

5-2016

# Effects of Host Plant Architecture on the Functional Response of *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae)

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EFFECTS OF HOST PLANT ARCHITECTURE ON THE FUNCTIONAL RESPONSE  
OF *CRYPTOLAEMUS MONTROUZIERI* MULSANT  
(COLEOPTERA: COCCINELLIDAE)

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A Thesis  
Presented to  
the Graduate School of  
Clemson University

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In Partial Fulfillment  
of the Requirements for the Degree  
Master of Science  
Entomology

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by  
Hechu Zhu  
May 2016

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Accepted by:  
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## ABSTRACT

Functional response describes the relationship between the number of prey consumed by a predator and the prey density. Three types of functional responses have been described based on the changes in prey consumption rates with increasing prey density. Plant architecture is one of several factors that can affect the searching efficiency, and thus functional response, of predators. The objective of this study was to investigate how the numbers of branches and leaves affected functional response of *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) when provided with *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) as prey. Greenhouse-grown chili peppers (*Capsicum annum* L., cv. ‘Jalapeño’) were manipulated to achieve desirable numbers of primary branches (2, 4 and 6) (the “branch experiment”) and numbers of leaves (5, 10 and 15) (the “leaf experiment”). The total surface areas were maintained constant in the two experiments to ensure that the detected differences in the functional response of *C. montrouzieri* were the results of varying branch or leaf number, not that of varying surface area. Results of this study suggested that *C. montrouzieri* exhibited Type II functional response on plants with 2, 4 and 6 branches, as well as on plants with 5 and 10 leaves. The lady beetle exhibited Type III functional response on plants with 15 leaves. Attack rates and handling time were not significantly different among treatments but slightly lower on plants with more leaves and branches, except the 15-leaved treatment had higher handling time than the 10-leaved treatment. Higher attack rates on plants with fewer branches and leaves suggested that *C. montrouzieri* was more efficient on plants of lower structural complexity. Higher handling time indicated that *C. montrouzieri* spent

more time searching and consuming prey. The results of the study allowed pest managers to make better predictions on potential efficiency of *C. montrouzieri* and population dynamics in this prey-predator system.

## DEDICATION

I would like to dedicate this thesis to my parents, Qing Huang and Haipeng Zhu, for the love and selfless support to complete this work. I hope that this achievement will complete the dream that you had for me all those years ago when you chose to give me the best education you could.

## ACKNOWLEDGMENTS

The completion of my thesis could not have been possible without the assistance and the participation of many people whose names may not all be enumerated. Their contributions are sincerely acknowledged and gratefully appreciated.

I would like to express my deepest appreciation to my advisor, Dr. Juang-Horng Chong, for his full support and expert guidance throughout this academic exploration. Without his counsel and patience, this thesis would not have been possible. In addition, I express my gratitude to Dr. Peter Adler and Dr. David Tonkyn for having served on my committee. Their thoughtful questions and comments were valued greatly.

I would also like to thank Dr. Peter Adler, Dr. John Morse, Dr. Matthew Turnbull, Dr. Mike Caterino and Dr. William Bridges Jr. for helping me with my coursework and academic research during my graduate study at Clemson University.

Thanks also go to my fellow graduate students at Department of Agricultural and Environmental Sciences and staff of the College of Agriculture, Forestry and Life Sciences and Pee Dee Research and Education Center. Special thanks go to my friends who helped me throughout my study and research.

Finally, I would like to thank my parents, grandparents and my boyfriend for their continuous love and support; I would not have been able to complete this work without their unconditional love and encouragement.

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# CHAPTER ONE

## LITERATURE REVIEW

### **Functional response**

A predator's functional response describes the relationship between the number of prey consumed by the predator and prey density (Solomon 1949). Functional response is an essential factor of the predator-prey systems and a key element of predator-prey population dynamics and interactions (Jeschke et al. 2002). Each predator-prey system can be described in part by a unique functional response (Jeschke et al. 2002, Hellström et al. 2014). Efforts to develop functional response models began as early as the 1920s (Holling 1966, Royama 1971). Holling (1959a, 1959b) categorized functional responses of predators into three types – Type I, II and III.

Predators displaying Type I functional response consume prey at a constant consumption rate until a maximum value or plateau is reached (Figure 1.1A, B). The slope of Type I functional response curve is equal to the predator's attack rate. Type I functional response is often expressed as

$$N_e = \frac{a N_0}{1 + a N_0} \quad \text{(Equation 1.1) (Holling 1959a)}$$
, where  $N_e$  is the number of prey consumed,  $N_0$  is the original prey number and  $a$  is the attack rate.

A "true" Type I functional response only occurs when handling time (time of the predator searching for and consuming preys) equals zero, which is not a realistic situation under natural conditions in most predator-prey systems (Begon et al. 1996).

Among the three types, Type II functional response is the most frequently observed (Hassell et al. 1976, Begon et al. 1996). In Type II functional response, as the prey density increases the number of prey consumed increases at a declining rate until the number of prey consumed reaches a maximum/plateau (Figure 1.1C). The proportion of prey consumed decreases with increasing prey density (Figure 1.1D). Holling (1959b) suggested that the proportion of prey consumed is the highest at the lowest prey density. As the prey density increases, prey become easier to find. But because handling time per prey remains constant, a predator spends more and more of its total foraging time in handling prey items. At a certain prey density, the predator spends all its time handling prey and has no time to search for additional prey; therefore, the number of prey consumed reach a maximum level.

Equation for Type II functional response, also known as Holling's disc equation (Holling 1959b), can be expressed as

———— (Equation 1.2) (Holling 1959b), where  $N_e$ ,  $N_0$  and  $a$  are defined as in the Type I functional response,  $T$  is the total experiment time, and  $T_h$  is the handling time.

Holling's disc equation assumes that prey consumed by predator can be immediately replaced so that prey density remains the same over time (Begon et al. 1996). However, the condition of constant prey density often does not occur in most experiments because the act of replenishing prey might disturb the predator and affect its foraging behavior. Thus, more sophisticated models are being developed to account

for realistic foraging behaviors (such as predator interference) and experimental conditions (such as depleting prey density) (Jeschke et al. 2002, Okuyama 2013).

In Type III functional response, the number of prey consumed increases slowly at low prey density but rapidly at intermediate prey density (Figure 1.1E). When the prey density reaches a threshold, the number of prey consumed approaches a maximum. Type III functional response curve resembles a logistic curve with sigmoid function (Figure 1.1F). The proportion of prey consumed increases as the density of prey increases in a logarithmic pattern up to the threshold, after which the proportion of the prey consumed begins to decrease. An increase in the predator's searching efficiency and a decrease in handling time occur when prey density increases, leading to Type III functional response. In other words, an increase in  $a$  or a decrease in  $T_h$  will make the curve to rise faster than the increase in prey density alone and shown as a hump-shaped curve.

