Hindawi Publishing Corporation
Psyche
Volume 2012, Article ID 503543, 5 pages
doi:10.1155/2012/503543

Research Article

Effect of Plant Characteristics and Within-Plant Distribution of Prey on Colonization Efficiency of *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae) Adults

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Received 2 October 2011; Accepted 20 December 2011

Academic Editor: Martin H. Villet

Cryptolaemus montrouzieri (Coleoptera: Coccinellidae) has been widely used in classical and inundative biological control of mealybugs, including the long-tailed mealybug, *Pseudococcus longispinus* (Hemiptera: Pseudococcidae). This study was conducted to investigate colonization and establishment efficiency of *C. montrouzieri* to manage *P. longispinus* on three different ornamental plant species (*Ficus elastica, Lilium longiflorum*, and *Dieffenbachia seguine*). Within-plant distribution pattern of *P. longispinus* and the colonization ecology of adult *C. montrouzieri* were investigated. Significantly more *P. longispinus* were found on the upper parts of the plants regardless of plant species, and *C. montrouzieri* adults discovered *P. longispinus* significantly faster when they were released on the top of the plants than on the bottom. Choice tests revealed that *C. montrouzieri* adults preferred smaller *P. longispinus* nymphs. The implications for utilization of *C. montrouzieri* for biological control of mealybugs on various ornamental plants are discussed.

1. Introduction

The long-tailed mealybug, *Pseudococcus longispinus* (Targioni-Tozzetti) (Hemiptera: Pseudococcidae), is a key pest of fruit trees and ornamental plants. *P. longispinus* feeds on various plant parts including roots, trunks, cordons, canes, leaves, and fruits, causing aesthetic damage on ornamental plants or yield loss of crops [1]. Fungal pathogens that grow on the honeydew excreted by *P. longispinus* can cause further damage. For example, high *P. longispinus* densities often cause leaf drop and reductions of crop quality and yield; Uygun [2] reported that yield loss of citrus due to *P. longispinus* could be up to 80–90%. Also, *P. longispinus* can transmit viral diseases in grapevines [3]. Chemical management 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 only effective when *P. longispinosus* is in the crawler stage and when host plants do not afford physical refuges from chemical sprays [4]. Therefore, biological control using natural enemies has been a major alternate method to manage *P. longispinus* [5].

Natural enemies utilized to manage *P. longispinus* include lady beetles, parasitic wasps, and lacewings [6]. Among the natural enemies, the mealybug destroyer, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae), is one of the key natural enemies of *P. longispinus*. *C. montrouzieri* is native to Australia and has been introduced to manage many mealybug species throughout the world [7, 8]. In the United States, *C. montrouzieri* was first imported in the late 1800s to manage mealybugs in California [9]. Since then, well-defined and efficient rearing techniques were developed [10], and thus *C. montrouzieri* has been commercially available to growers throughout the United States.

Cryptolaemus montrouzieri has been used for different biological applications: classical biological control [11] and augmentative biological control [12, 13]. In an established population, immature stages of *C. montrouzieri* dominate the stable age distribution, and most prey is consumed by the larvae [14]. However, adult stage of *C. montrouzieri* is released when biological control of *P. longispinus* is initiated because of their ability to disperse and colonize. Also, it is assumed that adults will lay eggs in suitable locations and give rise to another generation that provides the majority of pest suppression when they are in the larval stage.

Effectiveness of natural enemies is dependent upon the ability of the organism to establish populations in a given environment and find prey rapidly [15]. Previous studies showed that natural enemies' ability to establish and search for prey was affected by plant structure and size [16–20]. Garcia and O'Neil [15] showed that plant size and variegation affected the searching efficiency of *C. montrouzieri,* and Merlin et al. [21] found that oviposition of *C. montrouzieri* was stimulated by wax filaments produced by its prey. Also, these studies indicated that successful biological control of *P. longispinus* would be affected by how efficiently newly released *C. montrouzieri* adults search for *P. longispinus*. Specifically, there is a high chance for *C. montrouzieri* adults to successfully establish when they can start to search and find the suitable prey as soon as they are released. In addition, prey-size choice could affect successful colonization of predators [22]. Therefore, key factors influencing prey search efficiency of *C. montrouzieri* may include release location of *C. montrouzieri* based on within-plant distribution of *P. longispinus*, plant characteristics, and stages of *P. longispinus* that *C. montrouzieri* adults prefer.

