column for a given prey type followed by different letters are significantly different ($p<0.05$) using Least Significant Difference (LSD) procedures.

[2] Differences in predation between males and females were determined through an ANOVA. Overall experiment-wide differences in male and female predation were also determined with an ANOVA and were found to be significant on small larvae ($F = 16.5_{1, 67.5}$, $P = 0.0001$) and large larvae ($F = 6.33_{1, 74}$, $P = 0.0141$).

Table 2.3. Coefficients of determination (r^2 values) for *X. flavipes* functional response to *O. surinamensis*

Arena	Life stage	Type	Sex	r^2
Empty jars	**Small larvae**	I	Male	0.9629
		I	Female	0.9551
		II	Male	0.9837*
		II	Female	0.9871*
		III	Male	0.9643
		III	Female	0.9589
Empty jars	**Large larvae**	I	Male	0.9134
		I	Female	0.9312
		II	Male	0.9780*
		II	Female	0.9889*
		III	Male	0.9135
		III	Female	0.9407
Wheat jars	**Small larvae**	I	Male	0.9358
		I	Female	0.9337
		II	Male	0.9774*
		II	Female	0.9782*
		III	Male	0.9358
		III	Female	0.9337
Wheat jars	**Large larvae**	I	Male	0.9100
		I	Female	0.9225
		II	Male	0.9670*
		II	Female	0.9717*
		III	Male	0.9134
		III	Female	0.9231

- * Model that best fits the observed data.

Table 2.4. Predation by *X. flavipes* on *Oryzaephilus surinamensis* at varying densities in empty jars

Density	Mean No. killed (SE) [1]		Sex difference [2]
	Male	Female	P - value
Small larvae:			
10	7.00a (0.52)	8.00a (0.47)	0.2831
20	10.80b (0.63)	13.50b (0.50)	0.0044
30	16.10c (0.53)	19.00c (0.67)	0.0023
40	21.40d (0.83)	23.10d (0.71)	0.0694
50	22.20d (0.66)	23.80d (0.84)	0.0872
60	22.50d (0.65)	24.30d (0.73)	0.0548
	$F = 103.31$ df = 5, 54 $P = 0.0001$	$F = 98.61$ df = 5, 54 $P = 0.0001$	
Large larvae:			
10	7.20a (0.42)	7.30a (0.37)	0.9000
20	10.70b (0.56)	11.70b (0.58)	0.2104
30	12.80c (0.63)	15.40c (0.50)	0.0014
40	14.30cd (0.65)	16.80d (0.47)	0.0021
50	14.80d (0.84)	17.20d (0.55)	0.0031
60	15.10d (0.64)	17.40d (0.34)	0.0045
	$F = 23.15$ df = 5, 54 $P = 0.0001$	$F = 71.80$ df = 5, 54 $P = 0.0001$	

[1] Means in a column for a given prey type followed by different letters are significantly different ($p<0.05$) using Least Significant Difference (LSD) procedures.

[2] Differences in predation between males and females were determined through an ANOVA. Overall experiment-wide differences in male and female predation were also determined with an ANOVA and were found to be not significant on small larvae ($F = 2.80_{1, 1185}$, $P = 0.0966$) but were significantly different for large larvae ($F = 7.21_{1, 118}$, $P = 0.0083$).

Table 2.5. Predation by *X. flavipes* on *Oryzaephilus surinamensis* at varying densities in wheat jars

Density	Mean No. killed (SE) [1]		Sex difference [2]
	Male	Female	p - value
Small larvae:			
5	4.10a (0.23)	4.50a (0.17)	0.5783
10	7.90b (0.35)	9.10b 0.28)	0.0973
15	10.90c (0.38)	11.40c (0.54)	0.4874
20	11.80cd (0.57)	12.90cd (0.64)	0.1282
25	12.40cd (0.69)	13.40d (0.65)	0.1663
30	12.70d (0.56)	13.70d (0.65)	0.1663
	$F = 97.51$ df = 5, 22.9 $P = 0.0001$	$F = 127.07$ df = 5, 16.6 $P = 0.0001$	
Large larvae:			
5	4.30 a (0.21)	4.30 a (0.21)	1.0000
10	8.60 b (0.31)	8.90 b (0.31)	0.7123
15	10.20 c (0.53)	10.90 c (0.62)	0.3902
20	11.50 c (0.65)	12.10 c (0.67)	0.4612
25	11.80 c (0.77)	12.50 c (0.69)	0.3902
30	11.60 c (0.75)	12.80 c (0.70)	0.1421
	$F = 69.37$ df = 5, 16.8 $P = 0.0001$	$F = 85.18$ df = 5, 18 $P = 0.0001$	

[1] Means in a column for a given prey type followed by different letters are significantly different ($p<0.05$) using Least Significant Difference (LSD) procedures.

