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A THESIS PRESENTED TO THE GRADUATE SCHOOL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2010
To my parents, Monty and Lichy; my brothers, Alcides and Joshua; and my family
I would like to acknowledge my advisory committee, my chair Dr. Ronald D. Cave, Dr. Susan Webb, and Mr. Edward Skvarch. I am thankful to Dr. Cave for his continued support, encouragement, and guidance during the course of my studies as a graduate student, and also for providing me financial assistance to participate in state and national meetings. I am also grateful to Dr. Webb and Mr. Skvarch for their critiques and suggestions that made substantial improvements to this study. I would like to thank Dr. Patrick De Clercq at Ghent University in Belgium for providing valuable information to my thesis. I give thanks to José Castillo, Daniel Mancero, Dafne Serrano, and Bradley Smith for their help conducting my field research.

Thanks go out to the Ministry of Economy and Finances of Panama for sponsoring my master’s education in entomology at the University of Florida, and the Florida Specialty Crop Foundation to support my field research. Thanks to all my friends and colleagues at the entomology department in Gainesville, but especially to Veronica Santillan, Diana Castillo, Daniel Carrillo, Andres Sandoval, and Sebastian Padrón for their unconditional friendship. Thanks to Dr. Pasco Avery at IRREC for sharing his knowledge, and providing equipment. I would also like to thank Valerie Quant for letting me collect beetles from her organic farm in Vero Beach.

I am grateful to my best friends, Rodrigo Díaz and Verónica Manrique, for their professional advices regarding my research, but also for always being there for me as a family in Ft. Pierce. I also want to thank Dr. Cave’s family, Vilma, Eloise, and Jonathan, for letting me be part of their special moments during this time. Finally, I would like to give special thanks to my family for their loving encouragement and unconditional support, which motivated me to complete my study.
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The yellowmargined leaf beetle, Microtheca ochroloma Stål (Coleoptera: Chrysomelidae) is a pest of cruciferous crops in the southern United States since its first detection in the country in 1945. Little information is available in the literature about the natural enemies of this pest. Therefore, the goal of this study was to evaluate the efficacy of the predator Podisus maculiventris (Say) (Hemiptera: Pentatomidae) and the fungus Isaria fumosorosea (Brown and Smith) Wize (Hypocreales: Cordycipitaceae) to control populations of M. ochroloma. Preliminary results demonstrated that P. maculiventris preys on all stages of M. ochroloma. The first experiment of this thesis measured the predation rate, fresh weight, and developmental time of P. maculiventris feeding on M. ochroloma larvae at constant temperatures of 10, 15, 20 and 25°C in the laboratory. There was no development of 1st instars or egg hatch at 10°C. The nymphal stage of P. maculiventris preyed on a mean total of 65 ± 6.3 4th instars of M. ochroloma during 99 ± 4.6 days at 15°C, 53 ± 1.3 4th instars of M. ochroloma during 31 ± 0.3 days at 20°C, and 59 4th instars or 741 eggs of M. ochroloma in 23 ± 0.3 and 25 ± 0.3 days, respectively, at 25°C. Adults preyed on a mean total of 5.0 ± 2.1 4th instars of
*M. ochroloma* during 10 days at 15°C, 45.1 ± 2.3 4th instars at 20°C, and 64 ± 2.9 4th instars at 25°C. Adult females weighed more than males at 20°C.

The second experiment of this study was to determine a field release guideline for *P. maculiventris* by measuring its predation potential on *M. ochroloma* in field cages. Four, 10, and 16 1st instar *P. maculiventris* were separately released in cages containing an initial population of 130 1st instars *M. ochroloma* on six turnip plants. The release rate of 16 *P. maculiventris* per six large (≥7 leaves/plant) turnip plants significantly reduced the *M. ochroloma* population over time compared to the other two release rates. For six small (≤6 leaves/plant) turnip plants, a release rate of 10 *P. maculiventris* significantly reduced the *M. ochroloma* population over time compared to the lowest release rate, but it’s pest population suppression capabilities were not significantly different from the higher release rate.

The third experiment of this research was to evaluate the infectivity and lethal time (LT) of *I. fumosorosea* (commercial name: PFR-97™) on *M. ochroloma* at the concentration of 1g of PFR-97™ in 100 ml of distilled water in the laboratory. The larval stage is more susceptible to PFR-97™ than eggs, pupae, and adults. Infectivity rates of 17 and 20% were confirmed in the 1st and 3rd instars of *M. ochroloma*, respectively. The LT_{10} for 1st and 3rd instars of *M. ochroloma* were 4 and 3 days, respectively. Concentrations of 1, 2, 3, and 4 g of PFR-97™ in 100 ml of distilled water were applied to 1st instars of *M. ochroloma* to compare infectivity, LT, and lethal concentrations (LC). Confirmed infectivity rates for 1, 2, 3, and 4 g concentrations were 2, 5, 10, and 27%, respectively. The LT_{10} and LT_{25} for the 4 g concentration were 3.4 and 5.7 days,
respectively. The LC$_{10}$ and LC$_{25}$ were 1.4 g and 5.5 g per 100 ml of distilled water, respectively.

The results of my research suggest that $P. \text{maculiventris}$ is a promising biological control agent of $M. \text{ochroloma}$. This predator can be used in an augmentative biological control program in cruciferous crops to control $M. \text{ochroloma}$ on organic farms. $\text{Isaria fumosorosea}$ (PFR-97$^{\text{TM}}$), on the other hand, does not show any clear evidence of being a potentially effective biological control agent of $M. \text{ochroloma}$. 

PEST MANAGEMENT PROGRAMS ON ORGANIC FARMS ARE BASED ON ECO-FRIENDLY STRATEGIES. THE NATIONAL ORGANIC PROGRAM (NOP) STATES IN THE ELECTRONIC CODE OF FEDERAL REGULATIONS (eCFR) (STANDARDS) THAT “THE PRODUCER MUST USE MANAGEMENT PRACTICES TO PREVENT CROP PESTS, INCLUDING, BUT NOT LIMITED TO: 1) AUGMENTATION OR INTRODUCTION OF PREDATORS OR PARASITES OF THE PEST SPECIES; 2) DEVELOPMENT OF HABITAT FOR NATURAL ENEMIES OF PESTS; 3) NONSYNTHETIC CONTROLS SUCH AS LURES, TRAPS, AND REPELLENTS” (USDA 2010). MY RESEARCH FOCUSED ON THE FIRST AND THIRD OF THESE FEDERAL REGULATIONS TO PREVENT CROP PESTS. THIS WAS ACHIEVED BY EVALUATING THE EFFICACY OF A GENERALIST PREDATOR, PODISUS MACULIVENTRIS (SAY), AND THE INFECTIVITY OF AN ENTOMOPATHOGENIC FUNGUS, ISARIA FUMOSOROSEA WIZE (BROWN AND SMITH), AGAINST AN INVASIVE PEST,
Microtheca ochroloma Stål, commonly called the yellowmargin leaf beetle, in cruciferous crops.

Podisus maculiventris was chosen because it was observed in cruciferous crops feeding on larvae and adults of M. ochroloma, and because it can be used for augmentative biological control (Hough-Goldstein and McPherson 1996; De Clercq et al. 1998). Isaria fumosorosea is known worldwide as a microbial insecticide due to its diversity of infective strains and broad host range (Smith 1993). The commercial strain Apopka 97, registered as PFR-97™ 20% WDG, was chosen for this study because it is local to Florida (Vidal et al. 1998), and because more information about its host range needs to be known.
CHAPTER 2
LITERATURE REVIEW

*Microtheca ochroloma* Stål

Origin and Distribution

The yellowmargined leaf beetle, *Microtheca ochroloma* Stål (Coleoptera: Chrysomelidae), was previously known as *Microtheca punctigera* Achard. Jolivet (1950) revised the genus *Microtheca* and recognized genitalic differences between these two species. *Microtheca ochroloma* is an invasive insect from South America, specifically Argentina, Brazil (Rio de Janeiro and Rio Grande do Sul) (Silva et al. 1968; Racca Filho et al. 1994), Chile, and Uruguay. In the U.S., it was first reported from New Orleans in 1945, occurring in imported grapes coming from Argentina (Chamberlin and Tippins 1948). Since then, this pest has spread to Texas (Balsbaugh 1978), Alabama (Chamberlin and Tippins 1948), Mississippi (Rohwer et al. 1953), North Carolina (Staines 1999), Louisiana (Oliver 1956), Georgia (Guillebeau 2001), and Florida. In 1972, it was reported on watercress at an aquatic nursery in Tampa, Florida (Woodruff 1974).

Biology and Host Range

*Microtheca ochroloma* receives the common name “yellowmargined leaf beetle” because its elytra are brownish to black with yellow margins and four prominent rows of punctures on each elytron. The eggs are yellow to orange, oval, and often laid in the soil (Fig. 2-1A). The larva is yellow to brown, with a sclerotized head capsule (Woodruff 1974) (Fig. 2-1B). The immature stage takes 26.6 d at 20°C to complete its development from egg to adult, and the larval stage usually has four instars. However, laboratory experiments showed that 5% of the population passes through a fifth instar
(Ameen and Story 1997b). When the larvae are ready to pupate, they build a brownish, net-like pupal case (Fig. 2-1C) to surround themselves on dry old leaves (Woodruff 1974). The pupal stage lasts from 5 to 6 d at 20°C (Ameen and Story 1997b). The adult of *M. ochroloma* is about 5 mm long; usually the females are larger than the males (Fig. 2-1D). Newly emerged adults stay on the dry leaves for 2 d before moving to green foliage (Capinera 2001). Adults live to as many as 186 d when fed radish plants (Ameen and Story 1997b).

In Florida, *M. ochroloma* is present in the field during the cool months of October to April, which corresponds to the production season of many of its vegetable hosts (Bowers 2003). The host range of this pest includes all members of the Brassicaceae family, such as cabbage, collards, turnip, radish, and watercress (Chamberlin and Tippins 1948). Laboratory studies showed that turnip is the preferred host, since higher average fecundity (490 eggs per female) was obtained on this host plant compared to other plant species (198 eggs per female) (Ameen and Story 1997a).

**Damage and Summer Activity**

*Microtheca ochroloma* is a serious pest of high value crops such as leafy *Brassica* greens. Larvae and adults feed on the plant foliage and can completely defoliate their host plants (Fig. 2-2A, B). When the beetle populations are very high, feeding on the tubers of turnips can occur (Fig. 2-2C, D). Leaf quality is also affected by the abundant frass produced by the larvae. Bowers (2003) lists three reasons why *M. ochroloma* is a problem in Florida: (1) the host plants thrive in the cool months from October to April, concurring with the growing season for organic farmers in Florida; (2) hard frosts or freezes are unlikely to occur; and (3) there are no known predators or parasites in the U.S.
Although the damage caused by *M. ochroloma* occurs during in the coolest months of the year, Bowers (2003) believes that *M. ochroloma* is present during the summer in a reproductive quiescence, since adults only were found in the same spots in which turnips were planted before, also suggesting that the beetle passes the summer near its feeding sites. To corroborate these observations, some adult beetles were collected from the field in summer and after 24 hours of exposure to laboratory conditions females began laying eggs (Bowers 2003). This indicates that *M. ochroloma* does not require a lengthy period of time to reactivate its reproductive functions compared to other insects that do need several days to end the diapause process.

Diapause is a physiological resting period mediated by environmental factors that are unfavorable to the insect, so that it can survive certain adverse conditions such as extreme temperatures, and is often triggered by photoperiod (Danks 1987). To resume activity, the insect first must complete the diapause development mediated by favorable conditions (Danks 1987). Quiescence is a dormancy that is mediated directly by extreme environmental factors (*e.g.*, scarcity of food, extreme temperatures). The insect can respond to favorable environmental factors and resume normal activities immediately without having to pass through a pre-programmed period as in diapause (Danks 1987).

**Control Methods**

**Chemical control.** Control of *M. ochroloma* has been reported by growers in Texas using products such as carbaryl (Sevin®) and diazinon or mevinphos (Phosdrin®) (Drees 1990). In Brazil, Bastos-Dequech et al. (2008) studied the effect of plant extracts on *M. ochroloma*. One hundred percent larval mortality was achieved after 5 d in the laboratory at 27 ± 1°C by applying pó-de-fumo (*Nicotiana tabacum* L.,
Solanaceae) and ramo de cinamomo (*Melia azedarach* L., Meliaceae). Mortality in adults reached 82 and 74% when DalNeem (a commercial product based on *Azadirachta indica* A. Juss, Meliaceae) and pô-de-fumo were applied, respectively.

