

EVALUATION OF THE GREEN LACEWING *Chrysoperla rufilabris* BURMEISTER  
(NEUROPTERA: CHRYSOPIDAE) AS A BIOLOGICAL CONTROL AGENT OF THE  
YELLOWMARGINED LEAF BEETLE *Microtheca ochroloma* STÅL (COLEOPTERA:  
CHRYSOMELIDAE)

By

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To my dedicated parents, Alejo and Ligia, and my lovely family

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Abstract of Thesis Presented to the Graduate School  
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EVALUATION OF THE GREEN LACEWING *Chrysoperla rufilabris* BURMEISTER  
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The production of crucifers in organic farms in the southern United States has been highly affected by the yellowmargined leaf beetle, *Microtheca ochroloma* Stål (Coleoptera: Chrysomelidae). This insect was detected for first time in the country in 1945, and until now no host-specific natural enemy has been reported in the area. Therefore, the goal of this study was to evaluate the effectiveness of the generalist predator *Chrysoperla rufilabris* Burmeister (Neuroptera: Chrysopidae) as a biological control agent for the management of the yellowmargined leaf beetle in organic farms.

*Chrysoperla rufilabris* larvae have been reported feeding on *M. ochroloma* eggs and larvae. Therefore, the first experiment of this thesis quantified the development time and predation rate of *C. rufilabris* larvae feeding on eggs and first instars of *M. ochroloma* at constant temperatures of 10, 15, 20 and 25°C. The predator was able to develop at temperatures between 15 and 25°C. *Chrysoperla rufilabris* spent, from egg hatch to adult emergence,  $75.5 \pm 1.7$  and  $54.0 \pm 1.6$  days at 15°C,  $34.7 \pm 0.7$  and  $31.7 \pm 0.9$  days at 20°C, and  $26.6 \pm 0.6$  and  $21.4 \pm 0.7$  days at 25°C when fed eggs and first

instars of *M. ochroloma*, respectively. During the larval stage, the predator killed a mean total of  $633.9 \pm 38.5$  eggs and  $217.7 \pm 18.3$  first instars at 15°C,  $592.1 \pm 30.5$  eggs and  $224.8 \pm 18.9$  first instars at 20°C, and  $496.1 \pm 28.7$  eggs and  $218.5 \pm 17.6$  first instars of *M. ochroloma* at 25°C.

Prey preference of *C. rufilabris* among *M. ochroloma* eggs and first instars and *Myzus persicae* Sulzer (Hemiptera: Aphididae) nymphs was evaluated in the second experiment of this thesis. The predator showed a 5.2-fold preference for aphids over *M. ochroloma* eggs and larvae. When only eggs and larvae of *M. ochroloma* were offered in Petri dishes, the predator did not show any preference. However, when the immature stages of *M. ochroloma* were offered to the predator on bok choy plants, *C. rufilabris* killed more first instars than eggs due to differences in the location of the prey.

In the third experiment of this thesis, the performance of *C. rufilabris* feeding on *M. ochroloma* larvae under field conditions on an organic farm was evaluated. All possible combinations of cage-uncaged bok choy plants and with-without predator were evaluated. The release of the predator did not cause an important effect on *M. ochroloma* populations. Differences in the number of prey on the last sampling period were more related to the use of cages.

The results of my research suggest that *C. rufilabris* might not be a promising biological control agent of *M. ochroloma*. Augmentative releases of *C. rufilabris* to control *M. ochroloma* in organic farms probably would not be cost effective for the growers. Efforts should be focused on attracting, enhancing, and conserving natural populations of *C. rufilabris* present in the organic farms.

## CHAPTER 1 GENERAL INTRODUCTION

The organic food industry has grown rapidly in the United States during the last two decades. Since 1990, the demand for organic food has increased 20% or more per year, and around 73% of the fresh production is sold in traditional markets (Nguyen et al. 2008). In the 2007 Census of Agriculture, there were 172 organic farms in Florida, of which 57 produced vegetables, potatoes, and melons. Among the vegetables produced, crucifers constituted a large and important group which includes broccoli, cabbage, cauliflower, collards, kale, mustard, radish, turnips, and some Asian vegetables (e.g. bok choy, Chinese broccoli) (Webb 2010, Lamberts et al. 2011). Crucifer crops are conventionally and organically cultivated for various purposes, including human food (cooked or fresh), oil, medicinal benefits (Fenwick et al. 1982), and animal fodder.

In order for a product to qualify as organic, it must meet the standards of the National Organic Program (NOP) (Ferguson 2004, Nguyen et al. 2008). According to the NOP, arthropod pest management in organic crops must incorporate ecological approaches, including augmentation or introduction of natural enemies, development of suitable habitat for natural enemies, and non-synthetic control methods (Ferguson 2004, Zehnder et al. 2007). Since the use of synthetic pesticides is not an alternative for organic growers, it is vital to develop information about more eco-friendly control strategies.

Organic growers must deal with many insects that infest crucifers. The yellowmargined leaf beetle, *Microtheca ochroloma* Stål (Coleoptera: Chrysomelidae), has become a major pest of turnips, mustard, and Chinese cabbage in organic farms (Webb 2010). This insect is a problem for organic growers because: 1) it is active during

cool seasons (late fall, winter, and early spring) when crucifers are produced in Florida and the state becomes an important supplier for other parts of the country due to a reduced production of other states (Olson 2011); 2) it feeds on leaves which are the marketed part of the plant, thus reducing the quality of the product (Chamberlin and Tippins 1948, Ameen and Story 1997a); 3) it is an invasive species, and no effective, host-specific natural enemy is present in Florida; and 4) organic growers are not allowed to use foliar insecticides which are commonly used to control this pest (Bowers 2003).

Biological control is used to manage many economically important insect pests. Montemayor (2010) and Montemayor and Cave (2011, 2012) evaluated the generalist predator *Podisus maculiventris* Say (Hemiptera: Pentatomidae) as a control agent against *M. ochroloma*. They found that *P. maculiventris* fully develops when feeding on the immature stages of *M. ochroloma* at temperatures above 10°C, and has good potential to diminish populations of this pest in the field. My laboratory and field studies evaluated another generalist predator, *Chrysoperla rufilabris* (Burmeister), commonly known as the green lacewing. This predator is often used in augmentative programs (Ridgway and Jones 1968, New 1975), is commercially available, and has been used successfully against other foliage-feeding beetles, including the Colorado potato beetle (*Leptinotarsa decemlineata* Say).

Green lacewing larvae feed on a diversity of prey, principally small, soft-bodied arthropods (Tauber et al. 2000). However, Obrycki et al. (1989) indicated that even if a prey is consumed by the predator, this does not mean it is suitable prey for survival and development of the predator. For a prey type to be optimal, it must: 1) coincide in time

and space with the predator, 2) be easily recognized and captured by the predator, and 3) enable adequate predator reproduction and development (Canard et al. 1984).

*Chrysoperla rufilabris* larvae have been observed on crucifers infested by *M. ochroloma* (R. D. Cave, personal communication), and laboratory bioassays confirmed that they feed on larvae and eggs of the pest (Montemayor and Cave 2009). But no information is available about the suitability of yellowmargined leaf beetle eggs and larvae as nutritional resources for the predator's development.

My overall research goal was to understand further the effectiveness of *C. rufilabris* as an applied biological control agent for the management of the yellowmargined leaf beetle in organic farms. My specific objectives were to:

1. Quantify the development time and predation rate of *C. rufilabris* larvae feeding on eggs and larvae of *M. ochroloma* at four different temperatures.
2. Evaluate the prey selection preference of *C. rufilabris* when exposed to immature stages (eggs and larvae) of *M. ochroloma* and the green peach aphid, *Myzus persicae* Sulzer.
3. Evaluate the performance of *C. rufilabris* feeding on *M. ochroloma* larvae under field conditions on an organic farm.

## CHAPTER 2 LITERATURE REVIEW

### ***Microtheca ochroloma* Stål (Coleoptera: Chrysomelidae)**

#### **Distribution, Biology, and Ecology**

*Microtheca ochroloma*, commonly known as the yellowmargined leaf beetle (Woodruff 1974), is a non-indigenous pest that feeds on crucifers (Chamberlin and Tippins 1948, Oliver and Chapin 1983). It was reported for the first time in the United States in 1945 (Chamberlin and Tippins 1948) and has become established in Texas, Louisiana, Mississippi, Alabama, Georgia, North Carolina, California, and Florida (Chamberlin and Tippins 1948, Balsbaugh 1978, Oliver and Chapin 1983, Staines 1999, Guillebeau 2001, Gilbert et al. 2011). It also has been reported as a serious pest in Brazil, Argentina (where it is native), and Uruguay (Chamberlin and Tippins 1948).

The adult stage of this beetle is characterized by its small size (about 5 mm long) and its black or dark brown color with a conspicuous, pale yellow or white border and four rows of punctures on the elytra (Chamberlin and Tippins 1948, Woodruff 1974) (Fig. 2-1d). The female lays elongate, bright orange eggs, singly or in small clutches, on the soil or under fallen leaves (Woodruff 1974, Bowers 2003) (Fig. 2-1a). The larvae vary from gray to yellow-brown, are covered with fine hairs, and have a dark, sclerotized head capsule (Woodruff 1974, Fasulo 2005) (Fig. 2-1b). The yellowmargined leaf beetle goes through four instars (Ameen and Story 1997a). After the last instar, the insect ceases to feed and spins a pupal cocoon of a fibrous material that is excreted through the anal opening (Oliver and Chapin 1983). The pupal cocoon is spun on the undersides of dry leaves (Woodruff 1974, Fasulo 2005) (Fig. 2-1c). The pharate adult remains in the cocoon until its cuticle becomes sclerotized and fully pigmented (Oliver and Chapin

1983). Males are smaller than females and their posterior abdominal segment is decurved. For more precise sex recognition, the presence of two knob-like structures on the last abdominal segment can be observed in females during the pupal stage (Oliver and Chapin 1983, Ameen 1996). Copulation occurs 5-6 days after emergence (Oliver and Chapin 1983, Fasulo 2005). Ameen and Story (2007) determined that the life cycle from egg to adult lasts about 27 days at 20°C, with an average of 7.8 days for eggs, 10 days for larvae, three days for prepupae, and 5.6 days for pupae.

*Microtheca ochroloma* is an important pest of crucifers, including cabbage (*Brassica oleracea* L. var. *capitata*), napa cabbage (*B. pekinensis* Lour.), collard (*B. oleracea* var. *acephala*), mustard (*B. juncea* Cosson), radish (*Raphanus sativus* L.), turnips (*B. rapa* L.), mizuna, mibuna (*B. rapa* L. subsp. *Japonica*, Japanese leafy vegetables), and watercress (*Nasturtium officinale* L.) (Chamberlin and Tippins 1948, Woodruff 1974, Ameen and Story 1997b, Bowers 2003, Balusu and Fadamiro 2011). Although the yellowmargined leaf beetle feeds on a wide variety of crucifers, it shows a preference for turnips over other crucifers (Chamberlin and Tippins 1948, Ameen and Story 1997b, Balusu and Fadamiro 2011).

Plants of the family Brassicaceae contain secondary metabolites called glucosinolates (Kjær 1981, Mithen 2001). Glucosinolates are hydrolyzed by the enzyme myrosinase to produce different compounds that may serve as a defense against bacteria, fungi, and insects, or as attractants for crucifer-specialist pests (Sutherland 1977, Fenwick et al. 1982). Hopkins et al. (2008) reviewed the role of glucosinolates in multitrophic interactions. Many factors affect the quantity and quality of glucosinolates among and within plants, including variety, climate, cultivation conditions, and plant tissue (Fenwick et al. 1982). As a result, the products formed through hydrolysis of

glucosinolates differ among plants, which is the case of differences in the volatile profiles of turnips compared to cabbage and collards (Balusu and Fadamiro 2011). According to Balusu and Fadamiro (2011), *M. ochroloma* preference for turnips is mediated by plant volatiles; preliminary studies suggest isothiocyanate as the attractant.

Both larvae and adults of *M. ochroloma* consume foliage (Chamberlin and Tippins 1948, Ameen and Story 1997b). Feeding injury consists of holes chewed in the leaf; larvae produce large holes in foliage because they feed collectively (Chamberlin and Tippins 1948, Woodruff 1974).

