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Investigation of ecological behavior of two Coccinellidae beetle adults for biological control

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INVESTIGATION OF ECOLOGICAL BEHAVIOR OF TWO COCCINELLIDAE BEETLE ADULTS FOR BIOLOGICAL CONTROL

Folukemi Ebunoluwa Adedipe

Thesis submitted to the College of Agriculture, Forestry and Consumer
Sciences, Division of Plant and Soil Sciences at West Virginia University
in partial fulfillment of the requirements for the degree of

Master of Science
in
Plant and Soil Sciences – Entomology

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Morgantown, West Virginia
2009

ABSTRACT

INVESTIGATION OF ECOLOGICAL BEHAVIOR OF TWO COCCINELLIDAE BEETLE ADULTS FOR BIOLOGICAL CONTROL

Folukemi Ebunoluwa Adedipe

Biological control is the use of natural enemies, which is an effective strategy to control and suppress pest populations in a given agroecosystem; however farmers are not motivated to adopt this method because of the high cost and temporal unavailability of natural enemies.

This thesis is based on reports on findings of a series of laboratory experiments carried out to investigate ecological behavioral of two major natural enemies (i.e. *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) and *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae). Firstly, study was conducted to determine if *H. axyridis* could be attracted into cropping areas using companion plants with different olfactory and visual cues. Ten potential plants that can attract and provide *H. axyridis* with nectar and pollen were selected for a preference test. The result of the olfactory preference test showed that there was significant difference in the plant preference by *H. axyridis*; sunflower was the most preferred attractant plants. In a visual preference test, *H. axyridis* preferred yellow to other colors. Secondly, study was conducted to investigate within-plant distribution of long-tailed mealybug, *Pseudococcus longispinus* (Targioni-Tozzetti) (Hemiptera: Pseudococcidae) to quantify the searching and handling time of *C. montrouzieri* on three different plants heights and to determine preference of *C. montrouzieri* to the size of *P. longispinus*. *P. longispinus* were found more on the upper parts of the plants regardless of the height. The time *C. montrouzieri* spent to find the first *P. longispinus* was significantly ($P < 0.05$) different among the three different plant heights at two different releasing points. However, there were no significant differences in handling time and cleaning time. *C. montrouzieri* preferred smaller size of *P. longispinus* to medium and larger size. The finding of results in this thesis suggested that *H. axyridis* and *C. montrouzieri* can potentially be used as economical and sustainable biological control agents.

DEDICATION

To my lovely children Iseoluwa and Ewaoluwa Adedipe, they are the source of my joy.

&

To all that have contributed to my academic successes. God bless you all.

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I believe that I could not have done this with my strength alone; however he made all things beautiful in his time. I acknowledge the Almighty God for exercising his loving kindness and power over my life.

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My deepest gratitude goes to my parents for always struggling to live up to their responsibilities against all odds and constraints. I owe a large debt of gratitude to my husband Oluwatosin Adedipe, who has been there for me and endured this process all along the way. I'm really amazed at how much he loves and sacrifices for me. I could not have done this without him.

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CHAPTER 1: INTRODUCTION THESIS ORGANIZATION

This thesis is organized into four chapters. Chapter 1 is a general introduction to the project and a literature review. Chapter 2 discusses the screening and selecting of plant odor and colors that potentially attract *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae). Chapter 3 addresses within-plant distribution of *Pseudococcus longispinus* (Targioni-Tozzetti) (Hemiptera: Pseudococcidae), quantifies the searching and handling time, and determines preference of *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) to sizes of *P. longispinus*. Chapter 4 provides a general conclusion for this study. This thesis was prepared according to the publication guidelines established by the Entomological Society of America.

GENERAL INTRODUCTION

Effective pest management techniques will go a long way in striking a balance between meeting the demands of food supply for the growing world population and the social concerns of the effects of pesticides on humans and the environment at large. Pest management is the act of controlling pest populations which are considered to be a major barrier in crop production by reducing yields. Many methods have been adopted in controlling pest populations, but the most common method still is the use of pesticides. Pesticides, specifically insecticides, are chemicals that injure or kill insects; however spraying pesticides has a significant negative impact on humans and the environment. These may include serious human health risks, large-scale destruction of non target organisms and plants, and severe environmental impacts within the ecosystem. Additionally, pest populations have often developed resistance to the commonly used pesticides (Saxena 1982, Swezey and Salamanca 1987), and hence there are needs for alternative control strategies. A main alternative pest control tactic to pesticides is biological control, the use of natural enemies; biological control is one of the most important environmentally-friendly pest

management strategies. In this thesis, ecological behavioral of two major natural enemies (i.e. *H. axyridis* and *C. montrouzieri*) was investigated to improve biological control of two major horticultural pests, aphids and mealybugs.

OBJECTIVES OF STUDY

The goal of this study was to investigate the potential of using two generalist natural enemies, *H. axyridis* and *C. montrouzieri* as biological control agents. The objectives of this research were:

1. To develop a method to attract *H. axyridis* using companion planting by screening and selecting plants whose odor and color attract *H. axyridis* (Chapter 2).
2. To determine the searching and handling time and preference of *C. montrouzieri* using *P. longispinus* as prey at various plant heights (Chapter 3).

LITERATURE REVIEW

Life history of *H. axyridis*: The multi-colored Asian lady beetle, *Harmonia axyridis* Pallas, is an introduced biological control agent native to western Asia. It was first introduced into North America in 1916 and 1982 (Colunga-Garcia and Gage 1998) and the intention was to introduce a new beneficial predator. *Harmonia axyridis* is a highly voracious predator on a variety of insect pests, including aphids in food crops, trees and landscape plantings (Hukusima and Kamei 1970). In the eastern United States, *H. axyridis* has been used to control aphids in pecan orchards and has spread throughout the east coast. *H. axyridis* has become the dominant lady beetle species in many parts of the United States. Despite these successes in agroecosystems, *H. axyridis* has received more attention because it is a problem in late summer, when adults feed on ripening fruit in some geographic locations (Koch et al. 2004), and in autumn, when large numbers alight on buildings then move into interior spaces, looking for dark, protected fissures to settle for

the winter.

