Prey Preference of Chrysoperla Rufilabris (Burmeister) (Neuroptera: Chrysopidae) For Three Common Pest Species of Greenhouse Crops

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PREY PREFERENCE OF Chrysoperla rufilabris (BURMEISTER) (NEUROPTERA: CHRYSPIDAE) FOR THREE COMMON PEST SPECIES OF GREENHOUSE CROPS

A Thesis
Presented to
the Graduate School of
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In Partial Fulfillment
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ABSTRACT

*Chrysoperla rufilabris* (Burmeister) (Neuroptera: Chrysopidae) larvae are voracious generalist predators and important biological control agents on greenhouse crops. This study investigated the prey preference of second-instar *C. rufilabris* for three common greenhouse pests, namely the twospotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), the Madeira mealybug, *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae), and the melon aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae). This study also investigated the influence of prey density ratios on the preference of *C. rufilabris* larvae for *T. urticae* and *A. gossypii*. Prey species preference was evaluated in no-choice, two-choice, and all-choice laboratory bioassays. In the no-choice bioassays, *C. rufilabris* larvae demonstrated the same propensity to consume mealybugs (27.8% of offered individuals consumed), spider mites (26.1%), and aphids (22.4%). In the two-choice bioassays, *C. rufilabris* larvae did not exhibit preference for any particular species when two prey species were offered in pairs. In the all-choice bioassay, *C. rufilabris* larvae did not demonstrate preference for aphids (6.6 out of 10 individuals consumed), mealybugs (6.3 individuals consumed), or spider mites (5.8 individuals consumed). When the predator was offered *A. gossypii* and *T. urticae* at the density ratios of 75:25, 50:50, and 25:75, they did not exhibit detectable preference for either prey species. Results of this study suggest that second-instar *C. rufilabris* was truly a generalist predator which fed on the melon aphid, the Madeira mealybug, and the twospotted spider mite at similar consumption rates.
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CHAPTER ONE
LITERATURE REVIEW

Major arthropod pests of greenhouse ornamental and vegetable crops

Greenhouse vegetable and ornamental crops are susceptible to many arthropod pests. Over 140 different pest species are known to invade and cause crop damage in greenhouses (Heinz et al., 2004). Some significant pest groups include spider mites, thrips, whiteflies, aphids, mealybugs, armored scale insects, and lepidopteran larvae (Messelink et al., 2021). In a 2020-2021 survey of ornamental plant growers and Extension personnel by the IR-4 Project, mites and spider mites, scales and mealybugs, thrips, aphids, borers and beetles, lepidopterans, gall mites, adelgids, midges, symphylla, turf pests, and whiteflies were ranked as the top twelve pests (IR-4 Environmental Horticulture, 2021). These pests are successful because greenhouses present an ideal environment (e.g., consistent temperature and humidity, abundant host plants, and the absence of natural enemies) that benefit a pest’s life history and population growth (e.g., high fecundity, short developmental time, and high propensity to develop pesticide resistance) (Knapp et al., 2020).

In the following paragraphs, only three of the arthropod pests previously mentioned, i.e., aphids, spider mites, and mealybugs, will be discussed in more detail because they are used in the experiments described in this thesis.

*Aphids*

Aphids (Hemiptera: Aphididae) are common pests of greenhouse ornamental (Chau & Heinz, 2004) and vegetable crops (Blümel, 2004). *Aphis gossypii* Glover (the melon or cotton aphid) and *Myzus persicae* (Sulzer) (the green peach aphid) are two of the most important pest species in greenhouses. Aphids are small, typically around 1.5 mm in length
(Tilmon et al., 2011), with bodies that are generally oval with two cornicles at the end of their abdomen (Vilcinskas, 2016). Aphids display wing polyphenism, where some aphids have wings for dispersal while others do not (Yang et al., 2014). The presence of wings is determined by population and environmental factors, such as overcrowding, temperature, and the presence of natural enemies and diseases (Hatano et al., 2012). Aphids develop through four instars before becoming adults (Ebert & Cartwright, 1997). Since aphids can reproduce both sexually and asexually, they can amass a large population very quickly (Chau & Heinz, 2004). Their fecundity is dependent on many factors, such as plant species or cultivars. For instance, Razmjou et al. (2006) investigated the influence of five different cotton (Gossypium hirsutum L.) cultivars (Varamin, Sealand, Bakhtegan, Sahel, and Siokra) on aphid reproduction. The highest daily number of offspring produced was 2.21 on the cultivar Sahel and the lowest was 1.43 on the cultivar Sealand.

Aphids feed through piercing-sucking mouthparts and damage plants in multiple ways (Chau & Heinz, 2004). Infested plants can be stunted when an aphid population removes a large amount of nutrients essential to normal growth and physiological functions. Aphids also secrete a large amount of honeydew, which is the sugary phloem sap excreted after nutrients have been extracted in the aphids’ digestive tracts (Wäckers et al., 2008), on which black sooty mold can grow. The sooty mold on leaves can reduce the photosynthetic rate and plant growth (Chau & Heinz, 2004). Aphids can also vector plant diseases. For example, A. gossypii, M. persicae, and Macrosiphum euphorbiae (Thomas) are vectors of lettuce mosaic virus and cucumber mosaic virus (Chau & Heinz, 2004).
**Spider mites**

A non-insect pest of greenhouse ornamental and vegetable crops is spider mites (Acari: Tetranychidae) (Gillespie & Raworth, 2004; Tanigoshi et al., 2004). Spider mites do not have wings or antennae, and most species are equipped with silk glands (Tanigoshi et al., 2004). The twospotted spider mite, *Tetranychus urticae* Koch, is the most common spider mite species in greenhouses (Tanigoshi et al., 2004). The average life span for a female spider mite is 2–4 weeks (Fasulo & Denmark, 2009). Each female can produce several hundred eggs, which are affixed to silk strands or webbing produced by the females. Eggs hatch in 3 days and spider mites develop through one larval stage and two nymphal stages before becoming adults. Developmental and maturation times of the twospotted spider mite decreases as temperature increases. The ovipositional period has also been shown to decrease with increasing temperatures. For example, the ovipositional period for spider mites decreased from 40 days at 59°F to 10 days at 95°F (Tanigoshi et al., 2004). All females are diploid but all males are haploid; unmated females can produce males which they can mate with to produce female offspring (Tanigoshi et al., 2004).

Spider mites have piercing-sucking mouthparts. Damage caused by spider mites appears as light-colored stippling on the leaves. If feeding continues, the discoloration can progress to yellow, gray, or bronze (Fasulo & Denmark, 2009). Feeding by a high density of spider mites can also lead to stunting and eventually death of plants (Alatawi et al., 2007).

**Mealybugs**

Mealybugs (Hemiptera: Coccoidea: Pseudococcidae) are between 1 and 4 mm in length as nymphs (Baker, 1994). First instars are usually referred to as the "crawlers" because they are mobile. The legs and antennae of crawlers are more prominent than in
other instars or life stages (McKenzie, 1967). The bodies of many species are oval-shaped and typically pink or yellow (Baxendale & Shetlar, 1995), with wax filaments projecting from the sides of body and dorsum (Mani & Shivaraju, 2016). The nymphs and adult females are often covered in a white powdery or mealy wax secretion (Baker, 1994).

Some common mealybug species in greenhouses are the citrus mealybug 
*Planococcus citri* (Risso), the longtailed mealybug *Pseudococcus longispinus* (Targioni Tozzetti), the obscure mealybug *Pseudococcus viburni* (Signoret), the grape mealybug *Pseudococcus maritimus* (Ehrhorn), and the Madeira mealybug (*Phenacoccus madeirensis* Green) (Blumberg & Van Driesche, 2001; Chong et al., 2003). Adult males, which develop through four instars, have one pair of wings and two waxy projections from the posterior end of the abdomen (McKenzie, 1967; Mani & Shivaraju, 2016). Females develop through three instars before becoming adults (Mani & Shivaraju, 2016), which look similar to the nymphs (Downie & Gullan, 2004) but are larger (8-9 mm in length) (Mani & Shivaraju, 2016).

Most mealybug species reproduce sexually but some reproduce through parthenogenesis, while others may reproduce in both modes (Downie & Gullan, 2004). The developmental time and adult longevity are different between male and female mealybugs. For example, the pink hibiscus mealybug *Maconellicoccus hirsutus* (Green) takes 28.7 and 30.3 days to develop from egg to adult male and female, respectively, and adult females live between 13 and 16 days and males only 3-5 days (Subramanian et al., 2021). The developmental time can vary with environmental conditions and species; for example, female and male Madeira mealybugs develop from eggs to adults in 66.2 and 74.8 days, respectively, at 15°C, and 29.8 and 32.6 days, respectively, at 25°C (Chong et al., 2003).
Most mealybug species lay 150-600 eggs per female in a cottony ovisac (Krishnan et al., 2016; McKenzie, 1967); however, some species, such as the longtailed mealybug, are ovoviviparous (produce nymphs) and do not produce egg sacs (Daane et al., 2012).

Over 20% of mealybug species are polyphagous; for example, the citrus mealybug has been reported to feed on over 200 plant species (Watson, 2016). Adult females and immature mealybugs have piercing-sucking mouthparts, whereas adult males are non-feeding (McKenzie, 1967). Damage to the plants is caused by the nymphs and adult females sucking sap from the phloem. Some symptoms of mealybug feeding are chlorosis, defoliation, stunting, and wilting of the infested plants (Subramanian et al., 2021). Mealybugs also secrete honeydew, which can lead to sooty mold growth (Subramanian et al., 2021).