This study was conducted to investigate colonization efficiency of *C. montrouzieri* to manage *P. longispinus* on three different types of ornamental plants. The objectives of this study were (1) to investigate within-plant distribution of *P. longispinus*, (2) to quantify the searching time of *C. montrouzieri* related to release location, and (3) to determine preference of *C. montrouzieri* adults for the size of *P. longispinus*.

2. Materials and Methods

All experiments were conducted in the greenhouse and the entomology laboratory of West Virginia University, Morgantown, WV, U.S.A.

2.1. Within-Plant Distribution of P. longispinus. We obtained three common species of ornamental plants from the greenhouse at West Virginia University (Monongalia County, WV, USA). The ornamental plants in this study include *Ficus elastica* (Urticales: Moraceae) (86–94 cm in height), *Lilium longiflorum* (Liliales: Liliaceae) (42–61 cm in height), and *Dieffenbachia seguine* (Alismatales: Araceae) (23–27 cm in height). These plants were selected because they are very common ornamental plants produced in the greenhouse. These plants had been infested with *P. longispinus* for at least one year before experiments to obtain moderate-to-high density of *P. longispinus*. Five plants of each plant species with similar infestation levels were selected, and the total numbers of *P. longispinus* nymphs and adults were counted on the upper and lower halves of each plant. Densities of *P. longispinus* on upper and lower parts of each plant species were compared using two-way ANOVA at 5% error rate [23].

2.2. Prey Searching Time of C. montrouzieri Adults on Three Different Plant Species. C. montrouzieri adults were maintained in ventilated cages with *P. longispinus* and a honey-water solution under laboratory conditions of 25°C and 16 : 8 (L : D) h photoperiod. *C. montrouzieri* were reared on *P. longispinus*. All *C. montrouzieri* adults were denied prey but had access to water for the 12 h period preceding all experiments. One randomly chosen *C. montrouzieri* adult was introduced onto either top (i.e., top shoot) or bottom (i.e., bottom part of stem within 2 cm above the soil line) of a plant. Once one *C. montrouzieri* adult was introduced to a plant, searching time of *C. montrouzieri* was measured. Searching time was measured as the duration between introduction and finding the first prey. This experiment was replicated five times for each plant species and each release location. Each *C. montrouzieri* adult was used for the test only once. The searching time of *C. montrouzieri* was recorded and analyzed with two-way (i.e., releasing locations and plant species) ANOVA at 5% error rate (SAS Institute, 2008).

Because we used the same plants for repeated release of different *C. montrouzieri* adults in the experiment, any leftover chemical cues by previously used *C. montrouzieri* adults could affect the next adults introduced to the plant. Therefore, we examined the effect of leftover chemical cues by previously used *C. montrouzieri* adults on the next adults introduced to the plant by dividing the data into two groups: first ten and last ten introductions of *C. montrouzieri*. The searching time of the two groups was compared ANOVA at 5% error rate [23].

2.3. C. montrouzieri Preference to Prey Body Size. A total of 20 *C. montrouzieri* adults were denied prey but had access to plain water for the 12 h period preceding the experiment. Preference of *C. montrouzieri* for three different sizes of *P. longispinus* (0.3 ± 0.07, 1.3 ± 0.10, and 3.0 ± 0.14 mm) was investigated using an empty 9-cm-diameter petri dish (LAB-TEK Division Miles Laboratories, Inc., Naperville, IL, USA) containing an excised leaf of *F. elastic* on the bottom of the Petri dish. Three *P. longispinus* with different body sizes were 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*. *C. montrouzieri* adults' choice among the three different sizes of *P. longispinus* and handling and cleaning time was recorded. Handling time was measured from the start of first contact of *P. longispinus* by *C. montrouzieri* to cessation and included feeding. Cleaning time was measured as duration of waxy residue removal from the body. This choice test was run until the first nymph was consumed and replicated 20 times. Searching, handling, and cleaning times were compared using ANOVA at 5% error rate [23], and the first choices by *C. montrouzieri* adults to feed on the three different body sizes of *P. longispinus* were compared using Chi-square test [24].