[2] Differences in predation between males and females were determined through an ANOVA. Overall experiment-wide differences in male and female predation were also determined with an ANOVA and were found to be not significant on small larvae ($F = 1.81_{1, 118}$, $P = 0.1807$) or large larvae ($F = 0.93_{1, 118}$, $P = 0.3378$).

Table 2.6. Coefficients of determination (r^2 values) for *X. flavipes* functional response to *P. interpunctella*

Arena	Life stage	Type	Sex	r^2
Empty jars	**Eggs**	I	Male	0.8368
		I	Female	0.8405
		II	Male	0.9902*
		II	Female	0.9883*
		III	Male	0.8443
		III	Female	0.8523
Empty jars	**Larvae**	I	Male	0.9260
		I	Female	0.9239
		II	Male	0.9863*
		II	Female	0.9865*
		III	Male	0.9292
		III	Female	0.9288
Wheat jars	**Eggs**	I	Male	0.8947
		I	Female	0.8902
		II	Male	0.9945*
		II	Female	0.9953*
		III	Male	0.9292
		III	Female	0.9128
Wheat jars	**Larvae**	I	Male	0.9241
		I	Female	0.9259
		II	Male	0.9207
		II	Female	0.9649*
		III	Male	0.9598*
		III	Female	0.9287

* Model that best fits the observed data.

Table 2.7. Predation by *X. flavipes* on *Plodia interpunctella* at varying densities in empty jars

Density	Mean No. killed (SE) [1]		Sex difference [2]
	Male	Female	P - value
Eggs:			
10	9.50a (0.22)	8.60a (0.27)	0.3945
50	19.80b (0.90)	20.10b (0.67)	0.7762
150	24.60c (0.70)	23.90c (1.21)	0.5075
200	25.30d (0.79)	25.20cd (0.74)	0.0314
300	26.80d (0.65)	27.20d (0.70)	0.7047
	$F = 326.73$ df =4, 21.1 $P = 0.0001$	$F = 285.86$ df = 4, 21 $P = 0.0001$	
Small larvae:			
10	9.40a (0.22)	9.70a (0.15)	0.7690
20	16.70b (0.40)	17.10b (0.60)	0.6955
30	20.10c (0.67)	21.10c (1.11)	0.3286
40	21.30cd (0.83)	22.40c (0.78)	0.2828
50	22.20d (0.71)	23.10c (0.64)	0.3791
60	23.40d (1.12)	23.70c (0.70)	0.7690
	$F = 163.28$ df = 5, 16.3 $P = 0.0001$	$F = 224.46$ df = 5, 18.1 $P = 0.0001$	

[1] Means in a column for a given prey type followed by different letters are significantly different ($p<0.05$) using Least Significant Difference (LSD) procedures.

[2] Differences in predation between males and females were determined through an ANOVA. Overall experiment-wide differences in male and female predation were also determined with an ANOVA and were found to be not significant on eggs ($F = 0.21_{1, 98}$, $P = 0.6517$) or small larvae ($F = 0.47_{1, 118}$, $P = 0.4931$).

Table 2.8. Predation by *X. flavipes* on *Plodia interpunctella* at varying densities in wheat jars

Density	Mean No. killed (SE) [1]		Sex difference [2]
	Male	Female	P - value
Eggs:			
50	23.90a (1.08)	27.10a (0.63)	0.0069
100	30.20b (0.83)	33.10b (0.74)	0.0141
150	33.10c (0.86)	36.60c (0.64)	0.0032
200	35.20cd (0.71)	38.90cd (0.67)	0.0019
250	36.90de (0.74)	40.20d (1.18)	0.0054
300	37.70e (0.60)	41.40d (1.01)	0.0042
	$F = 39.79$ df = 5, 54 $P = 0.0001$	$F = 40.87$ df = 5, 54 $P = 0.0001$	
Small larvae:			
5	4.40a (0.22)	4.70a (0.15)	0.7694
10	8.10b (0.38)	9.40b (0.22)	0.2055
15	10.30c (0.70)	11.80c (0.66)	0.6252
20	12.00c (0.86)	12.90cd (0.80)	0.3798
25	12.80c (0.73)	14.00cd (1.00)	0.2423
30	13.40c (0.79)	14.70d (1.02)	0.2055
	$F = 64.70$ df = 5, 18 $P = 0.0001$	$F = 110.89$ df = 5, 12.2 $P = 0.0001$	

[1] Means in a column for a given prey type followed by different letters are significantly different ($p<0.05$) using Least Significant Difference (LSD) procedures.

[2] Differences in predation between males and females were determined through an ANOVA. Overall experiment-wide differences in male and female predation were also determined with an ANOVA and were found to be significant for eggs ($F = 11.39_{1, 118}$, $P = 0.0010$) but were not significant for small larvae ($F = 1.61_{1, 118}$, $P = 0.2066$).