**Integrated pest management (IPM) in greenhouse ornamental and vegetable crops**

IPM is a pest management approach that utilizes multiple tools to protect crops from pests without overusing pesticides and causing harm to the environment, humans, and non-target organisms (Gray et al., 2009). Growers of ornamental crops routinely employ some elements of IPM. According to a survey of growers and Extension personnel in 2021, 30% of the responded growers used IPM, 22% used biological control, and 23% sprayed at threshold (IR-4 Environmental Horticulture Program, 2021). However, the percentage of ornamental plant growers who utilize IPM has remained fairly consistent over the years. The percentages of IR-4 survey participants who had self-reported utilizing IPM were 23% in 2019, 23% in 2017, 25% in 2015, and 34% in 2013 (IR-4 Environmental Horticulture Program, 2019). Survey results indicate that efforts are still needed to increase the adoption
of IPM in greenhouse ornamental crop production. It is unclear how many greenhouse vegetable growers practice IPM.

General IPM strategies include (1) “Do nothing,” (2) reduce pest densities, (3) reduce host susceptibility to pest injury, and (4) reduce both pest populations and host susceptibility (Gray et al., 2009). These strategies employ tactics such as prevention, host plant resistance, cultural control, chemical control, and biological control (Naranjo & Luttrell, 2009).

**Monitoring and scouting**

Consistent monitoring of the presence, density, and damage of insect and mite pests is the cornerstone of an IPM program. Growers can implement a successful IPM program only when the presence of pests and their densities are known. Monitoring and scouting can be accomplished in many ways, such as using insect traps, visual plant inspections, and sentinel plants (Casey et al., 1999). Visual inspection of pest presence, abundance, and damage on plants, once or ideally twice a week (Schnelle & Rebek, 2017), is practiced by ornamental and vegetable crop growers on a regular basis (Yano, 2004).

Insect traps are good tools to detect the presence and estimate the densities of mobile pests in greenhouse crops (Yano, 2004). Some traps use pheromones or other semiochemicals to lure insects (Rizvi et al., 2021), whereas sticky cards take advantage of attractiveness of certain colors to some insects (Schnelle & Rebek, 2017). Sticky traps are typically yellow or blue; yellow is attractive to many insects but blue is more attractive to thrips. Placement and arrangement of sticky traps in the greenhouse are important for obtaining a representative sample of the insects present in the greenhouse. A general recommendation is one sticky trap for every 1000 sq. ft. of crop growing space, and the traps
should be arranged in a grid (Schnelle & Rebek, 2017). The sticky card should be positioned no more than 16 inches above the plant canopy (Schnelle & Rebek, 2017). The sticky traps should be checked and the insects captured on the traps should be counted (and recorded) at least once a week (Schnelle & Rebek, 2017).

Sticky traps are not appropriate for sampling immature, non-flying, or stationary insects (Daughtrey & Buitenhuis, 2020). Non-flying or stationary pests are monitored by observing for pest presence and density on the plants visually, aided by a magnifier, hand lens or microscope. A general recommendation is to inspect 20 or more plants for every 1000 sq. ft. of growing area (Schnelle & Rebek, 2017). Visual inspections of crop plants should begin by checking the substrate and working up the plant. The inspector should look at several leaves, both the underside and the tops, for the presence of arthropods and record their numbers when found (Schnelle & Rebek, 2017). A beat sheet is another method for collecting and monitoring arthropods in a crop, particularly mirid bugs (Hemiptera: Miridae), southern green stink bug \([Nezara viridula\) (L.); Hemiptera: Pentatomidae], and beneficial or predatory insects (Deutscher et al., 2005).

Sentinel plants are non-crop plant species that are more attractive to the pest than the crop plant. For example, flowering verbena can be used as a sentinel plant for thrips in foliage plant production because the verbena flowers are more attractive to the thrips than crop foliage (Frank, 2013). Checking the sentinel plant allows easy and early detection of pests infesting the crops because the pests will appear on the sentinel plants first (Sullivan & Skinner, 2013). Treatment can be applied after pest detection and the infested sentinel plants can be replaced.
Pest presence, density, and damage are used to make management decisions. Economic threshold and economic injury level are two key concepts in making IPM decisions (Bryant & Reay-Jones, 2020). Economic injury level is the minimum number of pests that will cause yield or monetary loss that is equivalent to the cost of taking action against the pests (Hunt, 2015). Economic threshold is the point at which pest-suppressive action must be taken to prevent pest densities from reaching the economic injury level (Hunt, 2015). Economic threshold depends upon crop phenology, arthropod pest species, pest population growth, and the rate at which the pests cause damage to the crop (Hunt, 2015). A certain level of pest activity is allowed in the crop before economic damage and loss of yield occurs; a grower would implement the "do nothing" strategy when pest activity is below the economic threshold (Gray et al., 2009).

**Prevention**

Prevention aims to stop pests from entering the greenhouse or infestations from occurring, therefore, prevents the pest population from ever reaching economic threshold (van Lenteren & Nicot, 2020). Many of these preventive measures are taken before the growing season begins, including disinfecting seeds, inspecting for pest presence and infestation, quarantining plants before planting, sterilizing soil, rotating crops, and using insect screens (van Lenteren & Nicot, 2020).

Quarantine and insect screening are two of the most commonly employed preventative measures in greenhouses. A period of quarantine for plant materials (such as cuttings, transplants, plugs, bulbs, and corms) entering the greenhouse is done to ensure that no arthropod pest or plant disease, if present on these materials, is introduced into the greenhouse (Kruidhof & Elmer, 2020). Quarantine is particularly important for thrips
because they can be very small and easy to miss when inspecting the plants and tend to
inhabit small spaces that also make it hard to spot them visually during inspection (Knapp et
al., 2020). Plants are quarantined and scouted for a period long enough for the symptoms of
infestation or infection to become evident. Plants that are infested or infected can be treated
or disposed in the quarantine area before being moved into the production fields or areas.

Insect screening over the doors, windows and air intakes of a greenhouse can be an
excellent barrier for keeping the insects from entering the greenhouse; however, the mesh
size needs to be very small—typically less than 200 microns—to exclude pests such as
thrips. It is important to recognize that screen capable of excluding small insects also
reduces airflow into the greenhouse (Schnelle & Rebek, 2017), which can lead to increased
humidity levels (Harmanto et al., 2006) and subsequently unwanted fungal growth (Seginer
& Zlochin, 1997) and plant diseases (Hand, 1988). Increasing the surface area of the
exclusion screen can compensate for this reduction in airflow (Schnelle & Rebek, 2017).

**Host plant resistance**

Host plant resistance is another way to prevent pest populations from causing too
much damage. Reduction of host plant susceptibility can be achieved, for example, through
genetic modification or selective breeding (Smith, 2009). Host plant resistance decreases the
negative effects or damages the pest may have on the host plant, and thus decreases the
amount of yield loss even in the presence of pest infestation (Gray et al., 2009).

The three methods by which host plant resistance is conferred are antibiosis,
antixenosis, and tolerance (Stout, 2013). Antibiosis is when the host plant resistance
negatively affects the arthropod pest's physiological processes and is sometimes referred to
as post-ingestive effects (Stout, 2013). These effects can impact the arthropod's growth,
survival, and fecundity (Funderburk et al., 1993; Stout, 2013). Plant characteristics important in antibiosis include plant tissue hardness, phenology, toxins, deterrents, and nutritional resistance (Vänninen, 2005). Antixenosis is when the pest's behavior is negatively affected by plant traits, which causes the pest to have a decreased preference or acceptance of a plant as a viable host (Stout, 2013). Some morphological features or chemical factors (for instance, waxy leaf surface, thicker plant tissue, and chemical deterrents such as saponins, condensed tannins, and cucurbitacins) may cause a plant to lose its attractiveness or acceptability to the herbivore (Funderburk et al., 1993). Tolerance is the ability of the plant to withstand or recover from plant damage (Stout, 2013). This does not affect the insect’s behavior or physiology. Under the same amount of plant damage, tolerant plants are able to produce higher crop yields compared to plants that are not tolerant.

Cultural control

Cultural control encompasses production or maintenance practices a grower can adopt during cropping to reduce or prevent pest populations (Kruidhof & Elmer, 2020; van Lenteren & Nicot, 2020). Some examples of cultural control include the use of repellants or attractants, mating disruption with sex pheromones, trap crops, barriers (for instance, insect mesh screening), UV or heat treatments, manipulation of the timing and amount of irrigation, tillage, and many more. Some of these, including the use of traps and pheromones, are employed by ornamental plant and vegetable growers.

Trap crops are non-crop plants that are more attractive to arthropod pests than the crop plants. Trap crops attract and concentrate pests in a particular area for localized eradication (Shelton & Badenes-Perez, 2006). For example, alfalfa (Medicago sativa L.) has been used as a trap crop to attract lygus bugs (Lygus hesperus Knight; Hemiptera: Miridae)
away from cotton crops (Godfrey & Leigh, 1994; Shelton & Badenes-Perez, 2006; Stern et al., 1964). Squash (Cucurbita pepo L.) was used to attract the sweetpotato whitefly, Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae), from tomato plants (Lycopersicon esculentum Mill) (Schuster, 2004).

The performance of pests (i.e., developmental time, adult longevity, body size, number of offspring) and their population densities can be influenced by the manipulation of nitrogen fertilization (Bar-Shmuel et al., 2020; Chow et al., 2009). Nitrogen is a limiting factor in the growth and development of arthropods; therefore, an increase in plant tissue nitrogen contents (through fertilization) can increase the performance of insect herbivores (Bar-Shmuel et al., 2020; Mattson, 1980). For instance, the nymphal developmental rate, body size, adult longevity, fecundity, and intrinsic rate of population increase of melon aphids were greater on fertilized greenhouse-grown cucumbers (Cucumis sativus L.) (Hosseini et al., 2010). Excessive nitrogen fertilization may increase the attractiveness and susceptibility of plants if the herbivores prefer host plants with higher levels of nitrogen. For instance, Ramachandran et al. (2020) demonstrated that greenhouse-grown tomato plants' ability to resist sweetpotato whitefly attack was improved when nitrogen fertilization rate was reduced.