*Microtheca ochroloma* is considered a cool season pest (Ameen 1996). In Florida, this beetle is active from October to May (Bowers 2003, Balusu 2011). Aestival behavior has been reported for the yellowmargined leaf beetle during hot weather seasons (Chamberlin and Tippins 1948, Oliver and Chapin 1983, Bowers 2003). During the fall, *M. ochroloma* adults migrate from wild mustard plants, considered their summer aestivation sites, to cruciferous crops (Balusu 2011). According to Bowers (2003), *M. ochroloma* resumes feeding and oviposition when favorable environmental and resource conditions are present, which indicates that it undergoes quiescence rather than a true diapause. Laboratory experiments showed that at 30°C food consumption and survivorship of immature *M. ochroloma* decrease, which may be due to aestivation (Manrique et al. 2012).

### **Control Methods**

The application of synthetic insecticides has been the main control option for this pest (Menezes Jr. et al. 2005). However, the use of synthetic foliar insecticides is not allowed in organic production (Bowers 2003, Ferguson 2004). Various biopesticides and botanical insecticides approved by the Organic Material Review Institute (OMRI) have

been evaluated against *M. ochroloma*. According to Balusu and Fadamiro (2012), Entrust® WP and PyGanic® applied in the field were the most effective formulations against larvae and adults, causing 100% mortality within 24 h. In general, the entomopathogenic formulations showed slow activity and the maximum mortality obtained was 50%. Plant extracts, such as pó-de-fumo (*Nicotiana tabacum* L., Solanaceae), ramo de cinamomo (*Melia azedarach* L., Meliaceae), and DalNeem (a commercial product extracted from *Azadirachta indica* A. Juss, [Meliaceae]), have been shown to cause high mortality of yellowmargined leaf beetle larvae and adults (Dequech et al. 2008).

Cultural control strategies for pest management in crops involve implementation of modified standard agricultural practices to prevent pest problems. Two cultural methods, intercropping and the use of straw mulch, have been evaluated to reduce the impact of *M. ochroloma* on crucifers. Bowers (2003) tested an intercropping system using mizuna (*B. rapa*, var. Kyona) and oak leaf lettuce (*Lactuca sativa* L. var. Berenice), but it did not prevent beetle colonization of host plants. Similarly, Manrique et al. (2010) evaluated the use of straw mulch to reduce the abundance of *M. ochroloma* in turnips. However, an opposite effect was observed, with a higher population density of the pest in plots with straw mulch.

There are no reports of host-specific natural enemies of *M. ochroloma*. However, in Florida the indigenous generalist predators *P. maculiventris* (Say) (Hemiptera: Pentatomidae), *C. rufilabris* (Burmeister) (Neuroptera: Chrysopidae), and *Hippodamia convergens* (Say) (Coleoptera: Coccinellidae) have been observed preying on larvae, adults and pupae of the yellowmargined leaf beetle (Montemayor and Cave 2009). *Stiretrus decastigmus* Herrich-Schaeffer (Hemiptera: Pentatomidae) and *Toxomerus*

*duplicatus* Wiedemann (Diptera: Syrphidae) have been reported preying on adults and larvae of *M. ochroloma* in Brazil (Poncio et al. 2010, Sturza et al. 2011).

*Podisus maculiventris* is able to complete its development by feeding on eggs and larvae of *M. ochroloma* (Montemayor and Cave 2011). Developmental time, consumption rate, and effectiveness of this predator as a biological control agent of *M. ochroloma* were evaluated in laboratory and field-cage experiments. The results showed that *P. maculiventris* might play an important role in the management of this pest in organic farms (Montemayor and Cave 2011).

### ***Chrysoperla rufilabris* Burmeister (Neuroptera: Chrysopidae)**

#### **Distribution and Biology**

Commonly known as the green lacewing, *C. rufilabris* is a cosmopolitan, generalist predator that is commonly found in man-altered ecosystems (New 1975, Tauber and Tauber 1983). It is abundant in the southeastern United States and Mexico where it is adapted to humid conditions and considered a late-season (late July and August) species (Dinkins et al. 1970, Tauber and Tauber 1983).

*Chrysoperla rufilabris* lays eggs that are oval and posted on a slender, pliable stalk glued to the substrate (Canard et al. 1984) (Fig. 2-2a). The larva is campodeiform with setae along the body, and the head is strongly chitinized with curved mandibles and maxillae that protrude forwards, forming a channel for passage of food (Fig. 2-2b) (Canard et al. 1984). The green lacewing larva goes through three instars. Two molts occur during the active period and the last molt occurs inside an ovoid cocoon made by numerous layers of silk which protect the pupa (Fig. 2-2c). The green lacewing pharate adult undergoes a final molt within minutes or hours after emerging from the cocoon (Duelli 1984). After emergence, the adult takes flight, irrespective of the presence of

food. Apparently, this first flight is required to induce oviposition, therefore few females lay eggs in the habitat in which they emerge (Canard et al. 1984, Duelli 1984, Ventura et al. 2007). The adults are light green with large and broadly oval wings that have a rich and regular venation (Canard et al. 1984). The antennae are long, filiform, and multisegmented. The head has no ocelli, but has golden and prominent compound eyes. Adults are not predaceous; they feed on honeydew or nectar and receive essential amino acids from mutualistic yeasts (Burke and Martin 1956).

Time of development of *C. rufilabris* from hatching to adult emergence ranged from 16 to 27 days at temperatures between 24°C to 30°C when *Aphis gossypii* Glover was its prey (Burke and Martin 1956). According to Butler and Ritchie (1970), the closely related species *Chrysoperla carnea* (Stephens) is not able to reach adulthood at a constant temperature of 15°C and the duration of the immature stages takes 40 days, whereas at 25°C it takes approximately 24 days to develop from egg to adult.

### ***Chrysoperla rufilabris* as a Biological Control Agent**

Green lacewing larvae feed on a diversity of prey, principally small, soft-bodied arthropods (Tauber et al. 2000). This predator is considered an excellent aphid killer (Hydorn and Whitcomb 1979, Canard et al. 1984, Dean and Schuster 1995, Chen and Liu 2001, Knutson and Tedders 2002); however, it has been evaluated as a biological control agent against other economically important pests, including many species of whiteflies (Legaspi et al. 1994, Dean and Schuster 1995), moths (Lingren et al. 1968, Hydorn and Whitcomb 1979), leafhoppers (Daanel and Yokota 1997), and mites (Canard et al. 1984).

Montemayor and Cave (2009) reported *C. rufilabris* feeding on eggs and larvae of *M. ochroloma*. However, the efficacy of this prey for green lacewing development is

unknown. Two species of green lacewing, *C. rufilabris* and *C. carnea*, have been documented feeding on other beetle eggs and young larvae (Nordlund 1991, Hough-Goldstein et al. 1993, Sablon et al. 2013). *Chrysoperla rufilabris* larvae preyed on and killed *L. decemlineata* eggs and larvae, and they reduced the beetle population by 99.7% and 97.9% when 5 and 10 larvae/cage were released, respectively (Nordlund 1991). The predator also reduced the pest's population on average by 84% in field experiments (Nordlund 1991). Laboratory experiments showed that all stages of *C. carnea* consumed mainly eggs and first and second instars of *L. decemlineata* (Sablon et al. 2013). The green lacewing third instar killed four times more prey than the first two instars, and the prey handling time increased as prey size increased. No studies have been done to determine if *C. carnea* is able to complete its life cycle feeding only on *L. decemlineata*.

Hydorn and Whitcomb (1979) found that using *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) to rear *C. rufilabris* significantly reduced the predator's survival to maturity and fecundity, and the length of the larval stage was prolonged.

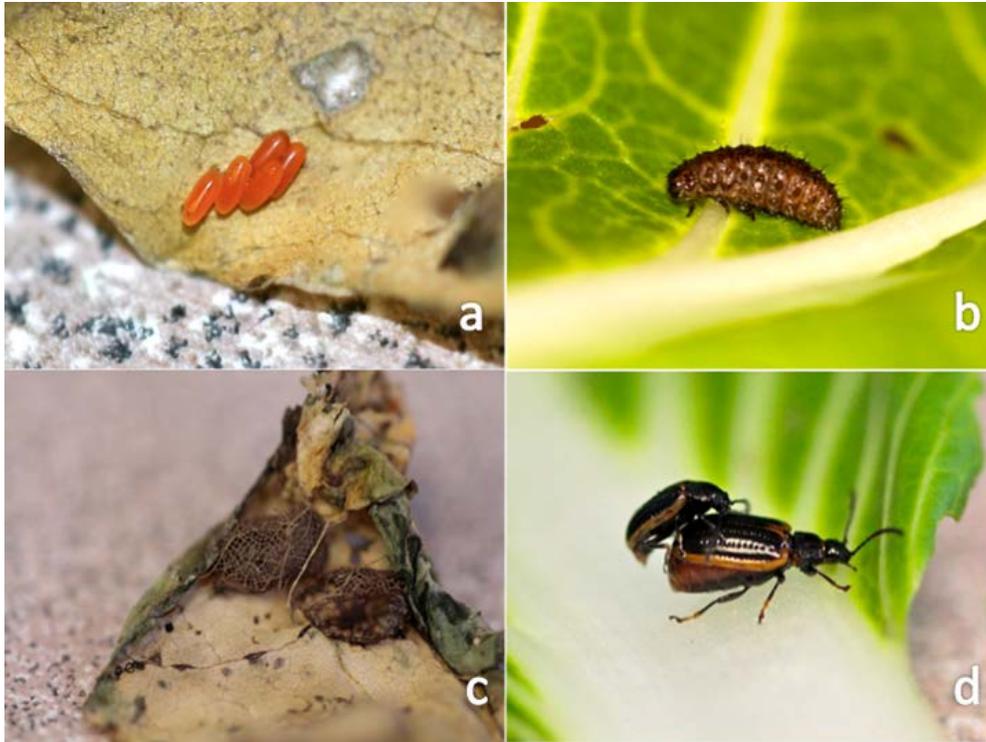


Figure 2-1. Developmental stages of *Microtheca ochroloma*: (a) eggs, (b) larvae, (c) pupae, and (d) adults. Photos by Angie Niño.

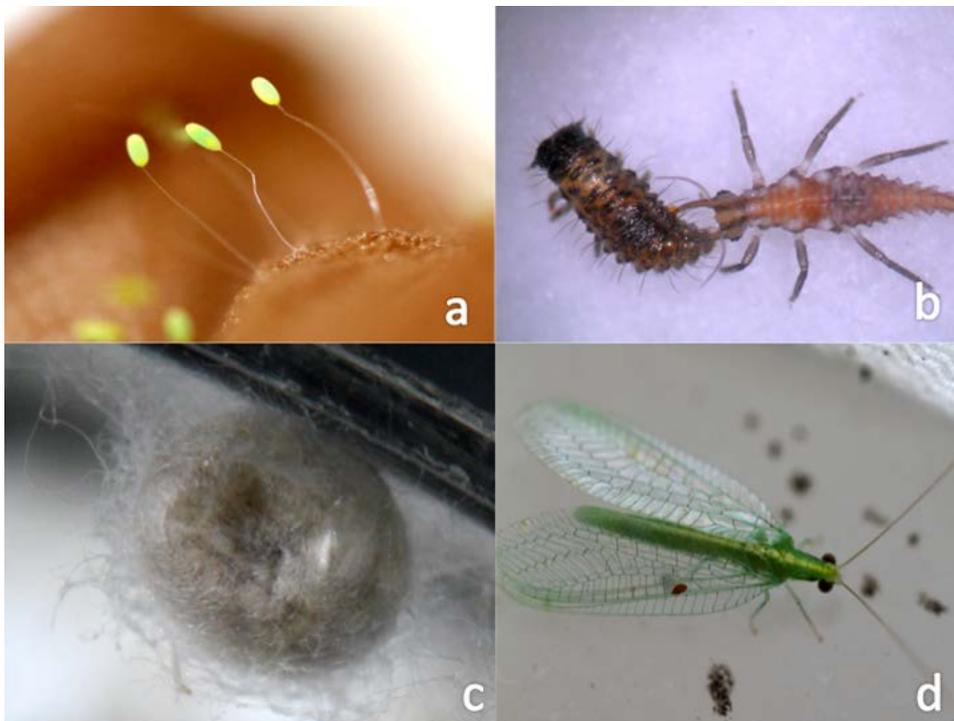


Figure 2-2. Developmental stages of *Chrysoperla rufilabris*: (a) eggs, (b) larvae, (c) pupae, and (d) adults. Photos by Angie Niño.