Type III functional response can be expressed as

$$\frac{aN_0}{1 + aN_0 + bN_0^2} \quad \text{(Equation 1.3) (Hassell et al. 1977), where } N_0,$$

$N_0$ ,  $T_h$  and  $T$  are defined as in the Type I and II functional response equations. The parameters  $b$ ,  $c$  and  $d$  can estimate the attack rate in the equation

$$\frac{aN_0}{1 + aN_0 + bN_0^2} \quad \text{(Equation 1.4) (Hassell et al. 1977).$$

A very important difference between Type II and Type III functional responses is the ability of a Type III predator to switch from one prey type to another (known as prey switching) (Hellström et al. 2014). In a multiple-prey system, predators are likely

to switch from one prey type of lower abundance to another of higher abundance, which could lead to Type III functional response (Murdoch 1969, Fryxell and Lundberg 2012).

Functional response plays a key role in understanding the behavior of biological control agents (Madadi et al. 2007); therefore, it is an important factor in selecting appropriate predators for biological control programs (Lester and Harmsen 2002). Predators displaying Type III functional response have positive density-dependent impact on prey populations, which can lead to a stable predator-prey system and a more effective biological control system (Fernández-Arhex and Corley 2003). Therefore, the Type III predators can be more effective biological control agents (Pervez and Omkar 2005). In contrast, predators with Type II functional response impose negative density-dependent impact on prey populations, which can lead to a less stable system and less effective biological control program (Fernández-Arhex and Corley 2003). It is therefore essential to determine the type of functional responses a potential biological control agent exhibits (Pervez and Omkar 2005). Practically, we can gain useful information on the predators' potential as biological control agents from studying their functional responses (Cabral et al. 2009).

Despite its theoretical negative density dependency, there are many examples of successful biological control programs that employ Type II predators and parasitoids (Fernández-Arhex and Corley 2003, Hughes et al. 1992). One of the main reasons leading to the occurrence of such discrepancy between functional response type and field performance is the artificiality of experimental conditions. Predators that are shown to exhibit Type II functional response are often evaluated under laboratory

conditions, which are less realistic than field conditions (Fernández-Arhex and Corley 2003). There are also other factors, for example, predation, competition, host features and environmental complexity, which could affect predator foraging behavior, functional response and attacking efficiency (Pervez and Omkar 2005). By studying how these factors influence functional response, we could be able to obtain a better understanding of the functioning of the predator-prey system. I am interested in how plant architecture may influence predatory efficacy.

### **Plant architecture**

Physical characteristics of plants can affect the efficiencies of natural enemies by influencing their searching pattern (Price et al. 1980). The morphological structures of plants are known to affect directly the foraging behavior of a natural enemy (Price et al. 1980). Plant architecture is frequently defined based on the arrangement, number, and size of plant parts (Gontijo et al. 2011). Plant morphology is heterogeneous in different spatial scales (Li and Reynolds 1994, Reynolds and Cuddington 2012) and has significant influence on population dynamics of prey (Hauzy et al. 2010). Spatial heterogeneity influences a predator-prey system in two ways (Hauzy et al. 2010). First, complex plant architectural structure provides partial or full refuges for prey, thus reducing predator attack rates. Plant architecture also influences the number of prey a predator can find by limiting the rate or pattern of predator movement. Spatial heterogeneity can affect the functional response of predators through these indirect influences on predator foraging behavior and efficiency.

In this study, small cage clips on each selected leaf were used to maintain relatively even distribution and abundance of prey and to eliminate the prey's natural tendency to seek refuges. Thus, the influence of refuges would not be considered. I only considered the impact of plant architecture, particularly the number of branches and leaves, on predator functional response.

Plant morphological complexity creates obstacle. Obstacles could reduce encounter rate with suitable prey, leading to a lower attack rate by the predator (Hauzy et al. 2010, Campbell Grant et al. 2007). Campbell Grant et al. (2007) explained that plants with a large number of branches create a highly complex spatial structure, which limits the movement of predators, resulting in greater searching time and lower attack rate.

Many previous studies investigated how plant structure influences predator foraging behavior and functional responses; however, few of them assessed how the numbers of branches and leaves affected the functional response of *C. montrouzieri*. Cloyd and Sadof (2000) showed that increasing plant size, height, leaf surface area, leaf number and branch number were negatively correlated with the attack rate of *Leptomastix dactylopii* (Howard) (Hymenoptera: Encyrtidae). Legrand and Barbosa (2003) reported that *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) foraging efficiency decreased with increasing plant structural complexity. Reynolds and Cuddington (2012) reported decreased searching efficiency and predation rate of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) on smaller leaves and branches. Garcia and O'Neil (2000) showed that *Cryptolaemus montrouzieri* Mulsant (Coleoptera:



Coccinellidae) attack rates decreased as the leaf number, plant height, and leaf surface area increased.

Unlike the study of Garcia and O'Neil (2000), this study will focus on the effects of both leaf number and branch number, at constant leaf surface area, on the functional response of *C. montrouzieri*. Many related studies explored the influence of a single plant architectural character, such as plant canopy (Hoddle et al. 1998, Madadi et al. 2007), leaf surface area (Need and Burbutis 1979, Maini et al. 1991, Wang et al. 1997), or height (Ables et al. 1980), on functional response. The influence of plant architecture, particularly the number of leaves and branches, on the functional response of *C. montrouzieri* has not been studied well. Because the structural complexity of all plants is increased during growth, the effects of increasing numbers of leaves and branches on the functional responses of natural enemies can be useful in predicting the efficiency of the natural enemies in future biological control programs.

The foraging behavior of predators is one of the most important factors affecting prey distribution and populations, and an understanding of this factor may improve the efficiency of biological control programs (Gontijo et al. 2012). Plant structural complexity promotes the efficacies of some biological control agents, for instance, the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Gontijo et al. 2012), the lady beetle *Harmonia axyridis* Pallas (Reynolds and Cuddington 2012), the spider *Nesticodes rufipes* (Rossi et al. 2006), and the ants (Riihimaki et al. 2006). This study will provide important ecological information for the use of *C. montrouzieri* as biological control agents in the future.

### *Cryptolaemus montrouzieri*

The model organism in this study is *C. montrouzieri*, a coccinellid native to Australia but has been used as biological control agent of mealybugs (Hemiptera: Pseudococcidae) and soft scale insects (Hemiptera: Coccidae) in at least 64 countries (Olivero et al. 2003, Al-Khateeb and Asslan 2009, Kairo et al. 2013). Outdoor distribution of *C. montrouzieri* in US is restricted to southern California and central and southern Florida (Gordon 1985). However, this species is released in greenhouses throughout the country for management of mealybugs.