Table 1: Mean (±SD) number of *P. longispinus* on the upper and lower parts of three different plants. Note that there were no significant differences within columns.

Plant species	Upper part of plant	Lower part of plant
Ficus elastica	415 ± 264.5a*	110 ± 93.2b
Lilium longiflorum	564 ± 172.4a	51 ± 26.8b
Dieffenbachia seguine	441 ± 154.1a	51 ± 40.4b

*Means within rows followed by the same letter are not significantly different (F test, $P < 0.05$).

3. Results

3.1. Within-Plant Distribution of P. longispinus. There were no significant differences in the total number of *P. longispinus* among the plants used in this study ($F = 1.21$; df = 2,57; $P > 0.05$). However, 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 plant species (Table 1). There were no significant differences in *P. longispinus* densities among the three plant species when *P. longispinus* densities on the upper and lower parts were compared: upper parts of plants ($F = 0.46$; df = 2,14; $P = 0.650$) and the lower part of plants ($F = 0.94$; df = 2,14; $P = 0.443$).

3.2. Prey Searching Time of C. montrouzieri Adults on Three Different Plant Species. The time *C. montrouzieri* spent to find the first *P. longispinus* was significantly different among the three different plant species ($F = 7.9$; df = 2,24; $P = 0.002$) and the two different release points (top and bottom) ($F = 29.73$; df = 1,24; $P < 0.001$). Because interactions between plant species and release points ($F = 7.37$; df = 2,24; $P < 0.001$) were significant, we separately compared the time *C. montrouzieri* spent to find the first *P. longispinus* between two release locations for each plant height category. We found that *C. montrouzieri* spent significantly more time searching for *P. longispinus* when they were released from the bottom of the plants regardless of plant species (Figure 1). This indicates that prey-searching time for *C. montrouzieri* adults to find the first *P. longispinus* can be reduced by releasing them from the top of the plant.

We found that there were no differences in the searching ($F = 1.93$; df = 1,18; $P > 0.05$), handling, and cleaning time ($F = 1.21$; df = 1,58; $P > 0.05$) between the first ten *C. montrouzieri* introduced to the plants and the next ten introduced. This indicates that there were no significant effects of chemical cues left, if any, on the searching behavior of *C. montrouzieri* adults in this study.

3.3. C. montrouzieri Preference to the Body Size of P. longispinus. The results of the preference test of *C. montrouzieri* adults for three different sizes of *P. longispinus* showed that there was significant ($\chi^2 = 9.109$; df = 2; $P < 0.05$) preference of *C. montrouzieri* adults for smaller *P. longispinus* compared to medium ($\chi^2 = 7.619$; df = 1; $P < 0.01$) and larger ($\chi^2 = 8.314$; df = 1; $P < 0.01$) sizes. Although there were no significant differences in handling time of *C. montrouzieri* feeding on different sizes of *P. longispinus* ($F = 3.25$; df = 2,17; $P = 0.064$), there were significant differences in cleaning time among *C. montrouzieri* feeding on different sizes of *P. longispinus* ($F = 8.14$; df = 2,17; $P = 0.003$) (Table 2). This result indicates that *C. montrouzieri* adults choose smaller *P. longispinus* more frequently and spend significantly more time to clean after feeding on larger *P. longispinus*.