H. axyridis has four distinct life stages: egg, larva, pupa and adult (Wang 1986, Hodek 1973). Adults of *H. axyridis* are oval, about 6 mm (1/4 inch) long and range in color from yellow to orange to red, with or without black spots on the wing covers (LaMana and Miller 1996); the number of spots varies drastically, ranging from zero to at least twenty. The head is usually concealed from above and the white pronotum generally has a black "M"-like shape, which is a key feature for species identification (Chapin and Brou 1991). *Harmonia axyridis* larvae are elongate, flattened and covered with minute spines (Chapin 1965, Kuznetsov 1997, Sasaji 1971). The larvae can rapidly move around leaves and branches, where they eat aphids and other soft-bodied insects. The pupa is orange with black spots and similar in size and shape to the adult. Eggs are yellow, oval shaped and laid upright in clusters of about 20, usually on the undersides of leaves.

Harmonia axyridis is bivoltine and overwinters as an adult in cracks and crevices in late October (Kidd et al. 1995, LaMana and Miller 1996). *Harmonia axyridis* adults begin laying eggs on host plants in early spring. Eggs hatch in about three to five days, and larvae begin searching on plants for aphids and other soft-bodied arthropods on which to feed (Osawa 2000, Sakurai et al. 1992); adults and larvae typically feed upon the same prey. Larvae molt four times to become pupae and pupation lasts five to six days until adults emerge (El-sebaey and El-Gantiry 1999). Adults typically live 30 to 90 days depending on temperature (El-Sebaey and El-Gantiry 1999, He et al. 1994, Soares et al. 2001). Development from egg to adult requires 267.3 degree days above a lower developmental threshold of 11.2° C in the United States (LaMana and Miller 1998). In October and November adults congregate to find overwintering sites and they attempt to return to their outdoor habitat in February and March. Generally adults mate before

dispersing from overwintering sites (LaMana and Miller 1996). Sexual activity of *H. axyridis* and other Coccinellidae was reviewed by Hodek and Ceryngier 2000.

In the United States, *H. axyridis* has been used for biological control in pecans (Teddners and Schaefer 1994) and red pines (McClure 1987). *Harmonia axyridis* contributes more to biological control of the pecan aphid complex in the southeastern United States than in the southwestern United States (Rice et al. 1998, Tedders and Schaefer 1994). In apple orchards, *H. axyridis* provides effective biological control of *Aphis spiraecola* (Brown and Miller 1998). The biological control of several citrus pests may also be benefiting from the establishment of *H. axyridis* (Michaud 1999, Michaud 2000, Michaud 2001a, Michaud 2001b, Michaud 2002, Stuart et al. 2002). In sweet corn, *H. axyridis* controls *Ostrinia nubilalis* eggs and *Rhopalosiphum maidis* (Musser and Shelton 2003). *Harmonia axyridis* has also been documented in alfalfa (Buntin and Bouton 1997, Colunga-Garcia and Gage 1998), cotton (Wells et al. 2001), tobacco (Wells and McPherson 1999), and winter wheat (Colunga-Garcia and Gage 1998) as a major biological control agent.

Life history of *Cryptolaemus montrouzieri*: The mealybug destroyer, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), was imported into the United States in 1891 from Australia to control citrus mealybug in California (Sadof 1995). *Cryptolaemus montrouzieri* is a small (about 3-4 mm long), dark brown lady beetle with a tan to orange head. The larvae grow to 1.3 cm in length and have woolly appendages of wax, which makes them resemble mealybugs. *Cryptolaemus montrouzieri* eggs are laid among the cottony egg sack of adult female mealybugs. Eggs hatch into larvae in about 5 days at 27°C (80°F) and the three larval stages last from 12-17 days. The larvae feed on mealybug eggs, young crawlers, and the honeydew produced by mealybugs (Yigit and Canhilal 1998). *Cryptolaemus montrouzieri* pupates in

sheltered places on stems or on greenhouse structures. Adults emerge after 7-10 days and live four months. Four days after emerging, adult females begin to lay eggs, and total number of eggs laid in a lifetime may be as many as 400 (Sadof 1995).

Life history and management of *P. longispinus*: The long-tailed mealybug *Pseudococcus longispinus* (Targioni-Tozzetti), (Hemiptera: Pseudococcidae), eggs are yellow at first and get dark before hatching (McKenzie 1967). The eggs may hatch as soon as they are laid, suggesting that young are born rather than hatching. Females lay 20 to 240 eggs under lab conditions (Furness 1976). The larvae are flattened, oval and light yellow with smooth bodies. Soon after beginning to feed, they exude a white, waxy covering over their bodies to camouflage themselves (Metcalf and Flint 1939). The female body is oval and covered with waxy filaments (McKenzie 1967) and they live 2 to 3 months. The males are tiny and winged, necessary for reproduction but live only a few days (Metcalf & Flint 1939).

Control of *P. longispinus* with insecticides is difficult because they hide in bark crevices and other inaccessible places and secrete thick layers of protective wax (Joyce et al., 2001). *Cryptolaemus montrouzieri* is a natural enemy of mealybug in both the larval and adult stages; a single larva may consume up to 250 small mealybugs. They are most effective when mealybug populations are high, and repeated releases may be necessary if mealybug populations are low (Sadof 1995). Although adults and young larvae prefer to feed on mealybug eggs, older larvae will attack any mealybug stage.

Preference test (choice test) for coccinellid beetles: In the last several decades, response of coccinellid beetles to different odors was investigated in a Y-tube olfactometer as described by Souissi et al.1998. Dicke et al. (1990) showed that mealybug-infested plants, damaged plants, and unparasitized mealybugs are significantly attractive to *Exochomus*

flaviventris Mader (Coleoptera: Coccinellidae). The attraction of *E. flaviventris* resulted from an increased production of volatiles or a change in the volatile blend. Bahlai et al. 2008 examined the roles of visual and olfactory cues from selected components of a hedgerow ecosystem in the foraging of *H. axyridis*. Olfactory bioassays were performed in a Y-tube olfactometer and they tested the response of *H. axyridis* to the odor of buckthorn leaves (*Rhamnus cathartica* L) and apple leaves (*Malus domestica* Borkh.). They found no differences between the numbers of beetles moving toward the odor of buckthorn artificially infested with *A. glycines* and uninfested buckthorn, but *H. axyridis* preferred naturally infested buckthorn over uninfested buckthorn. The results of this study provided information regarding the cues used in foraging of *H. axyridis*.