Table 2.9. Coefficients of determination (r^2 values) for *Xylocoris flavipes* functional response to *Rhyzopertha dominica*

Arena	Life stage	Type	Sex	r^2
Empty jars	**Eggs**	I	Male	0.7933
		I	Female	.08041
		II	Male	0.9710*
		II	Female	0.9805*
		III	Male	0.7936
		III	Female	0.8052
Empty jars	**Larvae**	I	Male	0.9373
		I	Female	0.9541
		II	Male	0.9752*
		II	Female	0.9653*
		III	Male	0.9373
		III	Female	0.9574
Wheat jars	**Eggs**	I	Male	0.7637
		I	Female	0.7840
		II	Male	0.9750*
		II	Female	0.9728*
		III	Male	0.7643
		III	Female	0.7840
Wheat jars	**Larvae**	I	Male	0.9143
		I	Female	0.9342
		II	Male	0.9354*
		II	Female	0.9462
		III	Male	0.9327
		III	Female	0.9472*

* Model that best fits the observed data.

Table 2.10. Predation by *X. flavipes* on *Rhyzopertha dominica* at varying densities in empty jars

Density	Mean No. killed (SE) [1]		Sex difference [2]
	Male	Female	P - value
Eggs:			
10	8.30a (0.45)	9.70a (0.15)	0.3377
25	14.70b (0.91)	15.60b (0.99)	0.5372
50	19.00c (1.15)	21.90c (0.92)	0.0486
100	21.40cd (1.39)	25.70d (1.16)	0.0038
150	23.10d (1.27)	24.80cd (1.05)	0.2449
200	22.60d (1.05)	26.40d (1.18)	0.0102
	$F = 28.21$ df =5, 54 $P = 0.0001$	$F = 147.67$ df = 5, 20.6 $P = 0.0001$	
Larvae in wheat kernels:			
5	4.40a (0.22)	4.80a (0.13)	0.6466
10	6.70b (0.45)	7.00b (0.37)	0.7309
15	9.30c (0.52)	10.50c (0.75)	0.1709
20	10.4cd (0.50)	15.40d (0.83)	0.0001
25	11.30d (0.42)	16.30d (0.83)	0.0001
30	11.60d (0.69)	16.60d (1.01)	0.0001
	$F = 34.34$ df = 5, 54 $P = 0.0001$	$F = 102.55$ df = 5, 12.4 $P = 0.0001$	

[1] Means in a column for a given prey type followed by different letters are significantly different ($p<0.05$) using Least Significant Difference (LSD) procedures.

[2] Differences in predation between males and females were determined through an ANOVA. Overall experiment-wide differences in male and female predation were also determined with an ANOVA and were found to be significant for eggs ($F = 4041_{1, 118}$, $P = 0.0378$) and small larvae ($F = 13.29_{1, 94.7}$, $P = 0.0004$).

Table 2.11. Predation by *X. flavipes* on *Rhyzopertha dominica* at varying densities in wheat jars

Density	Mean No. killed (SE) [1]		Sex difference [2]
	Male	Female	P - value
Eggs:			
10	7.40a (0.40)	8.10a (0.43)	0.5871
25	13.80b (0.80)	14.20b (1.02)	0.7562
50	17.50c (1.02)	19.60c (1.13)	0.1051
100	17.90c (0.75)	20.50c (0.96)	0.0455
150	19.30c (0.87)	21.40c (1.12)	0.1051
200	18.60c (0.93)	22.00c (1.10)	0.0094
	$F = 30.25$ df = 5, 54 $P = 0.0001$	$F = 30.29$ df = 5, 54 $P = 0.0001$	
Larvae in wheat kernels:			
5	3.40a (0.40)	3.60a (0.22)	0.8437
10	5.30a (0.42)	5.20b (0.49)	0.9214
15	8.10b (0.75)	7.90c (0.71)	0.8437
20	10.60c (0.96)	11.30d (0.83)	0.4905
25	11.20c (0.88)	12.50d (0.81)	0.2015
30	10.90c (0.80)	12.10d (0.89)	0.2382
	$F = 19.90$ df = 5, 54 $P = 0.0001$	$F = 52.65$ df = 5, 16.3 $P = 0.0001$	

[1] Means in a column for a given prey type followed by different letters are significantly different ($p<0.05$) using Least Significant Difference (LSD) procedures.

[2] Differences in predation between males and females were determined through an ANOVA. Overall experiment-wide differences in male and female predation were also determined with an ANOVA and were found to be not significant for eggs ($F = 3.70_{1, 118}$, $P = 0.0567$) or small larvae ($F = 0.52_{1, 118}$, $P = 0.4706$).