In Australia, adults of *C. montrouzieri* are released to augment the existing populations of other predators in controlling the exotic citrus mealybug, *Planococcus citri* (Risso), in citrus and custard apple (*Annona* sp.) orchards (Llewellyn 2002). In 1892 and 1930, *C. montrouzieri* was introduced from Australia to California as the primary biological control agent of the citrus mealybug (Gordon 1985). *Cryptolaemus montrouzieri* is also a predator of the mealybugs in the genera *Pseudococcus*, *Phenacoccus* and *Ferrisia*, and the coccids in the genus *Pulvinaria* (Gordon 1985). Proven to be one of the most effective natural enemies of mealybugs, rearing of *C. montrouzieri* was established in 1891 in California (Bartlett 1978).

Both adult and larva search for all stages of mealybugs actively on host plants (Clausen 1978, Kairo et al. 2013). Adults of *C. montrouzieri* have the capability to detect prey by vision or olfaction, while larvae seemingly searching prey only by random physical contact (Heidari and Copland 1992). The success of *C. montrouzieri*

will depend on its search strategy (more searching and consuming time on larger plants), the number of prey (more consumption at higher density), and the characteristics of the plant (lower attack efficiency at higher plant structural complexity) (Garcia and O'Neil 2000).

*Cryotolaemus montrouzieri* adults are approximately 3 to 4 mm in length. The color of its forewings and abdomen are black. The head and thorax is reddish to light brown color. Males have reddish-yellow forelegs, whereas females have nearly black forelegs (Booth and Pope 1986).

Under laboratory conditions of  $28 \pm 2^{\circ}\text{C}$ ,  $44 \pm 5\%$  RH and 16:8 (L: D) hours photoperiod, the developmental period of *C. montrouzieri* is 33.3 days when reared on the Madeira mealybug, *Phenacoccus madeirensis* Green (Al-Humiari et al. 2011). Egg development ranged from 3 to 5 days (Al-Humiari et al. 2011). Ghorbanian et al. (2011) also studied the development of *C. montrouzieri* in laboratory conditions on coleus when provided with citrus mealybug. The first to fourth instars completed development in 3.0, 2.4, 2.9 and 4.7 days, respectively. The pre-pupal and pupal periods were 2.4 and 7.8 days, respectively. The pre-oviposition period was 5.6 days. The oviposition period ranged from 46 to 109 days with an average of 70.4 days, and the post-oviposition period of 2.9 days. The adult longevity of *C. montrouzieri* was 79.0 days. The average number of eggs per female over her lifetime was 433.1 and mean eggs per female per day were 3.8.

### **Mealybug**

Mealybugs are increasingly problematic pests of agricultural and ornamental crops worldwide (Afifi et al. 2010). The target species of this study is the citrus mealybug, *P. citri*, a worldwide polyphagous pest species (Williams and Watson 1988).

The citrus mealybug is native to Asia but currently has a cosmopolitan distribution (CABI/EPPO 1999). *Planococcus citri* has been found as a serious pest and called “the greenhouse mealybug” in Europe since 1813, and recognized in the United States since 1879 (Anonymous 2007). In the USA, the citrus mealybug occurs outdoor and in greenhouse in 17 states: Alabama, Arizona, Arkansas, California, Florida, Hawaii, Kansas, Louisiana, Maryland, Massachusetts, Missouri, New Mexico, Ohio, South Carolina, Tennessee, Texas, and Virginia (CABI/EPPO 1999). As one of the major pests in greenhouses and nurseries, citrus mealybug damages a wide variety of ornamental plants, including annual and perennial flowering, foliage and woody plants (James 1937, McKenzie 1967, Malais and Ravensberg 2004). The common host crops of citrus mealybug in the world include apple, cassava, chili pepper, eggplant, potato, tomato, avocado, citrus, English ivy, ficus, gardenia, jasmine, oleander, persimmon, "pothos" (*Scindapsis* sp.), pittosporum and rhododendron (Ahmed and Abd-Rabou 2010, Ben-Dov 2013). In the greenhouses, the citrus mealybug is the most common mealybug species and causes the greatest amount of damage attacking coleus, bulbs, ferns and other ornamentals (Blumberg and van Driesche 2001).

The citrus mealybug develops four to five overlapping generations on citrus trees in the field (Bartlett and Lloyd 1958). When reared on sprouted potatoes at 27 °C, the development durations of the citrus mealybug are approximately 5 days for the first

instar, 9 days for the second instar, and 6 days for the third instar (Bartlett and Lloyd 1958). Females begin to produce eggs 14 days after eclosion (Bartlett and Lloyd 1958). Goldasteh et al. (2009) reported that female citrus mealybugs can complete development in 18 to 45 days under temperatures ranging from 18 to 32 °C, with the shortest developmental time (18 days) occurring at 25 °C, and the longest development time (46 days) occurring at 18 °C. Completion of development of males occurred 1 to 10 days after females achieved adulthood (Goldasteh et al. 2009). At 18°C, the average number of egg that each female can lay is over 400 (Copland et al. 1985).

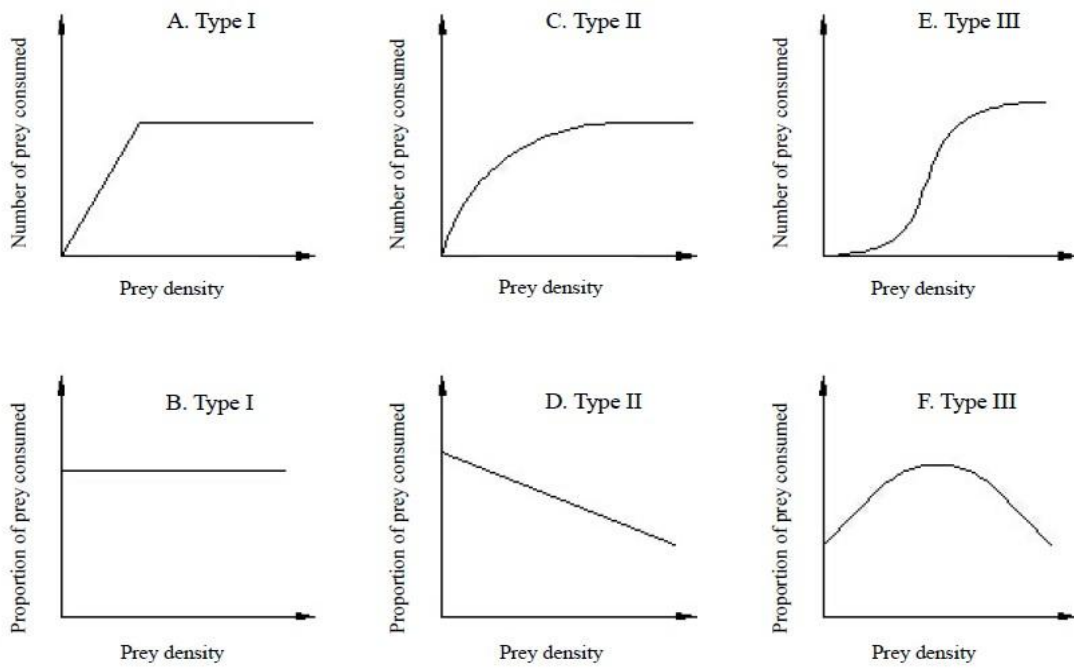
The nymphs and female citrus mealybug possess piercing-sucking mouthparts, which they use to suck fluids and remove nutrients from the phloem (Hogendorp et al 2009). The plants can be seriously damaged when sap is extracted by a high number of mealybugs (Goldasteh et al. 2009). Infested plants become wilted, stunted, distorted and chlorotic, leading to premature leaf drop, vigor reduction, and even death of entire or parts of the infested plants (Ahmed and Abd-Rabou 2010). The citrus mealybug can excrete sticky, sugary sap called honeydew, which stimulates the growth of black sooty mold, leading to reduced photosynthesis and inducing plant stress (Malais and Ravensberg 1922, Hill 1983). Feeding of citrus mealybug on fruits can cause premature fruit drop, reduce fruit production, discolor fruits and develop hard lumps on fruits (Griffiths and Thompson 1957). The citrus mealybug is a vector of many common grapevine viral diseases (Cabaleiro and Segura 1997). The citrus mealybug can be dispersed by both active (e.g. crawling among plants) and passive means (e.g. transported by wind, bird's feet and farm machinery and workers) (Kerns et al. 2004).