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CHAPTER 2: VISUAL AND OLFACTORY PREFERENCE OF *HARMONIA AXYRIDIS* PALLAS (COLEOPTERA: COCCINELLIDAE) ADULTS TO VARIOUS FLOWERING ATTRACTANT PLANTS

Abstract: Using natural enemies to suppress pest population within cropping systems, suitable habitat must be provided. This study was conducted to determine if *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) could be attracted into cropping areas using companion plants with different olfactory and visual cues. Ten potential plants that can attract and provide *H. axyridis* with nectar and pollen were selected for a preference test. These plants included dill (*Anethum graveolens*), yarrow (*Achillea* spp.), butterfly weed (*Asclepias tuberosa*), dandelion (*Taraxacum officinale*), bugleweed (*Ajuga reptans*), marigold (*Tagetes tenuifolia*), tansy (*Tanacetum vulgare*), goldenrod (*Solidago* spp.), morninglory (*Ipomoea* spp.), and sunflower (*Helianthus annuus*). The result of the olfactory preference test showed that there was significant difference in the plant preference by *H. axyridis*; sunflower and dill were the most preferred attractant plants. In a visual preference test, *H. axyridis* preferred yellow to other colors. This study suggests that the odor of certain companion plants and the color of the plants (olfactory and visual cues) could assist in the use of biological control by attracting high populations of *H. axyridis* and keeping them within the cropping area.

Keywords: choice test, visual test, preference, *H. axyridis*

The multi-colored Asian lady beetle, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), is an introduced biological control agent to the U.S.A. (Chapin and Brou 1991). *H. axyridis* is native to East Asia and multiple introductions into North America were made in 1916 and 1982 (Koch 2003, Colunga-Garcia and Gage 1998). Since the first introduction, *H. axyridis* has become a dominant lady beetle species in many parts of North America (Teddars and Schaefer 1994, LaMana and Miller 1996, Colunga-Garcia and Gage 1998). Currently, *H. axyridis* is found in various habitats including meadows, semi-natural areas, nurseries, orchards, vegetable gardens and crop fields (Koch 2003,). *H. axyridis* generally moves among such habitats by using olfactory and visual cues of aphid host plants (Koch 2003, Nalepa et al. 2000).

Natural enemies can be spatially relocated by providing habitat for them (Helenius 1998, Landis et al. 2000). A farm management technique used to enhance the presence and abundance of beneficial insects (e.g. natural enemies and pollinators) by manipulating the agricultural ecosystem is referred to as farmscaping, a whole-farm ecological approach to farm management (Dufour 2000). Farmscapes can provide food resources and habitats required by beneficial insects with the objective of increasing their populations on the farm (Pickett and Bugg 1998). Previous studies showed that the addition of companion plants providing nectar and pollen near crops enhanced biological control (Seagraves and Yeargan 2006). A few studies found that sown weed strips within cropped areas increased populations and activities of natural enemies in crops (Wyss 1995, Nentwig 1998, Schoenig et al. 1998, Wratten et al. 1998). Jones and Gillett (2005) found that sunflowers attracted numerous beneficial insects and Dufour (2000) listed many flowering plants that could attract and provide supplemental food source for beneficial insects. These previous studies indicated the potential of farmscaping using companion plants to attract natural enemies.

Although many natural enemies have been used to suppress pest populations in various agroecosystem, there have been two major barriers to the adoption of the strategy by farmers. First, specialist natural enemies (e.g. parasitic wasps) are widely used for biological pest control. However, they are frequently unavailable and expensive to growers due to temporal shortage. Second, no good methods for growers to utilize generalist natural enemies (e.g. soldier bugs and lady beetles) have been developed. In fact, many generalist natural enemies that can potentially control many insect pests already reside in agricultural ecosystems during the growing season. One way to take advantage of such generalist natural enemies existing on the farm is using farmscapes to attract them into the area where target pests are aggregated and cause damage on crops. *H. axyridis* is a generalist predator of a variety of pests including aphids (Koch 2003) and European corn borer eggs (Musser and Shelton 2003). Our observation and previous studies (Hukusima and Kamei 1970, Park and Obrycki 2004) implied that *H. axyridis* resided both inside and outside of cropping areas on the farm, but populations were generally not high in the cropping area in certain periods during the growing season. Many flowering plants have been listed to attract beneficial insects (Dufour 2000) and previous studies showed that *H. axyridis* responded to plant cues (Koch 2003, Bahlai et al. 2008, Berkvens et al. 2008).

This study was conducted to test ten potential companion plants that can be used for farmscapes to attract *H. axyridis* into cropping areas based on olfactory and visual response of *H. axyridis* to the companion plants. This study involved a series of laboratory choice tests to determine suitable plants that attract *H. axyridis*.

MATERIALS AND METHODS

Experimental insects and plants. This study involved a series of laboratory experiments to determine plant odors and colors that attract *H. axyridis* using choice tests. *Harmonia axyridis*

adults were collected in Morgantown, West Virginia, United States between April and June 2008 and maintained in the entomology laboratory at West Virginia University (Morgantown, West Virginia, United States) and fed with aphids (*Aphis* spp.). Ten plants that can attract and provide beneficial insects with nectar and pollen were selected. These plants included dill (*Anethum graveolens*), tansy (*Tanacetum vulgare*), goldenrod (*Solidago* spp.), sunflower (*Helianthus annuus*), yarrow (*Achillea* spp.), butterfly weed (*Asclepias tuberosa*), morninglory (*Ipomoea* spp.), dandelion (*Taraxacum officinale*), bugleweed (*Ajuga reptans*), and marigold (*Tagetes tenuifolia*). These plants were chosen because they bloom when *H. axyridis* are active.

Olfactory meter setup. The response of *H. axyridis* to different plant odors was investigated with a Y-tube olfactometer as described by (Souissi et al. 1998, Takabayashi and Dicke, 1992). It consisted of a Y-shaped 2.5-cm-diameter glass tube. The base and the two arms of the Y-tube measured 28 and 13 cm long, respectively (Fig 1). Each arm was connected to a flowmeter which gives the same amount of air to the two arms and an odor source container made of a glass cylinder (25 cm in diameter and 40 cm high), which was large enough to hold the plant. The air circulated in each olfactometer arm was 40 psi. Female and male predators were able to walk upwind toward the arms of the tube and sometimes performed short flight attempts.