CHAPTER 3  
DEVELOPMENTAL TIME AND KILLING RATE OF *CHRYSOPERLA RUFILABRIS*  
(BURMEISTER) (NEUROPTERA: CHRYSOPIDAE) FED *MICROTHERCA OCHROLOMA*  
(STÅL) (COLEOPTERA: CHRYSOMELIDAE)

**Introduction**

The organic food industry demands the development of pest control strategies that can be adopted by farmers. Since synthetic pesticides are not an alternative for organic growers, it is necessary to originate methodologies that incorporate ecological approaches for the management of pest problems (Ferguson 2004, Zehnder et al. 2007). The production of crucifers in organic farms has been highly affected by the presence of the yellowmargined leaf beetle, *M. ochroloma* Stål (Webb 2010). This invasive species, native to Argentina, was first detected in the United States in 1945 (Chamberlin and Tippins 1948) and is now established in several states. *Microtheca ochroloma* feeds on leaves of plants of the family Brassicaceae, including turnips, mustard, and Chinese cabbage among others (Chamberlin and Tippins 1948, Woodruff 1974, Ameen and Story 1997b, Bowers 2003, Balusu and Fadamiro 2011). The insect is active in late fall, winter, and early spring (Ameen 1996, Bowers 2003), coinciding with the time of crucifer production in Florida. Restrictions on the use of synthetic foliar insecticides and the lack of effective host-specific natural enemies in Florida leave organic growers with few alternatives to reduce the economic impact of this pest in their crops.

In search for a more eco-friendly alternative, Montemayor and Cave (2009) examined crucifer plants in organic farms to find native natural enemies that could be used against *M. ochroloma*. A variety of generalist predators were observed preying on the beetle, including *P. maculiventris* Say (Hemiptera: Pentatomidae), *H. convergens*

Say (Coleoptera: Coccinellidae), and *C. rufilabris* Burmeister (Neuroptera: Chrysopidae). My study evaluates the potential of *C. rufilabris* as a control agent against *M. ochroloma*. *Chrysoperla rufilabris*, commonly known as the green lacewing, because it has been used in pest control programs involving augmentative releases (Ridgway and Jones 1968, New 1975), is mass-produced by commercial insectaries, and has been successfully used against other foliage-feeding pests (Nordlund 1991).

The green lacewing larva, which is the predacious stage, feeds on a diversity of prey, principally small, soft-bodied arthropods (Tauber et al. 2000). Laboratory bioassays confirmed that *C. rufilabris* feeds on larvae and eggs of *M. ochroloma* (Montemayor and Cave 2009). However, Obrycki et al. (1989) point out that even if a prey is consumed, it is not evidence of its suitability for the survival and development of the predator. An optimal prey type must coexist in time and space with the predator, be easily recognized, captured, and accepted, and enable adequate predator reproduction and development (Canard et al. 1984). No information is available about the suitability of yellowmargined leaf beetle as a nutritional resource for the development of *C. rufilabris*. Therefore, the objective of this study was to quantify the developmental time and killing rate of *C. rufilabris* larvae when eggs and first instars of *M. ochroloma* were offered at four constant temperatures.

## **Materials and Methods**

### **Plant Material**

Turnip and bok choy (*Brassica rapa* L.) were used as host plants to rear *M. ochroloma*. Seeds were planted in 72-cell trays containing sterilized soil mix (Fafard super fine germinating mix). Two-week-old seedlings were transplanted into 3.8-L plastic pots containing a mixture of soil and fertilizer (Osmocote Classic® 14N, 14P, 14K).

## **Stock Colonies**

Adults and larvae of *M. ochroloma* were collected in the field and transported to the Biological Control Research and Containment Laboratory at the Indian River Research and Educational Center in Ft. Pierce. The insects were confined in Bug Dorms (60 × 60 × 60 cm) with two host plants which were changed twice weekly. To obtain eggs and first instars, 20 pairs of males and females were collected from the colony and placed in a plastic box (18 × 13.5 × 9 cm) with a screen mesh cloth in the lid for ventilation. *Microtheca ochroloma* females oviposited the eggs on the edges of white paper towels or Kimwipes (Kimberly-Clark®) which were collected every two days. Turnip or bok choy leaves were offered as food.

Adults of *C. rufilabris* were purchased from Rincon-Vitova Insectaries, Inc. (Ventura, CA). The company packed the adults in a cylindrical container with available food and a piece of moistened sponge and placed in a cooler. The number of adults alive was 4 times the quantity expected. After arrival, 30 pairs were placed in plastic containers and fed an artificial diet. The eggs collected from the colony were placed singly in plastic vials and stored in an environmentally controlled chamber at 25°C, 75% RH, and 12L:12D photoperiod until emergence.

## **Experimental Design**

An individual larva of *C. rufilabris* was housed in a plastic Petri dish (5.5-cm diameter) with a hole (2.5-cm diameter) in the top covered by screen mesh cloth. Each Petri dish contained a piece of moistened, white filter paper placed at the bottom to maintain appropriate humidity. A piece of bok choy leaf (3 cm<sup>2</sup>) was also provided as a food source or substrate for the prey and changed daily.

Eggs or first instars of *M. ochroloma* were offered *ad libitum* to each instar of *C. rufilabris*. Natural mortality of the prey was recorded by comparison with the control treatment, which consisted of a fixed number of prey in a Petri dish without the predator. In all treatments, dead prey were replaced daily. Prey mortality in the treatments with a predator was corrected by the mortality in the controls (Abbott 1925). Treatments at each of the three test temperatures had 20 replicates.

Killing rate was recorded as the number of prey killed per instar per day, total number of prey killed per instar, and total number of prey killed per larva. Developmental time for the predator was measured in number of days to complete development from egg hatch to adulthood. Mortality of the predators was also recorded.

### **Statistical Analyses**

Data on killing rate and development time was analyzed using analysis of variance (ANOVA). Treatment means were compared using the Tukey- Kramer HSD test. Mortality was analyzed using a Chi-square test. The level of association for mortality and temperature was based on the Pearson coefficient. JMP 8 (SAS Institute Inc. 2008) with a significance level of 5% was used for all statistical analyses.

### **Results**

*Chrysoperla rufilabris* larvae were able to complete their development at 15, 20, and 25°C, but not at 10°C. First instars at 10°C lived on average  $39 \pm 6.8$  d. Therefore, no results for killing rate and developmental time were obtained at this low temperature.

### **Developmental Time**

The number of days spent by *C. rufilabris* in each stadium and stage when fed *M. ochroloma* eggs decreased as temperature increased (Table 3-1). The time spent to develop from egg hatch to adult emergence decreased from 75 d at 15°C to about 27 d

at 25°C. Mean developmental times of all instars and the pupa at 15°C were significantly longer than at the other two temperatures ( $F=199.99$ ;  $df=2,48$ ;  $P<0.0001$  for first instar,  $F=191.43$ ;  $df=2,48$ ;  $P<0.0001$  for second instar,  $F=64.29$ ;  $df=2,42$ ;  $P<0.001$  for third instar,  $F=165.13$ ;  $df=2,27$ ;  $P<0.0001$  for pupae,  $F=371.35$ ;  $df=2,27$ ;  $P<0.0001$  for total development time). No significant difference was detected for third instar developmental time at 20 and 25°C. For the first and second instars and the pupa, the predator took about 1.3-1.4 times longer to develop at 20°C than at 25°C.

The number of days from egg hatch to adult emerge of *C. rufilabris* larvae fed *M. ochroloma* first instars also decreased as temperature increased, but *C. rufilabris* larvae held at 15°C developed more rapidly on first instars than on eggs of *M. ochroloma* (Table 3-2). The number of days spent in each instar and the pupal stage varied significantly among temperatures ( $F=146.20$ ;  $df=2,57$ ;  $P<0.0001$  for first instar,  $F=136.05$ ;  $df=2,49$ ;  $P<0.0001$  for second instar,  $F=13.96$ ;  $df=2,41$ ,  $P<0.0001$  for third instar,  $F=237.74$ ;  $df=2,23$ ;  $P<0.0001$  for pupae,  $F=183.79$ ,  $df=2,23$ ,  $P<0.0001$  for total development). There was no significant difference in developmental time of third instars between 20°C and 25°C. For the other stages, the predator took 70% longer to develop from egg hatch to adult stage at 15°C than at 20°C, which was 48% longer than at 25°C. A significant difference was detected for time of development between larvae fed with eggs and larvae fed with first-instar *M. ochroloma* at all three temperatures ( $F=133.12$ ;  $df=1,3$ ;  $P=0.0014$  at 15°C,  $F=7.8$ ;  $df=1,20$ ;  $P=0.011$  at 20°C,  $F=27.66$ ;  $df=1,27$ ;  $P<0.0001$ ). Mean developmental time of *C. rufilabris* fed eggs at 15°C was 40% longer than the mean developmental time of the predators fed larvae. At 20 and 25°C, the mean developmental time of the predators fed eggs was 9% and 24% longer, respectively, than it was for predators fed larvae.

## **Mortality**

The number of individuals of *C. rufilabris* that completed development feeding on eggs and first instars of *M. ochroloma* was reduced at the lowest temperature (Fig. 3-1). The Pearson coefficient of association showed a relationship between mortality and temperature ( $X^2=19.6$ ;  $df=2$ ;  $P<0.0001$  for *C. rufilabris* fed on eggs;  $X^2=12.35$ ;  $df=2$ ;  $P=0.0021$  for *C. rufilabris* fed on larvae). The highest mortality occurred during the pupal stage for all temperatures regardless the type of prey offered. When *C. rufilabris* was fed *M. ochroloma* eggs, only 10% of the initial cohort reached adulthood at 15°C, whereas at 20 and 25°C, 65% and 75% survived, respectively (Fig. 3-1a).

For *C. rufilabris* fed *M. ochroloma* first instar, the highest mortality occurred at 15°C, at which only 15% of individuals pupated whereas 45% and 70% reached pupal stage at 20 and 25°C, respectively (Fig 3-1b). For those individuals that emerged from the pupa, only two at 15°C, none at 20°C, and two at 25°C became adults; all other individuals died as pharate adults (Fig. 3-2).

## **Killing Rate**

The number of *M. ochroloma* eggs killed per day by *C. rufilabris* larvae increased as the temperature and instar increased (Table 3-3). One-way ANOVA detected significant differences among temperatures for the mean numbers of prey killed per day per instar ( $F= 21.79$ ;  $df= 2,49$ ;  $P<0.0001$  for first instar;  $F=12.99$ ;  $df=2,46$ ;  $P<0.0001$  for second instar;  $F=15.62$ ;  $df=2,41$ ;  $P<0.0001$  for third instar). The mean daily killing rates of the second and third instar at 20 and 25°C were not significantly different, but they were significantly higher than the mean daily killing rate of both stages at 15°C. First-instar predators killed on average about 3-4 prey eggs per day at 20 and 25°C, but less than three per day at only 15°C. Second instar predators killed on average about 10-11

prey eggs at the two highest temperatures, but no more than six per day at 15°C. Third instar predators killed on average about 66 prey eggs per day at 20 and 25°C but less than 32 per day at only 15°C.

The number of *M. ochroloma* first instars killed per day by *C. rufilabris* larvae increased as the temperature and instar increased (Table 3-4). Significant differences for mean daily killing rate of first-instar prey per predator instar were found among temperatures ( $F= 18.59$ ;  $df=2, 57$ ;  $P<0.0001$  for first instar,  $F=13.34$ ;  $df= 2,49$ ;  $P<0.0001$  for second instar,  $F=24.97$ ;  $df=2,41$ ;  $P<0.0001$  for third instar). The mean daily killing rate of first-instar prey by second instar predators did not differ between 20 and 25°C. However, the mean number of prey killed per day by predator larvae held at those two temperatures was significantly greater than at 15°C. The number of prey killed per day increased with temperature; the predator killed about two times more prey per day at 25°C than at 15°C.