Chapter III: Performance of the Predatory Insect *Xylocoris flavipes* (Reuter) (Hemiptera: Anthocoridae) Fed a Meat-Based Artificial Diet

Abstract

Xylocoris flavipes(Reuter), a known predator of many stored-products pests, is commercially reared for release as a biological control agent. The expense of rearing predatory insects is due to the extensive labor required to maintain these beneficial organisms on their natural food sources. Artificial diets are often employed to help alleviate some of this expense. The objective of this study was to determine whether developmental time, survivorship and fecundity of *X. flavipes* reared on a meat-based artificial diet differed substantially from those reared on a natural prey source. A recently patented diet was used for these experiments and was presented to the predators in Parafilm® domes. *Xylocoris flavipes* fed the artificial diet throughout their lives had a significant increase in nymphal developmental time from 13.00 d in the control to 16.97 d for those fed the diet ($P<0.05$). There was also a significant increase in nymphal mortality from 4.55% in the control to 18.60% in those fed the diet throughout their lives ($P<0.05$). The control had a significantly higher intrinsic rate of increase than all treatments reared on the diet ($\alpha = 0.05$). However, there was no significant decrease in fecundity or net reproductive rate ($P>0.05$). The artificial diet's effects on nymphal mortality caused the reduction in each treatment's intrinsic rate of increase, but did not affect the insects' performance enough to outweigh the monetary savings of not having to rear an additional natural food source for this predatory insect.

Introduction

Increases in pesticide usage have caused numerous environmental problems, which in conjunction with legislation such as the Food Quality Protection Act (FQPA) may force stored products managers to turn to other non-chemical options such as biological control in the near future. Stored product managers had few pest control options other than pesticides until legislation, passed in 1992, exempted certain beneficial insects from the tolerance standards. The EPA exempted from previous standards all genera of parasitoids and predators known commonly to attack stored food pests. It is now acceptable to use beneficial insects in stored raw whole grains and packaged food in warehouses so long as the insects do not become a component of the final food product (Anonymous 1992). Included on that list of tolerated beneficial organisms is the stored products predator *Xylocoris flavipes* (Reuter) (Hemiptera: Anthocoridae) (Subramanyam and Hagstrum 1996).

Biological control of pest species through inundative releases of natural enemies requires an abundant and reliable source of high quality beneficial organisms. One of the major difficulties is mass propagation of prey species to serve as food for the beneficial organisms. A natural rearing system utilizes the predator's natural prey, which has also been reared on one of its natural food sources. The labor costs for rearing plants and herbivores make some biological control programs too expensive to compete with chemical controls or at least too expensive to use on lower value crops. Thus, alternative food sources are often sought at either the plant or herbivore trophic level to make these programs more economically competitive (Van Driesche and Bellows 1996). The development of nutritionally adequate artificial diets devoid of insect components has

helped make biological control programs more feasible (Cohen 1985, Cohen and Urias 1986, Greenberg et al. 1994, Guerra and Martinez 1994, Guerra et al. 1994, Legaspi et al. 1994, Ogura and Hosoda 1995, Rojas et al. 1996, Xie et al. 1997, Greany et al. 1998).

The diet of biological control agents can influence their size, vigor, fecundity, sex ratio, and host recognition ability, which can in turn influence their effectiveness. The natural diet of an insect is generally assumed to be the best diet for the production of vigorous individuals with natural behaviors (Cohen 1985). The primary goal of this study was to determine if *X. flavipes* could be mass reared on a meat-based artificial diet and to determine whether the diet adversely affected the natural enemy's performance. This was ascertained by comparing developmental time, survivorship and fecundity of insects reared on the diet, compared to those reared on a natural food source.

Methods and Materials

Insects. *Xylocoris flavipes* used in this study were obtained from a commercial producer of beneficial insects (Biofac Inc., Mathis, TX) and a preexisting colony from the University of Florida at Gainesville. The colonies were combined and fed eggs of the Indianmeal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), ad libitum. The predators were kept at 30 C° and 65% RH with a photoperiod of 16:8 (L:D). All experiments were conducted under the same conditions. *Plodia interpunctella* used for the experiments were reared at Oklahoma State University following established procedures (e.g., Howe 1991). Fresh *P. interpunctella* eggs were harvested daily and frozen for at least 48 h at -20° C to ensure they would not hatch during the experiment.

Artificial Diet. Preliminary studies were conducted with a diet developed by Cohen and Urias (1986) for *Geocoris punctipes* (Say) (Hemiptera: Lygaeidae) presented to *X. flavipes* in Parafilm® sachets. Sachets were made by stretching a 5cm x 5cm piece of Parafilm® to approximately 9cm x 9cm to ensure it was as thin as possible to make penetration of the sachets easier. The diet was then added to the center of the Parafilm® (1-1.5ml) and sealed in the shape of a sausage by pressing the Parafilm® together. The predator would not initiate feeding on this diet so no further assays were conducted. The artificial diet used for the remainder of the study was developed by Greany and Carpenter (1998). The diet recipe and directions can be found in table 2.1. *Xylocoris flavipes* was initially exposed to this diet via the same method as the Cohen and Urias (1986) diet. Because of this diet's success in preliminary assays, a machine that made Parafilm®, dome shaped diet packets was used during the experiment to reduce the time required to make individual packets and to achieve uniformity.