Experiment 1: Single plant olfactory preference test. A laboratory experiment was conducted to select suitable plants that attract *H. axyridis* adults based on olfactory preference. Male and female *H. axyridis* were separated into a petri dish and starved for 24h before their use in the experiments. Each plant (flowering part) was field collected at bloom time and choice tests were carried out using the Y-tube olfactory meter. One flower was placed in one of the arms with no plant in the other arm (Fig 1). One adult beetle was introduced at the start point in the Y-tube

olfactory meter. The direction of movement toward the two arms of the olfactory meter was determined when a predator reached the far end (finish line) of one of the arms. This was repeated ten times with ten different adult beetles and the results of each trial were tested for statistical differences using a chi-square test (Sokal and Rohlf 1998).

Experiment 2: Two plants olfactory comparison test. The same choice test was conducted as described in experiment 1, with the exception of testing two different plants using the olfactory meter. The following pairs of flowers were compared:

1. bugleweed versus dandelion
2. yarrow versus marigold
3. dill versus butterfly weed
4. morning glory versus goldenrod
5. tansy versus sunflower
6. sunflower versus marigold
7. sunflower versus dandelion
8. sunflower versus yarrow
9. sunflower versus bugleweed
10. sunflower versus dill

One beetle was introduced at the start point in the Y-tube olfactory meter and its choice to a plant was recorded. This test was repeated ten times with male and female *H. axyridis* separately. The results of each trial were analyzed for significance by a chi-square test (Sokal and Rohlf 1998).

Experiment 3: Visual preference test. A field study was conducted to test visual preference of *H. axyridis* adults. Six different colored rectangle cardboard papers measuring 5.6

inches by 5.2 inches were painted with tanglefoot (Tanglefoot Company, Grand Rapids, MI) and randomly arranged in a rectangle form on a Plexiglas frame (Fig. 2). These colors included yellow (R: 189, G: 166, B: 44), Blue (R: 89, G: 123, B: 178), Green (R: 50, G: 132, B: 88), Red (R: 206, G: 50, B: 71), Orange (R: 219, G: 76, B: 40) and white (R: 183, G: 188, B: 196). Ten *H. axyridis* adults were released in the center of the box to see which color would attract them. The number of adults on each color cardboard was recorded 30 minutes after release. This experiment was replicated ten times with different insects, and preference of *H. axyridis* to different colors was analyzed with ANOVA (SAS Institute 2008).

RESULTS

Experiment 1: Single plant olfactory preference test. When two choices (plant vs. no plant) were tested for preference by *H. axyridis* adults, they exhibited a significant ($P < 0.05$) preference for dandelion, bugleweed, marigold, butterfly weed, dill, and sunflower (Table 1). However, there were no significant preferences for yarrow, morninglory, goldenrod and tansy, although these plants were also known as potential attractant plants in the literature (Dufour 2000).

Experiment 2: Two plants olfactory comparison test. *Harmonia axyridis* adults exhibited a significant preference for bugleweed over dandelion ($X^2 = 3.2$; $df = 1$; $P = 0.0736$), however, there was no difference between males and females attracted to bugleweed (Fig. 3). *H. axyridis* adults exhibited a significant statistics preference for marigold over yarrow ($X^2 = 5.0$; $df = 1$; $P = 0.0253$), with a significant difference between males and females attracted to marigold (Fig. 4). Lady beetles exhibited a significant preference for dill over butterfly weed ($X^2 = 9.8$; $df = 1$; $P = 0.0017$), and a significant statistics difference was found between males and females *H. axyridis* attracted to dill plant (Fig. 5). *H. axyridis* adults did not exhibit a significant preference

for morning glory over goldenrod ($X^2 = 1.8$; $df = 1$; $P = 0.1797$), although there was a significant statistics difference between males and females attracted to goldenrod (Fig. 6). *H. axyridis* adults exhibited highly significant preference for sunflower over tansy ($X^2 = 12.8$; $df = 1$; $P = 0.0003$) and there was a significant statistics difference between males and females attracted to sunflower (Fig. 7). *H. axyridis* adults exhibited extremely significant preference for sunflower over yarrow ($X^2 = 9.8$; $df = 1$; $P = 0.0017$), however, there was no significant statistics difference between males and females attracted to sunflower (Fig. 8). *H. axyridis* adults exhibited highly significant preference for sunflower over bugleweed ($X^2 = 12.8$; $df = 1$; $P = 0.0003$), however, there was no significant statistics difference between males and females attracted to sunflower (Fig. 9). *H. axyridis* adults exhibited extremely significant preference for sunflower over dandelion ($X^2 = 5.0$; $df = 1$; $P = 0.0253$), and there was a significant statistics difference between males and females attracted to sunflower (Fig.10). *H. axyridis* adults exhibited a highly significant preference for sunflower over marigold ($X^2 = 7.2$; $df = 1$; $P = 0.0073$), however, there was no significant statistics difference between males and females attracted to sunflower (Fig. 11). *H. axyridis* adults exhibited extremely significant preference for sunflower over dill ($X^2 = 7.2$; $df = 1$; $P = 0.0073$), and there was a significant statistics difference between males and females attracted to sunflower (Fig.12). Because the flowering time of each plant is different throughout the year (Table 1), suitable companion plants can be selected based on when *H. axyridis* adults are needed for aphid control. For example, bugleweed can be used early in the year and goldenrod late in the year.

Experiment 3: Visual preference test. Among the six colors that were tested in this research, (Yellow, blue, red, orange, green and white), *H. axyridis* adults exhibited a significant preference for for yellow over others color ($F = 62.25$; $df = 5,59$; $P < 0.0001$) (Table 2). We

found that the number of *H. axyridis* females found on yellow was significantly higher ($F = 19.92$; $df = 5,59$; $P < 0.0001$) (Table 2). However, *H. axyridis* male were not significantly attracted to yellow ($F = 9.06$; $df = 5,59$; $P < 0.0001$) (Table 2).