Moreover, some ants have the habit to carry them from one plant to another to collect the honeydew that is produced by the citrus mealybugs (Watson 1918).

The traditional management approach against the citrus mealybug is to use contact and systemic insecticides (Hatting and Tate 1996, Dreistadt 2001). Pesticides can reduce the population of citrus mealybugs rapidly to below the injurious level, and prevent outbreaks in the future (Hudson et al. 1996). However, the hydrophobic waxy coating produced by the third instars and adult females protects them from penetration of contact pesticide sprays. Therefore, additional management methods are of interest to pest managers (Copeland et al. 1985, Walker 2000). Many natural enemies have been identified, including parasitoids (*Chrysoplatycerus splendens* Howard, *L. dactylopii* and *Anagyrus pseudococci* (Girault)) and predators (*Chrysopa lateralis* Gu éin, *Laetitia coccidivora* Comstock and *C. montrouzieri*) (Griffiths and Thompson 1957, Cloyd and Sadof 2000, Anonymous 2007). *Cryptolaemus montrouzieri* are more capable of reducing heavy mealybug populations than are other species (Bartlett and Lloyd 1958).

### **Objective**

The objective of my study is to determine the type of functional responses of *C. montrouzieri* on chili pepper plants with different numbers of branches (2, 4 and 6) and leaves (5, 10 and 15) when provided varying densities of the citrus mealybugs.



**Figure 1.1.** Relationship between the number of prey consumed and prey density (A, C, E) and the relationship between the proportion of prey consumed and prey density (B, D, F) in Type I, II and III functional responses.

## CHAPTER TWO

### RESEARCH DESIGN AND METHODS

#### **Growing and maintaining the study plants**

Chili peppers (*Capsicum annum* L., cv. 'Jalapeño') were grown in a greenhouse at Pee Dee Research and Education Center (PDREC), Florence, SC. Seeds from a certified supplier (Park Seeds, Greenwood, SC) were sown in 0.3 × 0.6 m trays filled with multipurpose potting medium (3B Mix, Fafard, Anderson, SC). Seedlings were irrigated with tap water daily and transplanted into plastic pots (15.4 cm diameter) filled with the same potting medium when there were four fully expanded leaves (approximately 14 days after sowing). One teaspoon (about 10 g) of Osmocote (Scotts Company, Maryville, OH), a slow-release fertilizer, was applied evenly onto the medium surface of each pot immediately after transplant. A water-soluble fertilizer (Miracle-Gro® Water Soluble All Purpose Plant Food, N:P:K = 24:8:16, Scotts Company Marysville, OH) was applied every two days during irrigation at 313 ppm nitrogen, 104 ppm phosphorus and 208 ppm potassium. The fertilizer solution was delivered through a handheld hose-end applicator. Plants were arranged in three rows on benches in two greenhouses, and spaced 30 cm (from main stem to main stem) apart to allow full growth and expansion of the canopy.

#### **Structuring the plants**

I manipulated plants mechanically to obtain plants with desirable numbers of branches and leaves. Terminal shoots were pinched when the transplanted seedlings had eight fully expanded leaves. Top pinching promoted the growth of more branches and



leaves from the main stem. Only primary branches (branches developed from the main stem) and leaves on the main stem and primary branches were retained for the experiments.

This study included two experiments. One experiment (termed the “branch experiment”) was designed to detect the influence of the numbers of primary branches (2, 4 and 6) on the functional response of *C. montrouzieri*. The second experiment (termed the “leaf experiment”) assessed the influence of the numbers of leaves (5, 10 and 15) on the functional response of the lady beetle. I strived to maintain similar total stem length (sum of lengths of main stem and branches; 45 cm), leaf surface area (335 cm<sup>2</sup>), stem surface area (85 cm<sup>2</sup>) and total surface area (420 cm<sup>2</sup>) in the branch experiment. The desirable total stem length, leaf surface area, stem surface area and total surface area for the leaf experiment were 23 cm, 410 cm<sup>2</sup>, 50 cm<sup>2</sup> and 460 cm<sup>2</sup>, respectively. This experimental design ensured that the detected differences in the functional response of *C. montrouzieri* were the results of varying branch or leaf number, not that of varying surface areas.

We developed predictive models to estimate the leaf and stem surface areas so that only leaves, main stem and primary branches that allowed for similar surface areas were selected during the pruning process. We assumed that the stems and primary branches approximated circular truncated cones. Therefore, an equation for circular truncated cones was used to estimate surface areas of stems and primary branches,

(Equation 2. 1),

where  $\pi = 3.14$ ,  $l$  = length,  $r$  = top radius and  $R$  = base radius. The lengths and radius of primary branches and main stems were measured, and their surface areas were estimated and summed.

A model used to estimate the surface area of leaves was developed with data collected from ‘Jalapeño’ chili pepper plants grown in the greenhouses of PDREC in 2014 under procedures identical to this study. Three hundred fully expanded leaves were selected from the upper, middle and lower parts of 30 randomly selected plants. Mid-vein lengths (excluding petiole) of the selected leaves were measured. The actual one-side leaf surface areas were measured by scanning the leaves with an area meter (LI-3100 Area Meter; LI-COR, Inc., Lincoln, NE) three times per leaf and averaged. Leaf surface areas were plotted against mid-vein lengths using Sigmaplot (Systat Software, Inc. San Jose, CA), and linear, quadratic, cubic, 1-, 2-, 3-parametered exponential, 3-, 4-, 5-parametered sigmoid, 3- and 4-parametered logistic regression models were created. The logistic regression equation with 4 parameters

$$\text{————— (Equation 2.2),}$$

where  $y$  = the predicted leaf surface area and  $x$  = the actual length of the leaf mid-vein, provided the best fit for the data ( $R^2 = 0.9826$ ,  $y_0: P = 0.0718$ ;  $x_0: P < 0.0001$ ;  $a: P = 0.0002$ ;  $b: P < 0.0001$ ). All estimated leaf surface areas were doubled because each value presented only one side of the leaf.