Basulu and Fadamiro (2008) evaluated the effect of OMRI biorational insecticides against *M. ochroloma* in the field. PyGanic® (extract of chrysanthemum flowers), Aza-Direct® (azadirachtin from neem plant), and Mycotrol O® [entomopathogenic fungus *Beauveria bassiana* (Balsmo) Vuillemin] were not effective in reducing populations of *M. ochroloma*, but Entrust® (spinosad for organic production) did lower pest densities and mean damage ratings. Similar results were obtained when Overall and Edelson (2007) evaluated organic insecticides in the field. Results indicated that Entrust® and PyGanic® reduced populations of *M. ochroloma* by 96 and 63%, respectively.

**Cultural control.** The need to develop ecological methods to control *M. ochroloma* is evident to organic growers. Currently, there is little information about the ecology of the pest. Bowers (2003) studied emergence and host finding behavior and evaluated intercropping as a tactic for reducing the severity of outbreaks. Results of her studies showed that there were more adult beetles in the interior of the plots than in the border (55 versus 8 beetles, respectively), suggesting that a large number of adults oversummer in the production field. Intercropping failed to be a useful control strategy for this pest. Bowers (2003) found that intercropping host plants (mizuna: *Brassica rapa* L., var. Kyona, Cruciferae) among non-host plants (oak leaf lettuce: *Lactuca sativa* L. var. Berenice, Asteraceae) was not effective in preventing *M. ochroloma* from finding host plants.
Biological control. Currently, there are no natural enemies of *M. ochroloma* reported in the literature (Bowers 2003). However, a generalist predator, the spined soldier bug, *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae), was observed in the field preying on the larvae, adults, and pupae of *M. ochroloma*. In addition, laboratory bioassays showed that larvae of the green lacewing, *Chrysoperla rufilabris* (Burmeister) (Neuroptera: Chrysopidae) prey on eggs and larvae of *M. ochroloma* (personal observation, C. Montemayor).

*Podisus maculiventris* (Say)

Origin and Distribution

The distribution of *P. maculiventris* includes North America from Quebec to British Columbia in Canada and from Florida to Arizona in the U.S.A. (McPherson 1982). In northern Florida, the occurrence of *P. maculiventris* is temporal; adults emerge in April-May and are active in the field throughout the spring and summer until October-November when the overwintering period begins (Herrick and Reitz 2004).

Biology and Host Range

Various aspects of the biology, behavior, and life history of *P. maculiventris* are well documented in the literature (Wiedenmann and O’Neil 1990; O’Neil 1997; Legaspi 2004). Depending on the photoperiod and temperature, the life cycle can vary from 27 to 38 d. Eggs are 1 mm in diameter with long projections around the operculum. The nymphs pass through five instars of which all except the first are predaceous. Cannibalism is common from the second instar to adult. Total developmental time from egg to adult at 27 ± 1°C is 27 d (Richman and Whitcomb 1978). This predator can feed on more than 75 prey species within 8 orders, mainly Coleoptera and Lepidoptera (McPherson 1980), on a wide diversity of agricultural crops. Frequently utilized prey
include the Colorado potato beetle \textit{Stilodes decemlineata} (Say), the asparagus beetle \textit{Crioceris asparagi} L., the three-lined potato beetle \textit{(Lema trilinea} Olivier), the Mexican bean beetle \textit{(Epilachna varivestis} Mulsant), the fall webworm \textit{(Hyphantria cunea} Drury), and the diamondback moth \textit{(Plutella xylostella} Curtis) (Landis 1937).

\textbf{Searching Behavior}

According to O’Neil (1997), the searching strategy of \textit{P. maculiventris} differs between laboratory and field conditions. In the laboratory, the attack rate increases as prey density increases, but the estimated area searched decreases. The decline in the area searched was associated with accumulated handling time of the prey. In the field, \textit{P. maculiventris} maintains a constant low rate of attack even when prey density increases. In a potato field experiment, he found that the estimated area searched declined as Colorado potato beetle density increased, but it can not be attributed to the accumulated handling time of the prey because the predator attacked few prey in the field (<1 prey per day). This searching behavior in the field may permit \textit{P. maculiventris} to survive periods of low prey densities using their stored body lipids and trading off reproduction for survival (Wiedenmann and O’Neil 1990).

\textbf{Biological Control}

\textit{Podisus maculiventris} plays an important role in augmentative and conservation biological control of agricultural pests (Biever and Chauvin 1992; Hough-Goldstein 1996; Tipping et al. 1999). It is commercially available and is commonly used for augmentative releases in the field to control the Mexican bean beetle (O’Neil, 1988; Wiedenmann, 1991), the Colorado potato beetle (Stamopoulos and Chloridis, 1994; Tipping et al. 1999), and the viburnum leaf beetle \textit{[Pyrrhalta viburni} (Paykull)] (Desurmont 2008). The efficiency of \textit{P. maculiventris} as a predator can vary among
host plant species and ecosystems. For example, the presence of allelochemicals in the host plant or in the prey’s diet can directly or indirectly affect the performance of *P. maculiventris* due to its facultative plant-feeding behavior (Desurmont 2008). In addition, the size and shape of the host plant (or leaves) can decrease the efficiency of the predator by providing more leaf surface or more refugia to the prey (Desurmont 2008). The use of generalist native predators in agroecosystems to suppress pest populations may reduce risks to non-target organisms by avoiding the introduction of exotic biological control agents and their non-target impacts in the field. In addition, growers can easily obtain these generalist predators because they are commercially available.

*Isaria fumosorosea Wize* (Brown and Smith)

**Origin and Distribution**

The entomopathogenic fungus *Isaria fumosorosea* Wize (Brown and Smith) (=*Paecilomyces fumosoroseus*) (Hypocreales:Cordycipitaceae) has a worldwide distribution in temperate and tropical zones. Different strains of *I. fumosorosea* are listed in 27 countries in the catalogue of “USDA-ARS Collection of Entomopathogenic Fungal Cultures” (ARSEF) (Humber et al. 2009). According to Domsch et al. (1980), *I. fumosorosea* is well distributed from Europe to Africa and Asia, and occurs naturally in soil in the Netherlands, Germany, Canada, and Brazil. It has been isolated from the surface of dead insects (Greif and Currah 2007). In Florida, it was isolated from *Phenacoccus* sp. (Hemiptera: Pseudococcidae), and named the Apopka 97 strain (Vidal et al. 1998).

*Isaria fumosorosea* was described in 1832 by Fries and later by Wize in 1904. After the study of the genus *Paecilomyces* Samson by Samson (1974), *I. fumosorosea*
was included in a new section called *Paecilomyces* section *Isariodea*, and since then the fungus was named *Paecilomyces fumosorosea* (Wize) Brown & Smith for several years (Zimmerman 2008). However, phylogenetic studies of species belonging to this new section showed that it is polyphylectic within the Hypocreales (Ascomycota), and the *Isaria* clade, which includes *I. fumosorosea*, was elevated to genus rank (Luangsa-ard 2005).

**Biology and Host Range**

Abiotic environmental factors are important in the growth, viability, germination, and insecticidal activity of *I. fumosorosea* (Vidal and Fargues 2007). Vidal et al. (1997b) determined that temperature is important in the optimal growth of *I. fumosorosea*, in which it can range from 20 to 30°C, depending on the isolate. In Europe (French and Italian isolates), the optimal growth of *I. fumosorosea* is from 20 to 25°C, with higher tolerance to lower temperatures from 8 to 15°C. In tropical and subtropical areas (Texas, Florida, Cuba, and California isolates), optimal growth is from 25 to 28°C, with a greater tolerance to higher temperatures from 32 to 35°C. Indian isolates have optimal growth at 30°C, with higher tolerance to higher temperatures compared to the European and tropical and subtropical isolates (Vidal et al. 1997b). Solar radiation and low relative humidity are other abiotic factors than can negatively affect the performance of this fungus in the field (Fargues et al. 1996). Germination of conidia or blastospore growth of mycelia are inhibited by allelochemicals (tomatine, solanine, camptothecin, xanthotoxin, and tannic acid) produced by the host plant (Poprawski and Jones 2001; Lacey and Mercadier 1998).

*Isaria fumosorosea* produces the toxin beauvericin, which is also produced by *B. bassiana* and *Fusarium* species to kill insects. In addition, dipicolinic acid was isolated
from *I. fumosorosea* and confirmed to have an insecticidal effect on third instar *Bemisia tabaci* (Gennadius) type B (Bernardini et al. 1975; Asaff et al. 2005). *Isaria fumosorosea* has a broad host range in the field, including the beetles *S. decemlineata* (Bajan 1973), *Hypothenemus hampei* Ferrari, and *Popillia japonica* Newman (Humber et al. 2009), the moths *Y. maculipennis*, *Galleria mellonella* (L.), and *Spodoptera frugiperda* Smith (Smith 1993), and the flies *Musca domestica* L. (Humber et al. 2009) and *Crossocosmia zebina* (Walker) (Smith 1993).

**Biological Control**

There are several commercial products based on *I. fumosorosea* available in the market against arthropod pests. For example, Multiplex Mycomite® and Priority® are for control of mites; Micobiol® HE is for control of beetles, moths, and nematodes; Pae-Sin® controls whiteflies (Zimmerman 2008); and PFR-97™ 20% WDG® (Apopka strain) controls piercing-sucking insects in greenhouses (Zimmerman 2008).

*Isaria fumosorosea* strains have been used successfully used against whitefly populations in enclosed environments such as greenhouses (Osborne et al. 2008). In field evaluations, the strain CKPF-095 gave effective control of 2nd instar diamondback moth at $1 \times 10^9$ conidia/g (Maketon et al. 2008). In Florida’s citrus groves, Meyer et al. (2008) discovered the strain *Ifr AsCP* infesting adults of Asian citrus psyllid (*Diaphorina citri* Kuwayama). *Ifr AsCP* was compared to PFR-97™ and results showed that *Ifr AsCP* is different from, but related to, PFR-97™. Later, Hoy et al. (2010) determined that *Ifr AsCP* is highly pathogenic to the adult Asian citrus psyllids when the insects were exposed to spores collected from dead psyllids stored at -74°C.
PFR-97™ 20 % WGD is registered in the U.S.A. and manufactured by Certis USA. It is used in greenhouses and nurseries to control aphids, mites, and whiteflies on ornamental plants. However, PFR-97™ has been studied in the laboratory against various pests in food crops in order to be approved for application in the field. The overall goal of my thesis research is to provide organic farmers with environmentally friendly and effective control measures to suppress *M. ochroloma* populations in the field by using a native generalist predators and/or an entomopathogenic fungus.

**Objectives of Master of Science Thesis Research**

1. Measure the development time and predation rate of *Podisus maculiventris* when feeding on *Microtheca ochroloma* in the laboratory

2. Evaluate predation potential of *Podisus maculiventris* on larvae of *Microtheca ochroloma* in the field

3. Assess the infectivity by *Isaria fumosorosea* on *Microtheca ochroloma* in the laboratory
Figure 2-1. Life stages of *Microtheca ochroloma*. A) Eggs, B) Larva, C) Pupa, and D) Adults.
Figure 2-2. Damage by *Microtheca ochroloma*. A), Larvae feeding on leaves, B) Total defoliation of the turnip plant, C) Severe root damage by the larvae, D) Larvae feeding on the turnip’s roots.
CHAPTER 3
DEVELOPMENT TIME AND PREDATION RATE OF PODISUS MACULIVENTRIS (SAY) (HEMIPTERA: PENTATOMIDAE) PREYING ON MICROTHECA OCHROLOMA STÅL (COLEOPTERA: CHRYSMELIDAE)

Introduction

The yellowmargined leaf beetle, Microtheca ochroloma Stål, is a pest in crucifer crops in the southern United States. It is native to Argentina, Uruguay and Brazil (Chamberlin and Tippins 1948; Dos Anjos et al. 2007). Its preferred host is turnip (Brassica rapa L.), followed by mustard (Brassica juncea Cosson) (Ameen and Story 1997a). The main damage caused by M. ochroloma is defoliation, but when infestations are severe and leaves are entirely consumed, the beetle will feed on the roots (C. Montemayor, personal observation). There are no specialist natural enemies reported in the literature that can contribute to the control of this pest on organic farms (Fasulo 2005). Nevertheless, there are generalist predators present in agricultural ecosystems that may contribute to the management of M. ochroloma.

The spined soldier bug, Podisus maculiventris (Say) (Hemiptera: Pentatomidae: Asopinae), is a generalist predator present in many agroecosystems, where it preys primarily on Coleoptera and Lepidoptera larvae (McPherson 1980). In laboratory studies, nymphs and adults of P. maculiventris showed high potential predation rates against different life stages of the beet armyworm, Spodoptera exigua (Hübner) (De Clercq and Degheele 1994). On organic farms, P. maculiventris has been observed on crucifer crops, preying on all stages of M. ochroloma. The use of P. maculiventris in augmentative biological control may serve as a tool to reduce populations of M. ochroloma. However, no studies have been conducted to evaluate the efficacy of this predator as a biological control agent of this pest. Therefore, the objective of this study
was to know if *M. ochroloma* would be a suitable prey by quantifying the rate of predation of *M. ochroloma* larvae by *P. maculiventris* and the developmental time of the predator at four constant temperatures under laboratory conditions.