DISCUSSION

Compared to specialist natural enemies, generalist natural enemies can feed on a broad range of prey species in multiple genera, which is a major advantage of using them to control multiple pest insects in cropping systems. For example, soldier bugs feed on more than 75 insect pests including Colorado potato beetles, Mexican bean beetles, earworms, cutworms, cucumber beetles, armyworms, flea beetles, weevils, and stink bugs (McPherson 1980). Lady beetles can feed on aphids, mealybugs, scale insects, and eggs of moths (Dufour 2000, Park and Obrycki 2004). Another advantage of using generalist natural enemies is that they commonly reside on the farm throughout the growing season and can utilize plants as energy sources for reproduction and survival (Jones and Gillett 2005). Lady beetles can inhabit a farm even during scarcity of prey insects by feeding on pollen, nectar, or plant sap (Landis et al. 2005, Spellman et al. 2006, Fiedler and Landis 2007). Companion plants can provide such food and shelter for natural enemies as well as stimulate relocations of natural enemies into the cropping areas where they can control insect pests (Johanowicz and Mitchell 2000, Mensah 1999, Nentwig 1998, Schoenig et al. 1998, Wratten et al. 1998, Rodenhouse et al. 1992).

Olfactory cues of companion plants could play an important role in attracting natural enemies into cropping areas. Some natural enemies use plant volatiles to search for prey (Schaller and Nentwig 2000) and such chemicals can provide an adaptive advantage to natural enemies by shortening search time required to find prey (Hattingh and Samways 1995). In this study we found that *H. axyridis* were highly attracted to olfactory cues of sunflower, which was

consistent with field observations by Jones and Gillett (2005) who showed that sunflower plants attracted natural enemies when sunflowers reached a minimum height of 2.4 cm. The results of our study provide evidence that *H. axyridis* adults were highly attracted to volatiles from bugleweed, dill, marigold, sunflower, and goldenrod; volatiles associated with the plants were able to attract *H. axyridis* adults even in the absence of prey although many of these plants are acceptable hosts for aphids.

Visual cues also play important roles in host-finding activity of *H. axyridis*. The result of our study suggested that adult *H. axyridis* could be attracted by color even when scent cues are eliminated. *H. axyridis* exhibited a significant preference for yellow over other colors, which was consistent with field observation by Mondor and Warren (2000) who showed that both male and female *H. axyridis* were attracted to yellow color. This could be related to aphids that are commonly found on plants with yellow flowers. The result of our study showed that blue and red were the least preferred color to *H. axyridis* adults. We also found that both male and female *H. axyridis* showed preferences to different colors (Table 2); some *H. axyridis* males were attracted to orange and red while females were not. Additionally, when *H. axyridis* search for overwintering sites, they are attracted to and land on white or bright, reflective surfaces (Koch 2003, Obata et al. 1986).

Knowledge about how natural enemies utilize both visual and olfactory cues may lead to development of strategies to attract *H. axyridis* populations for aphid biological control (Bahlai et al. 2008). The result of this study provides two important implications for biological control with *H. axyridis*. First, *H. axyridis* potentially can be spatially relocated to cropping areas by using some companion plants. *H. axyridis* could be attracted by olfactory and visual cues of a few companion plants and yellow color, respectively. In this study, we found that volatiles of

bugleweed, marigold, butterfly weed, dill, and sunflower attracted *H. axyridis* and these plants with the exception of bugleweed have yellow flowers, indicating that *H. axyridis* adults can be attracted to both visual and chemical cues of the plants. The companion plants can also be used to keep *H. axyridis* in the cropping area even when there are no aphids, because they can feed on pollen and nectar of the companion plants.

Second, depending on crop species, selective planting of attractant plants can be done to attract *H. axyridis* season long. In this study, we found that five species of flowering plants attracted *H. axyridis* with olfactory cues and those plants can be systematically planted for targeting a series of bloom throughout the season; because the blooming time of each plant is different throughout the year, suitable companion plants can be selected based on when *H. axyridis* adults are needed for aphid control. For example, in addition to sunflower blooming during the middle of the growing season, bugleweed and goldenrod can be used early and late in the growing season, respectively, to attract *H. axyridis* adults.

Future work should examine the response of *H. axyridis* to specific volatiles associated with potential companion plants as well as chemical analyses of the volatiles. Important issues related to aphid control with *H. axyridis* include timing when aphid and *H. axyridis* populations generally first appear, when food sources (nectar and pollen from companion plants) are available and how long they last, and number of plantings and plant species mixes to enhance attraction of *H. axyridis*.

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Table 1: Preference of *H. axyridis* to ten selected plants with no plant, Chi-square test was used to test the preference

Plant	X^2	df	P	Flowering Time
Dandelion	11.520	1	0.007	June
Bugleweed	13.520	1	0.000	June
Marigold	3.920	1	0.047	June
Yarrow	0.000	1	1.000	September
Butterflyweed	13.520	1	0.000	August
Dill	5.120	1	0.023	October
Morning glory	0.080	1	0.777	October
Goldenrod	0.720	1	0.396	September
Sunflower	11.520	1	0.001	October
Tansy	2.000	1	0.157	September

Table 2: Average ($\pm$ SE) number of male, female and combined of *Harmonia axyridis* found on six different colors

Color	Male	Female	Combine
Yellow	2.4 $\pm$ 1.26a*	2.8 $\pm$ 1.23a	5.2 $\pm$ 1.23a
Green	1.3 $\pm$ 1.16b	1.5 $\pm$ 1.27b	2.8 $\pm$ 1.19b
Orange	0.4 $\pm$ 0.52c	0.0 $\pm$ 0.00c	0.4 $\pm$ 0.41dc
Blue	0.1 $\pm$ 0.32c	0.2 $\pm$ 0.63c	0.3 $\pm$ 0.49d
Red	0.2 $\pm$ 0.42c	0.0 $\pm$ 0.00c	0.2 $\pm$ 0.31d
White	0.7 $\pm$ 1.25bc	0.4 $\pm$ 0.52c	1.1 $\pm$ 0.94c

*Mean within each variable in each column followed by the same letter are not significantly different ($P > 0.05$; Tukey's standardized range test).