Plants assigned to the two-branch treatment were manipulated to have two primary branches (one in the middle and one near the base of main stem) with four leaves on the main stem (two near the top and two near the base) and four leaves on the primary

branches (one on each top and one in each middle) (Figure 2.1A). Plants assigned to the four-branch treatment retained four primary branches (two in the middle and two near the base of main stem) with two leaves on the main stem (one near the top and one near the base), two leaves on the upper primary branches (near the top) and four leaves on the lower primary branches (one near each top and one in each middle) (Figure 2.1B). Plants assigned to the six-branch treatment retained six primary branches (two near the top, two in the middle and two near the base symmetrically) with two leaves on the upper branches (one near each top), two leaves on the middle branches (one near each top) and four leaves on the lower branches (one near each top and one in each middle) (Figure 2.1C). All the plants assigned to the branch experiment had eight leaves per plant.

Only main stems were retained on plants assigned to the leaf experiment. Plants assigned to the five-leaf treatment retained five leaves (one on the top, two in the middle and two near the base) (Figure 2.1D). Plants assigned to the 10-leaf treatment retained 10 leaves (three on the top, three in the middle and four near the base) (Figure 2.1E). Plants assigned to the 15-leaf treatment retained 15 leaves (five on the top, five in the middle and five near the base) (Figure 2.1F). Leaves on each section of the main stem were evenly distributed on all directions in the leaf experiment.

Plants were pruned approximately 6 weeks after top pinching and two days prior to the experiments. Spare leaves and branches were removed from the main stem to obtain the desirable numbers of branches and leaves. Each number of branches/leaves  $\times$  prey density combination was replicated 11 times. Because of the time required to prune

the plants and collect the data, only one replicate of the leaf and branch experiment was conducted in each week in the summer of 2015.

### **Sources of insects**

Citrus mealybugs were selected from a laboratory colony initiated with individuals collected from a greenhouse at PDREC. Sprouted potatoes (*Solanum tuberosum* L.) were used as host plants and were introduced to the colony weekly. The colony was maintained under laboratory conditions of  $22.6 \pm 1.8$  °C,  $47.5 \pm 15.8\%$  R.H. and 16:8 (L:D). The colony was maintained in  $0.4 \times 0.55 \times 0.2$  m plastic tubs with a thin layer of Vaseline (Unilever US, Inc. Englewood Cliffs, NJ) on the inner sides to prevent crawlers from escaping. Old, wilted or rotting potatoes were replaced immediately. Pre-reproductive adult females (2-3 mm in length) were provided to *C. montrouzieri* in both experiments at densities of 2, 4, 8, 12 or 16 mealybugs per plant.

*Cryptolaemus montrouzieri* were purchased from two biological control agent suppliers. The lady beetles used in Replicate 1 – 4 and 11 were purchased from Biobest USA Inc. (McFarland, CA), whereas those used in Replicate 5 – 10 were purchased from IPM Laboratories, Inc. (Locke, NY). Preliminary data analysis suggested that adults from the two suppliers were not different in the numbers of mealybugs they consumed over a 24-hour period (Table 3.3 in Chapter 3). Adult females were selected one day prior to the experiments, and kept in glass vials individually with a moist cotton ball but no food and in the dark for 24 hours before being introduced onto the plants.

Voucher specimens of adult female *C. montrouzieri* (CUAC number: 24392 - 24394) and adult female *P. citri* (CUAC number: 24395) are deposited in the Clemson University Arthropod Collection, Clemson, SC.

### **Introduction of insects and collection of data**

The experiments were conducted under laboratory conditions ( $22.6 \pm 1.8$  °C,  $47.5 \pm 15.8\%$  R.H and a 14:10 (L:D) photoperiod). Mealybugs were carefully removed from the laboratory colony and transferred onto the pruned plants, using a fine paintbrush. Even distribution of the mealybugs was achieved by confining one or several mealybugs onto a leaf using a small clip cage (made with chiffon and foam and fixed onto leaves with alligator pins). Mealybugs were allowed to feed and settle on the plants for 24 hours before the plants were put in cages (30 × 30 × 60 cm; BioQuip Products, Rancho Dominguez, CA) and *C. montrouzieri* was introduced. The plants, harboring both the mealybugs and the lady beetles, were caged individually for 24 hours, after which the cages were removed, the lady beetles were collected, and the numbers of surviving mealybugs were counted.

### **Statistical analysis**

We first analyzed the data to determine if ANOVA's assumption of normal data distribution were met with Shapiro-Wilk test in JMP (JMP Pro 12, SAS 2015). In the branch experiment, the plant data were not normally distributed based on the results of Shapiro-Wilk test (total stem and branch length:  $W = 0.955253$ ,  $P < 0.0001$ ; leaf surface area:  $W = 0.949937$ ,  $P < 0.0001$ ; stem surface area:  $W = 0.942677$ ,  $P < 0.0001$ ; total surface area:  $W = 0.978669$ ,  $P = 0.0120$ ). In the leaf experiment, the estimated total

stem and branch lengths, stem and total surface areas were not in normally distributed (total stem and branch length:  $W = 0.951725$ ,  $P < 0.0001$ ; stem surface area:  $W = 0.919417$ ,  $P < 0.0001$ ; total surface area:  $W = 0.964541$ ,  $P = 0.0003$ ), whereas the leaf surface areas were normally distributed (leaf surface area:  $W = 0.989020$ ,  $P = 0.0976$ ). The estimated total stem and branch lengths, leaf, stem and total surface areas collected from the leaf and branch experiments were analyzed with one-way ANOVA and the means were separated with Tukey's Honest Significant Difference Test (HSD) at  $\alpha = 0.05$  respectively (JMP Pro 12, SAS 2015).

Data on the numbers of mealybugs consumed did not meet the assumption of normal distribution based on Shapiro-Wilk test (branch experiment:  $W = 0.935778$ ,  $P < 0.0001$ ; leaf experiment:  $W = 0.957338$ ,  $P < 0.0001$ ) (JMP Pro 12, SAS 2015).

Transformation of plant and insect data was not successful in normalizing the data distribution. Therefore, raw data were used in statistical analysis. Data from the leaf and branch experiments were analyzed separately.

Treatment effects on prey consumption by *C. montrouzieiri* were first analyzed with three-way ANOVA (different prey densities, different suppliers, different leaf or branch numbers, and interactions among the treatments) at  $\alpha = 0.05$  (JMP Pro 12, SAS 2015). When the numbers of mealybugs consumed were found to be similar between the two suppliers (Table 3.2), all plant parameters and prey consumption data from the two suppliers were pooled and reanalyzed with two-way ANOVA (different prey densities, different leaf or branch numbers, and interactions among the treatments) (JMP Pro 12,

SAS 2015). Tukey's HSD test was used to separate means when significant differences among means were detected by ANOVA at  $\alpha = 0.05$ .