**Materials and Methods**

**Stock Colonies.** Adults and larvae of *M. ochroloma* were brought from White Rabbit Acres certified organic farm located in Vero Beach, FL to the laboratory at the Biological Control Research and Containment Laboratory (BCRCL) at the Indian River Research and Educational Center (IRREC) in Ft. Pierce. The colony was established and maintained in plastic boxes (27 × 15 × 8 cm, Ziploc®) with screen mesh openings in the walls for ventilation. Boxes were kept in an environmentally controlled chamber at 25°C, 50% RH, and 10L:14D photoperiod.

A bottle of 250 eggs of *P. maculiventris* was purchased online from Rincon-Vitova Insectaries, Inc. (Ventura, CA [www.rinconvitova.com]) and shipped with overnight delivery. After arrival, eggs were divided into groups of 30 and each group was placed in a Petri dish (60 × 75 mm, Fisherbrand®) with moistened filter paper (55 mm Ø [diameter], Whatman®). The Petri dishes were sealed with Parafilm® and stored in an environmentally controlled chamber at 25°C, 50% RH, and 10L:14D photoperiod.

**Plant Material.** Turnip Seven Top (Greens) (*Brassica rapa* L. var. *rapifera*) seeds were seeded in 72-hole trays containing sterilized soil mix (Fafard® germination mix, Agawam, MA) inside a greenhouse. Seedlings were transplanted 2 weeks later into 3.8 L plastic pots containing soil mixture (Fafard® #3B mix). The plants were fertilized weekly with 400 ml per pot of liquid fertilizer (Miracle Grow® 24N-8P-16K).

**Experimental Design.** An individual neonate *P. maculiventris* was housed in a 7-cm³ plastic box (Fig. 1) with a hole in the top sealed with screen mesh. To maintain
proper humidity in the box, a piece of white filter paper (90 mm Ø, Whatman®) moistened with water was placed on the bottom of the cage. Each box held a 60 mm Ø piece of turnip leaf that was replaced daily. The number of 4th instar *M. ochroloma* provided daily to *P. maculiventris* varied among instars: five prey were provided to 2nd and 3rd instar *P. maculiventris* and 10 prey were provided to 4th and 5th instar *P. maculiventris*. The boxes with insects were held in environmentally controlled chambers at each of four constant temperatures, 10, 15, 20, and 25°C, with 50% RH and 10L:14D photoperiod. HOBO® data loggers were placed inside each chamber to monitor the temperature and humidity. Each treatment had at least 12 replicates.

Number of *M. ochroloma* larvae killed daily by each predator nymph was recorded. Total predation per instar was thus determined and the total predation per nymph was measured as the total predation through all instars. Total development time for each predator instar and for the total nymphal stage was measured in days. Predation by the adult stage of *P. maculiventris* was evaluated for the first 10 d of adult life. Fresh weights of newly ecdysed nymphs and adults were measured using an Ohaus Adventurer AR2140 analytical scale. A control treatment with three replicates consisted of five to ten 4th instar *M. ochroloma* (number varied gradually with *P. maculiventris* development in other treatments) in the absence of *P. maculiventris* to record natural mortality. Dead larvae in the control were replaced daily. Mortality in the control treatment was used as a correction factor for the mortality in the predator treatment.

Development time and daily and total predation of eggs of *M. ochroloma* by 2nd to 5th instar *P. maculiventris* were measured in Petri dishes at 25°C. Each Petri dish (60 × 75 mm, Fisherbrand®) contained one predator, eggs of *M. ochroloma*, and a white
moistened filter paper. The number of eggs of *M. ochroloma* provided daily to *P. maculiventris* varied among instars: 50 eggs were provided to 2nd, 100 eggs to 3rd instar, 200 eggs to 4th instar, and 500 eggs to 5th instar *P. maculiventris*. Turnip leaves were not provided.

**Statistical Analysis.** Data were analyzed using analysis of variance (ANOVA). Treatment means were separated using the Student–Newman–Keuls (SNK) test (SAS Institute, 1999) at a significance level of 5%. Means are reported with their standard error.

**Results**

Two-way ANOVA indicated a significant interaction between temperature and instar of *P. maculiventris* for all variables evaluated (developmental time, fresh weight, daily and total predation) (*F* = 25.03; df = 17,181; *P* < 0.0001 for developmental time; *F* = 12.49; df = 14,150; *P* < 0.0001 for fresh weight; *F* = 39.72; df = 14,128 *P* < 0.0001 for daily predation; *F* = 53.95; df = 14,128; *P* < 0.0001 for total predation). At 10°C, eggs did not hatch. First instar *P. maculiventris* reared at this same temperature died about 26 ± 2.9 d after hatching at 25°C, therefore no data was obtained to measure developmental time at 10°C. The 1st instar of *P. maculiventris* is a non-feeding stage, thus no daily and total predation rates were determined for this stadium.

**Development Time.** Mean development times of *P. maculiventris* eggs and instars varied with temperature (Table 3-1). Mean developmental times of eggs, 1st, 2nd, 3rd, 4th, and 5th instars reared at 15°C were significantly higher than those at 20 and 25°C (*F* = 6170.37; df = 2,31; *P* < 0.0001 for egg; *F* = 503.16; df = 2,42; *P* < 0.0001 for 1st instars; *F* = 131.88; df = 2,30; *P* <0.0001 for 2nd instars; *F* = 315.93; df = 2,27; *P* <0.0001 for 3rd instars; *F* = 57.10; df = 2,26; *P* = < 0.0001 for 4th instars; *F* = 926.75; df =
Mean development times of eggs, 1<sup>st</sup> instars, and 5<sup>th</sup> instars at 20°C were significantly higher than those at 25°C; there were no differences between these two temperatures for the 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> instars. Mean total development times of nymphs varied significantly with temperature ($F = 814.5; \text{df} = 2,25; P < 0.0001$) (Table 3-5); the development time at 25°C was significantly less than the development time at 20°C, which was significantly lower than the development time at 15°C.

There were significant differences in development time among instars within each temperature (Table 3-1). At all experimental temperatures the mean development time of the 5<sup>th</sup> instar was significantly higher than the mean development times of the other four instars. At 15°C, mean development times of eggs, 2<sup>nd</sup> instars, 3<sup>rd</sup> instars, and 4<sup>th</sup> instars were not significantly different from each other, but they were significantly higher than that of the 1<sup>st</sup> instar ($F = 30.12; \text{df} = 5,49; P < 0.0001$). At 20 and 25°C, mean development time of eggs was significantly higher than those of 2<sup>nd</sup> and 4<sup>th</sup> instars, which were significantly higher than mean development times of 1<sup>st</sup> and 3<sup>rd</sup> instars ($F = 99.02; \text{df} = 5,66; P < 0.0001$ for 20°C; $F = 57.34; \text{df} = 5,66; P < 0.0001$ for 25°C).

Mean development times of 2<sup>nd</sup> to 5<sup>th</sup> instar <i>P. maculiventris</i> feeding on <i>M. ochroloma</i> eggs were 3.9 ± 0.1 d, 3.8 ± 0.1 d, 4.0 ± 0.0 d, and 5.7 ± 0.2 d, respectively. These means were not significantly different from those of nymphs feeding on <i>M. ochroloma</i> larvae at 25°C (Table 3-1) ($P = 0.1325$). However, the total development time of the nymphal stage of the predator feeding on 4<sup>th</sup> instars of <i>M. ochroloma</i> was significantly shorter (23 ± 0.3 d) than when feeding on eggs (25 ± 0.3 d) ($F = 36.51; \text{df} = 1,20; P < 0.001$).
**Fresh Weight.** Mean fresh weights of some instars varied significantly with temperature (Table 3-2). Mean fresh weights of 3rd and 5th instars reared at 25°C were significantly higher than those at 20°C, which were significantly higher compared to those at 15°C ($F = 14.23; \text{df} = 2,29; P < 0.0001$ for 3rd instars; $F = 19.29; \text{df} = 2,26; P < 0.0001$ for 5th instars). There were no significant differences in mean fresh weight of 4th instars and adults between 20 and 25°C, but their mean fresh weights at these two temperatures were significantly higher compared to those at 15°C ($F = 11.66; \text{df} = 2,27; P = 0.0002$). There were no significant differences in mean fresh weight of 2nd instars among the three temperatures. As with development time, there were significant differences in mean fresh weights among stages within each temperature (Table 3-2). Mean fresh weight at all experimental temperatures increased significantly with each successive stage.

There was no significant interaction between temperature and sex for fresh weight of *P. maculiventris* adults. Mean fresh weights of adult females and males were not significantly different at 25°C. However, at 20°C, the mean fresh weight of females was significantly higher than the mean fresh weight of males (Table 3-6) ($F = 16.76, \text{df} = 1,10; P = 0.0022$). Mean fresh weights of females and males reared at 15°C were not statistically compared due to low sample sizes, yet the female adult weight tended to be higher than male adult weight.

**Daily Predation.** There were no significant differences in mean daily predation by the 2nd and 3rd instars between 20 and 25°C (Table 3-3). However, mean daily predation by these instars at those two temperatures were significantly higher than mean daily predation at 15°C. Mean daily predation by 4th instars, 5th instars, and adults...
was significantly higher at 25°C than that at 20°C, and mean daily predation was significantly higher at 20°C than at 15°C ($F = 36.82$; df = 2,26; $P < 0.0001$ for 4th instars; $F = 78.92$; df = 2,25; $P < 0.0001$ for 5th instars; $F = 79.03$; df = 2,19; $P < 0.0001$ for adults).

Mean daily predation also varied significantly among stages at each temperature (Table 3-3). At 15°C, the adult stage had significantly lower mean daily predation compared to that of the 5th instar, however, it was not significantly different from the mean daily predation by the 2nd, 3rd, and 4th instars ($F = 3.00$; df = 4,24; $P = 0.0387$). At 20°C, mean daily predation increased significantly with each successive stage ($F = 123.00$; df = 5,66; $P < 0.0001$). At 25°C, there was no significant difference in mean daily predation between the 5th instar and adult ($F = 186.99$; df = 5,60; $P < 0.0001$). However, their rates were significantly higher than predation by the 4th instar, which was higher than that by the 3rd instar. Second instar mean daily predation was significantly the lowest among all stages at 25°C. Mean daily predation of eggs of *M. ochroloma* by *P. maculiventris* nymphs increased significantly with each successive instar ($F = 159.14$; df = 3,36; $P < 0.0001$) (Table 3-7). Embryonic fluids within the eggs were totally consumed by the predator.

**Total predation.** Mean total number of *M. ochroloma* larvae consumed by each *P. maculiventris* stage also varied significantly among temperatures (Table 3-4). There was significantly higher mean total predation by the 2nd instar at 15°C compared to 20 and 25°C ($F = 50.80$; df = 3,31; $P < 0.0001$). Mean total predation by the 3rd instar was significantly higher at 25°C than at 15 and 20°C ($F = 6.64$; df = 2,27; $P = 0.0045$). Mean total predation by the adults was significantly higher at 25°C than at 20°C which was
higher compared to predation at 15°C \((F = 79.03; \text{df} = 2,19; \ P < 0.001\) ). On the other hand, there were no significant differences in mean total predation by 4\textsuperscript{th} and 5\textsuperscript{th} instars at 15, 20, and 25°C. Mean total predation of prey by the nympha stage varied significantly with temperature \((F = 4.72; \text{df} = 2,25; \ P = 0.0182\) ) (Table 3-5); predation at 20°C was significantly less than predation at 15°C, but predation at 25°C was not significantly different from predation at the lower two temperatures.

Similar to mean daily predation, mean total predation by \textit{P. maculiventris} nymphs varied significantly among stages at each temperature (Table 3-4). Mean total predation by 5\textsuperscript{th} instars at 15°C was significantly higher than predation by 4\textsuperscript{th} instars, which was significantly higher than that by the adult stage; predation by the latter did not vary significantly from predation by the 2\textsuperscript{nd} and 3\textsuperscript{rd} instars \((F = 10.88; \text{df} = 4,24; \ P < 0.0001\) ). Mean total predation at 20°C was significantly higher by the adult stage compared to the 5\textsuperscript{th} instar, which was higher than the 4\textsuperscript{th} and 3\textsuperscript{rd} instars, and these consumed significantly more prey than the 2\textsuperscript{nd} instar \((F = 210.30; \text{df} = 5,66; \ P < 0.0001\) ). At 25°C, mean total predation was significantly higher by the adult stage than by the 5\textsuperscript{th} instar, which was higher than predation by the 4\textsuperscript{th} instar; the latter was significantly higher compared to the 2\textsuperscript{nd} and 3\textsuperscript{rd} instars \((F = 349.78; \text{df} = 5,60, \ P < 0.0001\) ). Mean total predation of eggs of \textit{M. ochroloama} by \textit{P. maculiventris} nymphs increased significantly with each successive instar \((F = 250.29; \text{df} = 3,36; \ P < 0.0001\) ) (Table 3-7). Total consumption of eggs of \textit{M. ochroloama} during the nympha stage of \textit{P. maculiventris} was 741.0 ± 24.8.