The total number of *M. ochroloma* eggs killed per *C. rufilabris* larvae at 15°C, 20°C and 25°C decreased as the temperature increased but increased from first to third instar (Table 3-5). The mean total number of prey eggs killed per instar and per larva was significantly different among temperatures ( $F=20.96$ ;  $df=2,49$ ;  $P<0.0001$  for first instar,  $F=8.18$ ;  $df=2,46$ ;  $P<0.0001$  for second instar,  $F=4.99$ ;  $df=2,46$ ;  $P=0.0113$  for third instar,  $F=4.86$ ;  $df=2,42$ ;  $P=0.0127$  for larval stage). The number of prey eggs killed at 20°C did not differ significantly from the number killed at 25°C. However, the total killing rate per instar and per larva was significantly higher at 15°C compared to 25°C. First, second, and third instar predators killed, on average, 35, 65, and 574 prey eggs, respectively, at 15°C, whereas they killed, on average, about 20, 47, and 471 prey eggs,

respectively, at 20 and 25°C. *Chrysoperla rufilabris* killed, on average, about 90 more prey eggs at 15°C than at 20 and 25°C during its larval stages.

The mean total number of prey killed per third instar predator and per larva for all three instars was not significantly different among temperatures (Table 3-6). Significant differences were detected for first and second instar predators among temperatures ( $F=4.58$ ;  $df=2,55$   $P=0.0144$  for first instar,  $F=3.31$ ;  $df=2,48$ ;  $P=0.0450$  for second instar). The mean total number of prey killed for both early predator instars was not significantly different between 15 and 20°C and 20 and 25°C. However, the total number of prey killed at 15°C was about 1.3 times higher than at 25°C for first and second instar predators. The total number of prey killed per *C. rufilabris* larva at all three temperatures ranged from 218 to 225.

## Discussion

One of the first steps in a biological control program is evaluation of the suitability of the target pest as prey for the natural enemy to be released. It is important to know the number of prey that the predator is able to consume or kill and the effect of the prey on the development and survival of the predator. Developmental time of *C. rufilabris* was significantly affected by temperature. The number of days spent in each stadium and the total time from egg hatch to adult emergence decreased at higher temperatures (Tables 3-1 and 3-2). For the most part, an insect's body temperature varies with environmental temperature, when it increases the metabolism of the insect speeds up, and, consequently, the rate of development also increases, causing a reduction in the length of each stage (Jervis and Kidd 1996).

Developmental time can also be affected by the quality of the larval food (Canard et al. 1984). *Chrysoperla rufilabris* spent more time developing from egg hatch to adult

when it fed on eggs than it did when it fed on first instars of *M. ochroloma*. Montemayor and Cave (2011) found that the developmental time of *P. maculiventris* was significantly shorter when the predator fed on fourth-instar *M. ochroloma* than when it fed on eggs. *Chrysoperla rufilabris* development was significantly prolonged when it was fed with *T. castaneum* pupae and prepupae than when fed eggs and larvae of the potato tuber moth, *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae) (Hydorn and Whitcomb 1979). Legaspi et al. (1994) found that developmental time of *C. rufilabris* larvae feeding on the sweetpotato whitefly, *Bemisa tabaci* (Gennadius) (Hemiptera: Aleyrodidae), was longer than for those larvae feeding on lepidopteran prey.

Temperature also influences the survival of the predator. *Chrysoperla rufilabris* was not able to complete development at 10°C when feeding on eggs of *M. ochroloma*. According to Honěk and Kocourek (1988), the lower temperature thresholds for eggs of different species of Chrysopidae range from 6.1 to 10°C with an average of  $8.9 \pm 1.4^\circ\text{C}$ , so the fact that *C. rufilabris* larvae died at 10°C indicates that the lower temperature threshold for larvae might be close to that value. My results showed that the survival of the predator is reduced at lower temperatures.

The quality of prey also plays an important role in the predator's survival. Some prey might be nutritionally inadequate, resulting in high mortality or abnormal development of the predator (Canard et al. 1984). At 25°C, 75% of the predators fed eggs of *M. ochroloma* reached the pupal stage and all of them reached adulthood. In the case of the predators fed with first-instar *M. ochroloma*, 70% pupated, of which only 13% became adults. When *C. rufilabris* larvae were fed the aphids *M. persicae* and *A. gossypii*, 100% of individuals survived to adulthood, whereas all predators fed *Lipaphis erysimi* Kaltenbach (Hemiptera: Aphididae) died before adult emergence (Chen and Liu

2001). *Chrysoperla rufilabris* larvae provided with eggs and larvae of *B. tabaci* never pupated (Legaspi et al. 1994), and the survival of the predator was significantly less for individuals reared on *T. castaneum* than for those given any other prey (Hydorn and Whitcomb 1979).

The quality of a prey can be highly influenced by their diet. Specialized herbivores develop many adaptations that allow them to resist, metabolize, or assimilate secondary plant substances (Francis et al. 2001). These adaptations vary from chemical modification of toxins into a nontoxic compounds to sequestration of toxins as defense substance against natural enemies. Plants of the family Brassicaceae are known to synthesize glucosinolates as secondary metabolites (Kjær 1981, Fenwick et al. 1982, Fahey et al. 2001). The degradation of glucosinolates by an enzyme present in specialized plant cells leads to the production of different compounds that act as info-chemicals in plant-insect interactions and can also have toxic effects against insects or fungi (Hopkins et al. 2009). It is unknown if *M. ochroloma* is able to sequester glucosinolates from its host plant and use them as defensive compound. However, it might explain the higher mortality and inability of the predator to develop when fed *M. ochroloma* first instars. Francis et al. (2001) reported that *Adalia bipunctata* L. (Coleoptera: Coccinellidae) was unable to reach the adult stage and higher mortality was observed when fed *Brevicoryne brassicae* L. (Hemiptera: Aphididae) compared to individuals fed a non-specialist aphid reared on two mustard species. Mortality was related to the presence of isothiocyanates, a product of glucosinolate degradation.

*Chrysoperla rufilabris* readily consumed eggs and first instars of *M. ochroloma*. The number of prey killed per day increased with temperature; the predator consumed twice as many prey at 25°C as it did at 15°C (Tables 3-3 and 3-4). A similar pattern was

reported for the generalist predator *P. maculiventris* (Montemayor and Cave 2011). The rate at which food passes through the gut is affected by temperature (Jervis and Kidd 1996). At high temperatures, food is processed faster and more prey are needed to fill the gut. An empty gut directly affects the predator's hunger, forcing it to consume more food, which translates to killing more prey.

When the total number of prey killed was compared among temperatures, the predator killed more eggs per instar and larval stage at 15°C than at 20 and 25°C, which can be attributed to the longer time required by the predator to complete development at the lowest temperature (Tables 3-5 and 3-6). Montemayor and Cave (2011) observed similar results for the total number of prey killed by *P. maculiventris*; the predator killed a more *M. ochroloma* larvae at 15°C than at 20°C. *Podisus maculiventris* killed 50% more eggs of *M. ochroloma* during its developmental period at 25°C than did *C. rufilabris*. One possible explanation for this difference between the two predators is the dissimilarity in the life history phases of these two predators. Despite having similar total developmental times (27 d for *C. rufilabris*, 23 d for *P. maculiventris*), *P. maculiventris* goes through five instars and molts directly to an adult without going through a pupal stage, whereas *C. rufilabris* only goes through three instars and then molts to a pupal stage in which it ceases feeding. Therefore, I conclude that *P. maculiventris* has greater potential as a biological control agent for *M. ochroloma* eggs and larvae than does *C. rufilabris*.

Similar results were found for first and second instars of *C. rufilabris* fed first-instar *M. ochroloma*; however, for the last instar and the total killing rate for larvae, the average total number of prey killed did not differ among temperatures (Table 3-6).

The inability of *C. rufilabris* to complete development when fed first-instar *M. ochroloma* suggests that the prey might be nutritionally inadequate. A diet of *M.*

*ochroloma* eggs allowed the predator to reach adulthood, which, along with the high number of eggs that the predator killed, suggests that the predator might have potential to control the egg population of *M. ochroloma* in crucifer crops. However, it is necessary to determine if the predator will search for and consume eggs under natural conditions. It is also recommended to evaluate the effect on the biology and survival of the predator when it is fed mixed diets, including eggs and larvae of *M. ochroloma* and other soft-bodied arthropods that it might encounter on crucifers. The best seasons to release this predator in Florida for the control of *M. ochroloma* are late fall and early summer when temperatures are warmer. Thus, performance of the predator should be evaluated at fluctuating temperatures and under field conditions.

Table 3-1. Mean ( $\pm$  SE) number of days for development of *Chrysoperla rufilabris* larvae feeding on *Microtheca ochroloma* eggs at three constant temperatures. Number within parentheses equals sample size.

<i>Chrysoperla rufilabris</i> stage					
Temperature (°C)	1st	2nd	3rd	Pupa	Total
15	15.5 $\pm$ 0.5 (14) a	10.5 $\pm$ 0.3 (14) a	15.9 $\pm$ 0.7 (11) a	36.0 $\pm$ 1.3 (2) a	75.5 $\pm$ 1.7 (2) a
20	5.7 $\pm$ 0.4 (19) b	5.2 $\pm$ 0.2 (18) b	7.6 $\pm$ 0.6 (16) b	16.8 $\pm$ 0.5 (13) b	34.7 $\pm$ 0.7 (13) b
25	4.3 $\pm$ 0.4 (19) c	3.9 $\pm$ 0.2 (18) c	6.1 $\pm$ 0.5 (18) b	11.6 $\pm$ 0.5 (15) c	26.6 $\pm$ 0.6 (15) c

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

Table 3-2. Mean ( $\pm$  SE) number of days for development of *Chrysoperla rufilabris* larvae feeding on first-instar *Microtheca ochroloma* at three constant temperatures. Number within parentheses equals sample size.

<i>Chrysoperla rufilabris</i> stage					
Temperature (°C)	1st	2nd	3rd	Pupae	Total
15	10.2 $\pm$ 0.3 (20) a	7.6 $\pm$ 0.2 (15) a	10.2 $\pm$ 0.7 (14) a	29.0 $\pm$ 0.8 (3) a	54.0 $\pm$ 1.6 (3) a
20	5.6 $\pm$ 0.3 (20) b	4.4 $\pm$ 0.2 (17) b	6.8 $\pm$ 0.7 (13) b	14.3 $\pm$ 0.4 (9) b	31.7 $\pm$ 0.9 (9) b
25	3.3 $\pm$ 0.3 (20) c	3.0 $\pm$ 0.2 (20) c	5.4 $\pm$ 0.6 (17) b	10.2 $\pm$ 0.4 (14) c	21.4 $\pm$ 0.7 (14) c

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

Table 3-3. Mean ( $\pm$  SE) number of *Microtheca ochroloma* eggs killed per day per *Chrysoperla rufilabris* larva at three constant temperatures. Number within parentheses equals sample size.

<i>Chrysoperla rufilabris</i> instar			
Temperature (°C)	1st	2nd	3rd
15	2.3 $\pm$ 0.2 (14) a	6.3 $\pm$ 0.9 (12) a	32.3 $\pm$ 5.4 (10) a
20	3.3 $\pm$ 0.2 (19) b	9.7 $\pm$ 0.7 (18) b	66.9 $\pm$ 4.1 (16) b
25	4.2 $\pm$ 0.2 (19) c	12.1 $\pm$ 0.7 (19) b	65.4 $\pm$ 3.9 (18) b

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

Table 3-4. Mean ( $\pm$  SE) number of *Microtheca ochroloma* first-instars killed per day per *Chrysoperla rufilabris* larva at three constant temperatures. Number within parentheses equals sample size.

<i>Chrysoperla rufilabris</i> instar			
Temperature (°C)	1st	2nd	3rd
15	1.3 $\pm$ 0.2 (20) a	3.6 $\pm$ 0.4 (15) a	18.8 $\pm$ 2.4 (14) a
20	2.0 $\pm$ 0.2 (20) b	5.7 $\pm$ 0.4 (17) b	29.4 $\pm$ 2.5 (13) b
25	2.8 $\pm$ 0.2 (20) c	6.4 $\pm$ 0.4 (20) b	41.9 $\pm$ 2.2 (17) c

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

Table 3-5. Mean ( $\pm$  SE) total number of *Microtheca ochroloma* eggs killed per *Chrysoperla rufilabris* larva at three constant temperatures. Number within parentheses equals sample size.

<i>Chrysoperla rufilabris</i> instar				
Temperature (°C)	1st	2nd	3rd	Total
15	35.5 $\pm$ 2.1 (14) a	64.6 $\pm$ 3.9 (12) a	573.8 $\pm$ 39.8 (11) a	633.9 $\pm$ 38.5 (10) a
20	20.5 $\pm$ 1.8 (19) b	49.8 $\pm$ 3.9 (18) b	520.7 $\pm$ 33.0 (16) ab	592.1 $\pm$ 30.5 (16) ab
25	18.8 $\pm$ 1.8 (19) b	44.8 $\pm$ 3.1 (19) b	422.2 $\pm$ 31.1 (18) b	496.1 $\pm$ 28.7 (18) b

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

Table 3-6. Mean ( $\pm$  SE) total number of *Microtheca ochroloma* first-instars killed per *Chrysoperla rufilabris* larva at three constant temperatures. Number within parentheses equals sample size.