Figure 1. Y-tube olfactory meter used for choice test examining the preference of *H. axyridis* in response to two compared plants.

Figure 2. Rectangle box used for visual test examining the preference of *H. axyridis* in response to six different colors (yellow, green, blue, orange, red and white).

Figure 3. Preference of male and female *H. axyridis* to bugleweed vs dandelion Chi-square test was used to test the preference.

Figure 4. Preference of male and female *H. axyridis* to yarrow vs marigold. Chi-square test was used to test the preference.

Figure 5. Preference of male and female *H. axyridis* to dill vs butterfly weed. Chi-square test was used to test the preference.

Figure 6. Preference of male and female *H. axyridis* to morning glory vs goldenrod. Chi-square test was used to test the preference.

Figure 7. Preference of male and female *H. axyridis* to tansy vs sunflower. Chi-square test was used to test the preference.

Figure 8. Preference of male and female *H. axyridis* to sunflower vs bugleweed. Chi-square test was used to test the preference.

Figure 9. Preference of male and female *H. axyridis* to sunflower vs yarrow. Chi-square test was used to test the preference.

Figure 10. Preference of male and female *H. axyridis* to sunflower vs dandelion.

Chi-square test was used to test the preference.

Figure 11. Preference of male and female *H. axyridis* to sunflower vs marigold. Chi-square test was used to test the preference.

Figure 12. Preference of male and female *H. axyridis* to sunflower vs marigold. Chi-square test was used to test the preference.

**CHAPTER 3: EFFECT OF WITHIN PLANT DISTRIBUTION AND SIZE OF
MEALYBUG (PREY) ON SEARCHING BEHAVIOR OF *CRYPTOLAEMUS
MONTROUZIERI* (COLEOPTERA: COCCINELLIDAE)**

Abstract: This study was conducted to investigate within-plant distribution of long-tailed mealybug, *Pseudococcus longispinus* (Targioni-Tozzetti) (Hemiptera: Pseudococcidae) to quantify the searching and handling time of *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae), and to determine preference of *C. montrouzieri* to the size of *P. longispinus*. Distribution of *P. longispinus* was investigated on three different heights of plants including *Ficus elastica* (Alismatales: Araceae) (tall plant category), *Lilium longiflorum* (Liliales: Liliaceae) (medium plant) and *Dieffenbachia seguine* (Alismatales: Araceae) (short plant). Preference of *C. montrouzieri* to the size of *P. longispinus* prey was also examined. On average, *P. longispinus* were found more on the upper parts of the plants regardless of the height. The time *C. montrouzieri* spent to find the first *P. longispinus* was significantly ($P < 0.05$) different among the three different plant heights at two different releasing points. This indicates that releasing *C. montrouzieri* adults from the top or on smaller plants could reduce searching time for *P. longispinus*. However, there were no significant differences in handling time and cleaning time. *Cryptolaemus montrouzieri* preferred smaller size of *P. longispinus* to medium and larger size. There were no significant differences in handling time, but there were significant differences in cleaning time that was observed among different sizes of *P. longispinus*. The implications for the use of *C. montrouzieri* in biological control are also discussed.

Keywords: Mealybug destroyer, long-tailed mealybug, choice test, preference, distribution

The long-tailed mealybug, *Pseudococcus longispinus* (Targioni-Tozzetti) (Hemiptera: Pseudococcidae), is a key pest of fruit trees and ornamental plants (Ben-Dov and Germain 2003). *P. longispinus* feeds on various plant parts including roots, trunk, cordon, canes, leaves and fruit, causing aesthetic damage or yield loss. Molds that grow on the honeydew excreted by *P. longispinus* can cause further economic damage on crops. High *P. longispinus* densities often cause leaf drop and reductions of crop quality and yield; Uygun (2001) reported that yield loss of citrus by *P. longispinus* could be up to 80-90%. *P. longispinus* also can transmit viral diseases in grapevines (Acheche et al. 1999). Chemical control of *P. longispinus* is difficult because it produces thick layers of protective wax and can hide in bark crevices, spurs, or canes. In general, chemical control is effective when *P. longispinosus* is in the crawler stage and when the host plants do not provide *P. longispinosus* with enough shelters for protection (Furness 1977). Therefore, biological control using natural enemies has been a major alternate method to control *P. longispinus* (Cooper 1985).

Natural enemies that can control *P. longispinus* include lady beetles, parasitic wasps, and lacewings. Among the natural enemies, the mealybug destroyer, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), is one of the key natural enemies of *P. longispinus* in the United States. *C. montrouzieri* is native to Australia and has been used to control many mealybug species throughout the world (Bartlett 1974, 1978). *C. montrouzieri* was first imported into the United States in the late 1800s to control citrus mealybugs in California (Sadof 1995). Since then, *C. montrouzieri* has been commercially available to control mealybugs throughout the United States. In an established population, immature stages of *C. montrouzieri* dominate the stable age distribution and most prey is consumed by the larvae. However, *C.*

montrouzieri adults are released to initiate biological control of *P. longispinus* because of their ability to disperse and search for mealybugs.

Effectiveness of natural enemies controlling pests is dependent upon how they can establish populations in a given environment and how effectively the natural enemy can find prey within a short period (Garcia and O'Neal 2000). Previous studies showed that natural enemies' ability to establish and search for prey was affected by plant structure and size (Price et al. 1980; Lawton 1983; Andow and Prokrym 1990; Kareiva and Sahakian 1990; Grevstad and Kleptka 1992). Garcia and O'Neal (2000) showed that plant size and variegation affected the searching efficiency of *C. montrouzieri* and Merlin et al. (1996) found that oviposition of *C. montrouzieri* was stimulated by wax filaments produced by its prey. These studies also indicated that successful biological control of *P. longispinus* would be affected by how efficiently *C. montrouzieri* adults search for *P. longispinus* after release. Key factors influencing prey search efficiency of *C. montrouzieri* finding *P. longispinus* may include release location of *C. montrouzieri* based on within-plant distribution of *P. longispinus*, the plant types, and stages of *P. longispinus* that *C. montrouzieri* prefers.