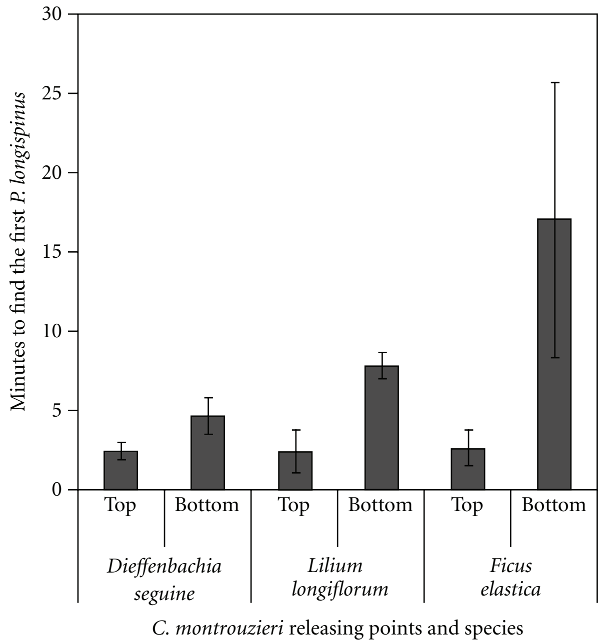

Figure 1: Effects of *C. montrouzieri* release locations and plant species on the time *C. montrouzieri* spent to find the first *P. longispinus*. Note that there were significant differences (F test; $P < 0.05$) between two release locations regardless of plant species.

Table 2: Handling and cleaning time (minutes ± SD) of *C. montrouzieri* adults feeding on different sizes of mealybug.

Mealybug size (mean ± SD)	Handling time	Cleaning Time	Total
Small (0.3 ± 0.07 mm)	6.9 ± 2.18a*	3.5 ± 2.03b	10.4 ± 4.21b
Medium (1.3 ± 0.10 mm)	10.3 ± 4.35a	4.5 ± 2.08b	14.8 ± 6.43ab
Large (3.0 ± 0.14 mm)	12.0 ± 7.00a	8.7 ± 1.53a	20.7 ± 8.53a

*Means within columns followed by the same letter are not significantly different (F test, $P < 0.05$).

4. Discussion

Within-plant distribution of pests is key information for determining where to release natural enemies. To maximize efficiency of biological control, prey-searching time could be reduced depending on where natural enemies are released [18, 25]. In this study, we observed that significantly more *P. longispinus* inhabited the upper part of plants regardless of plant species. This observation is in agreement with the finding by Flaherty et al. [1] who showed higher population and movement of mealybugs toward the top of grapevines. Because *C. montrouzieri* is known to be more effective when *P. longispinus* populations are high [8, 26], 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 species could affect prey-searching efficiency by natural enemies [15]. The results of our study indicated that effectiveness and establishment of *C. montrouzieri* increased when they were released on *Dieffenbachia seguine,* the smallest plant tested in this study. Therefore, the effectiveness of *C. montrouzieri* managing *P. longispinus* could be maximized when *C. montrouzieri* are released from the top and on smaller plants.

This study demonstrated that *C. montrouzieri* chose to feed on smaller *P. longispinus.* When *C. montrouzieri* fed on larger nymphs, they spent longer time handling and cleaning after feeding and before searching for another prey. This result is congruent with Merlin et al. [21] who found that *C. montrouzieri* consumed smaller *P. longispinus* nymphs first and then fed on larger nymphs or adults. Because establishment of natural enemies after release determines the success of augmentational biological control (i.e., inoculative release), finding the first prey by the natural enemies could increase the chance of establishment. Although a difference of 5–15 minutes in time to initial prey encounter may not make much of a difference to the final biological control outcome in a greenhouse, it could influence the chance of establishment because we frequently observed that *C. montrouzieri* adults could fly away to escape from the greenhouse when they cannot find the first prey in a reasonable period. Therefore, *C. montrouzieri* adults have higher chance to establish when the period for finding the first prey is shorter.

The results of this study suggest a major consideration for the use of *C. montrouzieri* to manage *P. longispinus.* The efficiency of *P. longispinus* management by *C. montrouzieri* depends on the location of *C. montrouzieri* release and the plant species. Our study showed that effectiveness and establishment of *C. montrouzieri* managing *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 are released where *P. longispinus* is abundant. Future study needs to investigate the effect of plant age and stage on vertical distribution of *P. longispinus* on the plant.

Acknowledgments

The authors thank Vicki Kondo and JB White at West Virginia University for their valuable support and suggestions related to this research. They also thank Sue Myers at West Virginia University for her help in identifying plant species used in this research. This project was partially supported by the Division of Plant and Soil Science and the Faculty Senate Grant at West Virginia University.

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