Diet Presentation. The machine used to make the dome shaped food packets was purchased from Analytical Research Systems (ARS) in Gainesville, Florida. The machine consists of a stainless steel box, 10.5 cm x 7cm, with holes 7mm in diameter cut in the top and an adapter on the side to attach a vacuum hose. Vacuum pressure is regulated by another machine also purchased from ARS. A 10cm x 5cm piece of Parafilm® is stretched to maximize thinness and placed over the holes. Vacuum pressure is then applied to the apparatus causing the Parafilm® to collapse into the holes forming the dome shapes. The domes are then filled with the diet using a 50 ml syringe. Wax-coated butcher paper is then placed wax side down over the filled diet domes and heat sealed to the Parafilm® with a brass hot roller purchased from ARS. Care was taken to heat the butcher paper only long enough to seal it to the Parafilm® to avoid cooking the diet. The resulting diet domes are 5-7mm in diameter and were stored frozen for up to 3 wk before being used. These domes were fed to the predator *X. flavipes* in three different feeding regimes.

Experiments. The first regime consisted of predators fed the artificial diet packets from egg hatch until death (ie. during nymph and adult stage, treatment NA). The second was predators fed *P. interpunctella* eggs as nymphs and artificial diet during the adult stage only (treatment A). In the third regime, nymphs were fed the artificial diet until adulthood and then switched to their natural diet of *P. interpunctella* eggs for the remainder of their lives (treatment N). The control treatment was *X. flavipes* reared on *P. interpunctella* eggs throughout their entire lives.

Xylocoris flavipes eggs were gathered from the colony within 24 h of oviposition. Upon egg hatch, first instar nymphs were placed singularly in 5.5 cm Petri dishes with

scratched bottoms to reduce static electricity. Each nymph received one diet packet or an unlimited number of *P. interpunctella* eggs depending upon treatment. Artificial diet packets and *P. interpunctella* eggs were changed every day to ensure nutritional quality. Nymphs were checked daily in order to determine the duration of each instar. Upon completion of nymphal development, bugs were sexed and mating pairs were assigned within treatments according to the date of adult emergence. Female *X. flavipes* that could not be matched with a male within a 24 h period were left unmated. Mating pairs were kept in 5.5cm Petri dishes with a small folded paper supplied for hiding purposes. The male and female remained together for the duration of their lives. Each treatment had no fewer than eight mating pairs in total by the end of experimentation.

The predator's eggs were counted and removed daily in order to determine fecundity and percentage egg hatch. Upon hatching the F_1 progeny were placed in colonies to determine if the predators could be further reared on the different treatment feeding regimes. Nymphs were place in 945ml glass jars and supplied with diet packets or *P. interpunctella* eggs every 3 d according to their treatment requirements. These nymphs from the treatment assays were kept together up to the fourth and fifth generations, approximately 3 mo.

Data Analysis. The developmental time of each instar, total nymphal development time, length of pre-oviposition, oviposition and post-ovipostional periods, fecundity, percentage egg hatch, and duration of adult stage were determined and statistically compared to the control figures using SAS PROC MIXED (SAS Institute 1989). Analysis of nymphal mortality was performed using Chi Squared methods with SAS PROC FREQ (SAS Institute 1989). Net reproductive rate and intrinsic rate of increase

(r) were computed using methods described by Elliott (1989) and then compared using multiple comparison analysis SAS PROC REG (SAS Institute 1989).

Results

When the Cohen and Urias (1986) diet was presented in Parafilm® sachets to *X. flavipes*, the predator disregarded them after minimal probing and would not feed. The predators were closely observed for 20 min and then watched an additional 10 min every hour for 5 hours. The predators were left in the Petri dishes until they starved to death, but feeding was never initiated. The Greany and Carpenter (1998) (Table 3.1) diet was offered in the same Parafilm® sachets in preliminary assays. The predators immediately probed the packets and began feeding.

Greany and Carpenter's artificial diet caused a significant increase in total nymphal development time. The control and treatment A, which were both fed eggs as nymphs, had significantly lower total nymphal developmental times of 13.00 and 12.83 d while the two treatments fed diet as nymphs (N and NA) had nymphal development times of 16.50 and 16.97 d. Individual instars were also examined. Treatments N and NA took a significantly longer time to develop than the control and treatment A for instars two through five, however there was no significant difference in developmental time of the first instar (Table 3.2). The highest nymphal mortality occurred in both treatments fed diet as nymphs, N and NA, and was 29.79 and 18.60% respectively but there was no significant difference between the two. The percentage nymphal mortality for treatment A and the control, both fed *P. interpunctella* eggs throughout immature stages, was 3.85 and 4.55%. Treatments N and NA were significantly higher than the control and treatment A (Table 3.2).

