

Prey preference of *Chrysoperla rufilabris* (Neuroptera: Chrysopidae) between immature stages of *Microtheca ochroloma* (Coleoptera: Chrysomelidae) and *Myzus persicae* (Hemiptera: Aphididae)

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Abstract

The prey preference of larvae of the green lacewing, *Chrysoperla rufilabris* Burmeister (Neuroptera: Chrysopidae), for consuming eggs and first instars of the yellowmargined leaf beetle, *Microtheca ochroloma* Stål (Coleoptera: Chrysomelidae), and nymphs of the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), was investigated in 2 laboratory experiments in 2012. When beetle eggs at the base of the plant or on the soil and beetle larvae on the foliage were offered to the predator on bok choy plants (*Brassica rapa* L.; Brassicaceae), *C. rufilabris* larvae killed 5.3 times more beetle larvae than eggs, likely due to prey location. Killed eggs were located on the base of the plant; no eggs placed on the soil, close to the base of the plant, were killed or damaged. In the simple environment of a Petri dish, the predator showed a 5.2-fold preference for aphids over *M. ochroloma* eggs and larvae presented separately. When 3 prey options were presented, first instar *C. rufilabris* killed 4.8 times more *M. persicae* nymphs than beetle eggs and 3.8 times more nymphs than beetle larvae. The killing rate of *M. ochroloma* eggs and larvae by *C. rufilabris* decreased about 73% when *M. persicae* nymphs were present. When only eggs and larvae of *M. ochroloma* were offered in Petri dishes, the predator did not exhibit any prey preference. Although *C. rufilabris* displayed a prey preference for *M. persicae* nymphs over immature *M. ochroloma* in the laboratory, it is important to evaluate the predation on these 2 types of prey in the field.

Key Words: predation; behavior; green lacewing; yellowmargined leaf beetle; green peach aphid

Resumen

Se investigó, en dos experimentos de laboratorio en 2012, la preferencia depredadora de larvas de la crisopa verde *Chrysoperla rufilabris* Burmeister (Neuroptera: Chrysopidae) para consumir los huevos y primeros estadios del escarabajo del margen amarillo, *Microtheca ochroloma* Stål (Coleoptera: Chrysomelidae), y las ninfas del áfido verde del melocotero, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). Cuando se ofrecieron huevos del escarabajo en la base de la planta o encima del suelo y larvas del escarabajo encima del follaje al depredador en plantas de bok choy (*Brassica rapa* L.; Brassicaceae), *C. rufilabris* mató 5.3 veces más larvas que huevos, probablemente debido a la ubicación de la presa. Los huevos muertos se ubicaron en la base de la planta; ningún huevo encima del suelo, cerca de la base de la planta, fue depredado ni dañado. En el entorno simple de un plato Petri, el depredador tuvo una preferencia 5.2 veces mayor para áfidos sobre huevos y larva de *M. ochroloma* presentados separadamente. Cuando se presentaron tres opciones de presas, *C. rufilabris* de primer estadio mató 4.8 veces más ninfas de *M. persicae* que huevos del escarabajo y 3.8 veces más ninfas que larvas de escarabajo. La tasa de mortalidad de huevos y larvas de *M. ochroloma* por *C. rufilabris* disminuyó cerca de 73% cuando las ninfas de *M. persicae* estuvieron presentes. Cuando se ofrecieron solamente huevos y larvas de *M. ochroloma* en platos Petri, el depredador no mostró ninguna preferencia de presa. Aunque *C. rufilabris* mostró una preferencia de presa para ninfas de *M. persicae* sobre los inmaduros de *M. ochroloma* en el laboratorio, es importante evaluar la depredación de estos dos tipos de presas en el campo.