<i>Chrysoperla rufilabris</i> instar				
Temperature (°C)	1st	2nd	3rd	Total
15	12.6 $\pm$ 0.8 (18) a	27.5 $\pm$ 2.2 (15) a	178.8 $\pm$ 16.7 (14) a	217.7 $\pm$ 18.3 (14) a
20	11.5 $\pm$ 0.7 (20) ab	23.7 $\pm$ 2.1 (16) ab	195.8 $\pm$ 17.3 (13) a	224.8 $\pm$ 18.9 (13) a
25	9.4 $\pm$ 0.7 (20) b	20.1 $\pm$ 1.9 (20) b	186.3 $\pm$ 16.1 (15) a	218.5 $\pm$ 17.6 (15) a

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

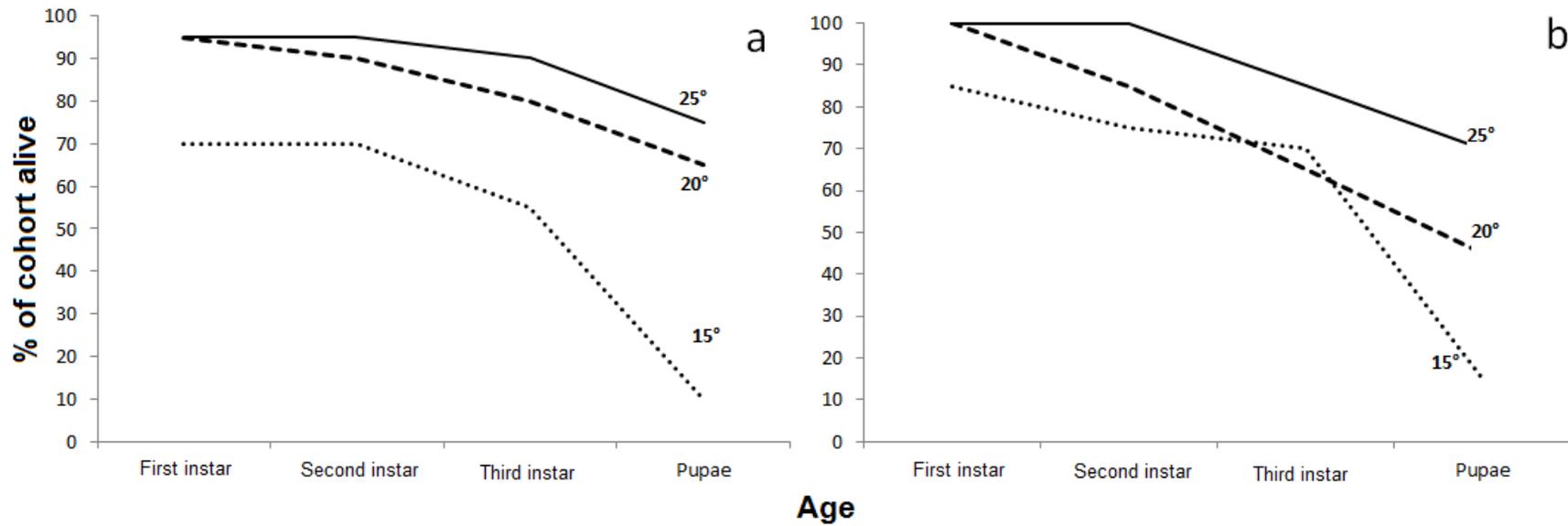


Figure 3-1. Percentage of *Chrysoperla rufilabris* that survived to the next developmental stage at three constant temperatures when fed *M. ochroloma* eggs (a) and first instars (b). Initial cohort consisted of 20 first-instar predators.



Figure 3-2. Dead *Chrysoperla rufilabris* pharate adult after pupal eclosion. Photo by Angie Niño.

CHAPTER 4  
PREY PREFERENCE OF *CHRYSOPERLA RUFILABRIS* (NEUROPTERA:  
CHRYSOPIDAE) BETWEEN IMMATURE STAGES OF *MICROTHECA OCHROLOMA*  
(COLEOPTERA: CHRYSOMELIDAE) AND *MYZUS PERSICAE* (HEMIPTERA:  
APHIDIDAE)

**Introduction**

The production of crucifers on organic farms in the southeastern US is threatened by the presence of the yellowmargined leaf beetle, *Microtheca ochroloma* Stål (Coleoptera: Chrysomelidae). This insect, indigenous to Argentina, was first detected in United States in 1945 (Chamberlin and Tippins 1948) and is now established in several states, including Texas, Louisiana, Mississippi, Alabama, Georgia, North Carolina, California, and Florida. *Microtheca ochroloma* consumes the foliage of plants of the family Brassicaceae (Chamberlin and Tippins 1948, Woodruff 1974, Ameen and Story 1997b, Bowers 2003, Balusu and Fadamiro 2011) and is active during late fall, winter, and early spring (Ameen 1996, Bowers 2003), which coincides with the time of crucifer production in Florida. The use of synthetic insecticides is the only effective method to control this pest (Menezes Jr. et al. 2005). The use of synthetic insecticides is not allowed in organic farming, however, growers require the development of methodologies that incorporate ecological approaches for the management of pest problems (Ferguson 2004, Zehnder et al. 2007). Biological control might have an important role as a potential alternative to control pests in organic farms, but no host-specific natural enemies of this pest have been reported in Florida. Therefore, it is necessary to evaluate the potential of native natural enemies that can be used against *M. ochroloma*.

The green lacewing, *Chrysoperla rufilabris* Burmeister (Neuroptera: Chrysopidae), was observed preying on the *M. ochroloma* on crucifers in organic farms in Florida (Montemayor and Cave 2009). The green lacewing larva, which is the

predacious stage, feeds on small, soft-bodied arthropods (Tauber et al. 2000). Laboratory bioassays confirmed that *C. rufilabris* feeds on eggs and larvae of *M. ochroloma* and that it is able to complete its development feeding on eggs of this pest (see Chapter 3). However, even if a prey enables adequate survival and development of the predator, it must also coexist in time and space with the natural enemy (Canard et al. 1984). Under natural conditions, *M. ochroloma* adults lay eggs at the base of the plant, on the soil, or under fallen leaves, and it is not known if the predator will look for and prey on them in these sites. It is also important to assess the effect that the presence of other insect pests might have on the performance and consumption rate of the predator. *Chrysoperla rufilabris* feeds on a wide variety of insects (Hydorn and Whitcomb 1979, Canard et al. 1984, Nordlund and Morrison 1990, Nordlund 1991, Legaspi et al. 1994, Tauber et al. 2000) but is usually considered to be an important predator of aphids. No information is available about the effect that the presence of aphids might have on the killing rate of immature stages of *M. ochroloma*. Therefore, the objective of this study was to evaluate the prey selection preference of *C. rufilabris* when exposed to immature stages (eggs and larvae) of *M. ochroloma* and the green peach aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae).

## **Materials and Methods**

### **Plant Material**

Turnip and bok choy (*Brassica rapa* L.) were grown as described in Chapter 3. Bok choy plants used in the experiments had six true leaves.

### **Stock Colonies**

Adults and larvae of *M. ochroloma* were collected in the field and used to establish the laboratory colony as described in Chapter 3.

Bok choy plants infested with all stages of *M. persicae* were confined in Bug Dorms (60 x 60 x 60 cm). Dead plants were replaced with new, clean plants when necessary.

Adults of *C. rufilabris* were purchased from Rincon-Vitova Insectaries, Inc. (Ventura, CA) and a colony was established in the laboratory as described in Chapter 3.

## **Experimental Design**

### **Predation on *M. ochroloma* Eggs and Larvae on Host Plants**

A bok choy plant was confined within a white insect rearing sleeve (60 cm wide x 70 cm long) (Fig. 4-1). On the plant, a first-instar *C. rufilabris* was offered one of the following prey items: (1) *M. ochroloma* eggs; (2) *M. ochroloma* eggs and first instars; or (3) *M. ochroloma* first instars. Each treatment had 10 replicates. Treatments with eggs or larvae only had 18 individuals per plant and treatments in which eggs and larvae were offered together had nine individuals of each type per plant. Eggs of *M. ochroloma* were placed at the base of the plant and on the soil around the base of the plant, whereas *M. ochroloma* larvae were placed on the leaf blades. The predator larvae were placed on the leaves of the plants. *Chrysoperla rufilabris* larvae were starved at least 12 hours prior to experimentation, with only water provided via a moistened cotton ball.

All cages were kept in a rearing room at constant 25°C, with 75% RH and 12L:12D photoperiod. The number of prey killed was counted at the end of 48 hours.

### **Prey Preference among *M. ochroloma* Eggs and Larvae and *M. persicae* Nymphs in a Petri Dish**

An individual first instar of *C. rufilabris* was housed in a Petri dish (diameter: 5.5 cm) with a hole in the top sealed with a screen mesh cloth. Predator larvae were starved at least 12 hours before experimentation; water was provided through a

moistened cotton ball. A piece of moistened, white filter paper (5.5-cm diameter) was placed at the bottom of each Petri dish to maintain appropriate humidity. A 3-cm<sup>2</sup> section of bok choy leaf was added as a food source or substrate for the prey.

Four combinations of prey were offered to first-instar *C. rufilabris*: (1) *M. ochroloma* eggs and first instars; (2) *M. ochroloma* eggs and *M. persicae* nymphs; (3) *M. ochroloma* first instars and *M. persicae* nymphs; and (4) all three types of prey together. Another three treatments were included in which only one of the three types of prey was offered to the predator. Natural mortality of the prey was recorded for each combination by holding a fixed number of prey in a Petri dish without the predator. There were seven and three replicates for each treatment with predators and control treatments without predators, respectively. The number of prey offered varied according to the type of prey. For treatments where a single prey type was provided, 18 individuals were offered. For treatments where two or three prey types were provided, 9 and 6 individuals of each type were offered, respectively.

The Petri dishes were kept in environmentally controlled chambers at constant 25°C, with 75% RH and 12L: 12D photoperiod. The number of prey killed was counted at the end of 24 hours.

### **Statistical Analyses**

For each experiment, the mean number of prey killed was compared with a one-way ANOVA. Prey preference data was analyzed using G-test for goodness of fit (Sokal and Rohlf 1995). In treatments in which three types of prey were offered, means were separated with a modified Tukey test for proportional data (Elliott and Reisch 2006)

## Results

### Predation on *M. ochroloma* Eggs and Larvae on Host Plants

*Chrysoperla rufilabris* first instars were able to find and prey on both eggs and first instars of *M. ochroloma* on bok choy plants. Given a choice, the predator attacked more *M. ochroloma* first instars eggs ( $G= 17.47$ ;  $\chi^2(0.05, 1) = 3.84$ ;  $P<0.001$ ).

*Chrysoperla rufilabris* preyed on a higher proportion of larvae ( $3.7\pm 0.6$ ) than eggs ( $0.7\pm 0.6$ ) of *M. ochroloma* within 48 hours. All eggs killed were located at the base of the plant; none of the eggs placed on the soil, close to the base of the plant, were killed or damaged. One-way ANOVA did not detect significant differences ( $F=0.087$ ;  $df=1, 16$ ;  $P=0.77$  for eggs,  $F=0.03$ ,  $df=1, 16$ ;  $P=0.86$  for larvae) among the treatments with a single prey type offered and the treatment with both prey types offered for the number of prey killed.

### Prey Preference among *M. ochroloma* Eggs and Larvae and *M. persicae* Nymphs in a Petri Dish

First instars of *C. rufilabris* preyed on eggs and first instars of *M. ochroloma* and nymphs of *M. persicae* when offered singly or in combination (Table 4-1). For the treatments in which only two prey types were offered, nymphs of *M. persicae* were always preferred over eggs or larvae of *M. ochroloma* ( $G= 20.58$ ;  $\chi^2(0.05, 1) = 3.84$ ;  $P<0.001$ ). When beetle eggs and larvae were offered as prey without aphids, the predator did not show a preference ( $G= 0.043$ ;  $\chi^2(0.05, 1) = 3.84$ ;  $P=0.83$ ). The killing rate of *M. persicae* nymphs by *C. rufilabris* was higher than that of eggs and larvae of *M. ochroloma*. In the paired prey treatments, first-instar *C. rufilabris* killed 5.5 times more aphid nymphs than beetle eggs or larvae.