The Greany and Carpenter diet had varying effects on the duration of the adult stage. Male and female predators were analyzed separately, and adult insects that were never

mated were excluded from analysis all together. Male predators fed the diet as nymphs (treatments N and NA) had the shortest adult life spans at 36.31 and 34.31 d. Treatment A males had a mean adult life span of 41.42 d while the control males lived for an average of 45.55 d. Only treatment NA and the control were significantly different from each other. The diet had almost an opposite effect on female predators. The control females' adult life span was the shortest at 22.90 d, while treatment NA had the longest at 47.31d; treatments N and A were intermediate to the others (Table 3.3).

The duration of the predators' complete life broke down in much the same way as the adult stage analysis did. In male predators, the control group lived the longest at an average of 58.40 d, while treatment NA was the shortest at 48.81 d with the two being significantly different. In female predators, the control group lived the shortest length of time at 36.00 d, while treatment NA lived the longest at 61.31 d (Table 3.3).

Adults fed eggs had the shortest pre-oviposition times of 2.65 (control) and 3.00 d (treatment N) and were not significantly different from each other (Table 3.4). Treatments A and NA, with adults fed diet, had longer pre-oviposition periods of 4.08 and 5.00 d respectively and were significantly different from each other as well as from the two other groups. Post-oviposition, defined here as the period of time from the end of oviposition until death, did not differ significantly different across treatments. The artificial diet provided in nymphal or adult stages lengthened the oviposition period of adult females compared to those in the control group (Table 3.4).

The artificial diet had no significant effect on the mean number of eggs laid per female nor on the percentage of those eggs that hatched (Table 3.5). Predators fed the artificial diet as adults (treatments A and NA) laid the highest mean number of eggs per

female at 89.83 and 87.62 followed by the control and treatment N at 81.95 and 70.93. Percentage egg hatch was almost the opposite with treatment N having the highest rate at 82.97% and treatment NA the lowest at 76.30% (Table 3.5).

The diet significantly affected predator egg cannibalism (Table 3.5). Treatment N and the control showed no significant difference, with each mating pair eating only 2.28 and 1.47% of the mean number of eggs laid, respectively. Treatment NA adults ate 11.64% of their eggs per mating pair while treatment A exhibited the highest per cent cannibalism eating an average of 21.63% of their eggs per mating pair (Table 3.5). I observed that cannibalism in this treatment occurred mostly during the first 10 d of adult life, after which the predator acclimated to the diet and cannibalism dropped to a similar rate as the other diet treatments and the control.

Population intrinsic rates of increase and net reproductive rates for *X. flavipes* reared on the different treatments were estimated to determine how much the colony could potentially grow. All treatments reared on the diet (treatments A, N and NA) had significantly lower intrinsic rates of increase than the control ($\alpha = 0.05$) with N being 0.1126, A 0.1335, NA 0.0988, and the control being 0.1571. However, there was no significant difference in net reproductive rate ($\alpha = 0.05$). For each individual female, treatment N increased by 23.92 individuals, treatment A by 42.71, treatment NA by 33.41 and the control by 38.35 individuals. Progeny from the four treatments were maintained on their respective diet regimes for up to 3 mo following the study. At the end of 3 mo, these colonies generated from the F_1 generation nymphs contained the following number of *X. flavipes*: 262 individuals in the treatment N colony, 374 in the NA colony, 497 in the A colony and 709 in the control colony.

Discussion

Xylocoris flavipes successfully completed development on the Greany and Carpenter (1998) diet and resulting females laid viable eggs at the same level as bugs reared on natural diet. However, when nymphs were fed the diet, their mortality and developmental time were significantly increased, which I believe caused a significant decrease in those colonys' intrinsic rate of increase. Treatment NA, in which the insects were fed the diet throughout their entire lives, was of highest concern since it would be the most practical prescription for mass propagation programs. Since there was no significant difference in the net reproductive rate, fecundity or fertility, it could be said that feeding *X. flavipes* the diet did not cause enough reduction in their nutritional requirements to hamper their reproductive performance. However, since treatment NA had lower colony numbers after 3 mo and a significantly lower intrinsic rate of increase, further studies should be conducted to determine how future generations would perform on the diet. Cohen (1985) and Cohen and Urias (1986) have taken their diet to up to 12 generations, testing fecundity over a 2 yr period. The artificial diet utilized here should be tested on successive generations of *X. flavipes* to determine if their reproductive performance would remain constant.