The statistical method used to discriminate among the three types of functional responses was based on logistic regression

—  $\text{—————}$  (Equation 2.3) (Juliano 2001).

The constant, linear, quadratic and cubic parameters ( $P_0$ ,  $P_1$ ,  $P_2$ , and  $P_3$ ) were estimated by inputting the numbers of prey consumed ( $N_e$ ) and the numbers of prey offered ( $N_o$ ) to the logistic regression. A Type I functional response will be described if  $P_1 = 0$ , which means the proportion of prey consumed is constant. A Type II functional response will be described if  $P_1 < 0$ , which means the proportion of prey consumed is negatively density dependent (Juliano 2001). A Type III functional response will be described if  $P_1 > 0$  and  $P_2 < 0$ , which means the proportion of prey consumed is positively density dependent. If the coefficient of the cubic term ( $P_3$ ) is non-significant ( $P < 0.05$ ), the coefficients of the logistic regression were re-estimated with a reduced model that eliminated the cubic term (Juliano 2001). If the coefficients  $P_1$ ,  $P_2$  are not significant, it is necessary to verify the type of functional response by plotting the proportion of prey consumed against the prey densities (Juliano 2001). Once the type of functional response was determined, the numbers of mealybug consumed were fitted to the functional response equation of the appropriate type to estimate the attack rates and handling times (PROC NLIN, SAS 2011).

The consumed prey were not replaced in this study, violating the assumption of Holling's disc equation (i.e. the prey is replaced immediately after being consumed) (Hellström et al. 2014). Thus, Roger's random-predator equation for Type II functional

response, which incorporates prey depletion, was used in this study. The functional response equations considered in this study were:

Type I:  $N_e = a \cdot N_0$  (Equation 2.4) (Holling 1959a),

Type II:  $N_e = N_0 \{1 - \exp [-a \cdot (T - T_h \cdot N_e)]\}$  (Equation 2.5) (Rogers 1972), and

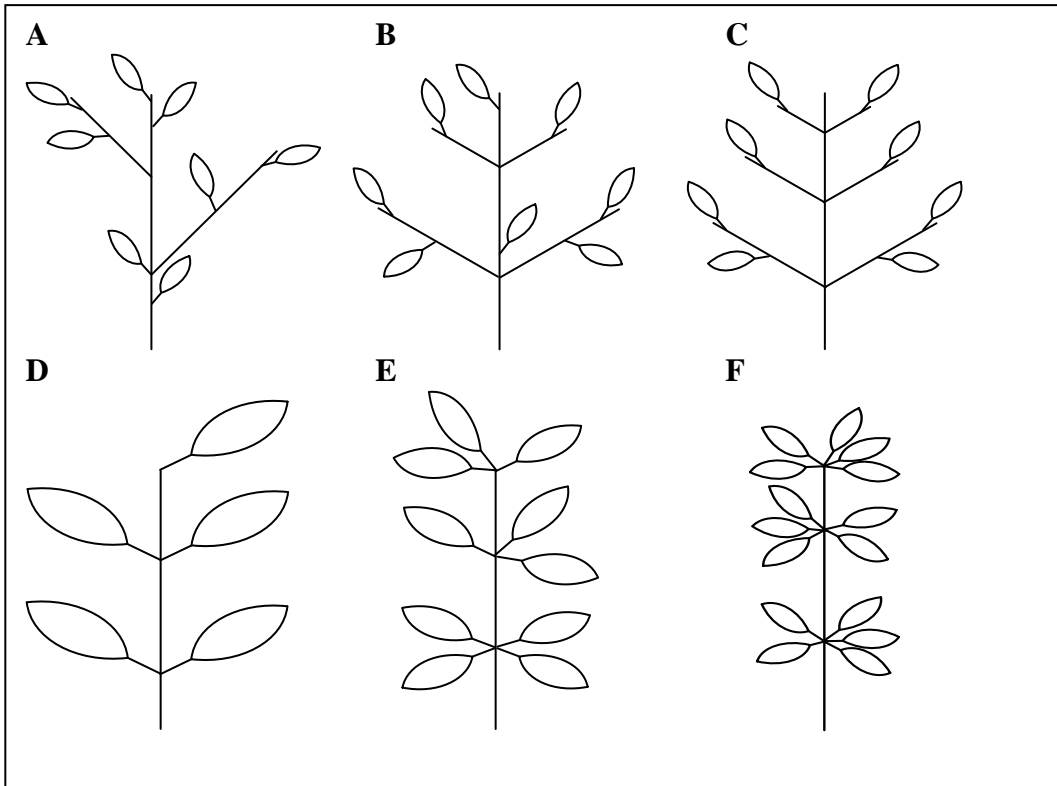
Type III:  $N_e = N_0 \left\{1 - \exp \left[ \frac{(d + b \cdot N_0) \cdot (T_h \cdot N_e - T)}{(1 + c \cdot N_0)} \right]\right\}$  (Equation 2.6) (Hassell et al. 1977),

where  $N_e$  is the number of prey consumed,  $N_0$  is the number of prey offered or the initial prey density,  $a$  is the attack rate,  $T$  is the total experimental time (fixed at 24 hours), and  $T_h$  is the handling time. The parameters  $b$ ,  $c$  and  $d$  in Equation 2.6 can be used to estimate the attack rate,

$a = \frac{d + b \cdot N_0}{1 + c \cdot N_0}$  (Equation 2.7) (Hassell et al. 1977). If a logistic regression analysis indicated

a Type III functional response, the three parameters ( $b$ ,  $c$  and  $d$ ) would define the relationship between attack rates and initial prey density. As a result, the parameter  $b$  in Equation 3 must be a positive value and the estimated values of  $c$  and  $d$  must be greater than or equal to 0. If the estimates of  $c$  and  $d$  were not significant, they could be eliminated from the full model of Equation 2.6 and 2.7 to obtain positive attack rates and handling times (Juliano 2001). Therefore, the minimum Type III functional response equation can be described as a model with only two parameters ( $b$  and  $T_h$ ).





**Figure 2.1.** A Approximate arrangement of 2 (A), 4 (B), 6 (C) primary branches and 5 (D), 10 (E), 15 (F) leaves.

## CHAPTER THREE

### RESULTS

#### **Plant architectural characteristics**

Despite my best efforts and care in selecting the most similar branches and leaves during the pruning process, the estimated total stem and branch length, leaf surface area, stem surface area and total plant surface areas were significantly different among the treatments in both leaf and branch experiments (Table 3.1). The 6-branched plants had on average 7% longer stems and 9-11% larger stem surface area than the 2- and 4-branched plants, but their leaf surface areas were 1% smaller than the 2 and 4-branched plants. As a result, the mean total surface area of 4-branched plants was similar to the 2- and 6-branched plants, but the total surface area of the 6-branched plants was significantly smaller than that of the 2-branched plants. The 5- and 10-leaved plants had 9% greater total length than the 15-leaved plants. Although 5-, 10- and 15-leaved plants had similar leaf surface areas, the stems of the 5-leaved plants were 8-11% thinner than the 10- and 15-leaved plants. As a result, the total surface area of the 5-leaved plants was the smallest.