When the predator was given three options of prey, a significant preference was detected ( $G = 19.50$ ;  $\chi^2(0.05, 1) = 3.84$ ;  $P < 0.001$ ). The Tukey test showed that *M. persicae* nymphs were preferred over eggs and larvae of *M. ochroloma* ( $\chi^2 = 30.84$ ;  $df = 2, 125$ ;  $P < 0.0001$ ; Tukey's  $q = 6.89$  and  $6.48$  for nymphs vs. eggs and nymphs vs. larvae respectively;  $q_{0.05} = 3.31$ ). First-instar *C. rufilabris* killed 4.5 and 4.0 times more *M. persicae* nymphs than eggs and larvae of *M. ochroloma*, respectively (Table 4-1).

The predator killed twice as many eggs and larvae of *M. ochroloma* when provided separately than it did when these two prey were provided together. The killing rate of eggs and larvae decreased about 73% when each prey was provided along with *M. persicae* nymphs.

## Discussion

Plant morphology has an important effect on the foraging behavior of Chrysopidae (Clark and Messina 1998). Patterns of searching behavior and predator consumption rates change considerably when predator and prey interact on a whole plant compared to less complex environments (e.g. Petri dishes) (Reynolds and Cuddington 2012b, a). When *C. rufilabris* first instars were fed eggs and larvae of *M. ochroloma* placed on a homogeneous arena (e.g., a bok choy leaf piece in a Petri dish) (see Chapter 3), the predator killed 89% and 40% more eggs and larvae, respectively, in 24 hours than the number of eggs and larvae killed when placed on a whole bok choy plant. This is similar to the observations of Reynolds and Cuddington (2012a) who reported that *C. carnea* consumed more pea aphids, *Acyrtosiphon pisum* Harris (Hemiptera: Aphididae), when housed in a Petri dish than on whole plants. Such differences might be explained by the fact that eggs were placed at the base of the plant or on the soil, natural oviposition sites, where apparently they are less accessible or

conspicuous to the predator. In the case of first instars of *M. ochroloma*, the predator has to explore a larger area, so a more time is required to locate the prey, which, together with the time needed to kill and consume it, reduces the time available to find and kill other prey. In my study, when both prey were offered simultaneously on a plant, the predator showed a preference for larvae, killing a higher number of first instars than eggs. The reason for this preference may be attributed to the intra-plant distribution of the predator in the field. Chrysopid larvae are usually found on the leaves of the plant; only 5% of chrysopids are collected from the soil surface compared to 95% of hemerobiids (Szentkirályi 1986). According to Canard et al. (1984), an optimal prey type must coexist in space with the predator. In the field, *C. rufilabris* might not provide good control of eggs of *M. ochroloma*, which are laid mainly on the soil and fallen leaves or at the base of the plant.

Prey preferences of *C. rufilabris* are affected by the type of prey offered and the age of the predator. *Chrysoperla rufilabris* has been reported feeding on a wide variety of soft-bodied arthropods (Hydorn and Whitcomb 1979, Canard et al. 1984, Nordlund and Morrison 1990, Nordlund 1991, Legaspi et al. 1994, Tauber et al. 2000). However, this predator has been used mainly in biological control of aphids. My results showed that when immature stages of *M. ochroloma* were offered as prey along with aphids in the confined space of a Petri dish, *C. rufilabris* first instar consumed unequal proportions of each prey, preferring aphids in all cases. Nordlund and Morrison (1990) evaluated the preference of second and third instars of *C. rufilabris* when provided with eggs and larvae of the tobacco budworm, *Heliothis virescens* (F.) (Lepidoptera: Noctuidae), and nymphs and adults of the cotton aphid, *A. gossypii*. The predator preferred tobacco budworm larvae over cotton aphids and aphids were preferred over *H. virescens* eggs.

When second and third instars of *C. carnea* were offered *Pieris brassicae* L. (Lepidoptera: Pieridae) eggs and larvae and *Brevicoryne brassicae* L. (Hemiptera: Aphididae) nymphs, *C. carnea* second instars preferred aphids over lepidopteran eggs and larvae, whereas the third instar preferred *P. brassicae* larvae over aphids and aphids over *P. brassicae* eggs (Huang and Enkegaard 2010). For a biological control program to be successful when generalist predators are used, it is important that the target pest be among the preferred prey (Nordlund and Morrison 1990). First instars of *C. rufilabris* did not choose any of the immature stages of *M. ochroloma* over aphids, however, it is not known if the prey preference of second and third instars of *C. rufilabris* is the same as that of first instars.

In my study, the number of eggs and larvae of *M. ochroloma* killed by first-instar *C. rufilabris* was reduced about 73% in the presence of *M. persicae* nymphs. Similar results were reported by Huang and Enkegaard (2010) who found that predation on eggs of *P. brassicae* was completely absent or reduced by 80% in the presence of *B. brassicae* when offered to second- and third-instar *C. carnea*, respectively. Second-instar *C. carnea* consumed fewer *P. brassicae* larvae in the presence of aphids.

Despite the preference of first-instar *C. rufilabris* for *M. persicae* nymphs over immature stages of *M. ochroloma* in the laboratory, it is important to evaluate the predation on these two types of prey in the field. A low population of aphids on bok choy plants in the field might not have a great impact on the killing rate of immature stages of *M. ochroloma* by the predator. Additionally, the presence of aphids in the crop can be beneficial for biological control by lacewings. In many cases, a low or moderate populations of other prey allows the predator to survive when numbers of the target pest are low (Ables et al. 1978). Studies have also showed that many species of the family

Chrysopidae are attracted by aphid's sex pheromones (Boo et al. 2003, Zhu et al. 2005, Koczor et al. 2010). According to Kunkel and Cottrell (2007), *C. rufilabris* laid more eggs on pecan seedlings infested with aphids than on uninfested plants.

Table 4-1. Mean number ( $\pm$  SE) of *Microtheca ochroloma* eggs and first instars and *Myzus persicae* nymphs killed by first-instar *Chrysoperla rufilabris*.

Prey killed	Treatments						
	N-L-E	N-L	N-E	E-L	N	L	E
Nymphs (N)	3.8 $\pm$ 0.4 a	4.7 $\pm$ 0.5 a	4.7 $\pm$ 0.6 a		7.6 $\pm$ 0.8		
Larvae (L)	1.0 $\pm$ 0.4 b	0.9 $\pm$ 0.5 b		1.6 $\pm$ 0.4 a		3.4 $\pm$ 0.4	
Eggs (E)	0.8 $\pm$ 0.4 b		0.9 $\pm$ 0.6 b	1.7 $\pm$ 0.4 a			3.4 $\pm$ 0.4

Means followed by the same lowercase letter within a column are not significantly different ( $P > 0.05$ ). Sample size equals 7 for all treatments

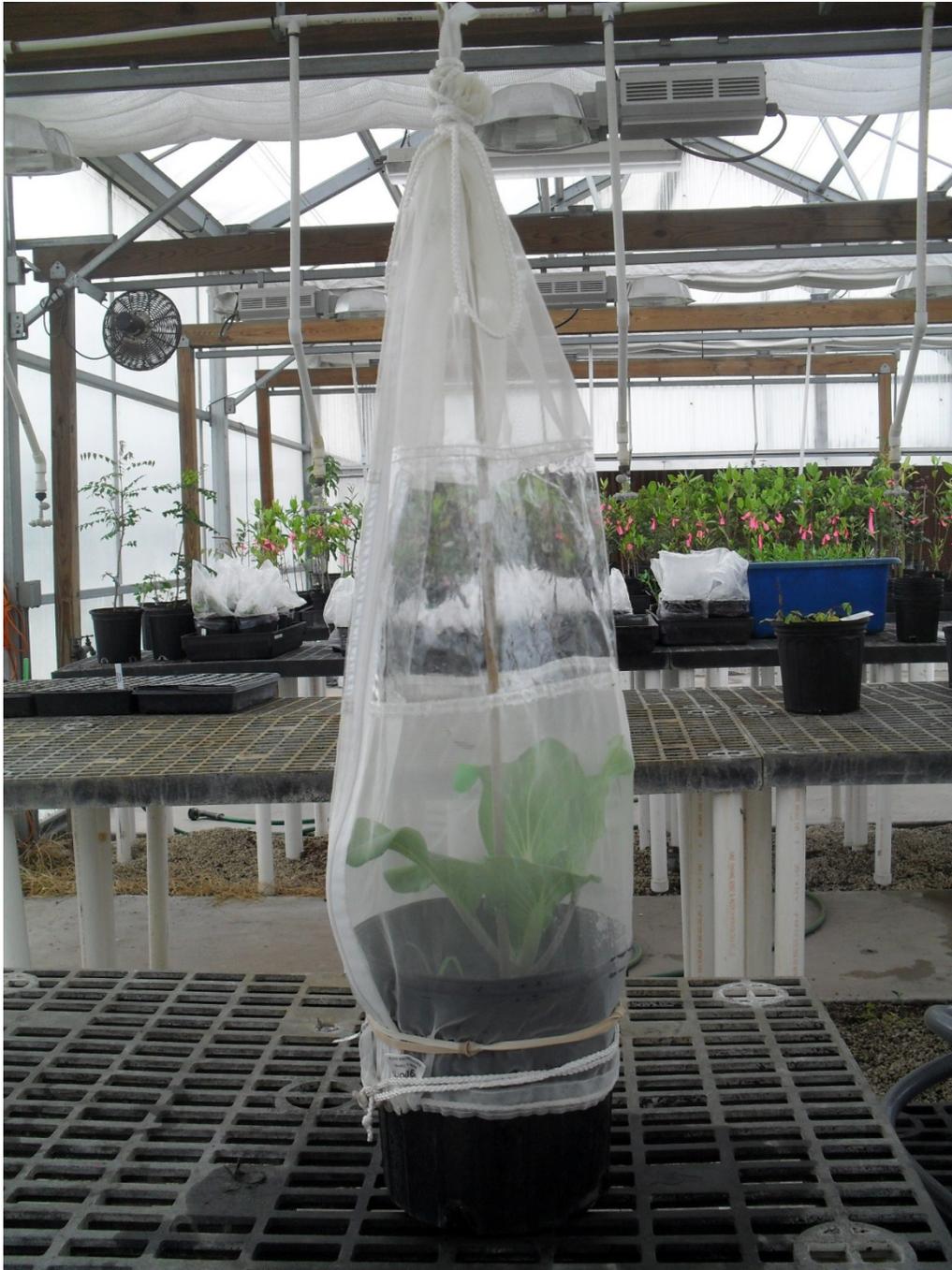


Figure 4-1. Bok choy plant confined in an insect rearing sleeve. A bamboo stick was used to support the sleeve and rubber bands around the edge of the pot prevented insect escape. Photo by Angie Niño.

CHAPTER 5  
PERFORMANCE OF *CHRYSOPERLA RUFILABRIS* (BURMEISTER) (NEUROPTERA:  
CHRYSOPIDAE) FEEDING ON *MICROTHERCA OCHROLOMA* (STÅL) (COLEOPTERA:  
CHRYSOMELIDAE) LARVAE UNDER FIELD CONDITIONS IN AN ORGANIC FARM

**Introduction**

The invasive species *Microtheca ochroloma*, commonly known as the yellowmargined leaf beetle, is considered one of the most detrimental pests in crucifer crops. This beetle feeds on economically important vegetables belonging to the Brassicaceae family, including cabbage (*Brassica oleracea* L. var. *capitata*), napa cabbage (*B. pekinensis* Lour.), collard (*B. oleracea* var. *acephala*), mustard (*B. juncea* Cosson), radish (*Raphanus sativus* L.), mizuna, mibuna (*B. rapa* subsp. *japonica*, Japanese leafy vegetables), watercress (*Nasturtium officinale* L.), and turnips (*B. rapa* L.), the latter being the most preferred (Chamberlin and Tippins 1948, Woodruff 1974, Ameen and Story 1997b, Bowers 2003, Balusu and Fadamiro 2011). The first *M. ochroloma* specimen reported in the United States was intercepted in Louisiana in a shipment of grapes coming from Argentina (Chamberlin and Tippins 1948). Now, *M. ochroloma* is established in several southern states, including Florida, where it is active during the coolest months of the year, October through April, the primary production season for crucifers in the state (Bowers 2003).