This study was conducted to investigate searching efficiency of *C. montrouzieri* to control *P. longispinus* on various ornamental plants. The objectives of this study were (1) to investigate within-plant distribution of *P. longispinus*, (2) to quantify the searching and handling time of *C. montrouzieri* related to releasing location, and (3) to determine preference of *C. montrouzieri* for the size of *P. longispinus*.

MATERIALS AND METHODS

Experiment 1: Within-plant distribution of *P. longispinus*. Three different categories of plants based on height (tall, medium, and short) were grown in the greenhouse at West

Virginia University (Monongalia County, WV). These plants included *Ficus elastica* (Alismatales: Araceae) (tall plant category), *Lilium longiflorum* (Liliales: Liliaceae) (medium plant) and *Dieffenbachia seguine* (Alismatales: Araceae) (short plant) and their heights were taken (92cm; 94cm; 91cm; 93cm; and 86cm), (61cm; 54cm; 44cm; 51cm; 42cm) and (23cm; 31cm; 25cm; 28cm; 37cm) respectively. Each plant was divided into two parts (top and bottom) and the number of *P. longispinus* were counted on each part of the plant. This experiment was replicated five times. Density of *P. longispinus* on top and bottom of the plants was analyzed with ANOVA and comparisons among plant heights were based upon Tukey's HSD test at 0.05% (SAS Institute 2008).

Experiment 2: Prey searching behavior of *C. montrouzieri* adults with respect to three different plant heights. *Cryptolaemus montrouzieri* adults were obtained from Peaceful Valley Farm Supply (Grass Valley, CA). *Cryptolaemus montrouzieri* were fed with *P. longispinus* collected from infested plants in the greenhouse at West Virginia University. *Cryptolaemus montrouzieri* were kept in ventilated cages with *P. longispinus* and a honey-water solution under laboratory conditions of 25°C and 16:8 (L:D) h photoperiod. All *C. montrouzieri* adults were denied access to prey but had access to water for the 12-h period preceding all experiments. Searching, handling and cleaning time of *C. montrouzieri* were measured on three different plants. These plants included *Ficus elastica* (Alismatales: Araceae) (tall plant category), *Lilium longiflorum* (Liliales: Liliaceae) (medium plant) and *Dieffenbachia seguine* (Alismatales: Araceae) (short plant) and their heights were taken (92cm; 94cm; 91cm; 93cm; and 86cm), (61cm; 54cm; 44cm; 51cm; 42cm) and (23cm; 31cm; 25cm; 28cm; 37cm) respectively. Searching, handling and cleaning time (initiation of feeding to cessation of feeding) was observed and recorded. Height was measured along the main stem, from the top of the soil to the

top of the plant. On each plant, one *C. montrouzieri* adult was released from the top and another from the bottom of the plant to compare the searching, handling and cleaning time of finding *P. longispinus*. This experiment was replicated five times using different *C. montrouzieri* adults. The searching, handling and cleaning time of finding *P. longispinus* was recorded and analyzed with ANOVA (SAS Institute 2008).

Experiment 3: Three way preference for different mealybug prey size. A total of 20 *C. montrouzieri* adults were denied access to prey but had access to water for the 12-h period preceding all experiments. Preference of *C. montrouzieri* for three stages of *P. longispinus* 1st instar (0.3 mm), 2nd instar (1.3 mm) and adult (3 mm) was investigated using a Petri dish (9cm diameter) containing a single leaf of *Ficus elastica* (Alismatales: Araceae) (Fig. 1). Three of each size of *P. longispinus* was randomly placed on the leaf for each replication. One *C. montrouzieri* adult was placed in the center of the Petri dish and allowed to search for *P. longispinus*. *Cryptolaemus montrouzieri* adults choice among three different stages of *P. longispinus* and handling time were measured. This experiment was replicated 20 times. The result of the experiment was analyzed for significance by a Chi-square test (Sokal and Rohlf 1998).

RESULTS

Vertical distribution of *P. longispinus* varied considerably on plants. There were no significant differences in density of *P. longispinus* on the top ($F = 0.46$; $df = 2,14$; $P > 0.6491$) and bottom ($F = 0.94$; $df = 2,14$; $P = 0.4427$) among the plants used in this study. However, we found that significantly ($F = 57.4$; $df = 1,29$; $P < 0.0001$) more *P. longispinus* were found on the upper parts of the plants regardless of the height (tall, medium and short) (Table 1.).

The time *Cryptolaemus montrouzieri* spent to find the first *P. longispinus* was significantly different among the three different plant heights (tall, medium and short) ($F =$

10.19; $df = 3,26$; $P = 0.0001$) and the two different releasing points (top and bottom) ($F = 12.06$; $df = 5,24$; $P = 0.001$). *Cryptolaemus montrouzieri* spent significantly more time searching for *P. longispinus* on taller plants when they were released from the bottom (Table 2). However, there were no significant differences in handling time ($F = 1.19$; $df = 3,26$; $P = 0.3319$) and cleaning time ($F = 0.43$; $df = 3,26$; $P = 0.7349$). This indicates that releasing *C. montrouzieri* adults from the top or on smaller plants could reduce time searching for *P. longispinus*.

The results of the preference test of *Cryptolaemus montrouzieri* adults for three different sizes of *P. longispinus* (0.3 mm, 1.3 mm and 3 mm), showed that there was significant preference of *C. montrouzieri* adults for *P. longispinus*. (Chi-square = 9.109, $df = 2$ and $P = 0.0105$). *C. montrouzieri* preferred smaller *P. longispinus* to medium and larger sizes. There were no significant differences in handling time of *C. montrouzieri* feeding on different sizes of *P. longispinus* ($F = 3.25$; $df = 2,17$; $P = 0.0639$), but there were significant differences in cleaning time among different sizes of *P. longispinus* ($F = 8.14$; $df = 2,17$; $P = 0.0033$). This result indicates that cleaning time of *C. montrouzieri* after feeding on larger sizes of *P. longispinus* was significantly longer than the cleaning time after feeding on medium and smaller sizes (Table 3).