**Physical control**

Physical control is any physical action or object a grower can do or use to reduce pest population and reduce damage. Physical control methods often involve changing the environment to decrease the risk of pest damage to the agricultural crops (Vincent et al., 2002). The tactics employed can be split into two different categories, passive and active (Vincent et al. 2002).
Passive approaches require no additional input for a specified amount of time after being set up. Passive tactics mostly involve some form of barrier to keep the pest from gaining access to the crop; examples include trenches, fences, mulch, particle films, inert dust, screens, and insect traps. Not all of these methods are suitable for greenhouse pest management. Trenches and fences, for example, would be utilized in field crops but not in greenhouses. Organic mulches, such as straw, bark chips, compost, and peat moss, can act as a barrier or repellent to pests and it has also been shown to indirectly reduce pest damage by promoting natural enemy population (Weintraub & Berlinger, 2004; Vincent et al. 2002). The repellant or attractant qualities of the different colored artificial mulches, composed of plastic or aluminum sheets, can be used to influence insect behavior (Csizinszky et al., 1995). For example, Wilson (1999) reduced aphid population with the use of silver-colored mulch.

Particle films are composed of chemically inert mineral particles (e.g., kaolin clay) that coat the plant surfaces to form a protective barrier against the pests. Many of these protective films can disrupt the pest’s ability to find their host plant, cause mortality when ingested, or repel the insect pests (Sharma et al, 2015). Dusts that are typically nontoxic, chemically unreactive, such as dusts of clay, wood ash, silica gels, mineral dusts (e.g., zinc oxide, calcium carbonate, and rock phosphate), and diatomaceous earth, can cause mortality when insects ingest the dust, or cause desiccation, asphyxiation, or excessive loss of lipids from their cuticle. However, this technique is more often applied to stored products like grains (Obeng-Ofori, 2010).

Some tactics previously discussed as preventative or cultural control can also be used as physical control, such as insect screens and traps. Insect screening can be used
preventatively, but it can also be used as a barrier to keep the pests away from the crop plant, which is physical control (Weintraub & Berlinger, 2004). In addition to being a tool for monitoring insect presence and abundance, insect traps can also be used to control pests in the greenhouse (Gu et al., 2008; Lu et al., 2012). Most commonly, yellow sticky traps are used for the mass trapping of whiteflies (Lu et al., 2012). Sticky traps work best when used in combination with another pest suppression method, such as biological control (Lu et al., 2012). For example, Gu et al. (2008) demonstrated superior control of sweetpotato whiteflies on tomato plants (var. ‘Jiafen 3’) when both yellow sticky cards (41 cards/acre) and parasitoids (*Eretmocerus* sp. nr. *rajasthanicus* Hayat; Hymenoptera: Aphelinidae) were employed.

Traps baited with pheromones can be utilized to monitor and control insect pest populations (Megido et al., 2013; Turchin & Odendaal, 1996; van Lenteren & Nicot, 2020). Pheromones are chemical substances secreted by organisms that cause a behavioral response in conspecifics. Because sex pheromone is generally female-produced and species-specific, mass trapping using sex pheromones will only work on male insects of the target species (Rodriguez-Saona & Stelinski, 2009). Sex pheromone lures are commonly used for the mass trapping and attracting and killing of Coleoptera and Lepidoptera; examples include species such as cotton boll weevil (*Anthonomus grandis grandis* Boheman), Japanese beetle (*Popillia japonica* Newman), cotton leafworm (*Spodoptera litura* F.), and tomato leafminer [*Tuta absoluta* (Meyrick)] (Witzgall et al., 2010). The sex or aggregation pheromone lures are used concertedly with a trap, such as delta traps or water traps, or paired with an insecticide (Megido et al., 2013; Turchin & Odendaal, 1996).
Active physical control approaches require consistent input. Some active tactics include cleaning, manipulation of sound, and temperature, electromagnetic control, and pneumatic control (Weintraub & Berlinger, 2004; Vincent et al., 2002). Cleaning is another tactic that can be classified under different control methods; it can be a preventative and a physical control measure. If cleaning and quarantine do not provide adequate control, they can be followed with soapy water and wax treatments that immobilizes the pest insects (Vincent et al., 2002). Sounds at low frequencies can disrupt insect development (Vincent et al., 2002). Pests can be controlled by increasing or decreasing the heat exceeds the pest’s tolerance but not extreme enough to damage the crops (Weintraub & Berlinger, 2004; Vincent et al., 2002). Electromagnetic radiation can be employed to kill or sterilize the pests, and possibly disrupt their vision depending on the type of radiation used (Weintraub & Berlinger, 2004). Pneumonic control involves arthropod pests being dislodged from the plants using aspirated or blown air. A more common pneumatic control tactic is vacuuming crop pests with various commercial insect vacuum devices, such as the BugVac, Ag-Vac, and Biovac (Chagnon & Vincent, 1996).

**Chemical control**

Chemical control involves the use of chemical pesticides to eradicate pests (Bhuler & Frank, 2022). Insecticides, in general, have five main modes of action: nerve and muscle action, growth and development regulators, effects on respiration, effects on midgut, and unknown/nonspecific (Insecticide Resistance Action Committee International MoA Working Group, 2022). Insecticides are typically either broad-spectrum or selective (Torres & Bueno, 2018). Broad-spectrum insecticides harm a wide range of insects indiscriminately, whereas selective insecticides are more effective against particular pest groups. Examples of
broad-spectrum pesticides include organophosphates, pyrethroids, and carbamates (Schnelle & Rebek, 2017).

Selective or target-specific pesticides affect physical or physiological processes in some organisms but not others (Torres & Bueno, 2018). An example is pymetrozine, which targets plant-sucking insects by paralyzing their chordotonal organs, thereby inhibiting their ability to feed and the insects die of starvation (Ausborn et al., 2005; Bextine et al., 2004; Kosari & Fazeli-Dinan, 2016). It has minimal impact on the survival and reproduction of natural enemies, such as parasitic wasps, ladybirds, hoverflies, and minute pirate bugs (Jansen et al., 2011; Kosari & Fazeli-Dinan, 2016).

Because of the selectivity of insecticides, it is important for a grower to correctly identify the pests that are causing the damage and understand the life history of these pests. Proper pest identification is important for selecting the most effective pesticides, whereas an understanding of the pests’ life histories will allow the growers to pinpoint or predict the appearance of the most vulnerable life stage, which should be the target of application.

Pesticide is a selection force that can drives the selection of certain traits among pest populations. One of such traits that can have significant implication for pest management efficacy is the development of pesticide resistance. Therefore, it is important to develop a plan to manage pesticide resistance. One of the most effective pesticide resistance management strategies is to rotate between pesticides with different modes of action (Chanda et al., 2016, Insecticide Resistance Action Committee, 2021).

**Biological Control**

Biological control employs natural enemies to control crop pests (Wraight & Hajek, 2009). There are three main biological control approaches, i.e., classical, conservation, and
augmentative biological control (Wraight & Hajek, 2004). Classical biological control (sometimes called introduction or importation biological control) relies on a non-native natural enemy that is purposefully introduced to combat a (typically non-native) pest (Gardiner et al., 2009). Classical biological control is not often practiced in greenhouse production; therefore, the following sections will focus on conservation biological control and augmentative biological control.

**Conservation biological control** (also referred to as natural biological control) utilizes and improves the effectiveness of existing natural enemy populations by manipulating the environment to benefit the natural enemies (Gardiner et al., 2009). Manipulations of the environment and natural enemy habitats may include providing supplemental food and shelter and using compatible insecticides. Some systems that provide both food and shelter, such as banker plants, are employed in greenhouses.

Providing supplementary shelter to natural enemies can be beneficial to sustaining and promoting their populations. Shelters or refuges can help conserve natural enemies when there are extreme environmental conditions (Landis et al., 2000), pesticide application (Gontijo, 2019), harvesting (Griffiths et al., 2008), for overwintering (González-Chang et al., 2019), for oviposition (Messelinke et al., 2014), or for larvae to avoid cannibalism (Messelinke et al., 2014). In greenhouses, non-crop plants and other objects may be adopted to provide oviposition sites and shelters to biological control agents. For example, plants with trichomes and domatia can be used as oviposition sites for beneficial arthropods like predatory mites (Schmidt, 2014). Faraji et al. (2002) found that ovipositing within the plant domatia protected the eggs of the predatory mite *Iphiseius degenerans* (Berl.) (Acari: Phytoseiidae) from intraguild predation. Parolin et al. (2013) demonstrated that when the
crop plant *Rosa sonia* Meilland var. ‘Sweet Promise’ (Rosaceae) was accompanied by plants possessing domatia (*Viburnum tinus* L. and *Vitis riparia* Michx. var. ‘Gloire de Montpellier’), the predatory mite *Neoseiulus californicus* McGregor (Acari: Phytoseiidae) was able to significantly suppress the population of *T. urticae* more than companion plants without domatia.

Biological control agents may be specialists and generalists (Snyder & Ives, 2009). Specialists typically utilize only one host or prey species for growth or reproduction. Many specialist biological control agents are parasitic wasps, such as the braconid parasitoid *Aphidius ervi* Haliday, which is used to control the pea aphid, *Acyrthosiphon pisum* Harris. Generalists will feed on a larger number of prey or host species (Snyder & Ives, 2009).

Some examples of generalist biological control agents include the predatory mite *Amblyseius swirskii* Athias-Henriot (Acarina: Mesostigmata: Phytoseiidae), which preys upon thrips, whiteflies, and mites (Lamb et al., 2008; Lee & Gillespie, 2011); nabis predatory bug, which prey upon aphids, potato leafhopper, and potato beetle (Östman & Ives, 2003; Koss et al., 2004); *Orius* spp., which prey upon spider mites, aphids, thrips, Lepidoptera eggs and others (Isenhour & Marston, 1981); and *Chrysoperla* spp., which prey on soft-bodied insects and mites (Taubet et al., 2000)

Provision of supplemental food or alternative prey can help conserve populations of some generalist or omnivorous natural enemies even when the host or prey density is low (Messelink et al., 2014). Omnivory occurs when an organism feeds on both plant material and prey items. This can be beneficial for many organisms as it provides additional nutrients, vitamins, and minerals that the organism would not typically get by being strictly an herbivore or a carnivore (Coll & Guershon, 2002). The addition of plant materials to a
typically predacious insect’s diet had been shown to increase predator longevity, development, and fecundity (Coll & Guershon, 2002).