Palabras Clave: depredación; comportamiento; crisopa verde; escarabajo del margen amarillo; áfido verde del melocotero

The production of crucifers on organic farms in the southeastern USA is seriously affected by the yellowmargined leaf beetle, *Microtheca ochroloma* Stål (Coleoptera: Chrysomelidae). The larvae and adults consume foliage and cause injury that consists of holes chewed in the leaf; larvae produce large holes in foliage because they feed collectively (Chamberlin & Tippins 1948; Woodruff 1974; Ameen & Story 1997a). The female lays elongate, bright orange eggs, singly or in small clutches, on the soil or under fallen leaves (Woodruff 1974; Bowers 2003). Ameen and Story (1997b) determined that the developmental

time from egg to adult is about 27 d at 20 °C, with an average of 7.8 d for eggs, 10 d for larvae, 3 d for prepupae, and 5.6 d for pupae.

Indigenous to Argentina, *M. ochroloma* was first detected in the US in 1945 (Chamberlin & Tippins 1948). It is now established in Texas, Louisiana, Mississippi, Alabama, Florida, Georgia, North Carolina, California, Illinois, and Pennsylvania (Chamberlin & Tippins 1948; Woodruff 1974; Balsbaugh 1978; Staines 1999; Gilbert et al. 2011; Marché 2013; Skvarla & Fleischer 2018). Larvae and adults of *M. ochroloma* consume only the foliage of plants in the family Brassicaceae, and they are economic pests on cabbage and collard (va-

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rieties of *Brassica oleracea* L.), turnip, bok choy, mizuna, and napa cabbage (varieties of *Brassica rapa* L.), mustard (*Brassica juncea* Cosson), radish (*Raphanus sativus* L.), and watercress (*Nasturtium officinale* L.) (Chamberlin & Tippins 1948; Woodruff 1974; Ameen & Story 1997a; Bowers 2003; Balusu & Fadamiro 2011). In Florida, the larvae and adults are active during late fall, winter, and early spring (Ameen 1996; Bowers 2003), which coincides with the time of crucifer production in the state. Laboratory experiments showed that food consumption by larvae, larval and pupal survivorship, and oviposition are significantly reduced at 30 °C, which may be responsible for the aestivation and reproductive quiescence during late spring and summer (Manrique et al. 2012).

The use of synthetic insecticides is the only effective method to control *M. ochroloma* (Menezes et al. 2005). However, applications of synthetic insecticides are not allowed in organic farming. Various biopesticides and botanical insecticides approved by the Organic Material Review Institute have been evaluated for control of *M. ochroloma*. According to Balusu and Fadamiro (2012), Entrust® WP and Py-Ganic® were the most effective formulations for control of larvae and adults in the field, causing 100% mortality within 24 h. In general, the entomopathogenic formulations that were tested 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 *M. ochroloma* larvae and adults (Dequech et al. 2008). Balusu et al. (2017) reviewed the literature on studies addressing sanitation, intercropping, and trap cropping. The use of straw mulch enhances *M. ochroloma* populations rather than facilitating predators of the pest; therefore, it is not recommended for use in organic crucifer production (Manrique et al. 2010).

Growers need methodologies that incorporate ecological approaches for the management of pest problems (Ferguson 2004; Zehnder et al. 2007). Biological control might have an important role in pest management of *M. ochroloma* for organic farms, but no host-specific natural enemies of the beetle occur in Florida. Therefore, it is necessary to evaluate the potential of native or commercialized natural enemies that can be used against the pest.

Larvae of the green lacewing *Chrysoperla rufilabris* Burmeister (Neuroptera: Chrysopidae) were observed preying on larvae of *M. ochroloma* on crucifers in organic farms in Florida (Montemayor & Cave 2009). Laboratory bioassays confirmed that *C. rufilabris* larvae can complete their development by consuming only *M. ochroloma* eggs or first instars (Niño and Cave 2015). However, even if a prey enables adequate survival and development of the predator, the natural enemy also must coexist in time and space with the prey to be an effective biological control agent (Canard et al. 1984). In the field, female *M. ochroloma* lay eggs at the base of the plant, on the soil, or under fallen leaves; it is not known if *C. rufilabris* larvae will search for and prey on them in these sites. It is also important to assess the effect that the presence of other prey species might have on the performance and consumption rate by the predator. Larvae of *C. rufilabris* feed on a wide variety of small, soft-bodied insects (Hydorn & Whitcomb 1979; Canard et al. 1984; Nordlund & Morrison 1990; Nordlund 1991; Legaspi et al. 1994; Tauber et al. 2000), especially aphids. No information is available about the effect that the presence of aphids might have on the killing rate of immature *M. ochroloma* by *C. rufilabris*. Therefore, the objective of this study was to evaluate the prey preference of *C. rufilabris* when exposed to eggs and larvae of *M. ochroloma* and the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae).