#### **Prey consumption rates**

No significant differences were found between the two suppliers or interactions that include this term in both the leaf and branch experiment (Table 3.2). Therefore, prey consumption data from the two suppliers were pooled and analyzed with two-way ANOVA.

The mean numbers of mealybugs eaten by *C. montrouzieri* were not significantly different among various branch and leaf numbers in both experiments, nor were there significant interactions between the leaf or branch numbers and prey densities (Table 3.3). In both the branch and leaf experiments, prey densities had significant effects on the numbers of mealybugs consumed by *C. montrouzieri* over a 24-hour period (Table 3.3). When mealybug density increased, a single *C. montrouzieri* gradually consumed more mealybugs until an upper asymptote was reached (Table 3.4, Figures 3.1 and 3.2). The numbers of mealybugs consumed at 8 mealybugs/plant were significantly higher than those at 2 and 4 mealybugs/plants but similar to those at 12 and 16 mealybugs/plants in both the leaf and branch experiments (Table 3.4). When 8 or more mealybugs were offered on each plant, *C. montrouzieri* consumed only 3.8-5.9 mealybugs (Table 3.4), suggesting that the numbers of mealybugs consumed by a single *C. montrouzieri* over a 24-hour period is about 4 mealybugs.

#### **Types of functional response, attack rates and handling times**

The cubic parameter estimates of the logistic regression were significant in the 15-leaved treatment only (Table 3.5). Thus, the maximum likelihood analysis was repeated without the cubic term in all treatments except the 15-leaved treatment (Juliano 2001).

In the 4-, 6-branched and 5-, 10-leaved treatments, a declining linear coefficient in the polynomial equation indicated that the proportions of prey consumed versus prey density were negative, indicating Type II functional response (Table 3.5). The declining slope in the proportion of prey consumed at varying densities indicated that Type II

functional response best fitted the data from 2-, 4-, 6-branched and 5-, 10-leaved treatments (Figure 3.1B, D, F; Figure 3.2B, D). The linear parameter of the 15-leaved study is positive and the non-linear term is negative, indicating that the data best fitted Type III functional response (Table 3.5). This result was confirmed through the examination of Figure 3.2F, where a curve approximating a hump-shaped curve was detected.

The best fitted Type II functional response equations of 2-, 4-, 6-branched and 5-, 10-leaved treatments can be described as

(Equation 3.1),

(Equation 3.2),

(Equation 3.3),

(Equation 3.4), and

(Equation 3.5).

The best fitted Type III functional response equation of 15-leaved treatment can be described as

\_\_\_\_\_ (Equation 3.6).

In the branch experiment, the attack rates and handling times of *C. montrouzieri* decreased with increasing branch numbers (Table 3.6). The 95% confidence intervals of attack rates and handling times on all branch numbers overlapped, suggesting that the attack rates and handling times of *C. montrouzieri* against citrus mealybugs were similar

among plants with 2, 4 and 6 branches. The lady beetles foraging on 6-branched plants had the lowest efficiency where attack rate was 36% and 65% lower than those on the 4-branched and 2-branched plants, respectively.

The estimated handling time of *C. montrouzieri* on plants with 15 leaves were not different from those on plants with 5 or 10 leaves, as seen in the overlapping 95% confidence intervals (Table 3.6). The attack rates of *C. montrouzieri* on plants with 15 leaves were best described as

$$a = 3.84 e^{-3} \cdot N_0 / (1 + N_0) \text{ (Equation 3.7).}$$

The attack rate increased with increasing prey density until it approached a plateau at 8 mealybugs/plant (Figure 3.3). The attack rates of *C. montrouzieri* on plants with 5 and 10 leaves were similar (Table 3.6). The highest attack rates of *C. montrouzieri* on plants with 15 leaves were 17% and 96% lower than those on plants with 5 and 10 leaves, respectively.

**Table 3.1.** Means ( $\pm$ SEM) of estimated plant part dimensions on manipulated chili pepper plants ‘Jalapeno’ used in leaf and branch experiments.

Branch number	Length (sum of main stem and branches) (cm)	Leaf surface area (cm <sup>2</sup> )	Stem surface area (cm <sup>2</sup> )	Total surface area (cm <sup>2</sup> )
2	43.2 $\pm$ 0.4b	338.5 $\pm$ 1.4a	83.80 $\pm$ 0.76b	422.3 $\pm$ 1.4a
4	43.7 $\pm$ 0.4b	339.5 $\pm$ 1.6a	81.01 $\pm$ 1.31b	419.6 $\pm$ 1.8ab
6	46.7 $\pm$ 0.6a	324.7 $\pm$ 1.8b	91.13 $\pm$ 1.12a	415.8 $\pm$ 1.9b
<i>F</i>	16.1662	25.3419	23.2067	3.5378
<i>df</i>	162	162	162	162
<i>P</i> -value	< 0.0001	< 0.0001	< 0.0001	0.0313
Leaf number	Length (sum of main stem and branches) (cm)	Leaf surface area (cm <sup>2</sup> )	Stem surface area (cm <sup>2</sup> )	Total surface area (cm <sup>2</sup> )
5	24.6 $\pm$ 0.4a	406.3 $\pm$ 1.4	47.4 $\pm$ 0.71b	453.7 $\pm$ 1.4b
10	24.1 $\pm$ 0.5a	405.3 $\pm$ 1.6	53.2 $\pm$ 1.14a	458.5 $\pm$ 1.6a
15	22.5 $\pm$ 0.5b	411.0 $\pm$ 2.1	51.7 $\pm$ 1.01a	462.7 $\pm$ 1.8a
<i>F</i>	5.7837	3.1195	43.9409	23.3412
<i>df</i>	162	162	162	162
<i>P</i> -value	0.0037	0.0508	< 0.0001	< 0.0001

Means within a column followed by the same letters are not significantly different among the leaf or branch numbers according to Tukey’s HSD at  $\alpha = 0.05$  (JMP Pro 12, 2015).

**Table 3.2.** Three-way ANOVA table for the numbers of mealybugs consumed by *Cryptolaemus montrouzieri* over 24 hours in leaf and branch experiments on chili peppers.