DISCUSSION

Within-plant distribution of pests is key information for determining where to release natural enemies to maximize efficiency of biological control because prey searching time could depend on the location for releasing natural enemies (Andow and Prokrym 1990, Yang and Sadof 1995). In this study, we showed that significantly more *P. longispinus* inhabited the upper part of plants regardless of plant types. This observation is in agreement with the finding by Flaherty et al. (1992) who showed higher population and movement of mealybugs toward the top

of the plant. Because *C. montrouzieri* is known to be more effective when *P. longispinus* populations are high (Bartlett 1978; Moore 1988), releasing *C. montrouzieri* adults from the top of the plants could reduce prey searching time because *P. longispinus* is abundant on the upper part of plants. In addition, plant height could affect effectiveness of biological control by affecting prey searching efficiency of natural enemies (Garcia and O'Neil 2000). The results of our study indicated that effectiveness of *C. montrouzieri* increased when they are released on smaller plants. Therefore, the effectiveness of *C. montrouzieri* controlling *P. longispinus* could be maximized when *C. montrouzieri* are released from the top and on smaller plants.

This study also demonstrated that *C. montrouzieri* preferred to feed on smaller *P. longispinus*. When *C. montrouzieri* fed on larger larvae, they spent longer time in handling and cleaning after feeding and before searching for another prey. This result is congruent with Merlin et al. (1996) who found that *C. montrouzieri* consumed smaller *P. longispinus* nymphs first and then fed on larger nymphs or adults. Adult *C. montrouzieri* is known to be able to detect their prey by visual and chemical stimuli (Heidari and Copland 1992).

In conclusion, the results of this study suggest a major consideration on the use of *C. montrouzieri* to control *P. longispinus*. The efficiency of *P. longispinus* control by *C. montrouzieri* depends on the location of *C. montrouzieri* release and the plant height. Our study showed that effectiveness of *C. montrouzieri* controlling *P. longispinus* could be maximized when *C. montrouzieri* were released from the top of the plants and on the smaller plants; *C. montrouzieri* adults can reduce prey search time when they were released where *P. longispinus* is abundant.

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Table 1: Average ($\pm$ SE) number of *P. longispinus* on the top and bottom part of plants of three different heights

Plant height*	Top	Bottom
Short	354 $\pm$ 207a**	90 $\pm$ 71b
Medium	470 $\pm$ 178a	57 $\pm$ 32b
Tall	418 $\pm$ 115a	49 $\pm$ 36b

* 23cm (Short), 51cm (Medium) and 92cm (Tall).

** Means within each variable in each row followed by the same letter are not significantly different ($P > 0.05$; Tukey's standardized range test).

Table 2: Searching, handling and cleaning time of *C. montrouzieri* adults on different plant heights at two different releasing locations

Plant height*	Releasing location on plant	Searching Time (min)	Handling Time (min)	Cleaning Time (min)	Total Time (min)
Short	Bottom	4.60±1.14bc**	4.00±2.00a	2.60±1.52a	11.2±4.66cb
	Top	2.40±0.55c	3.40±1.14a	2.20±1.64a	8.0±3.33c
Medium	Bottom	7.80±0.84b	4.40±1.14a	2.20±1.10a	14.4±3.08b
	Top	2.40±1.34c	4.80±1.48a	2.40±1.34a	9.6±4.16cb
Tall	Bottom	17.00±8.72a	5.20±0.84a	2.20±0.84a	24.4±10.40a
	Top	2.60±1.14c	3.80±1.48a	3.40±1.34a	9.8±3.96cb

* 23cm (Short), 51cm (Medium) and 92cm (Tall).

** Means within each variable in each column followed by the same letter are not significantly different ($P > 0.05$; Tukey's standardized range test).

Table 3: Handling and cleaning time of *C. montrouzieri* adults feeding on different sizes of mealybug

Mealybug size*	Handling time (min)	Cleaning Time (min)	Total (min)
Small	6.9±2.18a**	3.5±2.03b	10.4±4.21b
Medium	10.3±4.35a	4.5±2.08b	14.8±6.43ab
Large	12.0±7.00a	8.7±1.53a	20.7±8.53a

* Small (0.3 mm), Medium (1.3 mm) and Large (3 mm)

** Means within each variable in each column followed by the same letter are not significantly different ($P > 0.05$; Tukey's standardized range test)

Fig. 1. The behaviour of *C. montrouzieri* in a 3-way food preference choice test between three stages of *P. longispinus*: 1st instar (0.3 mm), 2nd instar (1.3 mm) and adult (3 mm).

Figure 2. Preference of *C. montrouzieri* to stages of *P. longispinus*: 1st instar (0.3mm), 2nd instar (1.3 mm) and adult (3 mm).

CHAPTER 4: CONCLUSION

Knowledge about how natural enemies use plant-based resources may lead to the ability to directly attract natural enemy populations to increase biological control. *Harmonia axyridis* adults reside on the farm during the growing season. Therefore, it is important to attract and keep them within the farm to control pest insects. *H. axyridis* can feed on aphids, mealybugs scale insects, and eggs of moths (Park and Obrycki 2004). Currently, *H. axyridis* has proved beneficial in suppressing pests such as corn leaf aphid (Seagraves and Yeargan 2006).

The result of this study (Chapter 2) indicates two major applications on how to attract *H. axyridis* adults. *H. axyridis* occurs in several agroecosystems and is considered an important predator on many significant economic pests. Our results suggest that it may be possible to attract this natural enemy to crops in which it already occurs by adding sunflower as a companion planting. Second, knowing the color of plants that *H. axyridis* prefers can also serve as a means of attracting them into cropping areas because when prey insects become scarce they tend to feed on pollen and nectar. Thus, such natural enemies can potentially be used as economical and sustainable biological control agents as long as they can be attracted and kept in the cropping areas within farms.

This study (Chapter 3) also reveals the implications of using *Cryptolaemus montrouzieri* as a biological control agent. Plant heights and position from which *C. montrouzieri* has been released for biological control has an impact on their effectiveness. For example, fewer predators would be needed to control pests on smaller plants when released from the top than on taller complex plants when released from the bottom. This indicates that releasing *C. montrouzieri* adults from the top or on smaller plants could reduce time spent searching for *P. longispinus*. This study also demonstrates that *C. montrouzieri* prefers to feed on 1st instars of *P. longispinus*.

and will spend longer time in handling and cleaning after feeding on larger instars before moving on to another prey. The result of this study shows that care must be taken when using this predator as a biological control agent.

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