\textbf{Discussion}

The 7-cm\(^3\) plastic boxes used as cages provided more space for \textit{P. maculiventris} nymphs and adults to seek and attack the larvae of \textit{M. ochroloama} in a more natural way
compared to Petri dishes. According to Wiedenmann and O'Neil (1991), attacks rates in Petri dishes will be limited by handling time, but in the field or more complex arenas they will be limited by search behavior. The *M. ochroloma* larvae that were attacked in my study were most often killed, then either abandoned unconsumed or partially consumed. De Clercq and Degheele (1994) reported similar observations when nymphs preyed on 5th instars of the beet armyworm.

Neither development of 1st instars nor egg hatching of *P. maculiventris* was successfully completed at 10°C. These findings match with the estimated lower developmental threshold for eggs 10.7°C and nymphs 11.7°C of *P. maculiventris*, as determined by De Clercq and Degheele (1992). There was a well defined inverse interaction between developmental time and temperature. Developmental time of all stages of *P. maculiventris* is longer at 15°C compared to higher temperatures (Table 3-1). The fifth instar of *P. maculiventris* requires more time to develop than earlier instars, regardless temperature (Table 3-1). More time in the 5th instar allows the daily and total predation rates of the predator to be greater compared to those of the earlier instars (Tables 3-3 and 3-4). Development times of nymphs reared at 20 and 25°C and fed 4th instar *M. ochroloma* are comparable to development times of nymphs reared on beet armyworm larvae at 23°C (De Clercq and Degheele 1994). Mahdian et al. (2006) reported similar trends of consumption rate and developmental time of *P. maculiventris* feeding on 4th instar *Spodoptera littoralis* (Boisdunval) at 18, 23, and 27°C. Desurmont and Weston (2008) found that nymphs of *P. maculiventris* consumed an average of 101 larvae and 17 adults of *Pyrrhalta viburni* (Paykull) (Coleoptera: Chrysomelidae) and the
total developmental time from 2nd instar to adult was 22 d at 22°C, which is very similar to the total developmental time (21 d) at 20°C in my study.

*Podisus maculiventris* is able to complete its life cycle by feeding on eggs only of *M. ochroloma*. This suggests that the egg of *M. ochroloma* is an adequate food source as is the 4th instar, and probably, too, the adult and pupa of the beetle since *P. maculiventris* also feeds on them (C. Montemayor, personal observation). It is yet unclear whether or not nymphs will search for eggs on dry leaves and in the soil; however, if they do, the population of *M. ochroloma* could be significantly reduced due to the predator’s high predation of eggs (Table 3-6). Two other studies have reported lower mean total consumption of other prey eggs by the nymphal stage of *P. maculiventris*: 293 eggs of *Stilodes decemlineata* Say (Coleoptera: Chrysomelidae) (Gusev et al. 1983) and 160 eggs of *Epilachna varivestis* (Mulsant) (Coleoptera: Coccinellidae) (Waddill and Shepard 1975). However, De Clercq and Degheele (1994) reported total nymphal consumption of 1,200 eggs of *S. exigua*.

Regarding to the weight measured of *P. maculiventris* in this study. The mean fresh weight of newly ecdysed 5th instar *P. maculiventris* fed on 4th instar *M. ochroloma* at 25°C is 30.98 mg. This weight was comparable to the 27.3 mg in the study of De Clercq and Degheele (1994), who fed the predator 4th instar *S. exigua* at 23°C.

The numbers of *M. ochroloma* larvae killed by nymphs and adults suggest that *P. maculiventris* has great potential to reduce populations of *M. ochroloma* under field conditions in Florida, in which the natural temporal occurrence of the predator (spring-summer) matches well with outbreaks of the pest in late spring (Herrick and Reitz 2004). In order to enhance the control of *M. ochroloma* in the field, augmentative
releases of the predator can be made in the late fall and early spring. Therefore, it is essential to evaluate its predation potential under field conditions (see Chapter 4).

Figure 3-1. Plastic boxes with screened windows used to house experimental insects.
Table 3-1. Mean (± SE) development time of *Podisus maculiventris* reared at three constant temperatures with 4th instar *Microtheca ochroloma* as prey. Number within parentheses equals sample size.

<table>
<thead>
<tr>
<th>Temp* (°C)</th>
<th>Mean development time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Egg</td>
</tr>
<tr>
<td>15</td>
<td>17.0 ± 0.0 (10) Ba</td>
</tr>
<tr>
<td>20</td>
<td>5.7 ± 0.1 (12) Bb</td>
</tr>
<tr>
<td>25</td>
<td>4.0 ± 0.0 (12) Bc</td>
</tr>
</tbody>
</table>

Means followed by the same uppercase letter within a row are not significantly different (*P* > 0.05). Means followed by the same lowercase letter within a column are not significantly different (*P* > 0.05). (*) Temperature.

Table 3-2. Mean (± SE) fresh weight of newly ecdysed *Podisus maculiventris* reared at three constant temperatures with 4th instar *Microtheca ochroloma* as prey. Number within parentheses equals sample size.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Mean fresh weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2nd instar</td>
</tr>
<tr>
<td>15</td>
<td>0.77 ± 0.0 (21) Da</td>
</tr>
<tr>
<td>20</td>
<td>0.84 ± 0.0 (12) Da</td>
</tr>
<tr>
<td>25</td>
<td>0.82 ± 0.0 (12) Da</td>
</tr>
</tbody>
</table>

Means followed by the same uppercase letter within a row are not significantly different (*P* > 0.05). Means followed by the same lowercase letter within a column are not significantly different (*P* > 0.05).
Table 3-3. Mean (± SE) daily predation of 4th instar *Microtheca ochroloma* by *Podisus maculiventris* reared at three temperatures. Number within parentheses equals sample size.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; instar</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; instar</th>
<th>4&lt;sup&gt;th&lt;/sup&gt; instar</th>
<th>5&lt;sup&gt;th&lt;/sup&gt; instar</th>
<th>Adult (10 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>0.7 ± 0.1 (10) ABb</td>
<td>0.7 ± 0.1 (6) ABb</td>
<td>0.8 ± 0.1 (5) ABc</td>
<td>1.2 ± 0.2 (4) Ac</td>
<td>0.5 ± 0.2 (4) Bc</td>
</tr>
<tr>
<td>20</td>
<td>1.1 ± 0.1 (12) Da</td>
<td>2.3 ± 0.2 (12) Ca</td>
<td>2.5 ± 0.2 (12) Cb</td>
<td>3.4 ± 0.1 (12) Bb</td>
<td>4.5 ± 0.2 (12) Ab</td>
</tr>
<tr>
<td>25</td>
<td>1.0 ± 0.1 (12) Da</td>
<td>1.9 ± 0.1 (12) Ca</td>
<td>3.4 ± 0.2 (12) Ba</td>
<td>6.5 ± 0.3 (12) Aa</td>
<td>6.4 ± 0.3 (6) Aa</td>
</tr>
</tbody>
</table>

Means followed by the same uppercase letter within a row are not significantly different (*P* > 0.05). Means followed by the same lowercase letter within a column are not significantly different (*P* > 0.05).

Table 3-4. Mean (± SE) total predation of 4th instar *Microtheca ochroloma* by *Podisus maculiventris* reared at three constant temperatures. Number within parentheses equals sample size.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; instar</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; instar</th>
<th>4&lt;sup&gt;th&lt;/sup&gt; instar</th>
<th>5&lt;sup&gt;th&lt;/sup&gt; instar</th>
<th>Adult (10 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>12.9 ± 1.1 (10) CBa</td>
<td>11.0 ± 1.8 (6) CBa</td>
<td>14.8 ± 2.4 (5) Ba</td>
<td>30.0 ± 5.8 (4) Aa</td>
<td>5.0 ± 2.1 Cc</td>
</tr>
<tr>
<td>20</td>
<td>4.9 ± 0.5 (12) Db</td>
<td>9.0 ± 0.7 (12) Ca</td>
<td>12.0 ± 0.9 (12) Ca</td>
<td>27.2 ± 1.0 (12) Ba</td>
<td>45.1 ± 2.3 (12) Ab</td>
</tr>
<tr>
<td>25</td>
<td>3.8 ± 0.2 (12) Db</td>
<td>6.5 ± 0.4 (12) Db</td>
<td>13.2 ± 0.7 (12) Ca</td>
<td>35.6 ± 2.0 (12) Ba</td>
<td>64.1 ± 2.9 (6) Aa</td>
</tr>
</tbody>
</table>

Means followed by the same uppercase letter within a row are not significantly different (*P* > 0.05). Means followed by the same lowercase letter within a column are not significantly different (*P* > 0.05).
Table 3-5. Mean (± SE) total development time and predation of 4th instar *Microtheca ochroloma* per nymph of *Podisus maculiventris*. Number within parentheses equals sample size.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Total development (days)</th>
<th>Total predation (prey)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>99 ± 4.6 (4) a</td>
<td>65 ± 6.3 (4) a</td>
</tr>
<tr>
<td>20</td>
<td>31 ± 0.3 (12) b</td>
<td>53 ± 1.3 (12) b</td>
</tr>
<tr>
<td>25</td>
<td>23 ± 0.3 (12) c</td>
<td>59 ± 2.1 (12) ab</td>
</tr>
</tbody>
</table>

Means followed by the same lowercase letter within a column are not significantly different (*P* > 0.05).

Table 3-6. Mean (± SE) fresh weight of newly ecdysed adults of *Podisus maculiventris* reared with 4th instar *Microtheca ochroloma* as a prey. Number within parentheses equals sample size.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Female Mean fresh weight (mg)</th>
<th>Male Mean fresh weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>43.00 ± 2.4 (2)</td>
<td>39.55 ± 2.0 (2)</td>
</tr>
<tr>
<td>20</td>
<td>63.50 ± 1.2 (8) a</td>
<td>50.45 ± 4.0 (4) b</td>
</tr>
<tr>
<td>25</td>
<td>61.98 ± 2.9 (6) a</td>
<td>54.00 ± 2.3 (6) a</td>
</tr>
</tbody>
</table>

Means followed by the same lowercase letter within a row are not significantly different (*P* > 0.05).
Table 3-7. Mean (± SE) predation of eggs of *Microtheca ochroloma* by *Podisus maculiventris* nymphs reared at 25°C. Number within parentheses equals sample size.

<table>
<thead>
<tr>
<th>Predation</th>
<th>2(^\text{nd}) instar</th>
<th>3(^\text{rd}) instar</th>
<th>4(^\text{th}) instar</th>
<th>5(^\text{th}) instar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily</td>
<td>6.3 ± 0.6 (10) d</td>
<td>18.2 ± 1.0 (10) c</td>
<td>50.2 ± 3.1 (10) b</td>
<td>83.5 ± 4.4 (10) a</td>
</tr>
<tr>
<td>Total</td>
<td>24.2 ± 2.1 (10) d</td>
<td>68.3 ± 3.6 (10) c</td>
<td>187.7 ± 12.6 (10) b</td>
<td>460.7 ± 13.8 (10) a</td>
</tr>
</tbody>
</table>

Means followed by the same lowercase letter within a row are not significantly different ($P > 0.05$).
CHAPTER 4
PREDATION POTENTIAL OF *PODISUS MACULIVENTRIS* (HEMIPTERA: PENTATOMIDAE) ON *MICROTHECA OCHROLOMA* (COLEOPTERA: CHRYSONELIDAE) IN THE FIELD

**Introduction**

The yellowmargined leaf beetle, *Microtheca ochroloma* Stål, is a cool seasonal pest in crucifer (Brassicaceae) crops, with turnips (*Brassica rapa* L.) and mustard (*Brassica juncea* Cosson) its preferred host (Amen and Story 1997a). In 1945, the first specimen to be found in the United States was reported in New Orleans on grapes coming from Argentina where it may also be a serious problem (Chamberlin and Tippins 1948). Since then, *M. ochroloma* has spread to and established in several southern states in the United States (Staines 1999), including Florida where the beetle was found on watercress in 1972 (Woodruff 1974). In Florida, *M. ochroloma* is present in the field during the coolest months (October through April), which corresponds with the primary production season for leafy greens (Bowers 2003).

For organic crucifer growers, it has been a challenge to maintain populations of *M. ochroloma* under tolerable levels, given that growers are restricted to using insecticides on the Organic Materials Review Institute (OMRI) list, and no known native specialist natural enemies have been reported (Bowers 2003). The manipulation of generalist predator populations to enhance control of *M. ochroloma* should be considered in developing an integrated pest management program for this pest.