Materials and Methods

Adults and larvae of *M. ochroloma* were hand-collected Sep 2012 from bok choy at Kai Kai Farm, Indiantown, Florida, USA, and used to establish a laboratory colony. The beetles were held in Bug Dorms (60 × 60 × 60 cm; Model BD2120-P, BugDorm Store, Taipei, Taiwan) containing potted bok choy plants that were replaced with fresh plants twice per wk. The cages were maintained in the laboratory at about 22 °C, 60% RH, and natural photoperiod at the time. Eggs and larvae were obtained by placing 20 pairs of male and female *M. ochroloma* in a plastic box (18 × 13.5 × 9 cm) with a screen mesh cloth in the lid for ventilation. Eggs were laid on the edges of white paper towels or Kimwipes (Model S-12814, Kimberly-Clark, Pleasant Prairie, Wisconsin, USA) and collected every 2 d. The eggs were placed on bok choy leaves and observed daily for hatching so that the first instars could be used in the experiments.

Adults of *C. rufilabris* were purchased from Rincon-Vitova Insectaries, Inc. (Ventura, California, USA). A colony of the predator was established in the laboratory. Upon arrival, 30 pairs were placed in plastic containers and fed a viscous liquid artificial diet consisting of whey, pollen, dry Brewer's yeast, sugar, and water (modified from Rincon-Vitova Insectaries, Inc.). Eggs collected from the container walls and lid were placed singly in clear plastic vials (2.5 cm long × 0.5 cm diam) and stored in an environmentally controlled chamber set for 25 °C, 75% RH, and 12:12 h (L:D) photoperiod. The eggs were observed daily for hatching so that the first instars could be used in the experiments.

Bok choy was grown as described by Niño and Cave (2015). This crucifer was chosen because it is abundantly cultivated on Florida organic farms where *M. ochroloma* is a pest. Plants used in the experiments had 6 true leaves. Bok choy plants infested with all stages of *M. persicae* were confined in Bug Dorms (60 × 60 × 60 cm). Dead plants were replaced with new, clean plants when necessary.

Our study was conducted in 2012 at the Biological Control Research and Containment Laboratory at the University of Florida's Indian River Research and Education Center, Ft. Pierce, Florida, USA. To evaluate prey preference between *M. ochroloma* eggs and larvae on a plant, a bok choy plant was confined within a white insect rearing sleeve cage (60 cm wide × 70 cm long) made with small mesh cloth. A bamboo stick supported the sleeve, and rubber bands around the pot prevented insect escape. On the plant, a first instar of *C. rufilabris* was offered one of the following prey items: *M. ochroloma* eggs; *M. ochroloma* eggs and first instars; or *M. ochroloma* first instars. Each treatment had 10 replicates. Treatments with eggs or larvae only had 18 prey items per plant, and treatments in which eggs and larvae were offered together had 9 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 after being starved at least 12 h 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 12:12 h (L:D) photoperiod. The numbers of inflated eggs and living larvae of *M. ochroloma* were counted at the end of 48 h.

To evaluate prey preference among *M. ochroloma* eggs and larvae and aphid nymphs in a simple environment, 1 first instar of *C. rufilabris* was housed in a Petri dish (5.5 cm diam) with a hole in the top sealed with a screen mesh cloth. Predator larvae were starved at least 12 h before experimentation; water was provided through a moistened cotton ball. Moistened, white filter paper (5.5 cm diam) was placed at the bottom of each Petri dish to maintain appropriate humidity. A 3 cm² section of bok choy leaf was added as a food source or substrate for the prey.