Efforts to develop strategies that reduce the economic impact of this pest and maintain populations at tolerable levels have not been fruitful. Restriction by the National Organic Program on the use of synthetic insecticides (Ferguson 2004, Nguyen et al. 2008), and the lack of specialist natural enemies in Florida, leave organic growers without alternatives to control *M. ochroloma* (Bowers 2003). A promising strategy for the management of this pest is the manipulation of native generalist predator populations.

One of the predators found by Montemayor and Cave (2009) preying on *M. ochroloma* in the field is the green lacewing, *Chrysoperla rufilabris* Burmeister (Neuroptera: Chrysopidae).

*Chrysoperla rufilabris* naturally occurs in many agroecosystems in North America (McEwen et al. 2007). This generalist predator consumes small, soft-bodied arthropods, including aphids, scales, whiteflies, eggs and larvae of moths and butterflies, thrips, and mites among others. This predator and the closely related species *C. carnea* have been used successfully to control the Colorado potato beetle, also a chrysomelid, under laboratory conditions (Nordlund 1991, Sablon et al. 2013). In field-cage experiments, both species also have controlled other pests effectively (Ridgway and Jones 1968, New 1975, Legaspi et al. 1994).

Laboratory experiments confirmed that *C. rufilabris* feeds on eggs and larvae of *M. ochroloma*, and according to temperature conditions in Florida, the predator's development when feeding on this pest might be favored during late fall and early summer which overlaps with the active season of the pest (see Chapter 3). However, the potential of *C. rufilabris* as a biological control agent of field populations of *M. ochroloma* is unknown. Therefore, the goal of this study was to evaluate the performance of *C. rufilabris* feeding on *M. ochroloma* larvae under field conditions on an organic farm.

## **Materials and Methods**

### **Plant Material**

Bok choy seeds (*Brassica rapa* L.), variety Joi choy, were seeded in 50-cell trays. Four-week-old seedlings were transplanted individually into 3.8-L plastic pots containing a mixture of soil and fertilizer (Osmocote classic® 14N, 14P, 14K). Four weeks after

transplanting, the plants were taken to the field and their pots were buried in a single row with 30 cm between adjacent plants, in a field bed at the White Rabbit Acres Organic Farm at Vero Beach, FL. Drip irrigation was provided to each plant through an inverted plastic bottle with holes in the lid.

### **Stock Colonies**

Adults and larvae of *M. ochroloma* were collected in the field, and a colony was established at the Biological Control Research and Containment Laboratory at the Indian River Research and Educational Center in Ft. Pierce, FL (as described in Chapter 3). Adults of *C. rufilabris* were purchased from Rincon-Vitova Insectaries, Inc. (Ventura, CA) and a colony was established in the laboratory (as described in Chapter 3).

### **Experimental Design**

Four treatments were evaluated: (1) plants confined in cages with predators; (2) plants confined in cages without predators; (3) uncaged plants with predators; and (4) uncaged plants without predators. For treatments 1 and 2, plants were housed inside a white insect rearing sleeve (60 cm wide × 70 cm high) sealed around the rim of the buried pot and secured with elastic bands to prevent the escape of insects. Bamboo sticks were used to tie the cage closed at the top and provide space around the plant (Fig. 5-1a). Ten first instars of *M. ochroloma* were placed on each plant using a fine paint brush. In the treatments with predators, five *C. rufilabris* first instars were released per plant. A second release of prey and predators was done two weeks after the first release. Each treatment had eight replicates. The experiment was conducted during 3-29 March 2013.

Treatments were randomized within the bed (Fig. 5-1b). All plants were monitored every four days for four weeks, and the numbers of *M. ochroloma* eggs,

larvae, and adults and *C. rufilabris* larvae per plant were recorded. Excess adults and eggs of *M. ochroloma* were removed to maintain similar densities of individuals on all plants. At the end of four weeks, the above-ground parts of each plant were taken to the laboratory and carefully examined. The numbers of prey and predators were counted. Each plant was gently washed and four damaged leaves were randomly chosen per plant to measure the leaf area removed as a result of *M. ochroloma* larval feeding. Each leaf was placed under a glass sheet and photographed into a digital format using an EOS Rebel T4i camera. Once all images were in digital format, the level of defoliation was measured using the image analysis software Scion Image Beta as described by O'Neal et al. (2002). Then, each plant was placed in a paper bag, dried at 45°C for two weeks, and the dry weight of the plant was measured on an Ohaus Scout Pro.

### **Statistical Analyses**

Number of prey found on the plants was transformed using square root. Arcsine transformation was used to convert percentage of defoliation. Untransformed data are reported in tables and figures. Means were compared using analysis of variance (ANOVA). Treatment means were separated using Tukey-Kramer HSD test. JMP 8 (SAS Institute Inc. 2008) with a significance level of 5% was used for all statistical analyses.

### **Results**

During the data collection period, the mean maximum temperature was  $22.9 \pm 4.3^\circ\text{C}$  and the mean minimum temperature was  $8.7 \pm 5.2^\circ\text{C}$ . Six of the eight replicates for the caged treatment without predators were severely infested with aphids, which reduced plant growth and masked the effect of *M. ochroloma* larvae on the plants. Therefore, this treatment was excluded from the statistical analysis.

During the first two weeks of sampling, the number of *M. ochroloma* larvae decreased with time and few eggs and adults were found in the treatments without cages (Fig. 5-2). After the second release of prey and predators, the numbers of *M. ochroloma* larvae and eggs tended to increase with time for the uncaged treatments but not the caged plants with predators. In the caged treatments, the number of individuals at each sampling period varied considerably due to the difficult access to some parts of the plant and the area around it, which did not allow thorough inspection of the plant. Therefore, mean comparisons among treatments were done only for the number of insects at the last sampling period. One-way ANOVA detected significant differences in the number of larvae of *M. ochroloma* that survived to the last sampling on bok choy plants ( $F= 3.80$ ;  $df= 2, 21$ ;  $P=0.0391$ ) (Table 5-1). Caged plants with predators had about three times fewer *M. ochroloma* larvae than uncaged plants with predators. In the treatment with caged plants with predators, the total number of *M. ochroloma* larvae placed on the plants was reduced by about 75%. The number of beetle larvae on uncaged plants did not differ between treatments.

The number of *C. rufilabris* larvae found on the plants decreased with time for both treatments in which the predator was released (Fig. 5-3). Only 20% and 10% of the predators released in the treatments with and without cages, respectively, were found in the last sampling. In the treatments without cages, the *C. rufilabris* larvae apparently were able to move between plants. Predators were found on the cages next to the plants where they were released. Only two predators reached adulthood, both on caged plants infested with aphids.

The percentage of foliar area removed by *M. ochroloma* was lower for caged plants with predators (Table 5-1). Significant differences among treatments were

detected for the percentage of foliage area loss ( $F=8.10$ ;  $df=2, 21$ ;  $P=0.0025$ ).

*Microtheca ochroloma* consumed three times more foliage from uncaged plants without predators than from caged plants with predators. Foliage area loss did not vary between uncaged plants with and without predators. Foliage area loss on uncaged plants with predators did not differ significantly from that of caged plants with predators.

Caged plants with predators had a significantly lower dry weight than uncaged plants with predators ( $F= 3.46$ ;  $df= 2, 21$ ;  $P=0.05$ ). Dry weight of uncaged plants with predators was 1.6 times more than that of caged plants with predators (Table 5-1). No significant differences were detected between the treatments without cages. Dry weight for uncaged plants without predators did not differ significantly from the dry weight of caged plants with predators. Four of eight caged plants with predators were infested with aphids and these plants had the lowest dry weight values for that treatment. Ants and lady beetles were observed preying on aphids on uncaged plants, and these predators apparently helped keep the aphid population at low numbers.

### **Discussion**

When the number of larvae of *M. ochroloma* was compared among treatments, the lowest number of the pest were found on caged plants with predators, while the highest number was observed on uncaged plants with predators, which suggests that the isolation of prey and predators through cages played an important role. On caged plants, the predator killed on average 75% of the larvae, whereas on plants without cages, the proportion of prey killed was about 22%. The differences in predation rates might be attributed to new oviposition by female *M. ochroloma* which had free access to the plants without cages and which were present at every sampling period (Fig. 5-2). Although adults and eggs of *M. ochroloma* were removed from the plants, larvae from

undetected eggs or larvae moving from nearby plants may have replaced the killed prey, or these could have allowed the prey populations on plants to reach high numbers so the predators were no longer effective at controlling them. According to Grasswitz and Burts (1995), releases of *C. rufilabris* were insufficient to keep densities of the green apple aphid, *Aphis pomi* De Geer, below damaging levels when plant growth was vigorous and aphid populations were high. Another possibility is that the predator's interplant movement on caged plants was limited, whereas on uncaged plants the *C. rufilabris* larvae were able to move to other plants. On two occasions, I found predators walking on the exterior of cages next to uncaged plants on which they were released.

When the number of *M. ochroloma* larvae was compared between the treatments without cages, no significant difference was detected. If predators had any impact on *M. ochroloma* populations, no evidence of additional mortality caused by the predator was apparent. Unfortunately, the two caged treatments could not be compared because the caged plants without predators were excessively infested with aphids. At one point, the population of aphids was so high that the plant leaves were covered with honeydew and *M. ochroloma* larvae were not able to feed on the plant tissue or move among the leaves.

The number of predators recorded on uncaged plants was very low (Fig. 5-3)., Only about 20% of the predators were found at the next sampling period after each release, and the numbers decreased as time passed, which explains the similarity in the number of prey present in the two uncaged treatments. A decrease in the predator numbers may have been due to a number of factors. One well-known aspect is the cannibalistic behavior displayed by *C. rufilabris*. The sibling species *C. carnea* showed strong cannibalistic tendencies as a response to food deprivation; this behavior allows

neonate larvae to survive when food is scarce (Duelli 1981). Another factor might be death by starvation. When the prey population is low, the predator might not be able to easily find sufficient prey. Many times I noticed that *M. ochroloma* larvae dislodged from the plant when disturbed, which helped them avoid predation. Knutson and Tedders (2002) demonstrated that starvation of first-instar *C. rufilabris* is an important source of mortality. Environmental conditions also play a very important role in the population dynamics of prey and predators. The mean minimum temperature during the experiment was 8.7°C, which is below the lower developmental threshold reported for *C. downesi* and *C. harrissi* larvae (McEwen et al. 2007). Temperatures below the mean minimum temperature were registered on 20 days during the sampling period, which may have decreased survivorship of *M. ochroloma* larvae.

Defoliation was lowest in plants with predators confined in cages. In that treatment, the defoliation was a result of feeding by *M. ochroloma* larvae only, and as the predator killed prey the damage did not increase significantly. On the other hand, in the treatments without cages, defoliation occurred by feeding of *M. ochroloma* larvae and adults. The predators might have been able to prevent damage caused by larvae by killing them, but they were not able to control adults because *C. rufilabris* larvae only feed on soft-bodied arthropods (Canard et al. 1984).

Dry weight of bok choy plants was lower for caged plants with predators than for uncaged plants with predators. In this case, the cage might have restricted plant growth by limiting space and sunlight to the plant. The cage also limited access to other natural enemies that control aphids. Ants and ladybeetles were found preying on aphids on uncaged plants, whereas on caged plants the only predators present that could control aphids were *C. rufilabris*. As a result, four plants confined in cages were highly infested

with aphids. The dry weights of these plants were the lowest among the replicates for that treatment. Aphids on crucifers cause yellowing and curling of leaves, and plants may be stunted or die (Webb 2010).

Further studies are needed to determine the effectiveness of *C. rufilabris* in organic farms. These studies should include the dispersal behavior of the adults. Species of *Chrysoperla* display two dispersal strategies: preoviposition flight and nocturnal movement during the reproductive period (McEwen et al. 2007). According to Duelli (1984), *C. carnea* undergoes obligatory flight after emergence before reacting to stimuli like food or oviposition sites that extend its dispersal capacity in large areas. Moreover, once the reproductive period starts, *C. carnea* flies and disperses during most nights of its life unless temperatures are too low. This dispersal behavior has been considered an important constraint to augmentative biological control by lacewings (Knutson and Tedders 2002).