Predatory stink bugs (Hemiptera: Pentatomidae: Asopinae) are commonly used in augmentative releases to control pests in agricultural ecosystems (Biever and Chauvin 1992; Hough-Goldstein 1996; Tipping et al. 1999). The spined soldier bug, *Podisus maculiventris* (Say), is a generalist predator native to North America (McPherson 1982).
Coleoptera and Lepidoptera larvae are its main prey (McPherson 1980). This generalist predator has been released in the field and in greenhouses to control pests in tomato and cotton with successful results (Lopez et al. 1976; Ables and McCommas 1982; De Clercq et al. 1998). Hough-Goldstein and McPherson (1996) reported that augmentative releases of *P. maculiventris* in small field plots reduced the larval population of the Colorado potato beetle, *Stilodes decemlineata* (Say). The study of predation rates in field-cage experiments demonstrated that the 5th instar of *P. maculiventris* could kill an average number of 15 3rd instar *S. decemlineata* (Stamopoulos and Chloridis 1994).

In central Florida, *P. maculiventris* has been observed preying on *M. ochroloma* (C. Montemayor, personal observation). However, little is known about its predation potential on field populations of *M. ochroloma*. In northern Florida, *P. maculiventris* is present beginning in March and starts overwintering in October (Herrick and Reitz 2004). This period of time permits natural populations of *P. maculiventris* to interact with the pest for the last couple of months of late spring in the crucifer growing season, since *M. ochroloma* is a problem during the cooler months of the year in Florida. Early augmentative releases of *P. maculiventris* in cruciferous crops in the field may contribute to control of *M. ochroloma*.

The present study evaluates the predation capacity of *P. maculiventris* at different densities in field cages containing *M. ochroloma*. The goal of this study is to provide growers a guideline for releasing *P. maculiventris*. This way the predator can be used as a new integrated pest management tool to control *M. ochroloma*. 

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Materials and Methods

Stock Colonies. Adults and larvae of *M. ochroloma* were obtained from the field and a laboratory colony was established and maintained as described in Chapter 2. The releases of *P. maculiventris* were designed to simulate as much as possible the way that growers would release the predator based on the recommendations of the vendor. Therefore, eggs of *P. maculiventris* were purchased and held in the laboratory as described in Chapter 2. When the first 100 1st instars emerged, they were held for 5 d at 15°C and then released into the field cages.

Plant Material. Small plants of Seven Top (Greens) variety turnips (*B. rapa* var. *rapifera*) were grown as described in Chapter 2. Three weeks after transplanting in pots, seedlings were transplanted into field beds at the IRREC. Four beds, each 100 m long and 0.70 m wide, were covered by white plastic mulch. Granulate fertilizer (8N-12P-20K with minor elements; Howard Fertilizer Co., Inc., Orlando, FL) was placed in the center of the beds at a rate of 85 g/m. Distance between plants was 30 cm, distance between rows was 30 cm, and distance between beds was 90 cm. Irrigation was provided by flooding between beds.

Experimental Design. Cage frames 1.35 m long, 0.90 m wide, and 0.70 m high were constructed of 1.904 cm (3/4 inch) PVC (polyvinyl chloride) tubing. Fine mesh cloth was sewn in a manner to fit tightly over each frame; around the base of the cloth cage was a 15-cm skirt. Each cage was placed over a field bed with six turnip plants. Soil was heaped on the skirt to avoid the entrance or exit of animals and anchor the cage in place. One hundred thirty-two 1st instars of *M. ochroloma* were introduced into each cage (22 larvae per plant). In 2009, the insects were introduced into the cage 20 d after the turnip plants were transplanted into the field; in 2010, the insects were
introduced into the cage 10 d after transplanting. On the same day as the introduction of *M. ochroloma* into the cages, one of three densities (4 = low, 10 = medium, or 16 = high) of 1st instar *P. maculiventris* was released evenly among the six plants in a cage. Each treatment (*P. maculiventris* density) was replicated four times. Rincon-Vitova Insectaries recommends releasing 25 eggs per “hot-spot” or 25 eggs per 10 m² for caterpillar control. In other words, Rincon-Vitova’s release recommendation for my study would be three 1st instars per 1.21 m² (area of the cage). However, by comparison, the actual release rates in my study were 33 1st instars in the low predator density treatment, 83 1st instars in the medium predator density treatment, and 132 1st instars in the high predator density treatment per 10 m².

Treatment arrangement was randomized within each replicate block (Fig. 1). The experiment was conducted twice, once in February-March 2009 and once in February-March 2010. All plants in each cage were monitored every four days for four weeks in 2009 and for five weeks in 2010, and the numbers of *M. ochroloma* larvae, pupae, and adults and *P. maculiventris* nymphs and adults per plant were recorded. At the end of both experiments, all plants were pulled out of the cages and brought into the laboratory for the last sampling (Fig. 2). A HOBO® data logger (Onset Computer Corporation, Bourne, Massachusetts) was placed inside a cage to monitor temperature and relative humidity during the experiments.

**Statistical Analysis.** The effect of predator density on the caged *M. ochroloma* population was analyzed by comparing the number of live *M. ochroloma* per cage with analysis of variance with repeated measures over time. Treatment means were
separated by time using the Student–Newman–Keuls (SNK) test (SAS Institute, 1999) at a significance level of 5%. Means are reported with their standard error.

Results

2009 Experiment

The mean temperature during the experiment was 16.7°C ± 0.1. The mean maximum temperature was 23.2°C ± 0.6. Fifteen days had a maximum above this temperature, with 27.9°C as the highest recorded temperature for three days between 1:00 and 1:30 p.m. The mean minimum temperature was 10.8°C ± 0.9. Fourteen days had a minimum below this temperature, with -0.2°C as the lowest temperature on one day between 6:00 and 6:15 a.m.

Although 132 1st instars were introduced into each cage at the beginning of the experiment, the highest mean number of *M. ochroloma* larvae per cage on the first sampling date was 33 ± 3.1 in the medium predator density treatment. On 18 February, the mean number of larvae per cage in the high predator density treatment was significantly lower than in the medium predator density, low predatory density, and control treatments (*F* = 5.69; *df* = 3,12; *P* = 0.0116). On 22 February, the mean number of *M. ochroloma* pupae per cage in the high and medium predator density treatments was significantly lower than in the low predatory density and control treatments (*F* = 8.58; *df* = 3,12; *P* = 0.0026) (Fig. 3-Pupae). On 3 March, the mean numbers of pupae per cage in all treatments with predators were significantly lower than in the control treatment (*F* = 4.37; *df* = 3,12; *P* = 0.0268) (Fig. 3-Pupae). Although SNK test could not separate the mean number of pupae per cage among treatments on 18 and 26 February, ANOVA did show significance differences among them. A real effect by the predator was apparent in the medium and high predator density treatments.
because these treatments consistently had lower mean number of pupae per cage compared to the low predator density and control treatments ($F = 4.08; \ df = 3,12; \ P = 0.0326$; $F = 4.34; \ df = 3,12; \ P = 0.0273$) (Fig. 3-Pupae). On 3 March, the mean number of adult *M. ochroloma* in the two higher predator density treatments was significantly lower than in the low predatory density and control treatments ($F = 8.87; \ df = 3,12; \ P = 0.0023$) (Fig. 3-Adults).

Overall, there were differences in the number of *M. ochroloma* larvae + pupae + adults per cage among treatments over time. The mean number per cage was significantly lower in the high predator density treatment than in the medium predator density, which was significantly lower compared to the low predator density treatment. There was no significant difference in the mean number per cage between the low predator density and the control. However, there was a significant interaction between treatment and time (treatment × time: $F = 5.10; \ df = 3,72; \ P = 0.0167$; treatment: $F = 18.37; \ df = 3,72$; time: $F = 61.07; \ df = 3,72; \ P < 0.0001$) (Fig. 3-Total). The medium and high predator treatments had the lowest mean number of *M. ochroloma* per cage from 18 February until the end of the experiment. On 26 February, there was a significant difference, according to the ANOVA, in the mean number of *M. ochroloma* per cage among the treatments, however, no differences were detected when the means were separated with SNK test ($F = 3.54; \ df = 3,12; \ P = 0.0482$).

Excluding eggs, the population of *M. ochroloma* per cage in the high predator density treatment at the end of the experiment was $91.7\% \pm 8.3$ adults and $8.3\% \pm 8.3$ pupae. In the medium predator density treatment, the proportions per cage at the end of the experiment were $88.1\% \pm 7.9$ adults and $11.9\% \pm 7.9$ pupae. In the low predator
density treatment, the *M. ochroloma* population (excluding eggs) per cage was 89% ± 3.8 adults and 9.9% ± 3.7 pupae, whereas in the control treatment the proportions were 75.3% ± 8.4 adults, 24.2% ± 8.2 pupae, and 0.5% ± 0.5 larvae.

Overall, there were differences in the number of *P. maculiventris* nymphs per cage among treatments (*F* = 3.87; df = 3,72; *P* < 0.0001) (Fig. 4). The number of nymphs per cage was significantly higher in the medium predator density treatment than in the high predator density treatment which was significantly higher than in the low predator density treatment; the control treatment, with no predators released, was significantly lower than all other treatments. However, there was a significant interaction between treatment and time (treatment × time: *F* = 3.87; df = 3,72; *P* < 0.0001; treatment: *F* = 79.12; df = 3,72; *P* < 0.0001; time: *F* = 7.10; df = 3,72; *P* < 0.0001) (Fig. 4).

On only one date, 6 and 22 February (4 d after introducing predators into the cages), the mean number of predator nymphs per cage was significantly higher in the medium predator density treatment compared to the high predator density treatment; on all the other sampling dates there was no significant difference between these two treatments. Beginning 3 March, the two higher predator density treatments maintained significantly higher numbers of *P. maculiventris* nymphs per cage compared to the low predator density treatment during the remainder of the study (*F* = 18.63; df = 3,12; *P* < 0.0001) (Fig. 4).

At the end of the experiment, the overall survivorship ([number of nymphs recovered/number of nymphs released] × 100) of *P. maculiventris* per cage among the three predator release treatments was 51.3% ± 5.4. In the low predator density treatment, predator survivorship was 62.5% ± 12.5; 75.0% ± 14.4 of the nymphs were in
the 3rd instar and 25% ± 14.4 were in the 4th instar. In the medium predator density treatment, predator survivorship was 52.5% ± 4.8; 65.4% ± 16.7 of the nymphs were in the 3rd instar and 34.6% ± 16.7 were in the 4th instar. Predator survivorship in the high predator density treatment was 39.1% ± 6.9; 83.7% ± 10.3 of the nymphs were in the 3rd instar and 16.3% ± 10.3 were in the 4th instar.

2010 Experiment

The mean temperature during the experiment was 14.8°C ± 0.6. The mean maximum temperature was 21.6°C ± 0.6. Twenty days had a maximum above this temperature, with 26.9°C as the highest recorded temperature for one day between 2:30 and 2:45 p.m. The mean minimum temperature was 8.3°C ± 0.8. Seventeen days had a minimum below this temperature, with 0.3°C as the lowest temperature on one day between 5:30 and 5:45 a.m.

The highest mean number of *M. ochroloma* larvae per cage on the first sampling date was 27 ± 7.6 in the control treatment. On 1 March, the mean number of larvae per cage in the high, medium, and low predator density treatments was significantly lower than in the control treatment \((F = 12.54; \text{df} = 3,12; P = 0.0005)\) (Fig. 5-Larvae). On 9 March, pupae were observed in the low predator density and control treatments, but not in the high and medium predator density treatments. However, no significant differences were detected among the four treatments (Fig. 5-Pupae). On 13 March pupae were seen only the in the control treatment, but again no significant differences were detected among all treatments (Fig. 5-Pupae). On 17, 22, and 25 March, the mean number of adults per cage in the three predator density treatments was
significantly lower than in the control treatment ($F > 21.75; \text{df} = 3,12; P < 0.0001$) (Fig. 5-Adults). All *M. ochroloma* became reproductive adults in all treatments after 36 d.

Overall, there were differences in the number of *M. ochroloma* larvae + pupae + adults per cage among treatments over time. The mean number of *M. ochroloma* per cage was significantly lower in the medium and high predator density treatments than in the low predatory density treatment, which was significant lower compared to the control treatment (Fig. 5-Total). However, there was a significant interaction between treatments and time (treatment × time: $F = 4.64; \text{df} = 3,96; P < 0.0001$; treatment: $F = 58.97; \text{df} = 3,96; P < 0.0001$; time: $F = 29.56; \text{df} = 3,96; P < 0.0001$) (Fig. 5-Total). On 1, 17, 22, and 25 March, there were no significant differences in the mean number of *M. ochroloma* per cage among the low, medium, and high predator density treatments, but all of them were significantly different from the control treatment ($F = 12.54; \text{df} = 3,12; P = 0.0005$; $F = 21.75; \text{df} = 3,12; P < 0.0001$; $F = 28.47; \text{df} = 3,12; P < 0.0001$; $F = 38.35; \text{df} = 3,12; P < 0.0001$) (Fig. 5-Total).