Four combinations of prey were offered to *C. rufilabris* larvae: *M. ochroloma* eggs and first instars; *M. ochroloma* eggs and *M. persicae* nymphs; *M. ochroloma* first instars and *M. persicae* nymphs; and all 3 prey types together. Another 3 treatments were included in which only 1 of the 3 prey types was offered to the predator. There were 7 replicates for each treatment. The quantity of prey offered varied according to the type of prey. For treatments with a single prey type, 18 individuals were offered. For treatments with 2 or 3 prey types, 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 12:12 h (L:D) photoperiod. The quantity of prey killed in each Petri dish was counted at the end of 24 h.

Mean numbers of prey killed were compared with a 1-way ANOVA ($P < 0.05$). Pairwise prey preference data were compared using the G-test for goodness of fit (Sokal & Rohlf 1995). In treatments in which 3 types of prey were offered, means were separated with a modified Tukey test for proportional data (Elliott & Reisch 2006).

Results

First instars of *C. rufilabris* were able to find and prey on eggs and first instars of *M. ochroloma* on bok choy plants. Given a choice, the predator attacked more *M. ochroloma* larvae (mean = 3.7 ± 0.6) than eggs (mean = 0.7 ± 0.6) ($G = 17.47$; $\chi^2_{(0.05, 1)} = 3.84$; $P < 0.001$). All eggs killed were located on 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 analysis of variance did not detect significant differences for total number of killed prey ($F = 0.087$; $df = 1, 16$; $P = 0.77$ 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.

First instar *C. rufilabris* preyed on eggs and first instars of *M. ochroloma* and nymphs of *M. persicae* when offered singly or in combination (Table 1) in a simple environment. *Myzus persicae* nymphs always were preferred by the predator over eggs or larvae of *M. ochroloma* ($G = 20.58$; $\chi^2_{(0.05, 1)} = 3.84$; $P < 0.001$). In the paired prey treatments, first instar *C. rufilabris* killed 5.2 times more aphid nymphs than beetle eggs or larvae. When beetle eggs and larvae were offered together as prey without aphids, the predator did not show a preference for either ($G = 0.043$; $\chi^2_{(0.05, 1)} = 3.84$; $P = 0.83$). The predator killed twice as many eggs and larvae of *M. ochroloma* when provided separately than when these 2 prey choices were provided together.

When the predator was given 3 prey options, a significant preference was detected ($G = 19.50$; $\chi^2_{(0.05, 1)} = 3.84$; $P < 0.001$). *Myzus persicae* nymphs were significantly preferred over eggs and larvae of *M. ochroloma*. First instars of *C. rufilabris* killed 4.5 and 4.0 times more *M. persicae* nymphs than eggs and larvae of *M. ochroloma*, respectively (Table 1). The killing rate of eggs and larvae decreased about 73% when the immature stages of *M. ochroloma* was offered together with *M. persicae* nymphs.

Table 1. Mean number (\pm SE) of *Microtheca ochroloma* eggs and first instars and *Myzus persicae* nymphs killed by first instar *Chrysoperla rufilabris* in 24 h in a Petri dish. Sample size equals 7 for all treatments. N = *Myzus persicae* nymphs; L = *Microtheca ochroloma* larvae; E = *Microtheca ochroloma* eggs; — = stage(s) not included in treatment.

Prey killed ^a	Treatments						
	N-L-E	N-L	N-E	E-L	N	L	E
Nymphs	3.8 ± 0.4 a	4.7 ± 0.5 a	4.7 ± 0.6 a	—	7.6 ± 0.8	—	—
Larvae	1.0 ± 0.4 b	0.9 ± 0.5 b	—	1.6 ± 0.4 a	—	3.4 ± 0.4	—
Eggs	0.8 ± 0.4 b	—	0.9 ± 0.6 b	1.7 ± 0.4 a	—	—	3.4 ± 0.4

^aMeans followed by the same lowercase letter within a column are not significantly different ($P > 0.05$; modified Tukey test for proportional data).