Omnivory in arthropods allows growers to maintain a predator population by supplementing the predators’ diet with an alternative food, such as ones provided by plants (i.e., pollen, nectar, and plant tissue) (Benson & Labbe, 2021). This supplementation could be accomplished by selecting crops that provide extra food resources, such as higher quantities of pollen (Messelink et al., 2014). Pollen can provide resources such as amino acids, proteins, sterols, lipids, and carbohydrates (Wäckers et al., 2007). Provision of pollens increased the fecundity of *Amblyseius cucumeris* Oudemans (Mesostigmata: Phytoseiidae), *Amblyseius fallacis* (Garmen) (Mesostigmata: Phytoseiidae), and *Orius minutus* L. (Hemiptera: Anthocoridae) (Lu et al., 2014). Predatory mites, such as *A. swirskii* and *Euseius scutalis* (Athias-Henriot) (Mesostigmata: Phytoseiidae), increased their populations when corn pollen (*Zea mays* L.) was supplied to the mites on twines (Adar et al., 2014). Pollen, such as those from cattail (*Typha angustifolia* L.) and commercially sold as Nutrimite®, has been shown to enhance population of predatory mites by increasing population density (Pijnakker et al., 2015). Bee-collected pollen is another viable, less expensive option for pollen (Messelink et al., 2014). The bee-collected pollen, however, is mixed with sugars and enzymes; this type of pollen is more prone to fungal growth and less nutritionally valuable to predatory mites compared to some other pollens.

Crops that provide plant nectar could also benefit some natural enemies. Nectar provides valuable resources, such as sugars, small amounts of amino acids, lipids, vitamin C, potassium, and sodium (Lu et al., 2014; Wäckers et al., 2007). The predatory midge, *Feltiella acarisuga* (Vallot) (Diptera: Cecidomyiidae), increased egg production when it was
supplemented with extrafloral nectar (Messelink et al., 2014). Adding nectar to the diet of adult parasitoids, syrphids, and gall midges increases their lifespan and improves flight activity and oviposition (Messelink et al., 2014).

In addition to plant-derived food, animal-derived food sources also can be used to enhance natural enemy establishment, density, development, reproduction, or for mass rearing (Messelink et al., 2014; Nguyen et al., 2014). Sterilized *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs and decapsulated cysts of *Artemia franciscana* Kellogg (Anostraca: Artemiidae) are both factitious food sources used to promote the establishment of a predator population and for mass rearing (Benson & Labbe, 2021). Both support reproduction and development in many natural enemies, such as *Orius* spp., predatory mites, predatory mirid bugs, and coccinellid beetles (Benson & Labbe, 2021; Castañé et al., 2006; Nguyen et al. 2014). Populations of *A. swirkii* increased when fed with a mixture of yeast, sugars, and proteins (Messelink et al., 2014). Longevity and establishment were enhanced when *Geocoris varius* (Uhler) (Hemiptera: Geocoridae) was fed a powdered artificial food source composed of ground pork, pork liver, egg yolk, sucrose solution, acetic acid, propionic acid, sorbic acid, and chloramphenicol (Igarashi et al., 2013).

Banker plants are commonly used for conserving natural enemies in greenhouses. Banker plants are non-crop alternative plant species that sustain populations of natural enemies by providing them with an alternative food (usually pollen or an alternative prey) and a shelter (for refuge or oviposition) (Huang et al., 2011). When a banker plant system provides alternative prey, it is common to select an alternative herbivorous prey species that is not a pest species to the crop. Banker plants can also serve as refuges for natural enemies (Messelink et al., 2014; Parolin et al., 2013). Banker plant systems geared towards predators
of aphids have been successful and are some of the first banker plant systems used commercially. Fava bean (*Vicia faba* L.) serves a banker plant for the parasitoid *Aphidius ervi* Haliday (Hymenoptera: Braconidae) by supporting the development of the parasitoids on a population of pea aphids. The parasitoid is then used for controlling potato aphid, *Macrosiphum euphorbiae* (Thomas), and foxglove aphid, *Aulacorthum solani* (Kaltenbach) (Osborne et al., 2023).

Another approach to providing both shelters and alternative food to sustain biological control agent population in greenhouses is the use of open rearing system. For example, rearing units for the biological control agent *Atheta coriaria* (Kraatz) (Coleoptera: Staphylinidae) are commercially available for control of shore fly (Diptera: Ephydridae) and sciarid fly (Diptera: Sciaridae) populations in the greenhouse (Bennison, 2010). Rearing boxes can be made by the grower or can be purchased from biological control agent suppliers. These rearing boxes can be ready to deploy to the greenhouse after a 4-week rearing period. A rearing box with 60 adults can generate around 2,400 *A. coriaria* after 4 weeks. With a slight modification of the rearing box (i.e., removal of ventilation screening and addition of foil lid with two holes cut out), it becomes a release box that can provide a continuous emergence of *A. coriaria* for weeks. *Atheta coriaria* can continue to breed in the boxes and also disperse through the crops to feed on the pest insects (Bennison, 2010).

Biological control agent populations in the greenhouses can also be preserved through the use of compatible pesticides. Compatible pesticides aim to circumvent some of the lethal and sublethal effects of pesticides on non-target natural enemies but still provide control of the pest (Messelink et al., 2014). Pesticide lethal effects cause mortality, while sublethal effects can affect insect development, adult longevity, fecundity, mobility, their
navigation and orientation abilities, feeding behavior, oviposition, feeding behavior, and more (Desneux et al., 2007).

Both broad-spectrum and selective pesticides can be used compatibly with biological control agents. Broad-spectrum pesticides can be used compatibly with natural enemies when applied in a manner that limits their direct contact with the natural enemies, such as through treating pest infestation hotspots, or using different application methods, such as soil and seed treatments (Torres & Bueno, 2018), drenches, granules, or combining the pesticide with a lure (Reddy, 2016). Systemic pesticides can also be an option, but whether the natural enemy feeds on plant materials, such as pollen and nectar, and if contaminated prey has an effect on the predator, need to be considered (Torres & Bueno, 2018). Selective pesticides, such as pymetrozine, can be used when they do not target the natural enemies (Jansen et al., 2011; Kosari & Fazeli-Dinan, 2016).

The timing for pesticide application can be an important factor for conserving natural enemies. Because some insect developmental stages are more susceptible to pesticides, the best application time for natural enemies is when they are at their most pesticide-resistant stage (Reddy, 2016). For instance, the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Mesostigmata: Phytoseiidae) were the least susceptible to the pesticide cyromazine in the egg stage compared to nymphal and adult stages (Blümel & Stolz, 1993; Reddy, 2016) and the egg stage of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) was more susceptible to pesticides compared to nymphs and adults (Reddy, 2016).

**Augmentative biological control** is the most commonly employed form of biological control in greenhouses (Jeffers & Chong, 2021). With augmentative biological control, natural enemies are periodically released with the intent of establishment
(inoculative) or no establishment (inundative) (Jeffers & Chong, 2021). Inundative augmentative biological control involves releasing a large number of natural enemies with the expectation that the natural enemies will not become established within the crop and repeated releases may be needed (Gontijo, 2019). Inoculative release involves a seasonal or periodical release of natural enemies with the expectation that the natural enemies will become established (O'Neil & Obrycki, 2009). Inundative biological control is more commonly practiced in greenhouses than inoculative biological control.

Situations in which inundative biological control is often employed are when damage thresholds are low and the crops are only short-term (Hajek, 2004; van Lenteren, 2000). There is no expectation that the released natural enemies will produce progeny and exert long-term control. However, if resources are still available for the natural enemies after the initial release, they are capable of persisting in the crop for a slightly extended period (Hajek, 2004). Growers can intentionally supply the natural enemies with resources they may need (i.e., through conservation biological control) in order to prolong the duration of the biological control agent's usefulness. This could be achieved with the addition of artificial food sources or banker plants to supplement the natural enemies when host prey numbers are low (Stinner, 1977). The use of compatible pesticides also is employed to preserve biological control agents introduced through inundative releases (Sivinski, 2013).

Inundative biological control is often used to quickly suppress a pest population in the early stage of infestation (Hajek, 2004). For the natural enemies to exert proper control over the pest population, the release must coincide with the most vulnerable or susceptible pest developmental stage (Hajek, 2004). The importance of carefully timed releases is illustrated in the control of lettuce aphid, *Nasonovia ribisnigri* (Mosely), infestations with
syrphid flies (Sivinski, 2013). Lettuce aphid prefers to inhabit the very densely packed leaves in the center of the lettuce head, where they are virtually inaccessible to predators and parasitoids. The syrphid flies should be released early when the aphids still occupy the outer leaves of the lettuce head, allowing the syrphid flies to exploit this brief window of vulnerability and exert control over the aphids.

The number of natural enemies released is highly variable and depends on the type and density of pest and natural enemy (Stinner 1977). For instance, when released against three different aphid species on potato crops, even a high rate of 33,993 Chrysopa sp. (Neuroptera: Chrysopidae) larvae per acre was only successful in reducing two of the three aphid species (Shands et al., 1972). When released in vegetable crops, the numbers of Trichogramma (Hymenoptera: Trichogrammitidae) parasitoids released can range from 5,000 to 200,000 individuals per acre depending on the level of infestation (Barbercheck, 2009). The release rates of Trichogramma vary from 20,234-40,468 individuals per acre against cabbage looper, Trichoplusia ni (Hübner) (Lepidoptera: Noctuidae), and 16,187-40,468 Trichogramma individuals per acre against European corn borer, Ostrinia nubilalis (Hübner) (Lepidoptera: Crambidae) (Stinner 1997). Successful management of A. gossypii by third-instar Cycloneda sanguinea L. (Coleoptera: Coccinellidae) was achieved at a ratio of 1 ladybeetle to 20 aphids (Hussey & Bravenboer, 1971).