Overall, there were differences in the number of *P. maculiventris* nymphs per cage among treatments ($F = 1.79; \text{df} = 3,96; P < 0.0001$) (Fig. 6). The number of nymphs per cage was significantly higher in the high and medium predator density treatments than in the low predator density treatment, which was significantly higher than the control treatment with no predators released ($F = 1.79; \text{df} = 3,96; P < 0.0001$). However, there was a significant interaction between treatment and time (treatment × time: $F = 1.79; \text{df} = 3,96; P < 0.0001$; treatment: $F = 34.70; \text{df} = 3,96; P < 0.0001$; time: $F = 5.11; \text{df} = 3,96; P = 0.01$) (Fig. 6). Significant differences were found amount treatments on all sampling dates except for 9 March ($F = 2.63; \text{df} = 3,96; P = 0.09$). The
mean number of nymphs per cage in the high predator density treatment decreased over time. On 25 March, the low predator density treatment was the only treatment significantly different from the control ($F = 4.24$; $df = 3,96$; $P = 0.0293$).

At the end of the experiment, the overall survivorship per cage of $P. \text{maculiventris}$ was $26\% \pm 8.1$. In the low predator density treatment, survivorship per cage was $56.3\% \pm 11.9$; $25.0\% \pm 28.9$ were in the 3rd instar, $50.0\% \pm 28.9$ were in the 4th instar, $12.5\% \pm 12.5$ were in the 5th instar, and $12.5\% \pm 12.5$ were adults (molting to adult on 25 March). In the medium predator density treatment, survivorship per cage was $17.5\% \pm 8.5$; $66.7\% \pm 16.7$ were in the 4th instar, and $33.3\% \pm 16.7$ were in the 5th instar. In the high predator density treatment, survivorship per cage was $3.1\% \pm 1.8$; $50.0\% \pm 50.0$ were in the 4th instar and $50.0\% \pm 50.0$ were in the 5th instar.

**Discussion**

**2009 Experiment**

Only seven sampling dates (five weeks) were evaluated due to the very large turnip plants in the cages at the end of the experiment (Fig. 7), which also made sampling more difficult. The notable reduction in the number of larvae of $M. \text{ochroloma}$ in the control treatment on 6 February, four days after the release of the insects into the cages, was likely due to the difficulty in detecting the small first instars by the visual sampling method. Temperature probably did not have a major influence on the mortality of $M. \text{ochroloma}$, since temperature ranged from 10.8 to 23.3°C during the experiment. Cold tolerance studies indicate that 1st instar $M. \text{ochroloma}$ can survive at least 2 d exposed to 0°C (unpublished data). The decrease in the number of $M. \text{ochroloma}$ per cage in all treatments from 6 to 18 February (Fig. 3-Total) can be attributed, in part, to the transition of larvae to pupae (Fig. 3-Larvae), but in the predator
density treatments it also can be attributed to predation by *P. maculiventris*, as evidenced by the significantly greater decrease in number of *M. ochroloma* in the medium and high predator density treatment versus the low predator density and control treatments. On 18 February, the difference between the mean number of larvae and pupae is probably due to the larvae behavior when it is ready to pupate (Fig. 3-Larvae and Pupae). When the larvae are ready to pupate, they move to dry leaves, tight places, and soil (Woodruff 1974; Bower 2003). In this study, the larvae moved under the plastic, making it difficult to find them. This confirms the disappearance of larvae after 22 February in the control treatment was due to pupation. In the treatments with predators, the reduced number of *M. ochroloma* larvae can be attributed to predation and pupation. On the last sampling date, the predation effect of *P. maculiventris* on the reduction in the number of *M. ochroloma* was more noticeable in the medium and high predator density treatments compared to the low predator density treatment (Fig. 3 Total). However, the overall number of *M. ochroloma* was kept lowest in the high predator density treatment during the 30-day experiment.

There was a drastic reduction of 1st instar *P. maculiventris* on 6 February, probably due to the visual sampling method or unexplained causes not measured. Temperature likely did not influence the number of 1st instars observed because De Clercq and Degheele (1992) estimated the lower threshold for development of *P. maculiventris* nymphs is 11.7°C, well below temperatures experienced during my field study. Although the survival of 1st instar *P. maculiventris* feeding on 1st instar *M. ochroloma* in the field has not been studied, a reduction in the survivorship could be expected. The observed survival of the predator per cage on 6 February was 40% ± 8.6, whereas the
actual survival determine on 3 March was 51% ± 5.4, suggesting that the survivorship of
the predator at the beginning of the experiment was underestimated by the sampling
method. Cannibalism may have been minimal or may not have occurred at all in this
experiment since the search area was high due to the large plant size and because the
populations of *M. ochroloma* were maintained at low levels, *i.e.* never driven to
extinction. If populations of *M. ochroloma* were to become limited, *P. maculiventris*
would have no problem surviving on other prey since it is a generalist predator, or even
on plants since it is a facultative herbivore (McPherson 1982; Valicente and O’Neil
1995). According to Wiedenmann and O’Neil (1990), the presence of plant material can
enhance survival of *P. maculiventris* at very low prey inputs.

**2010 Experiment**

Plant size (about four leaves per plant) in the 2010 experiment was considerably
smaller than it was in 2009 experiment (about seven leaves per plant), both at the
moment of the insect releases and at the end of the experiment (Fig. 8), because the
releases were made 10 d earlier after transplanting in 2010 than in 2009. This
difference extended the experiment to nine sampling dates in 2010. Plants in the
control treatment had to be replaced on 25 February due to complete defoliation by the
*M. ochroloima* larvae. Plants in the other treatments, on the other hand, did not have to
be replaced. This is evidence that predation by the *P. maculiventris* nymphs on *M.
ocroloma* significantly reduced the number of larvae in those treatments and,
therefore, the feeding damage on the plants.

On 21 February, four days after releasing the insects in the cages, there was a
drastic reduction in the number of *M. ochroloma* larvae as there was in 2009, again
probably due to the lack of detection by the sampling method. From 21 February to 5
March, the number of *M. ochroloma* decreased in all treatments (Fig. 5-Total), just as it did in 2009 and for the same possible reasons. It is important to observe that after 9 March, the number of *M. ochroloma* was virtually zero in the medium and high predator treatments for all stages (Fig. 5-Total), probably due to the effect of predation by *P. maculiventris*. This phenomenon did not happen in the 2009 experiment because the leaf surface area (larger plants) was higher than in 2010, therefore reducing the probability of the predator encountering and feeding upon the prey. Desurmont and Weston (2008) studied the effect of two host plant species, arrowwood (*Viburnum dentatum* L., Caprifoliaceae) and American cranberrybush (*Viburnum opulus* L. var. *americanum* Aiton, Caprifoliaceae), on consumption of the viburnum leaf beetle, *Pyrrhalta viburni* (Paykull), by *P. maculiventris*. Their results showed that prey consumption was inversely related to leaf surface area on one of the host plants. They hypothesized that the searching efficiency of the predator decreases as leaf surface increases because of the ability of the prey to hide or move around.

The 44% survivorship of *P. maculiventris* on 21 February is similar to the observed survival in 2009; however, survivorship rates at the end of the two experiments are quite different. The final survivorship in 2010 was 26% compared to 51% in 2009. Lower survival in 2010 could have been due to cannibalism since the leaf surface area was smaller and the number of *M. ochroloma* larvae was driven to zero after 9 March in the high and medium predator density treatments. Dispersal would be an option for the predators in an open field scenario, but not to a great extent because the nymphs do not have wings. According to the data gathered in this study, 1\textsuperscript{st} instar *P. maculiventris* would still be in the nymphal stage five weeks after being released in the field.
On the last sampling date (25 March), the effect of *P. maculiventris* nymphal predation on the number of *M. ochroloma* per cage is remarkable in all the predator density treatments. However, the number of *M. ochroloma* was kept lower for 36 d with at least 10 nymphs released per six plants. The release rate of three *P. maculiventris* 1\textsuperscript{st} instars per 1.21 m\textsuperscript{2} or 25 1\textsuperscript{st} instars per 10 m\textsuperscript{2} as recommended by Rincon-Vitova Insectaries is not adequate to control *M. ochroloma*. Therefore, two recommendations emerge from the overall results of my field-cage study:

- Release 16 1\textsuperscript{st} instars of *P. maculiventris* per six plants if the plants are expected to be large (≥7 leaves/plant) with at least 130 1\textsuperscript{st} instars of *M. ochroloma*.
- Release 10 1\textsuperscript{st} instars of *P. maculiventris* per six plants if the plants are expected to be small (≤6 leaves/plant) with at least 130 1\textsuperscript{st} instars of *M. ochroloma*.

Although there was no significant overall difference between the medium and high predator density treatments, the second recommendation is made from an economic point of view and the probability of cannibalism. In addition, increasing the number of biological control agents in the field does not always translate into greater pest control, but does increase the cost of using biological control (Van Driesche et al. 2002; Collier and Van Steenwyk 2004). Consequently, releasing an optimal number of biological control agents should result in a more efficient and economic augmentative biological control program (Crower 2007).
Figure 4-1. Cages in the field at the beginning of the 2009 experiment.

Figure 4-2. Plants being gathered for final sampling in the laboratory.
Figure 4-3. Number of *Microtheca ochroloma* per cage during the 2009 field experiment. Means followed by the same letter within each sampling date are not significantly different (P>0.05). An asterisk (*) over the bars on the same date indicates that differences among treatments were detected by ANOVA, but the means could not be separated by the SNK test.
Figure 4-4. Mean number of *Podisus maculiventris* nymphs per cage during the 2009 field experiment. Means followed by the same letter within each sampling date are not significantly different (P>0.05).
Figure 4-5. Mean number of *Microtheca ochroloma* per cage during the 2010 field experiment. Means followed by the same letter within each sampling date are not significantly different (P>0.05).
Figure 4-6. Number of *Podisus maculiventris* nymphs per cage during the 2010 field experiment. Means followed by the same letter within each sampling date are not significantly different (P>0.05).
Figure 4-7. Size of turnip plants in the 2009 experiment.

Figure 4-8. Size of turnip plants in the 2010 experiment.
CHAPTER 5
INFECTIVITY OF MICROTHECA OCHROLOMA STÅL (COLEOPTERA: CHRYSOMELIDAE) BY ISARIA FUMOSOROSEA WIZE (BROWN AND SMITH)

Introduction

Microtheca ochroloma Stål, the yellowmargined leaf beetle, is a serious pest in crucifer crops during the late fall and winter months in Florida. Since 1947, this adventive species has been established in most of the southern US states. Ameen and Story (1997a) studied the feeding preferences of the larvae and adults and concluded that turnip and mustard are their preferred host plants. Its main damage is caused by defoliation; however, roots can also be damaged when infestations are severe. On large-scale commercial farms, the most common and easy way to control this beetle is by applying insecticides, but the overuse of insecticides can lead to the development of resistance over time, as has occurred with of the Colorado potato beetle Stilodes decemlineata (Say) (Alyokhin et al. 2008). On organic farms, it is more difficult to control M. ochroloma due to the restricted use of insecticides, in addition to the lack of specific natural enemies of M. ochroloma in the United States (Fasulo 2005). Currently, there is no pest management program available for growers to control this pest in the United States. Bowers (2003) evaluated whether intercropping between host (mizuna) and non-host (oak leaf lettuce) plants can reduce the severity of M. ochroloma outbreaks, but still the beetles were able to find and colonize host plants among the non-host plants.

Biological control by entomopathogenic fungi may potentially be used to control M. ochroloma on organic farms. Anjos et al. (2007) reported infection of M. ochroloma in the field by Beauveria bassiana (Bals.) Vuilleman in Rio Grande do Sul, one of more southern states in Brazil. Isaria fumosorosea (=Paecilomyces fumosoroseus) Wize
(Brown and Smith) has a worldwide distribution and its effectiveness against many pest insects, especially whiteflies, is well documented (Osborne and Landa 1992; Vidal et al. 1997a; Lacey et al. 1999). This fungus has a broad host range, including chrysomelid beetles such as S. decemlineata (Bajan 1973), Pyrrhalta luteola (Mueller), and Spaethiella sp. (Humber et al. 2009).

In 1986, a strain of I. fumosorosea named Apopka 97 was isolated in Apopka (Orange County), FL from Phenacoccus sp. (Hemiptera: Pseudococcidae) (Vidal et al. 1998). The strain is registered under the commercial name PFR-97™ 20% WDG® [chemical family: microbial insecticide, chemical name: Paecilomyces fumosoroseus Apopka Strain 97 (ATCC 20874)] by the manufacturer Certis USA, Columbia, MD. It is recommended for use in greenhouses against aphids, citrus psyllid, spider mites, thrips, and whitefly [www.certisusa.com], but it is still being evaluated against field pests in food crops.