Discussion

Plant morphology has an important effect on the foraging behavior of Chrysopidae (Clark & 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 & Cuddington 2012a, 2012b). When *C. rufilabris* first instars were fed eggs and larvae of *M. ochroloma* placed on a homogeneous arena (i.e., a bok choy leaf piece in a Petri dish), the predator killed 89% and 40% more eggs and larvae, respectively, in 24 h than the number of eggs and larvae killed when placed on a bok choy plant for 48 h. These results are similar to the observations of Reynolds and Cuddington (2012a) who reported that *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) consumed more pea aphids, *Acyrtosiphon pisum* Harris (Hemiptera: Aphididae), when housed in a Petri dish rather than on whole plants. Such differences might be explained by the fact that eggs were placed on the base of the plant or on the soil, natural oviposition sites for *M. ochroloma*, where they are apparently less accessible or conspicuous to the predator. In the case of first instars of *M. ochroloma*, the predator must explore a larger area, so 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 our study, when *M. ochroloma* eggs and larvae were offered simultaneously on a plant, the predator showed a preference for larvae by 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 usually are found on leaves; only 5% of green lacewings are collected from the soil surface compared to 95% of brown lacewings (Hemerobiidae) (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 & Whitcomb 1979; Canard et al. 1984; Nordlund & Morrison 1990; Nordlund 1991; Legaspi et al. 1994; Tauber et al. 2000). However, this predator has been used principally in biological control of aphids. Our results show that when immature stages of *M. ochroloma* were offered together with aphids as prey in the confined space of a Petri dish, *C. rufilabris* first instars 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, *Chloridea virescens* (F.) (Lepidoptera: Noctuidae), and nymphs and adults of the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae). The predator preferred tobacco budworm larvae over cotton aphids,

and aphids were preferred over tobacco budworm eggs. When second and third instars of *C. carnea* were offered eggs and larvae of *Pieris brassicae* (L.) (Lepidoptera: Pieridae) and nymphs of *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae), *C. carnea* second instars preferred the aphids over the butterfly eggs and larvae, whereas the third instar preferred *P. brassicae* larvae over aphids and aphids over *P. brassicae* eggs (Huang & 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 & 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 our 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 would be relevant to evaluate the predation on these 2 types of prey in the field. A low population of aphids on bok choy plants in the field might not have a significant 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 of *M. ochroloma* by sustaining a population of green lacewings. In many cases, a low or moderate population of other prey allows the predator to survive when numbers of the target pest are low (Ables et al. 1978). Studies also have shown that many species of the family Chrysopidae are attracted to aphid sex pheromones (Boo et al. 2003; Zhu et al. 2005; Koczor et al. 2010), and this attraction may increase chrysopid populations on plants with a target pest. For example, Kunkel and Cottrell (2007) observed that *C. rufilabris* laid more eggs on pecan seedlings infested with aphids than on uninfested seedlings. Sustainment of a population of green lacewings feeding on aphids on organically produced crucifers might enhance the predator's biological control capability when *M. ochroloma* first appears in the crop and is in the initial stages of establishment during the fall season when temperatures are still relatively warm in Florida. However, Niño and Cave (2015) observed that the developmental rate and survivorship of *C. rufilabris* with *M. ochroloma* eggs and larvae as prey decreased at cooler temperatures and concluded that the effectiveness of *C. rufilabris* to suppress *M. ochroloma* populations will be less during the cooler mo of Nov to Apr when the beetle is rapidly reproducing and causing crop damage. Niño and Cave (2015) also found that pupal survivorship of *C. rufilabris* was low when the larvae preyed on *M. ochroloma* larvae, but not when *M. ochroloma* eggs were the prey. This stresses the importance of a suitable alternate prey, such as aphids, to maintain populations of *C. rufilabris* in early season leafy crucifer crops.

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