**Red-Lipped green lacewing, Chrysoperla rufilabris**

*Biology and ecology*

Chrysopidae (Neuroptera), commonly referred to as green lacewings, includes around 1200 species in three subfamilies (Nothochrysinae, Apochrysinae and Chrysopinae)
(Albuquerque et al., 2012; New, 1984). Chrysopinae is the most specious subfamily (New, 1984). Larvae of all chrysopid species are predaceous, but adults are non-feeding, predaceous or herbivorous (feed on pollen, nectar, and honeydew) depending on species (Canard & Principi, 1984). It is unclear when they were actually first implemented, but chrysopid species have been considered for biological control in greenhouses since 1742 (Senior & McEwen, 2001). Studies investigating the release of lacewings as control measures for arthropod pests have occurred in greater frequency since the late 1940s (Senior & McEwen, 2001; Tulisalo, 1984).

Larvae of red-lipped green lacewing, *Chrysoperla rufilabris* (Burmeister) (Neuroptera: Chrysopidae), are voracious predators commonly employed as biological control agents in North America (Woolfolk et al., 2004). *Chrysoperla rufilabris* larvae grow in length from 1 mm to 8 mm as they develop through three instars (Hoffmann & Frodsham, 1993). The mouth parts have pincer-like jaws that they can use to pierce prey (Albuquerque et al., 2012; Hoffmann & Frodsham, 1993). The jaw is composed of maxillae and mandibles, which come together to form a channel called the 'feeding tube.' Enzymes that digest prey materials are released from that channel, which the lacewing larvae also use to suck up the digested materials. Larvae are brown/brick red in color, with cream/yellow colorations on the margins of the body, but these colorations are most prominent in the third instars (Hoffmann & Frodsham, 1993; Smith, 1922). The third instars pupate singly in ovoid cocoons made of silk that the larvae produce (Gepp, 1984). The adults are 12-20 mm in body length, pale or bright green, and have membranous wings folded tent-like over their bodies (Hoffmann & Frodsham, 1993). *Chrysoperla* spp. diapause as adults under shortened photoperiod (10:14 hour L:D) (Tauber et al., 1993; Vitanović et al., 2019).
The larvae are active foragers (Woolfolk et al., 2004). Clark & Messina (1998) demonstrated that, in the absence of prey, *Chrysoperla carnea* Stephens larvae spent 69.9% of the observation time searching and only 30% of time turning (changing direction from the original path by 90° or greater) and resting. Bond (1980) found that larvae moved up to 0.731 cm/second when searching, and larvae that had been deprived of food had a higher velocity. Their searching efficiency depends on many factors, such as the type of plant they are foraging on. They spent 69.9% of their time searching on crested wheatgrass [*Agropyron desertorum* (Fisher ex Link) Schultes] but only 48.3% of time searching on Indian rice grass [*Oryzopsis hymenoides* (Roemer & Schultes) Ricker] (Clark & Messina, 1998). When foraging for aphids, they caught more aphids on mature Indian rice grass, but they also dislodged more than they caught. On mature crested wheatgrass, they were more likely to capture the aphids they made contact with than to dislodge them.

Development of *C. rufilabris*, which goes through an egg stage, three instars, a prepupal and a pupal stage (Pappas et al., 2011), was completed in about 24 days at 75% relative humidity, 22.2°C, and on a diet of *Sitotroga* eggs (Lepidoptera: Gelechiidae) (Tauber & Tauber, 1983). The duration of these developmental times varies depending on prey species. For example, Burke & Martin (1956) found that the eggs took between 3 and 7 days (average 4) to hatch, and the duration of the first instar was 2-4 days (average 2.4), second instar was 2-6 days (average 2.3), third instar was 2-7 days (average 3), prepupal was 2-6 days (average 2.7), and pupal was 4-11 days (average 6.5) when fed *A. gossypii* at 23-31°C. Adults have a longevity of around 25 days (Portilla et al., 2017). Adult females deposit their eggs on plant leaves or shoots, often singly (Smith, 1922), but can also be in clusters (Pappas et al., 2011). Similar to other chrysopids, *C. rufilabris* have white, yellow,
or green, oval-shaped eggs (0.7-2.3 mm) (Pappas et al., 2011) that are laid on thin hyaline stalks (Ruzicka, 1997) and are 4-8 mm long (Smith, 1921).

**Prey preference**

*Chrysoperla rufilabris* is reported to feed on soft-bodied prey, such as Lepidoptera eggs and larvae, and immature beetles, true bugs (including aphids, scales, giant scales, mealybugs, spittlebugs, leafhoppers, treehoppers, and whiteflies), and thrips (Albuquerque et al., 2012; Batista et al., 2022). Much of the literature refers to the larvae as voracious generalist predators that will consume any available soft-bodied arthropod prey in their path (Chen & Liu, 2001; Fréchette & Coderre, 2000; Fréchette et al., 2006; Gebiola & Stouthamer, 2019; Nordlund & Morrison, 1990; Woolfolk & Inglis, 2004).

*Chrysoperla rufilabris* is typically marketed as a biological control agent for aphids; some sources even describe them as primarily aphidophagous (Batista et al., 2022; Giles et al., 2000). However, many biological control suppliers (for example, Arbico Organics) stated in their marketing documents that, in addition to aphids, *C. rufilabris* larvae can also be used to control many other arthropod pests, including mealybugs, whiteflies, aphids, mites, thrips, and multiple beetle species. Very few published papers demonstrated conclusively that *C. rufilabris* could successfully suppress pest populations, and that they will even feed upon some of the species that biological control suppliers have listed in their marketing literature.

Host preferences of related species, such as *C. carnea*, have received more attention than those of *C. rufilabris* (Table 1). I believe this is possibly due, in part, to misidentification in many cases. *Chrysoperla carnea* was once thought to be the only species of green lacewing in the Holarctic region, but in fact, what was thought to be *C.*
was actually a group of many different species of *Chrysoperla* (Henry et al., 2002; Price et al., 2015).

Among the purported target pests, only a few studies evaluated *C. rufilabris’* efficacy against aphids, mites, and mealybugs. The literature contains several papers that show that *C. rufilabris* feeds on many different species of aphids. Batista et al. (2022), Chen & Liu (2001), and Giles et al. (2000) evaluated the performance (development, survival, and reproduction) of *C. rufilabris* on various aphid species such as *Rhopalosiphum padi* L., *Aphis oestlundi* Gillette, *A. gossypii*, *Aphis glycines* Matsumara, *Aphis nerii* Boyer de Fonscolombe, *Aphis fabae* Scopoli, *M. persicae*, *Lipaphis erysimi* (Kaltenbach), and *A. pisum*. Burke & Martin (1956) conducted feeding tests, investigated the life history and feeding habits, and observed the abundances and overwintering habits of *C. rufilabris*. The literature contains two papers that demonstrate *C. rufilabris* larvae feed on mites. Norton et al. (1999) investigated foraging behavior against two mite species, *Orthotydeus lambi* (Baker) and *Amblyseius andersoni* (Chant). Creary (2009) investigated the effects of a pesticide on *C. rufilabris* using spider mites *Eurytetranychus buxi* (Garman) and *Tetranychus schoenei* (McGregor) as prey. Goolsby (1994) evaluated the effectiveness of monthly inundative release of *C. rufilabris* in controlling the longtailed mealybug. None of these studies investigated preference for spider mites and mealybugs.

Only three papers explored the preference of *C. rufilabris* for two prey species in a choice test. Dean & Schuster (1995) found that *C. rufilabris* first, second, and third instars consumed 3.7, 6.4, and 6.9 sweetpotato whiteflies, respectively, and 0.9, 2.2, and 5.4 potato aphids, respectively, in mixed prey arenas but these results were not significant. The whiteflies were smaller than the aphids and, therefore, were less nutritionally valuable
according to the authors. Nordlund and Morrison (1990) reported that the second and third instars of *C. rufilabris* spent a greater percentage of time (11.3% and 43.4%, respectively) feeding on larvae of the tobacco budworm, *Heliothis virescens* (F.) (Lepidoptera: Noctuidae), than they did cotton aphids (1.7% and 4.0%, respectively). Niño et al. (2023) reported that *C. rufilabris* preferred *M. persicae* nymphs over *Microtheca ochroloma* Stål (Coleoptera: Chrysomelidae) eggs and larvae in both two-choice and all-choice tests.

No study has examined the prey selection behavior of *C. rufilabris* when three or more species are presented simultaneously. Studies on *Chrysoperla carnea*, *Chrysoperla externa* (Hagen) and *Chrysoperla plorabunda* (Fitch) demonstrated that *Chrysoperla* spp. demonstrated marked preference for specific prey species or prey life stages in multiple-choice experiments (Table 1). It is likely that an experimental design that provides more than two prey species simultaneously can better detect preference among multiple prey species.

**Table 1.** A summary of published results on the prey preference of *Chrysoperla* spp. Data in all studies were obtained via two-choice or all-choice tests involving multiple prey species. Only the results pertinent to the preference results are summarized.
There are many factors that can determine prey preference and previous research on other natural enemies [for example, *Orius insidiosus* Say (Hemiptera: Anthocoridae), *Hippodamia convergens* Guenn-Meneville (Coleoptera: Coccinellidae), and *Geocoris floridanus* Blatchley (Heteroptera: Geocoridae)] has speculated on what they might be (i.e., nutritional value, prey density, prey mobility, etc.) (Ables et al., 1978; Butler & O’Neil, 2008; Eubanks & Denno, 2002; Torres et al., 2004; Xu et al., 2006).

**Study objectives**

A lack of comparative study on the preference and efficacy of *C. rufilabris* against multiple prey species that could be present simultaneously in the same greenhouse hampers our ability to develop recommendations on how and where *C. rufilabris* can be utilized most effectively. A logical next step to fill the knowledge gap of what and if *C. rufilabris* has a preference between different prey species is to conduct an experiment designed to evaluate
C. rufilabris’ preference among its supposed prey species, such as mealybugs, aphids, and spider mites. Prey acceptability can be assessed in no-choice bioassays, whereas prey preference can be determined in choice bioassays where different combinations of prey species are offered simultaneously. This type of experiment will clarify the actual prey range of this generalist predator.