One pronounced affect of the diet was increased egg cannibalism by adult mating pairs, which occurred at the highest levels with adults that had been reared on *P. interpunctella* eggs as nymphs that were then moved to diet as adults (treatment A). Predators in this treatment required some time to adjust to the artificial diet after feeding on eggs as nymphs. Treatment A was the only feeding regime to go from being fed eggs as nymphs to diet as adults, and the time required for them to recognize the diet as food

led to the significantly higher cannibalism because they were eating their own eggs during this time. There was no apparent reverse learning effect when nymphs reared on artificial diet were then switched to *P. interpunctella* eggs as adults. This means that individuals fed artificial diet as nymphs immediately recognized the *P. interpunctella* eggs as food when placed on them during their adult stage. One obstacle when commercially mass rearing predators and parasites on artificial diets is that when later exposed to a natural food source in the field, they do not recognize natural hosts as prey (Sauls et al. 1979, Nordlund and Sauls 1981, Herard et al. 1988). The fact that egg cannibalism was lower for predators switching from artificial diet to eggs does not conclusively prove that these predators would recognized their hosts' cues in a natural setting. It does appear that rearing the predators on this artificial diet had minimal or no affect on predatory ability. Herard et al. (1988) determined that insects reared on an artificial diet could be exposed to their natural hosts immediately before release, in order to ensure that they would recognize their natural prey in the field and that doing so could even significantly increase their searching efficacy. So, if problems arise later and *X. flavipes* does lose the ability to recognize their natural prey, they could potentially be "rescued" as Herard et al. (1988) successfully did in their experiment.

Presentation of artificial diets to predaceous and parasitic insects has been the focus of many studies (Cohen 1981, Cohen 1985, Cohen and Urias 1986, Greenberg et al. 1994, Bratti and Coulibaly 1995, Ogura and Hosoda 1995, Smith and Wilson 1995, Richards and Schmidt 1996, Rojas et al. 1996, Hofstetter and Kenneth 1997, Xie et al. 1997). This research shows that the method of presenting artificial diet in Parafilm® domes is adequate for feeding *X. flavipes*. However, it is possible that the increased

nymphal mortality witnessed in treatments reared on the diet was a result of diet presentation. First instar *X. flavipes* are less than 1.0 mm in length, so it is possible that these nymphs had trouble accessing the diet through the Parafilm®. Further research needs to be conducted to determine if there is an alternative method of presentation that would be more appropriate for these smaller nymphs.

Problems associated with mass propagation of natural enemies have been identified as the critical constraints impeding the commercialization of augmentative biological control programs (King and Morrison 1984, King 1993). Approximately 1.0g of *P. interpunctella* eggs is required to feed 100 *X. flavipes* for one week. Maintaining a large colony of *P. interpunctella* to mass produce bugs is expensive and a cheaper alternative would clearly be advantageous for commercial rearing. The Greany and Carpenter diet is extremely cost effective without denying the insect valuable nutritional requirements. The diet costs approximately $2.50 per batch and one batch will feed about 100 *X. flavipes* for one month. The Greany and Carpenter (1998) diet has also been tested with other predaceous insects such as *Podisus maculaventrus* (Say) (Hemiptera: Pentatomidae) and others are currently being tested. The economic benefits of mass rearing several biological control agents on the same diet could make integrated pest management programs more economically feasible, and able to compete with the cheaper pesticide programs

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Table 3.1. Greany and Carpenter (1998) meat based artificial diet.

Ingredients

GIBCO BRL powdered media (Formula No. 95-053EA/Lot No.65P9055)

1 N Sodium hydroxide (1 N NaOH = 4.01g NaOH to 100ml water)

L-glutamine (Sigma No. G-7029)

Gentamicin (Sigma No. G-1397)

10% Potassium hydroxide

Sodium bicarbonate

Egg yolk (chicken)*

Fatty ground beef (30% fat)**

Beef liver**

* egg yolk needs to be separated out and the membrane around the yolk needs to be removed.

** beef and liver need to be thoroughly blended and strained to remove excess connective tissue.

Directions for Diet Preparation

1. Add 3.29g of powdered media and 0.65g of glutamine to 80ml distilled water. Stir with stirring bar until medium is dissolved.
2. Add 0.030g of sodium bicarbonate and bring up to 85ml with distilled water.
3. Add sodium hydroxide to the medium dropwise until a pH of 6.6 is attained.
4. Add 0.1ml of gentamicin and shake gently making sure all glutamine is dissolved.
5. Weigh out 26g of egg yolk (2 eggs) and place in a blender cup.
6. Blend egg yolk with 28.5g of ground beef until medium appears homogenous.
7. Add 85ml of media solution and 28.5g of pre-ground liver to egg/beef mixture and blend for approximately 1 minute. Take care not to heat the diet while blending.
8. Pour mixture into a large beaker and place on a stir plate and add potassium hydroxide until pH is brought up to 6.8.
9. Strain diet through a kitchen-type strainer to remove particulates.

Table 3.2. Longevity and survivorship of *X. flavipes* immature life stages when fed artificial diet or *P. interpunctella* eggs.