The use of microbial insecticides as a tool to control pests in agricultural ecosystems is becoming more popular among growers. Although there is no information available on commercial products to specifically control M. ochroloma, products that control other pest beetles should be evaluated on M. ochroloma. In this study, the infectivity of Apopka 97 was evaluated as a potential biological control agent of M. ochroloma.

Materials and Methods

Stock Colony. Eggs, larvae, and adults of M. ochroloma were obtained from the field and a laboratory colony was established and maintained as described in Chapter 2.

Fungus. PFR-97™ 20% WDG (a.i. Paecilomyces fumosoroseus Apopka strain 97 20%, inert ingredients 80%) was provided for research by Certis USA in a 0.45 kg bag.
(Lot: 0833004401) in the form of desiccated granules of *I. fumosorosea* blastospores. The bag contains $1 \times 10^9$ colony-forming units (CFU)/g.

**Plant Material.** Plants of Seven Top (Greens) turnips (*Brassica rapa* L. var. *rapifera*, Brassicaceae) were grown as described in Chapter 2.

**Experiment 1. Susceptibility of *Microtheca ochroloma* to Infection by PFR-97™**

The goal of this experiment was to determine the susceptibility of various stages of *M. ochroloma* to infection by PFR-97™. Five stages of *M. ochroloma*, egg, 1st and 3rd instars, pupa, and adult, were removed from the laboratory colony for exposure to a concentration of 1 g of PFR-97™ in 100 ml of sterile distilled water at 25°C, 10L:14D photoperiod, and 60% RH. The fungal suspension was prepared in a beaker and allowed to settle for 20 min until the supernatant containing blastospores and the inert sediment of the product separated. The suspension was applied (see below for method) to groups of 10 insects per stage housed in separate and sealed plastic Petri dishes (60 × 75 mm, Fisherbrand®) with moistened filter paper (55 mm Ø [diameter], Whatman®) on the bottom dish. A 2.5 cm² Ø piece of turnip leaf was placed on top of the filter paper. The Petri dishes were sealed with Parafilm®.

All fungal treatments consisted of 10 replicates with 10 pseudo-replicates per replicate. A pseudo-replicate was a single insect. A control treatment consisted of five replicates in which the test insect stages were sprayed with sterile distilled water only. Mortality was checked daily during the 7 d following the fungal application. Infectivity rate was determined by using the control mortality as a correction factor for the mortality in the fungus treatment. Morphological traits unique to *I. fumosorosea* in dead insects (see below for method) were used to confirm infection. The experiment was repeated one time.
Experiment 2. Infectivity of the Most Susceptible Stage of *Microtheca ochroloma* by Four Concentrations of PFR-97™

The goal of this experiment was to compare the infectivity of four concentrations of PFR-97™ in the most susceptible stage of *M. ochroloma*, which was determined in Experiment 1. The concentrations were 1, 2, 3, and 4 g of PFR-97™ per 100 ml of sterile distilled water. Each concentration was applied (see below for method) to groups of 10 1st instars housed in separate and sealed plastic Petri dishes with moistened filter paper on the bottom. A 2.5 cm² piece of turnip leaf was placed on top of the filter paper. The Petri dishes were then housed in an environmentally controlled chamber set to 25° C, 10L:14D photoperiod, and 60% RH. Each fungal treatment consisted of 10 replicates with 10 pseudo-replicates per replicate. A control treatment consisted of five replicates in which the test insects were sprayed with sterile distilled water only. Mortality was checked daily during the 7 d following the fungal application. Infectivity rate was determined by using the control mortality as a correction factor for the mortality in the fungus treatment. Confirmed infectivity rate was determined by morphological traits unique to *I. fumosorosea* in dead insects (see below for method). The experiment was repeated one time.

For both experiments, the initial blastospore concentration was determined by counting the number of blastospores per ml using a disposable plastic Neubauer hemocytometer, C-Chip DHC-N01, manufactured by Incyto (Korea). Each suspension was poured separately into 180-ml Nalgene™ (Rochester, NY) spray bottles for application to test insects. Each group of insects with their respective piece of leaf received 3 sec of application (~2.5 ml) on each side of the leaf. The sprayed leaf was not removed from the Petri dish and starting 3 d after treatment new non-sprayed
leaves were added daily to each Petri dish but not removed. Blastospore deposition density was determined by placing a plastic cover slip among the test insects during the application of the fungus, after which the number of blastospores per mm² was counted. Viability of blastospores was determined by taking 100µL from the 10⁻³ serial dilution, spreading it on potato dextrose agar (PDA) in Petri dishes, maintaining the dishes under the same environmental conditions as the insects, and counting the number of CFU after 7 d.

To confirm infectivity in Experiment 1, dead insects were removed daily and transferred directly to a Petri dish containing a mixture of PDA, odine, streptomycin, and chlrophenacol; dishes were then sealed with Parafilm® and stored at 25°C. The presence of hyphae morphologically unique to I. fumosorosea (Fig. 5-1) was recorded. In Experiment 2, dead insects were surface sterilized in 70% ethanol for a few seconds before being placed on the PDA-mixture plates. Unconfirmed infectivity was assigned to dead insects in which I. fumosorosea could not be identified because of its absence or contamination by other fungi.

**Statistical Analysis.** Data were arcsine-transformed prior to analysis. Mortality and infectivity rates were analyzed using analysis of variance, and treatment means were separated using Student-Newman-Keuls test. All tests were performed with PROC GLM in SAS v. 9.2 (SAS Institute Inc. 2002), with a significance level of 5%. Lethal concentration (LC₂₅) and lethal times (LT₁₀ and LT₂₅) were analyzed using PROC PROBIT® (SAS Institute Inc. 2002), and significant differences between treatments were identified using 95% confidence intervals (Tabashnick and Cushing 1987).
Results

Experiment 1

For 1 g of PFR-97™ in 100 ml of water, the concentration of the suspension was 3.0 ± 0.1 × 10⁷ blastospores/ml (Fig. 5-2A). Mean blastospore deposition density was 1,043 ± 181.5 blastospores/mm² (Fig 5-2B). Viability was 49 ± 1.0 CFU/ml⁻¹ in 10⁻⁴ serial dilution (Fig.5-2C).

The most susceptible stage of *M. ochrolooma* to the Apopka 97 strain of *I. fumosorosea* was the larval stage. The mean mortality rates of eggs, pupae and adults were not significantly different from their respective controls (Fig. 5-2). In contrast, mean mortality rates of the 1ˢᵗ and 3ʳᵈ instars were significantly higher (4.4 and 0.8 times, respectively) than their respective controls ($F = 14.39; \text{df} = 1,13; \ P = 0.0022; \ F = 8.12; \text{df} = 1,13; \ P = 0.0137$) (Fig. 5-3). Mean infectivity rate was at least 6.3 times higher in the 1ˢᵗ and 3ʳᵈ instars than in the egg, pupal and adult stages ($F=12.19; \text{df} = 4,35; \ P < 0.0001$) (Fig. 5-3). However, only 17 and 20% of the infectivity in the 1ˢᵗ and 3ʳᵈ instar, respectively, was confirmed (Figs. 5-4 and 5-9). Mortality of the 1ˢᵗ instar was observed beginning 3 d after treatment. By that time the 1ˢᵗ instars had already molted to 2ⁿᵈ instar. Mortality in the 3ʳᵈ instar was observed beginning 1 day after treatment.

The LT₁₀ for the 1ˢᵗ instar (4 d) was significantly higher than the 3ʳᵈ instar (2 d) ($P < 0.05$). No significant difference was apparent for the LT₂₅ between the two instars ($P > 0.05$) (Fig. 5-5). The LT₅₀ could not be determined due to the low mortality rate of larvae exposed to the 1 g treatment concentration of PFR-97™; however the LT₅₀ predicted by the model for the 1ˢᵗ and 3ʳᵈ instar were 8.6 (fiducial limits: 7.6-10.5) and 21.2 d (fiducial limits: 12.9-60.7), respectively. The LT models were not significant for eggs, pupae, and adults ($P > 0.05$).
**Experiment 2**

For this experiment, the concentration of blastospores, blastospore deposition, and viability for the four experimental concentrations of PFR-97™ are reported in Table 5-1. The 1st instar of *M. ochroloma* was selected for this experiment based on the results of Experiment 1. There was no mortality in the control treatment. Therefore, all mortality in the fungus treatments was considered to be caused by infection with *I. fumosorosea*. Mean infectivity rate was significantly higher by 2.6 times in the 4 g concentration treatment than in the 1, 2, and 3 g concentration treatments (*F* = 3.76, df = 3,36; *P* =0.0191) (Fig. 5-6). Confirmed infectivity rates were 2, 5, 10, and 27% in the 1, 2, 3, and 4 g concentration treatments, respectively (Fig. 5-6).

Based on fiducial limits (95%), the LT<sub>10</sub> and LT<sub>25</sub> in the 4 g concentration treatment were significantly lower compared to the other treatments (*P* < 0.05). The LT<sub>50</sub> predicted by the model was 10 d (fiducial limits: 8.4-13.7). There was no significant difference among the 1, 2, and 3 g concentration treatments (*P* > 0.05) (Fig. 5-7).

The LC<sub>10</sub> and LC<sub>25</sub> of PFR-97™ applied to 1st instar *M. ochroloma* were 1.4 (fiducial limits: 0.3-2.0) and 5.5 g (fiducial limitis: 3.6-39.0) per 100 ml of distilled water, respectively, on day 7. The LC<sub>50</sub> predicted by the model was 25.6 g per 100 ml of distilled water, on day 7 (intercept = -1.42 ± 0.16; slope = 1.0 ± 0.36; *X*² = 1.0 ± 0.36; *P* = < 0.0001).

**Discussion**

**Experiment 1**

The fungus had a low, insignificant ovicidal effect with an egg mortality rate of 3% (Figs. 5-4 and 5-8). Although the ovicidal effect was low in this experiment, the fungal residues on the eggs and on the leaf surface may have a significant impact on
the emerging neonates. There is a great deal of variability and discussion concerning
the ovicidal effect of *I. fumosorosea*. Rodrigues-Rueda and Fargues (1980) showed that
*P. fumosoroseus* has high ovicidal activity on eggs of the moths *Mamestra brassicae*
(Linnaeus) and *Spodoptera littoralis* (Boisduval). In contrast, Lacey et al. (1999)
reported a low, but significant mortality (10-20%) of eggs of *Bemisia tabaci* (Gennadius)
treated with of PFR-97™, but no significant ovicidal effect was reported on eggs of
*Yponomeuta xylostella* (Linneaus) (Maketon et al. 2008).

Larvae of *M. ochroloma* in the 1st and 3rd instars experienced the highest
infection rates among all the insect’s life stages. The unconfirmed infections may be
attributed to the procedure of transferring dead insects to Petri dishes without first
surface sterilizing the insects. This may have resulted in the rapid growth of
saprophagous fungi, thus slowing the growth of *I. fumosorosea* (if there was any) and
not allowing its appearance of diagnostic morphological features (Fig. 5-9). Larvae
infected by *I. fumosorosea* exhibited noticeable reduced growth (Fig. 5-10) and
unsuccessful molting in which the exuvium remained attached to the new integument
(Fig. 5-12). Similar studies by Hussain et al. (2009) have also shown a reduction in the
consumption and growth of all instars of *Ocinara varians* Walker (Lepidoptera:
Bombycidae) when *I. fumosorosea* strain 03011-C3.19A was applied. A reduction in
feeding was also reported by Fargues et al. (1994) in *S. decemlineata* attacked by *B.
bassiana*. Mortality and growth rate reduction may be attributed to the production of
toxins by the fungus, mechanical disruption of the structural integrity of membranes by
the growth of hyphae, and dehydration of cells from the loss of fluids (Ferron 1981;
Tefera and Pringle 2003; Assaf et al. 2005).
Microtheca ochroloma pupates within a net-like case which only has direct contact with the cuticle of the pupa at the apex of the body (Fig. 5-11). For this reason, low mortality and infectivity rates of pupae were observed, since the net-like case apparently serves as a physical barrier to the deposition of blastospores on the cuticle of the pupa, which is necessary to initiate infection.

Adults of *M. ochroloma* were not affected by *I. fumosorosea*, probably because the hard cuticle is composed primarily of a higher degree of cross-linked proteins and chitin than that of the immature stages, which provides greater strength and hardness to the exoskeleton and functions as a formidable barrier to blastospore germination (Klowden 2007). Only 4% mortality was recorded in the fungus treatment, compared to none in the control treatment (Fig. 5-3), but the mortality in the treatment cannot be confidently attributed to the fungus since there was no confirmed infectivity (Fig. 5-4). Michalaki et al. (2007) reported low mortality of adults of *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) exposed to *I. fumosorosea*.