Another gap in the literature is defining what drives the prey preference of C. rufilabris, if there is one. Many factors can shape a predator's prey preference and very little is known about what actually drives prey preference for C. rufilabris (Dean & Schuster, 1995; Nordlund & Morrison, 1990), particularly with the influence of varying densities of two prey species of different preferences. To my knowledge, there is no research on how the relative densities of multiple prey species can affect the prey preference and foraging behavior of C. rufilabris.

A careful examination of C. rufilabris foraging behavior when varying densities of multiple species are encountered simultaneously can also be an evaluation of optimal foraging theory (Pyke et al., 1977). Optimal foraging theory is based on the assumption that foraging behavior that maximizes the energetic or fitness benefit of a species will be favored by natural selection. It predicts that the preferred food items should be eaten first and then move on to less favored items in the order of their descending preference ranking. If optimal foraging theory is correct, then it can be expected that the prey item most preferred by C. rufilabris will be consumed before any other prey items, regardless of varying densities.
CHAPTER TWO
MATERIALS AND METHODS

Sources and maintenance of insects, mites, and plants

Green bean (*Phaseolus vulgaris* L., cv. “Lake Blue 274”; Fabaceae; seeds purchased from Dillon’s Seeds, Dillon, SC), French marigolds (*Tagetes patula* L., cv. “Bonanza”; Asteraceae; seeds purchased from Park Seeds Wholesale, Greenwood, SC), gerbera daisy (*Gerbera jamesonii* Bolus ex Hook. cv. “Jaguar Orange” and “Jaguar Yellow”; Asteraceae; plugs purchased from Raker-Roberta’s, Litchfield, MI), and non-transgenic cotton plants (seeds from USDA-ARS in Florence, SC; variety unkown) were germinated and grown in 6-inch plastic pots filled with potting media (Sungro Professional, Sun Gro Horticulture, Inc., Agawam, MA) in a greenhouse at Clemson University Pee Dee Research and Education Center (PDREC) in Florence, SC. The plants were fertilized with Nutriculture general purpose 20-20-20 soluble fertilizer (Plant Marvel Laboratories, Chicago Heights, IL) biweekly and irrigated once daily. The greenhouse was maintained at an average temperature of 78°F and 49% relative humidity. These plants were grown to satisfy insect colony maintenance, and the cotton plants were used for constructing experimental arenas.

Second-instar *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae), third-instar *Aphis gossypii* Glover (Hemiptera: Aphididae), and adult *Tetranychus urticae* Koch (Acari: Tetranychidae) were obtained from greenhouse and laboratory colonies. Colonies of *T. urticae* and *P. madeirensis* were maintained on French marigold and cotton plants, and a colony of *A. gossypii* was established on French marigold, gerbera daisy, and cotton plants in greenhouses at PDREC. Aphid colony was established by allowing aphids (originated with alates dispersing into the greenhouses through opened vents) to feed and build up,
whereas mealybug and spider mite colonies were established by allowing dispersal of individuals from infested plants in the same or nearby greenhouses. Laboratory colonies of aphids, mealybugs, and mites were also established to ensure ample numbers of each species for the project. Mealybugs were collected as ovisacs from the greenhouse colony and transferred onto potato sprouts kept in the laboratory. The mealybugs were allowed to develop on sprouted potatoes to the desirable life stage for this study. The laboratory colony of *T. urticae* was established and maintained by infesting green bean plants with mites collected from the greenhouse colony. Aphid colony in the laboratory was established with individuals collected from the greenhouse and maintained on French marigold and cotton plants.

*Chrysoperla rufilabris* larvae were purchased from a commercial biological control agent producer (Rincon-Vitova Insectaries, Ventura, CA). The larvae received were all hatched at the same time and mainly second instars at the time of arrival; the second and third instars were differentiated by body length (4.8 mm vs. 9 mm; Smith 1922). Each order of larvae was maintained in the laboratory at PDREC for 1 week, during which groups of larva were haphazardly selected and removed from a storage container for the experiments at different days. The insects were removed from the shipping containers and then housed in a storage container with crumpled paper to reduce cannibalism, similar to a method used by Rutledge & O’Neil (2005) for *Orius insidiosus* (Say) (Hemiptera: Anthocoridae). An excess amount of cabbage looper [*Trichoplusia ni* (Hübner); Lepidoptera: Noctuidae] eggs were purchased from Frontier Scientific (Newark, DE) and placed in the container to maintain the larvae at a satiated stage. A cotton ball moistened with deionized water also was placed in the container to ensure the larvae were properly hydrated. All insects were kept at 16:8
hours (light: dark) photoperiod (Huang & Enkegaard, 2010) using LED lighting and timer, and approximately 21.9°C and 20% relative humidity.

**Experimental arenas and data collection**

All experiments were conducted in 60-mm Petri dishes. A moistened filter paper and a cotton leaf disk were fit snugly in the bottom of each Petri dish, on top of which thirty individual prey were placed. *Aphis gossypii* and *T. urticae* were transferred from the greenhouse and laboratory colonies (from marigold and bean plant, respectively, for spidermites; marigold for aphids), whereas *P. madeirensis* were sourced from the laboratory colony. The prey were transferred individually from the colonies with a fine hairbrush. All prey were allowed 1 hour to acclimate and disperse in the Petri dish before a lacewing larva was added.

Data collection process was the same for all experiments in this study. The numbers of live and dead prey were recorded 3 hours after the introduction of the lacewing larvae. Live and dead prey can be distinguished with light prodding to the individuals to stimulate movement. There are four causes of mortality: complete consumption, partial consumption, killed but not consumed, and non-predatory causes. A prey that died from complete consumption was completely devoid of body contents and/or had a collapsed exoskeleton. Partially consumed prey often had a portion of their body volume missing and had obvious feeding wounds or punctures by the lacewing’s mouthparts. Both unconsumed prey and prey died from non-predatory causes had body shape and volume that were the same as the live prey. Unconsumed prey showed signs of mouthpart punctures, but prey that died from non-predatory causes did not have wounds. Only the numbers of prey that were completely consumed, partially consumed, and killed but not consumed were considered in the
calculation of mortality and data analyses. The ability to clearly identify individuals killed by the lacewing larvae excluded mortality caused by natural or other causes thereby eliminated the need for correction for control mortality (such as Abbott’s formula) typically performed in similar studies.

**Experiment 1: Preference for three prey species**

Three bioassays were conducted to determine the preference of *C. rufilabris* larvae for third-instar *A. gossypi*, second-instar *P. madeirensis*, and adult *T. urticae*. No-choice bioassays were conducted to determine if *C. rufilabris* would accept the prey species provided. Two-choice bioassays were conducted to determine the preference ranking of *C. rufilabris* among the three prey species. All-choice bioassays were conducted to validate the rank of preferences determined in the two-choice bioassays.

Ten Petri dishes (i.e., the number of replicates = 10) were prepared for the no-choice bioassays. Each Petri dish was populated with 30 individuals of one prey species and received one second-instar *C. rufilabris*. The numbers of prey killed by *C. rufilabris* or died from other causes were recorded as previously described. The percent killed of each species (i.e., completely consumed + partially consumed + killed but not consumed/total number) were analyzed with Kruskal-Wallis test at α = 0.05 (PROC NPAR1WAY; SAS 2017) with prey species as the independent variable and percent mortality as the dependent variable. When Kruskal-Wallis test detected significant difference among the prey species, the percent mortality data from all species were pooled, ranked, and subjected to Fisher’s least significant difference (LSD) test (Conover, 1999).

Each Petri dish contained two prey species (15 individuals of each species) in the two-choice bioassays; a total of six two-choice combinations were prepared. One second-
instar *C. rufilabris* was introduced into each Petri dish, and the numbers of live and dead prey of each prey species was recorded 3 hours after predator introduction. Each two-choice combination was replicated 10 times. The null hypothesis of no preference (i.e., mortality of 50% for each species) was tested with a binomial test at $\alpha = 0.05$ (PROC FREQ; SAS 2017) for each two-species combination. The rank order of preference among three prey species was determined based on the results of the binomial tests.

In the all-choice bioassays, 10 individuals of each prey species were introduced into a single Petri dish simultaneously. The numbers of live and killed prey were recorded 3 hours after the predator’s introduction as previously described. The experiment was replicated eight times. The null hypothesis of no preference (i.e., 33.33% mortality for each species) was analyzed with Chi-Square Goodness-of-Fit tests (PROC FREQ; SAS 2017) at $\alpha = 0.05$. To determine the preference ranking, individual pairings of the three prey species were analyzed with the Manly-Chesson index ($\beta$) (Chesson, 1983) where:

$$
\beta = \frac{\log\left(\frac{e_1}{A_1}\right)}{\log\left(\frac{e_1}{A_1}\right) + \log\left(\frac{e_2}{A_2}\right)}
$$

The $\beta$ is the preference for a prey species, $e_1$ and $e_2$ are the numbers of Species 1 and 2, respectively, after being exposed to the predator. $A_1$ and $A_2$ are the numbers of prey Species 1 and 2, respectively, before being exposed to *C. rufilabris*. Preference for Species 1 is indicated as $\beta_1$ and preference for Species 2 is indicated as $\beta_2$. The $\beta$ values were subjected to Kruskal-Wallis test at $\alpha = 0.05$ to detect significant differences among the preference for the three species.
Experiment 2: Influence of prey density ratios on prey consumption

The purpose of this experiment was to determine if the relative abundances of two prey species affects the prey consumption rate by *C. rufilabris*. This experiment was carried out in Petri dishes prepared as previously described. Each Petri dish contained the most preferred and the least preferred prey species, as determined in Experiment 1. Since no preferred prey species was identified in Experiment 1, I decided to select the prey species that had the highest and lowest numbers of individuals killed by *C. rufilabris* in Experiment 1, i.e., *A. gossypii* and *T. urticae*, respectively.