Releases of *C. rufilabris* to control *M. ochroloma* in organic farms probably would not be cost effective for the growers. Application systems can be an important cause of mortality of the predator (Daanel and Yokota 1997) and both larvae and adults disperse. Production and shipment of this predator by suppliers is expensive, so augmentative releases would not be economically viable if constant releases are needed to reduce a pest population. A preferred strategy might be to attract and enhance oviposition by *C. rufilabris* adults. The use of synthetic baits and artificial food sprays has been evaluated to manipulate lacewing populations (Hagen et al. 1976, Boo et al. 2003, Kunkel and Cottrell 2007, Koczor et al. 2010). Attracting, enhancing, and conserving *C. rufilabris* should be investigated for management of *M. ochroloma* in organic crucifer production.

Table 5-1. Mean ( $\pm$  SE) number of *Microtheca ochroloma* larvae, leaf damage percentage, and dry weight of bok choy leaves in field-cage study. Sample size for all means was eight.

Treatments	Number of <i>M. ochroloma</i> larvae	Leaf damage percentage	Dry weight
Predator- caged	5.0 $\pm$ 2.4 a	1.6 $\pm$ 0.2 a	18.3 $\pm$ 3.6 a
Predator- uncaged	15.7 $\pm$ 4.8 b	3.1 $\pm$ 0.8 ab	30.1 $\pm$ 2.7 b
No predator – uncaged	11.4 $\pm$ 3.1 ab	5.0 $\pm$ 0.7 b	22.5 $\pm$ 3.2 ab

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



Figure 5-1. Experimental setup of bok choy plants in the field (a) and distribution of the treatments within the bed (b). Photos by Angie Niño.

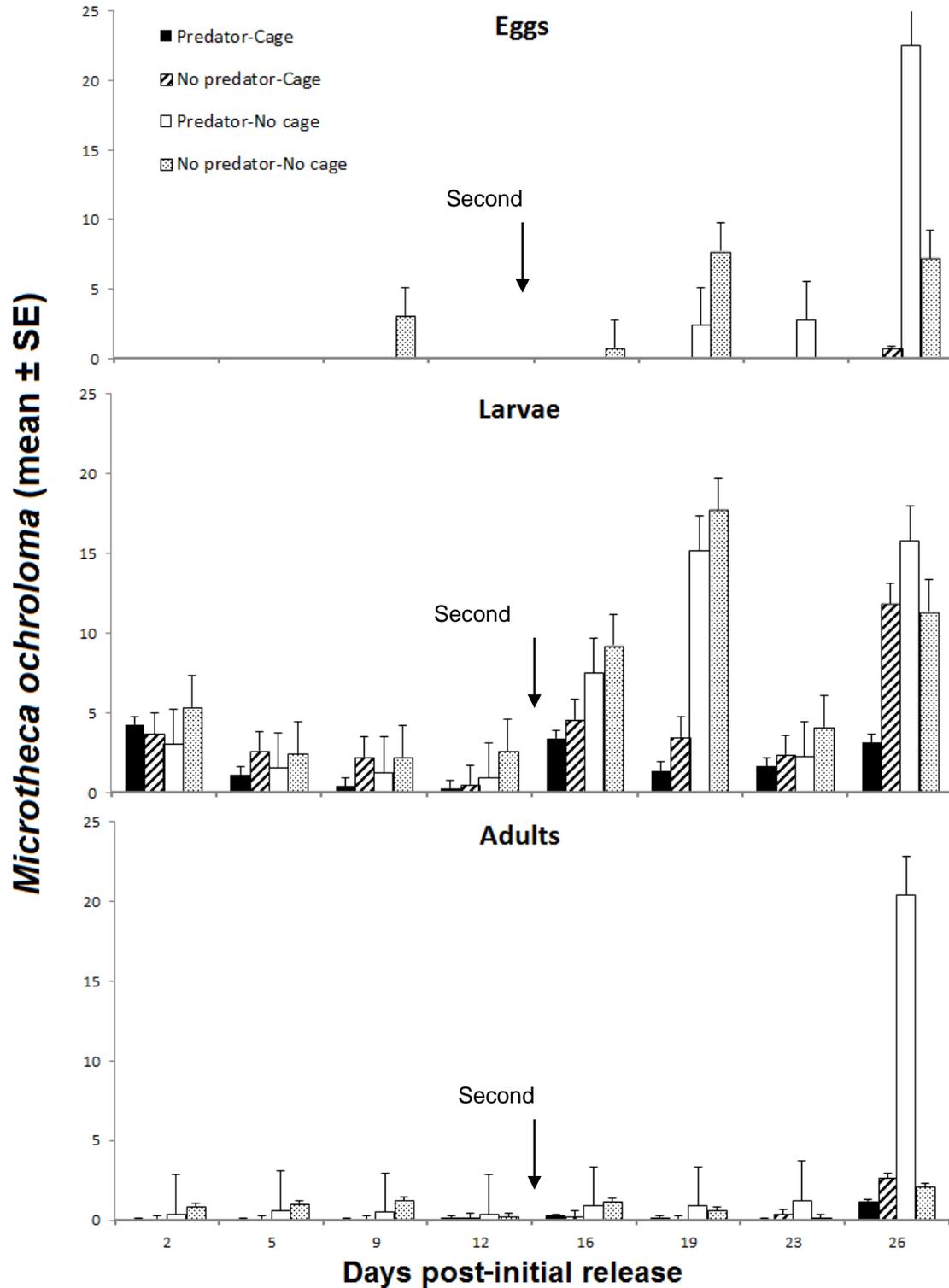


Figure 5-2. Number of *Microtheca ochroloma* eggs, larvae, and adults on caged and uncaged bok choy plants with and without larvae of *Chrysoperla rufilabris*. A second release of prey and predators was done two weeks after the initial release.

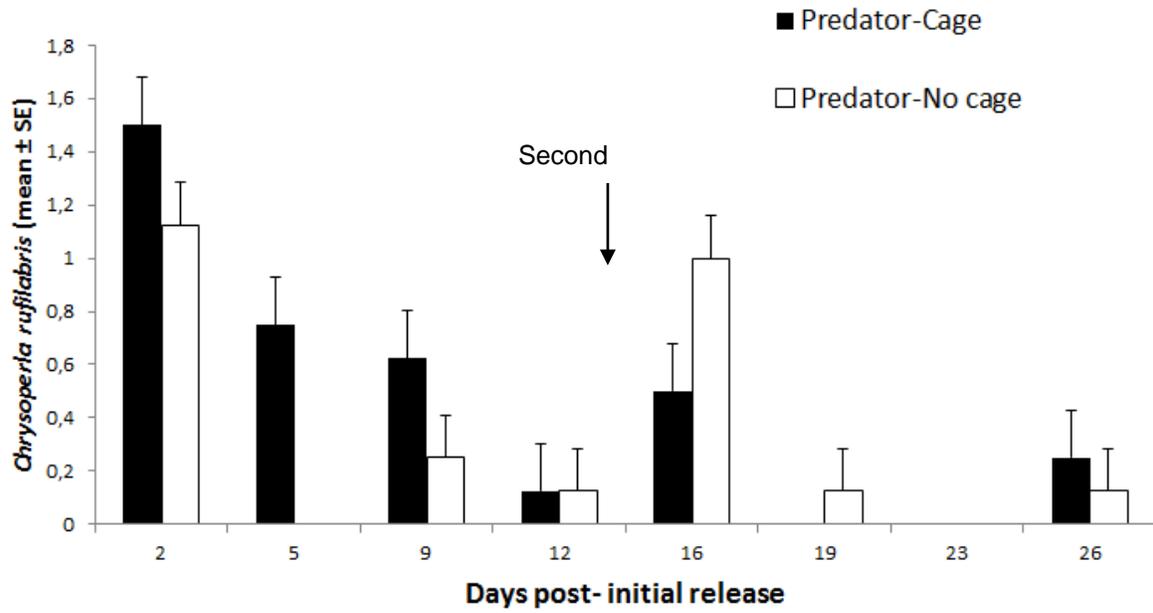


Figure 5-3. Number of *Chrysoperla rufilabris* larvae on caged and uncaged bok choy plants.

## CHAPTER 6 CONCLUSIONS

The main goal of my thesis was the evaluation of a potential biological control agent, *Chrysoperla rufilabris* Burmeister (Neuroptera: Chrysopidae), for management of the yellowmargined leaf beetle, *Microtheca ochroloma* Stål (Coleoptera: Chrysomelidae), on organic farms. I concentrated on three main aspects: (1) the suitability of immature stages (eggs and first instars) of *M. ochroloma* for the development and survivorship of the predator and its prey killing rate at three temperatures; (2) prey preference among eggs and larvae of *M. ochroloma* and aphid nymphs; and (3) performance of the predator in the field feeding on larvae of *M. ochroloma*.

*Chrysoperla rufilabris* is able to develop at temperatures between 15°C and 25°C, but a high mortality was registered at 15°C. The number of days spent by *C. rufilabris* from egg hatch to adult emergence decreased from 75 d and 54 d at 15°C to about 27 d and 22d at 25°C, when fed eggs and first instars of *M. ochroloma*, respectively. The mean developmental time of *C. rufilabris* fed eggs at 15°C was 40%, at 20°C was 9%, and at 25°C was 24% longer than the mean developmental time of the predators fed larvae.

The number of *M. ochroloma* eggs and first instars killed daily per instar of *C. rufilabris* increased with temperature. The predator killed twice as many prey daily at 25°C as it did at 15°C. However, the total number of eggs of *M. ochroloma* killed per *C. rufilabris* larvae was higher at 15°C compared to 20 and 25°C because the predator took more time to develop. When the predator was fed with first-instar *M. ochroloma*, the total number of larvae killed was similar regardless of temperature. The survival of

the predator was reduced at lower temperatures; only 10 and 15% of the initial cohort reached pupal stage at 15°C, whereas 75% and 70% did at 25°C when fed eggs and larvae of *M. ochroloma*, respectively. Pupae of *C. rufilabris* reared on a diet consisted only on *M. ochroloma* eggs, emerged as adults; whereas only a lower proportion of pupae fed first-instar *M. ochroloma* reached adulthood.

*Chrysoperla rufilabris* did not exhibit any preference when eggs and first instars of *M. ochroloma* were offered simultaneously in Petri dishes. However, when the predator was given a choice between immature stages (eggs and first instars) of *M. ochroloma* and nymphs of *M. persicae*, it preferred aphids in all cases. On a whole plant infested only with *M. ochroloma* eggs and first instars, *C. rufilabris* killed more first instars than eggs, which might be related to the location of the prey on the plant. The predator spends more time searching for prey on the leaves of the plant, which is where *M. ochroloma* larvae are usually found, whereas *M. ochroloma* eggs are usually oviposited at the base of the plant or on the surrounding soil.

In the field-cage experiment, differences in the number of *M. ochroloma* among treatments were more related to the effect caused by the cages. If predators had any impact on *M. ochroloma* populations, no evidence of additional mortality caused by the predator was evident. The number of predators found on the plants after initial release decreased with time. Only about 20% of the predators were founded at the next sampling period after release.

Based on my results, releases of *C. rufilabris* to control *M. ochroloma* in organic farms probably would not be cost effective for the growers. The poor survival of the predator when fed *M. ochroloma* first instars, and the fact that none of the immature

stages of *M. ochroloma* were preferred by the predator, suggests that this predator might not provide satisfactory control of this pest. Production and shipment of *C. rufilabris* by suppliers is expensive, so augmentative releases would not be economically viable if constant releases are needed to reduce a pest population. A preferred strategy might be to attract and enhance oviposition by *C. rufilabris* adults in the field.

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## BIOGRAPHICAL SKETCH

Angie Niño was born in Bogotá, Colombia. She graduated with a bachelor's degree in applied biology from the Universidad Militar Nueva Granada in Colombia in 2007. For her bachelor's thesis, she worked on biological control of mites in flower crops. From 2010 to 2011, she worked as a research assistant with the Biological Control Research group and the Biodiversity and Ecology of Wild Bees Research group at the Universidad Militar Nueva Granada. She began her Master of Science degree program in the Entomology and Nematology Department in August 2011. She received a Fulbright- Colciencias scholarship from the Departamento Administrativo de Ciencia, Tecnología e Innovación and Fulbright to support her during her study program. She is a member of the Sociedad Colombiana de Entomología, the Entomological Society of America, and the Florida Entomological Society. She presented talks about her research at the annual meetings of the Entomological Society of America in 2012 and at the Florida Academy of Sciences and the Sociedad Colombiana de Entomología in 2013.