**Experiment 2**

There was a well defined positive correlation between fungus concentration and mortality rates of 1st instars of *M. ochroloma*. The highest infectivity and confirmed infectivity rates were achieved with the 4 g concentration treatment, which corresponded to the highest concentration of blastospores/ml, deposition of blastospores/mm², and viability among all treatments (Table 5-1). However, the 4 g concentration treatment achieved only 29% infectivity in the laboratory; infectivity rates in the field may be expected to be lower. The model predicts the LC₂₅ is 5.5 g per 100 ml of water, which is equivalent to approximately $1.6 \times 10^8$ blastospores/ml, and the predicted LC₅₀ is 25.6 g per 100 ml of water, which is equivalent to approximately $7.3 \times$
$10^6$ blastospores/ml. Higher concentrations of PFR-97$^\text{TM}$ should be tested in the laboratory, since it seems that infectivity rates in the 1$^{\text{st}}$ instar increase as the concentration of blastospores/ml increases (Fig. 5-6), and the LT$_{10}$ and LT$_{25}$ are achieved faster with increasing concentration (Fig. 5-7). Once the product is registered for use in the field for fruiting crops, the concentration of CFU per bag probably will be higher; therefore, the amount of grams per 100 ml required for high infection rates would be lower.

The unconfirmed infectivity rate in Experiment 2 was lower compared to the unconfirmed infectivity rate in Experiment 1 because when the insects died in the former they were sterilized with alcohol for few seconds before placing them on the PDA. The unconfirmed infectivity rate in Experiment 2 may be reduced even more by using the polymerase chain reaction technique to identify the presence of PFR-97$^\text{TM}$ strain in dead insects, as has been done in other studies (Meyer 2007; Meyer et al. 2008; Hoy et al. 2010).

Once blastospores are deposited and germinate on the integument of the insect, death of the host will most likely occur within 3 d at any concentration. In both experiments, the appearance of fungal infection in the 1$^{\text{st}}$ instar of $M. \text{ochroloma}$ began 3 d following application. Similar results were reported by Tounou et al. (2003) in nymphs of the green leafhopper, Empoasca decipiens Paoli (Hemiptera: Cicadellidae), which began dying 3 d after treatment with $I. \text{fumosorosea}$ strain Pfr12. However, there will be a higher probability of deposition and germination of blastospores at higher concentrations of the fungus, thereby killing a greater number of insects compared to lower concentrations.
In summary, the larval stage of *M. ochroloma* is the most susceptible stage to *I. fumosorosea*. However, a concentration of PFR-97™ greater than those tested here is required to reach the LT$_{50}$ and LC$_{50}$ in 1$^{st}$ instars under laboratory conditions. Higher concentrations than those tested in the laboratory will need to be applied in the field as well.

Figure 5-1. Morphological characteristics of *Isaria fumosorosea* infecting *Microtheca ochroloma* larva.
Figure 5-2. Laboratory tests of *Isaria fumosorosea*. A) Blastospore concentration (blastospores/ml), B) Blastospore deposition density (blastospore/mm²), and C) Viability (CFU/g).
Figure 5-3. Mortality of *Microtheca ochroloma* by PFR-97\textsuperscript{TM} 20% WDG at $3.0 \times 10^7$ blastospores/ml 7 d after application. Bars with different letter within each stage are significantly different (SNK test, P < 0.05).
Figure 5-4. Infectivity of *Microtheca ochroloma* by PFR-97™ 20% WDG at $3.0 \times 10^7$ blastospores/ml 7 d after application. Bars with the same letter are not significantly different (SNK test, $P > 0.05$).
Figure 5-5. Lethal time of the first and third instars of *Microtheca ochroloma* treated with PFR-97™. LT10 and LT25 values within the same box are not significantly different in their 95% confidence intervals.
Figure 5-6. Infectivity of first instar *Microtheca ochroloma* at four concentrations of PFR-97™ 7 d after application. Bars followed by the same lowercase letter are not significantly different (P>0.05).
Figure 5-7. Lethal time of the first instar *Microtheca ochroloma* exposed to four concentrations of PFR-97™. LT$_{10}$ and LT$_{25}$ values within the same box are not significantly different in their 95% confidence intervals.
Figure 5-8. Eggs of *Microtheca ochroloma* infected by *Isaria fumosorosea*.

Figure 5-9. Unconfirmed and confirmed infectivity by *Isaria fumosorosea* in dead larvae of *Microtheca ochroloma*.
Figure 5-10. Reduction in the growth of larvae of *Microtheca ochroloma* infected by *Isaria fumosorosea*.

Figure 5-11. Net-like pupal case of *Microtheca ochroloma*.
Figure 5-12. Unsuccessful molting by a larva of *Microtheca ochroloma* infected with *Isaria fumosorosea*. A) Head partially out, B) Exuvia attached to the dorsal part of the body, and C) Larva starting to pull out from the tip of the abdomen.
Table 5-1. Laboratory tests of *Isaria fumosorosea* in Experiment 2.

<table>
<thead>
<tr>
<th>PFR-97™ (g)</th>
<th>Concentration ± SE(^a) (blastospores/ml)</th>
<th>Deposition ± SE (blastospores/mm(^2))</th>
<th>CFU(^b) ± SE (CFU/ml at 10(^{-4}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.2 ± 0.1 \times 10^7</td>
<td>779 ± 150.5</td>
<td>24.5 ± 2.5</td>
</tr>
<tr>
<td>2</td>
<td>3.8 ± 0.2 \times 10^7</td>
<td>1088 ± 174.1</td>
<td>55.5 ± 2.5</td>
</tr>
<tr>
<td>3</td>
<td>8.4 ± 0.7 \times 10^7</td>
<td>4157 ± 962.6</td>
<td>84.5 ± 1.5</td>
</tr>
<tr>
<td>4</td>
<td>1.1 ± 0.0 \times 10^8</td>
<td>6658 ± 881.6</td>
<td>146.5 ± 1.5</td>
</tr>
</tbody>
</table>

\(^a\) standard error. \(^b\) Colony-forming units.
CHAPTER 6
CONCLUSIONS

My thesis study focused on the evaluation of two potential biological control agents of the yellowmargined leaf beetle, *M. ochroloma*. The predator, *P. maculiventris*, was chosen because it 1) occurs naturally in the field, 2) can be acquired commercially by growers, and 3) preys on many agricultural pests. The fungus, *I. fumosorosea* (PFR-97™), was chosen because it 1) is a local strain and 2) has been under previous study for application to food crops in the field.

There was no development of 1st instars or egg hatching of *P. maculiventris* at 10°C. Development of the predator from egg to adult is shorter at 20 and 25°C (31 and 23 d, respectively) than at 15°C (99 d). The daily predation by *P. maculiventris* starting from the 3rd instar until 10 d of adulthood was higher at 25°C compared to 20°C, which was higher than at 15°C. However, the total predation by *P. maculiventris* on 4th instar *M. ochroloma* was higher at 25°C compared to 20°C, which was higher than at 15°C only during the 10 d of adulthood. *Podisus maculiventris* nymphs killed 59, 53, and 65 4th instars of *M. ochroloma* at 15, 20 and 25°C, respectively. Fresh adult females weigh more than males at 20°C, but not at 25°C. Predator nymphs also preyed on an average of 741 eggs of *M. ochroloma*, completing their development in 25 d at 25°C. Therefore, *P. maculiventris* can develop successfully on a diet of *M. ochroloma* eggs or larvae, despite the presence of secondary compounds (glucosinolates) in crucifers consumed by *M. ochroloma*. The nymphal stage of *P. maculiventris* develops faster and preys on more 4th instars of *M. ochroloma* at higher temperatures.

A two-year cage experiment addressed the field predation potential of *P. maculiventris* when 1st instars were released at three densities (4=low, 10=medium, and
16=high) on six turnip plants with a known initial population of *M. ochroloma* larvae. In 2009, the plants grew larger (≥7 leaves/plant). The high predator density treatment reduced the *M. ochroloma* population significantly more than the medium predator density treatment, which reduced the pest population significantly more than the low predator density treatment. The high predator density treatment reduced the *M. ochroloma* population by 96% and the overall survivorship of *P. maculiventris* was 51%. In 2010, the plants were smaller (≤6 leaves/plant). The medium and high predator density treatments reduced equally the *M. ochroloma* population, but significantly more than the low predator density treatment. The medium and high predator density treatments reduced the *M. ochroloma* population by 99% and the overall survivorship of *P. maculiventris* was 26%. Therefore, depending on the plant size, 10 or 16 nymphs per 1.21 m² (the approximate area covered by six plants) are recommended to release in the field to control *M. ochroloma* population.

Two experiments addressed the evaluation of the fungus on *M. ochroloma*. In Experiment 1 the larval stage was shown to be the most susceptible stage to PFR-97™. In Experiment 2 the most susceptible stage from Experiment 1 (i.e. 1st instar) was exposed to four concentrations of PFR-97™ (1, 2, 3, and 4 g of PFR-97™ each in 100 ml of distilled water). The 4 g concentration caused the highest infection rate (27% confirmed infectivity) on the 1st instars of *M. ochroloma* compared to the 1, 2, and 3 g concentration (2, 5, and 10% infectivity, respectively). Therefore, PFR-97™ is not recommended for use to control *M. ochroloma* in the field, due to the low rate of infection observed in the laboratory.
For the Growers

*Podisus maculiventris* is recommended for use in the field to control populations of *M. ochroloma*. The initial density of *M. ochroloma* used in this study is relatively low compared to densities that may be observed in organic farms. Therefore, releases of *P. maculiventris* should be made when infestations of *M. ochroloma* are low so that the predator may provide preventive control of increasing pest populations. For big plants (≥7 leaves/plant), a release of 16 1st instar *P. maculiventris* per six plants or 1.21 m² is recommended. For small plants (≤6 leaves/plant), a release rate of 10 1st instar *P. maculiventris* per six plants or 1.21 m² is recommended. A bottle of 250 eggs of *P. maculiventris* can be purchased online at [www.riconvitova.com](http://www.riconvitova.com) ($112.74), [www.arbico-organics.com](http://www.arbico-organics.com) ($133.50), or [www.planetnatural.com](http://www.planetnatural.com) ($118.95). The costs of releasing 10 and 16 nymphs per 1.21 m² are $4.50 and $7.21, respectively. A bottle of 250 eggs of *P. maculiventris* will cover 30 m² for the release density of 10 nymphs and 19 m² for the release density of 16 nymphs. A cheaper alternative for obtaining eggs is by collecting adults from the field and holding them indoors with prey. The prey can be mainly caterpillars or beetle larvae. However, the availability of prey and the time to feed the predators can be a disadvantage to this alternate method of obtaining eggs.

The product PFR-97™ containing blastospores of *I. fumosorosea* is not recommended for use in the field to control *M. ochroloma*, since higher concentrations than those tested in the laboratory are required to kill more than 50% of the population. The suspension preparation of PFR-97™ at concentrations higher than 4 g per 100 ml will increase pest management costs significantly, and the higher concentrated material could clog the nozzle during application of the product. The manufacturer, Certis
(www.certisusa.com), sells a 9.5-kg bag of PFR-97™ for $35.00. Even though PFR-97™ did not have a high rate of infection in *M. ochrocoma*, OMRI listed products such as Entrust® and PyGanic® can be another option to control populations of *M. ochrocoma*. 
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Cecil O. Montemayor Aizpurúa was born in David, Panamá. She received her bachelor’s degree at the Panamanian School of Agriculture, Zamorano, in Honduras in 2005. She conducted her degree internship at Chiquita Brands International Co. in La Ceiba, Honduras, working with entomopathogenic fungi to control pests in bananas. In 2006, she was an intern at the University of Minnesota, where she worked in wetlands restoration and in the biological control of soybean aphids. Since 2007, she has worked at the University of Florida’s Biological Control Research and Containment Laboratory (BCRCL) at the Indian River Research and Education Center in Ft. Pierce. At the BCRCL, she first worked as a short-term scholar, conducting research on biological control of insects, specifically the yellowmarginated leaf beetle, and processing specimens collected for an inventory of the arthropods on tree islands in the South Florida Water Management District Conservation Area. She then began her Master of Science degree program in the Entomology and Nematology Department in August 2008. She received a scholarship grant from the Ministry of Economy and Finances of Panama to support her during her study program. She is a member of the Entomological Society of America, Florida Entomological Society, and Florida State Horticultural Society. She was the president of the Statewide Student Association at University of Florida from 2009 to 2010. She has presented talks about her research at the annual meetings of the Florida State Horticultural Society (third place in the student competition), the Florida Entomological Society (first place in the student competition), and the Entomological Society of America.