The ratios (%) of third-instar melon aphids vs. adult twospotted spider mites in each Petri dish were 100:0, 75:25, 50:50, 25:75, or 0:100. The total number of prey items in a Petri dish was 30 individuals. One second-instar *C. rufilabris* larva was introduced into each Petri dish after the prey had acclimatized for 1 hour. The mortality of each prey species was determined 3 hours after predator introduction. This experiment was replicated 7 times.

Food selectivity coefficient $\beta$ at each density ratio was calculated based on the Manly-Chesson index ($\beta$) (Chesson, 1983). The coefficient ranges from 0 to 1, with $\beta = 0.5$ indicating nonselective feeding and $\beta < 0.5$ indicating that *C. rufilabris* consumed prey preferentially. Kruskal-Wallis test was used to determine if $\beta$ of two species differed among density ratios at $\alpha = 0.05$. The ratio of prey was the independent variable and the $\beta$ value was the dependent variable. The data was then pooled, ranked, and subjected to Fisher’s means separation test.
CHAPTER THREE
RESULTS AND DISCUSSION

Prey Species Preference of *Chrysoperla rufilabris*

Green lacewings are important generalist biological control agents used for managing pest populations on greenhouse crops. Species such as *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) and *Chrysoperla rufilabris* (Burmeister) (Neuroptera: Chrysopidae) are offered by many biological control agent suppliers for the management of many insect and mite pests. Some of the target pests mentioned by these suppliers include beet armyworm [*Spodoptera exigua* (Hubner); Lepidoptera: Noctuidae], California laurel aphid [*Euthoracaphis umbellularia*e (Essig); Hemiptera: Aphididae], Colorado potato beetle [*Leptinotarsa decemlineata* (Say); Coleoptera: Chrysomelidae], citrus mealybug [*Planococcus citri* (Risso); Hemiptera: Pseudococcidae], grape leafhopper (*Erythroneura elegantula* Osborn; Hemiptera: Cicadellidae), and sweetpotato whitefly [*Bemisia tabaci* (Gennadius); Hemiptera: Aleyrodidae] (Arbico Organics). The preference of *Chrysoperla* species for these pest species has been explored for the European species, *C. carnea*. Preference of the North American species, *C. rufilabris*, on the other hand, is poorly known. In particular, there are very few papers that explore the prey preference of *C. rufilabris* when multiple prey species are offered simultaneously in choice bioassays.

In this study, second-instar *C. rufilabris* did not exhibit a preference for any of the prey species offered. In the no-choice test, they killed similar numbers and percentages of *Aphis gossypii* Glover (Hemiptera: Aphididae), *Phenacoccus maderiensis* Green (Hemiptera: Pseudococcidae), and *Tetranychus urticae* Koch (Acari: Tetranychidae) (Table 2). On average, 25% of the offered prey were killed by *C. rufilabris* in the no-choice.
bioassays. In the two-choice test, the larvae killed numerically more *A. gossypii* than *T. urticae*, numerically more *A. gossypii* than *P. maderiensis*, and numerically more *P. maderiensis* than *T. urticae*; however, the binomial tests did not detect any significant preference for a particular prey species among the pairs of species (Table 3). When all prey species were provided simultaneously in the all-choice test, no significant difference was detected in the numbers of prey killed or the Manley-Chesson indices among the three prey species (Table 4). Numerically, *C. rufilabris* killed more *A. gossypii* and fewer *T. urticae*. However, *T. urticae* had the highest and *P. maderiensis* had the lowest Manly-Chesson beta index.

Larvae of *Chrysoperla* spp. have been shown to have marked preference for aphids (various species) over other prey species (Table 1). For example, Niño et al. (2023) reported a clear preference by *C. rufilabris* for the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), over the eggs and larvae of *Microtheca ochroloma* Stål (Coleoptera: Chrysomelidae) in all-choice bioassays. Based on results from Niño et al. (2023) and similar studies, I expected *C. rufilabris* to display a preference for *A. gossypii*. Contrary to my expectations, second-instar *C. rufilabris* did not display preference for any of the three prey species evaluated in this study. The result of non-preference I had observed in this study may be influenced by other factors related to the prey selection behavior of predators, or as the consequences of experimental design. Prey selection behavior may be influenced by prey abundance, mobility, size, color, odor, and nutritional quality compared to energetic cost (Eubanks & Denno, 2000). Experimental artifacts may include the size and confinement of the experimental arena (Michaud, 2002), the lack natural leaf habitat, and the short acclimation time for the predator.
Prey accessibility may have influenced the results of this study. Accessibility of the prey may be influenced by the prey’s mobility, their ability to evade detection or capture, and any defenses the prey may have against the predator (Allen & Flecker, 1988; Eubanks & Denno, 2000). Some predators prefer highly mobile prey; for example, *Geocoris punctipes* Say (Hemiptera: Geocoridae) displayed a preference for mobile *Acyrthosiphum pisum* (Harris) (Hemiptera: Aphididae) over immobilized individuals (Eubanks & Denno, 2000). Behavioral observations will need to be taken to assess if prey mobility is a determining factor for the preference displayed by *C. rufilabris*. The data collected in this study (i.e., the numbers of live and dead prey) did not provide sufficient information to determine if *C. rufilabris* indeed prefers a mobile prey item or species.

The ability of the prey to evade capture or detection can also have an effect on predator preference (Allen & Flecker, 1988). Mobile prey are more efficient at evading capture by the predator than sessile prey (Eshel et al., 2006). In this study, the leaf disks were fitted snugly, and aphids, mealybugs, and spider mites had limited opportunity to hide and evade capture in the experimental arena (e.g., hiding under the leaf disks); therefore, it is unlikely that prey evasion was a factor influencing the results. Past research has speculated that the mobility of the prey could be what drives the preference of *C. carnea* for their prey (El-Zahi, 2017; Huang & Enkegaard, 2010; Shrestha & Enkegaard, 2013). Further research with extensive observational data will be required to elucidate if the prey movement could be contributing to the prey preference of *C. rufilabris*.

Nutritional quality of prey, often in terms of prey body size, is another factor that could influence prey selection behavior. Dean & Schuster (1995) reported that *C. rufilabris* larvae consumed more whiteflies (*B. tabaci*) than aphids (*M. euphorbiae*). If prey preference
was determined purely on the number of prey killed, whiteflies would have been the preferred prey item. Dean & Schuster’s (1995) conclusion may have been skewed by the fact that B. tabaci is only 1/5th the size of M. euphorbiae. A lacewing larva would have to eat more B. tabaci to secure the same amount of resource as one aphid. In the current experiment, immature stages of P. madeirensis and A. gossypii were selected to remove some of the size disparity between them. The size of T. urticae was markedly smaller than aphids and mealybugs. It is reasonable to assume that more spider mites would need to be consumed in this study to satiate a lacewing larva the same amount as consuming a smaller number of the larger aphid or mealybug prey. Since the spider mites were consumed the same amount as aphids and mealybugs this could indicate they prefer the spider mites less that the other two prey option.

Lacewings are often labeled as aphidophagous (Giles et al., 2000; Fréchette & Coderre 2000), but the results of this experiment suggest differently. It is possible that, rather than a lack of any preference, the prey species offered to the C. rufilabris in this study may all be preferred equally. Unlike C. carnea, aphids might not be a preferred prey item for C. rufilabris. There are some aphid species that C. rufilabris cannot even complete development on. Batista et al. (2022) found that, when reared on 16 different aphid species, C. rufilabris could not complete development on Rhopalosiphum padi L., Uroleucon obscuricaudatus Olive, M. persicae, Aphis fabae Scopoli, and Aphis nerii Boyer de Fonscolombe. Only seven of the 16 aphid species were found to be optimal prey items based on completion of development, survival, emergence of adults, and egg production. Many of these aphid species were previously considered satisfactory prey by multiple papers cited by
Batista et al. (2022). It is important to continue to research and clarify the preferences and prey selection behavior of *C. rufilabris* to optimize management strategies for growers.

**Influence of prey density on consumption**

In this experiment, it was expected that prey density ratio would affect the preference of *C. rufilabris*. The original research plan called for selecting the two most preferred prey species from Experiment 1 as subject species in Experiment 2. However, since no preference was detected in Experiment 1, I chose the prey species that were consumed the most (*A. gossypii*) and the least (*T. urticae*) across all three bioassays in Experiment 1 with the hope that some level of preference could be detected.

First, the data from all five prey density ratios were analyzed. The analysis reported a significant difference between the different ratios for both $\beta_1$ and $\beta_2$ ($\chi^2 = 22.9318$, df = 4, $P < 0.001$). However, the two end ratios, i.e., 100:0 and 0:100, might not be most useful in assessing the influence of changing prey density ratio since there was only one species in each of these ratios. I therefore did a second analysis based on data from the three middle density ratios and found no significant difference in $\beta_1$ and $\beta_2$ ($\chi^2 = 0.794$, df = 2, $P > 0.9611$), indicating that varying relative aphid and spider mite density ratios did not affect their consumption (Figure 1). The results suggest that the relative density of each prey is not a factor influencing prey preference in *C. rufilabris*.

According to the optimal foraging theory, more specifically the optimal diet model, if one prey species is preferred over the other, the predator will seek out the preferred prey first before consuming the less preferred prey (Lacher, et al., 1982; MacArthur & Pianka, 1966). However, the behavior of the predator will change as the density of the preferred prey is reduced to a point where continuing to search for the preferred prey generates less
fitness or benefits than consuming the less preferred prey. For example, if Species 1 is the preferred prey species, a predator that feeds disproportionately more on Species 1 when encountering prey at a 75:25 (preferred: less preferred) density ratio will feed more on Species 2 when the prey density ratio is changed to 25:75. That means a greater abundance of the less-preferred prey will be consumed as the preferred prey becomes rarer and the predator takes more energy and time to find them (Lacher, et al., 1982; MacArthur & Pianka, 1966).