Treatment	Average duration of instars in days (SE) [1]					Total duration of nymphal stage in days (SE) [1]	Mortality during nymphal stage (%) [2]
	1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar		
fed diet as Nymphs Only	4.07a (0.12)	2.35b (0.09)	3.15b (0.23)	3.15b (0.20)	4.78b (0.32)	16.50b (0.38)	29.79b
fed diet as Adults Only	3.69a (0.17)	1.74a (0.08)	1.92a (0.04)	1.98a (0.05)	3.52a (0.08)	12.83a (0.17)	3.85a
fed diet as Nymphs & Adults	3.95a (0.08)	2.10b (0.09)	2.74b (0.14)	3.25b (0.17)	4.91b (0.19)	16.97b (0.30)	18.60b
Control fed *P. interpunctella* eggs	3.76a (0.17)	1.59a (0.09)	1.93a (0.06)	2.05a (0.03)	3.69a (0.09)	13.00a (0.17)	4.55a
	$F = 1.49$	$F = 14.62$	$F = 19.79$	$F = 27.13$	$F = 19.25$	$F = 71.58$	$F = 18.23$
	df = 3, 93.6	df = 3, 169	df = 3, 75.5	df = 3, 58.9	df = 3, 75.4	df = 3, 64.2	df = 3
	$P = 0.2219$	$P = 0.0001$	$P = 0.0001$	$P = 0.0001$	$P = 0.0001$	$P = 0.0001$	$P = 0.0001$

[1] Means in a column followed by different letters are significantly different ($p<0.05$) using Least Significant Difference (LSD) procedures.

[2] Mean in a column were analyzed using Chi-Squared methods and are significantly different ($p<0.05$)

Table 3.3. Longevity of *X. flavipes* fed artificial diet or *P. interpunctella* eggs as nymphs and/or adults.

Treatment	Mean duration of adult stage in days [1]		Mean lifetime longevity from nymph to adult [1]	
	Male	Female	Male	Female
fed diet as Nymphs Only	36.31ab (5.93)	30.73ab (3.79)	52.38ab (5.89)	47.07ab (4.86)
fed diet as Adults Only	41.42ab (2.17)	35.38b (2.99)	54.63ab (2.18)	47.83b (3.11)
fed diet as Nymphs & Adults	34.31a (3.52)	47.31c (4.07)	48.81a (3.48)	61.31c (2.52)
Control fed *P. interpunctella* eggs	45.55b (1.51)	22.90a (3.28)	58.40b (1.57)	36.00a (2.60)
	$F = 3.56$	$F = 7.62$	$F = 2.46$	$F = 16.37$
	df = 3, 33.80	df = 3, 68	df = 3, 33.9	df = 3, 38.9
	$P = 0.0242$	$P = 0.0002$	$P = 0.0002$	$P = 0.0001$

[1] Means in a column followed by different letters are significantly different ($p<0.05$) using Least Significant Difference (LSD) procedures.

Table 3.4. Duration (days) of pre-oviposition, oviposition and post oviposition periods in female *X. flavipes* fed artificial diet or eggs of *P. interpunctella*. [1]

Treatment	Mean duration of pre-oviposition period	Mean duration of oviposition period	Mean duration of post-oviposition period
fed diet as Nymphs Only	3.00a (0.32)	32.38b (4.02)	3.88a (1.43)
fed diet as Adults Only	4.08b (0.17)	29.00b (2.32)	3.33a (1.37)
fed diet as Nymphs & Adults	5.00c (0.24)	35.83b (3.29)	4.33a (1.52)
Control fed *P. interpunctella* eggs	2.65a (0.19)	19.90a (2.55)	2.90a (0.46)
	$F = 22.86$	$F = 5.72$	$F = 0.39$
	df = 3, 59	df = 3, 60	df = 3, 22.1
	$P = 0.0001$	$P = 0.0016$	$P = 0.7625$

[1] Means in a column followed by different letters are significantly different ($p<0.05$) using Least Significant Difference (LSD) procedures.

Table 3.5. Effect of artificial diet on fecundity, egg cannibalism and egg hatch in *X. flavipes*. [1]

Treatment	Mean number of eggs laid per female (SE)	Mean % of eggs cannibalized per mating pair (SE)	Mean % of eggs hatched per female (SE)
fed diet as Nymphs Only	70.93a (13.01)	2.28a (0.74)	82.97a (3.69)
fed diet as Adults Only	89.83a (10.29)	21.63c (2.69)	78.49a (2.13)
fed diet as Nymphs & Adults	87.62a (13.98)	11.64b (3.25)	76.30a (3.01)
Control fed *P. interpunctella* eggs	81.95a (11.27)	1.48a (0.61)	79.45a (2.33)
	$F = 0.47$	$F = 20.46$	$F = 0.69$
	df = 3, 68	df = 3, 17.8	df = 3, 60
	$P = 0.7028$	$P = 0.0001$	$P = 0.5646$

[1] Means in a column followed by different letters are significantly different ($p<0.05$) using Least Significant Difference (LSD) procedures.

VITA

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