Optimal foraging theory predicts that in the presence of a higher value prey, in this case the preferred prey, all lower value prey (less preferred prey) should be ignored (Lacher, et al., 1982; MacArthur & Pianka, 1966). In this study, contrary to the results from Experiment 1, numerically more *T. urticae* were consumed by *C. rufilabris*. Although the Manly-Chesson indices were numerically higher for *T. urticae*, second-instar *C. rufilabris* did not ignore *A. gossypii* in all the density ratios. The Manly-Chesson indices for the two species remained the same as the prey density ratio changed, suggesting that there was no switching behavior when the abundance of *T. urticae* was lower and encounter rates likely dropped in frequency compared to *A. gossypii*, supporting the conclusion that *C. rufilabris* has no preference.

**Conclusion**

In this project, I sought to determine if *C. rufilabris* consumed prey preferentially (among *T. urticae*, *A. gossypii*, and *P. maderiensis*) and if prey density drove preference for *T. urticae* and *A. gossypii*. *Chrysoperla rufilabris* larvae did not display preference for a particular species in no-choice, two-choice, and all-choice bioassays. The consumption of spider mites and aphids was not impacted by changing prey density ratios. The results from
this research indicate that *C. rufilabris* does not have a strong preference for aphids over other prey species. The results also did not meet my original expectations, pointing to the complexity of prey selection behaviors and the need for additional study on the foraging behavior of *C. rufilabris*. The divergences in the prey selection behavior of *Chrysoperla* congenerics are apparent. *Chrysoperla rufilabris* seems to feed in a much different manner than its morphologically similar relatives, such as *C. carnea*. Future research should consider investigating factors that drive differential prey preference in *Chrysoperla* spp. Future studies on prey preference of *Chrysoperla* spp. should also be conducted in multiple prey systems that are more indicative of greenhouse crop conditions. The next step for this particular research would be to conduct choice tests on a whole plant instead of a leaf disk in a Petri dish with these prey species.

The results of this study reveal the foraging behavior of *C. rufilabris* and how they select their prey. This species is not selective in term of prey species based on the results of this study. This has implication for IPM on greenhouse ornamental and vegetable crops since the prey preference of beneficial insects is important for predicting the efficacy of biological control agents (Eubanks & Denno, 2000). *Chrysoperla rufilabris* appears to be a generalist predator that does not show strong preference for aphids. It likely consumes any soft-bodied prey that it comes across during foraging. Growers wishing to use *C. rufilabris* in a biological control program may be able to use this species to manage multiple pest species, including aphids, mealybugs, and spider mites, as some biological control agent suppliers are claiming.
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Table 1. A summary of published results on the prey preference of Chrysoperla spp. Data in all studies were obtained via two-choice or all-choice tests involving multiple prey species. Only the results pertinent to the preference results are summarized.

<table>
<thead>
<tr>
<th>Chrysoperla Spp.</th>
<th>Prey</th>
<th>Summary</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. carnea</em> (Stephens)</td>
<td><em>Bemisia tabaci, Aphis gossyipii, Ameles devastan, Phenococcus solinogis, Lipaphis erysimi, &amp; Bagrada Picta</em></td>
<td>The all-choice tests show that <em>C. carnea</em> larvae have the highest preference for <em>A. gossyipii</em> followed by, in descending order, <em>P. solinogis</em> mealybugs, <em>B. tabaci</em> whiteflies, and <em>A. devastan</em> leaf hoppers on cotton plants. The most preferred prey on mustard crop in an all-choice test was <em>L. erysimi</em> aphids followed by <em>B. picta</em> stink bug eggs, and least preferred was <em>A. devastan</em>.</td>
<td>Solangi et al., 201</td>
</tr>
<tr>
<td><em>C. carnea</em></td>
<td><em>Pieris brassicae &amp; Brevicoryne brassicae</em></td>
<td>Third instar <em>C. carnea</em> preferred <em>P. brassicae</em> caterpillars over <em>B. brassicae</em> aphids, but second instar <em>C. carnea</em> preferred the aphids over the caterpillars.</td>
<td>Huang &amp; Enkegaard, 2010</td>
</tr>
<tr>
<td><em>C. carnea</em></td>
<td><em>Frankliniella occidentalis &amp; Nasonovia ribisnigri</em></td>
<td>Third instar <em>C. carnea</em> preferred <em>N. ribisnigri</em> aphids over <em>F. occidentalis</em> thrips significantly in two of the 5 prey ratios (10.80 &amp; 65.25), and overall consumed more aphids than thrips.</td>
<td>Shrestha &amp; Enkegaard, 2013</td>
</tr>
<tr>
<td><em>C. carnea</em></td>
<td><em>P. solinogis &amp; A. gossyipii</em></td>
<td>In a free choice feeding experiment, <em>C. carnea</em> larvae preferred <em>A. gossyipii</em> aphids over 1st, 2nd, and 3rd instar <em>P. solinogis</em> mealybugs. In no-choice experiment, they ate significantly more 1st instar mealybugs than aphids.</td>
<td>El-Zahi, 2017</td>
</tr>
<tr>
<td><em>C. carnea</em></td>
<td><em>B. brassicae, Myzus persicae, &amp; Lipaphis erysimi</em></td>
<td><em>C. carnea</em>’s consumption for both two-choice tests comparing <em>B. brassicae</em> vs. <em>M. persicae</em> and <em>L. erysimi</em> vs. <em>M. persicae</em> did not differ significantly between their consumption:encounter ratios</td>
<td>Jessie et al., 2015</td>
</tr>
<tr>
<td><em>C. externa</em> (Hagen)</td>
<td><em>Liothyrrys hyalinus, Macrosiphum euphorbiace &amp; Myzus simulans</em></td>
<td>Three two-choice experiments showed that <em>N. simulans</em> seed bugs were preferred over <em>L. hyalinus</em> scale insects, plant bugs, and <em>M. euphorbiace</em> aphids were preferred over both <em>L. hyalinus</em> and <em>N. simulans</em>.</td>
<td>Crues et al., 2022</td>
</tr>
<tr>
<td><em>C. plorabunda</em> (Fitch)</td>
<td><em>Diaphorne citri, Toxoptera citricida, &amp; Brevipalpus sp.</em></td>
<td>In the all-choice test, <em>C. plorabunda</em> showed a preference for <em>D. citri</em> psyllid. In two-choice tests, they showed a preference for <em>T. citricida</em> aphids over <em>Brevipalpus</em> sp mites.</td>
<td>(Palomares-Pérez et al., 2022)</td>
</tr>
</tbody>
</table>
Table 2. Mean (± standard error) percent and number of prey killed by *C. rufilabris* in no-choice tests. The initial number of prey offered were 30 individuals.

<table>
<thead>
<tr>
<th>Prey species</th>
<th>% of prey killed</th>
<th>Number of prey killed</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aphis gossypii</em></td>
<td>22.4 ± 7.7</td>
<td>6.2 ± 2.3</td>
</tr>
<tr>
<td><em>Phenacoccus maderiensis</em></td>
<td>27.8 ± 8.9</td>
<td>7.4 ± 3.1</td>
</tr>
<tr>
<td><em>Tetranychus urticae</em></td>
<td>26.1 ± 10.8</td>
<td>7.1 ± 2.4</td>
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</tbody>
</table>

Kruskal-Wallis statistics:

<table>
<thead>
<tr>
<th></th>
<th>$\chi^2$</th>
<th>df</th>
<th>$P$</th>
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<tbody>
<tr>
<td></td>
<td>0.9362</td>
<td>2</td>
<td>0.6262</td>
</tr>
<tr>
<td></td>
<td>0.4392</td>
<td>2</td>
<td>0.8028</td>
</tr>
</tbody>
</table>

Table 3. Mean (± standard error) number of prey consumed by *C. rufilabris* larvae in two-choice tests. The initial number of prey was 15 per prey species for a total of 30 individuals per dish. Data were analyzed with binomial tests.

<table>
<thead>
<tr>
<th>Pair</th>
<th><em>Aphis gossypii</em></th>
<th><em>Phenacoccus maderiensis</em></th>
<th><em>Tetranychus urticae</em></th>
<th>Z</th>
<th>Two-sided P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.7 ± 1.8</td>
<td>-</td>
<td>7.8 ± 1.5</td>
<td>0.7006</td>
<td>0.4835</td>
</tr>
<tr>
<td>2</td>
<td>11.8 ± 0.6</td>
<td>10.2 ± 0.9</td>
<td>-</td>
<td>1.0787</td>
<td>0.2807</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>6.8 ± 1.5</td>
<td>7.4 ± 1.7</td>
<td>0.5035</td>
<td>0.6146</td>
</tr>
</tbody>
</table>
Table 4. Mean (± standard error) number of prey consumed by *C. rufilabris* larvae in an all-choice test.

The initial number was 10 per prey species for a total of 30 individuals per arena.

<table>
<thead>
<tr>
<th>Prey species</th>
<th>Number of prey consumed</th>
<th>Manly-Chesson index (β)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aphis gossypii</em></td>
<td>6.6 ± 1.1</td>
<td>0.44 ± 0.10</td>
</tr>
<tr>
<td><em>Phenacoccus maderiensis</em></td>
<td>6.3 ± 0.9</td>
<td>0.40 ± 0.10</td>
</tr>
<tr>
<td><em>Tetranychus urticae</em></td>
<td>5.8 ± 1.0</td>
<td>0.50 ± 0.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Chi-square-Goodness-of-Fit</th>
<th>Kruskal-Wallis test</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\chi^2$</td>
<td>0.0001</td>
<td>1.6510</td>
</tr>
<tr>
<td>df</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>$P$</td>
<td>1.0</td>
<td>0.4380</td>
</tr>
</tbody>
</table>

Figure 1. Mean (± standard error) Manly-Chesson indices for *Aphis gossypii* ($\beta_1$) and *Tetranychus urticae* ($\beta_2$) at five prey density ratios (*A. gossypii: T. urticae*).