Branch experiment	<i>F</i>	<i>P</i> -value	<i>df</i>
Number of branches	0.1770	0.8380	2
Prey density	38.1730	< 0.0001	4
Suppliers	0.1130	0.8932	1
Number of branches × suppliers	0.2132	0.6450	2
Number of branches × prey density	0.4462	0.8912	8
Suppliers × prey density	0.5091	0.7291	4
Number of branches × prey density × suppliers	0.8950	0.5226	8
Error			135
<b>Leaf experiment</b>			
Number of leaves	1.3168	0.2714	2
Prey density	33.0059	< 0.0001	4
Suppliers	1.8098	0.1808	1
Number of leaves × suppliers	1.2264	0.2966	2
Number of leaves × prey density	0.8402	0.5689	8
Suppliers × prey density	0.1739	0.9515	4
Number of leaves × prey density × suppliers	0.5299	0.8324	8
Error			135

**Table 3.3.** Two-way ANOVA table for the numbers of mealybugs consumed by *Cryptolaemus montrouzieri* over 24 hours in the leaf and branch experiments on chili peppers.

Branch experiment	<i>F</i>	<i>P</i> -value	<i>df</i>
Number of branches	0.2099	0.8109	2
Prey density	40.3964	< 0.0001	4
Number of branches × prey density	0.4827	0.8670	8
<b>Leaf experiment</b>			
Number of leaves	1.1709	0.3129	2
Prey density	34.7190	< 0.0001	4
Number of leaves × prey density	0.8377	0.5709	8

**Table 3.4.** Mean numbers ( $\pm$ SEM) of citrus mealybugs consumed by each *C. montrouzieri* at varying prey densities on chili pepper plants of varying branch and leaf numbers.

Initial prey density ( $N_0$ )	Branch number		
	2	4	6
2	1.36 $\pm$ 0.50b	1.45 $\pm$ 0.21c	1.45 $\pm$ 0.24b
4	2.18 $\pm$ 0.44b	2.55 $\pm$ 0.43bc	2.45 $\pm$ 0.39b
8	4.64 $\pm$ 0.45a	4.73 $\pm$ 0.60a	5.18 $\pm$ 0.42a
12	5.00 $\pm$ 0.56a	4.09 $\pm$ 0.37ab	4.91 $\pm$ 0.51a
16	5.18 $\pm$ 0.57a	5.00 $\pm$ 0.59a	4.73 $\pm$ 0.45a
	Leaf number		
	5	10	15
2	1.82 $\pm$ 0.12c	1.82 $\pm$ 0.12c	1.18 $\pm$ 0.23c
4	2.73 $\pm$ 0.27bc	2.91 $\pm$ 0.37bc	2.55 $\pm$ 0.37bc
8	4.00 $\pm$ 0.40ab	4.64 $\pm$ 0.61ab	4.55 $\pm$ 0.53ab
12	5.27 $\pm$ 0.52a	4.45 $\pm$ 0.76ab	3.82 $\pm$ 0.40a
16	5.18 $\pm$ 0.63a	5.91 $\pm$ 0.51a	5.45 $\pm$ 0.51a

Means in a column followed by the same letters are not significantly different among the prey densities according to Tukey's HSD at  $\alpha = 0.05$  (JMP Pro 12, 2015).

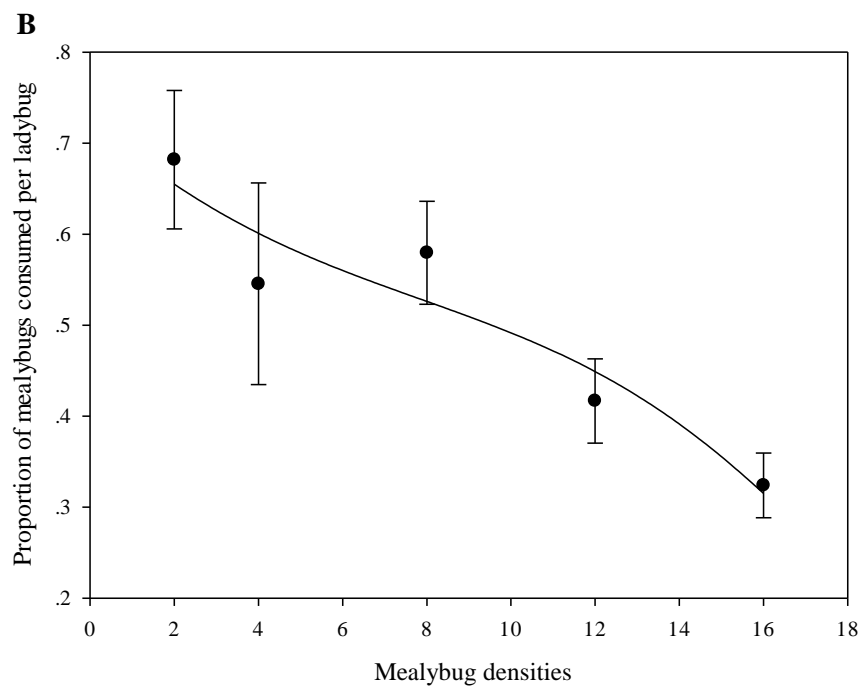
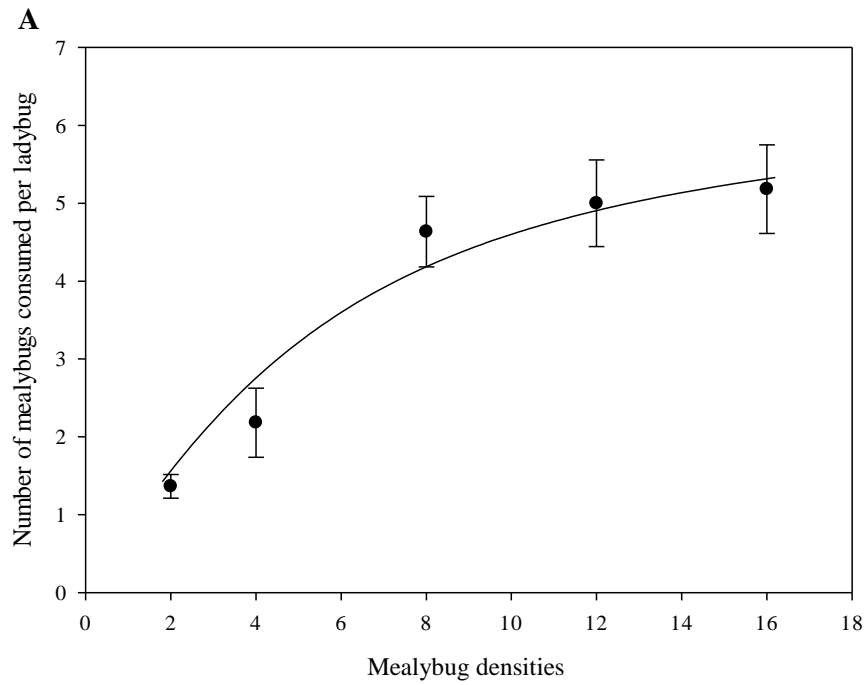


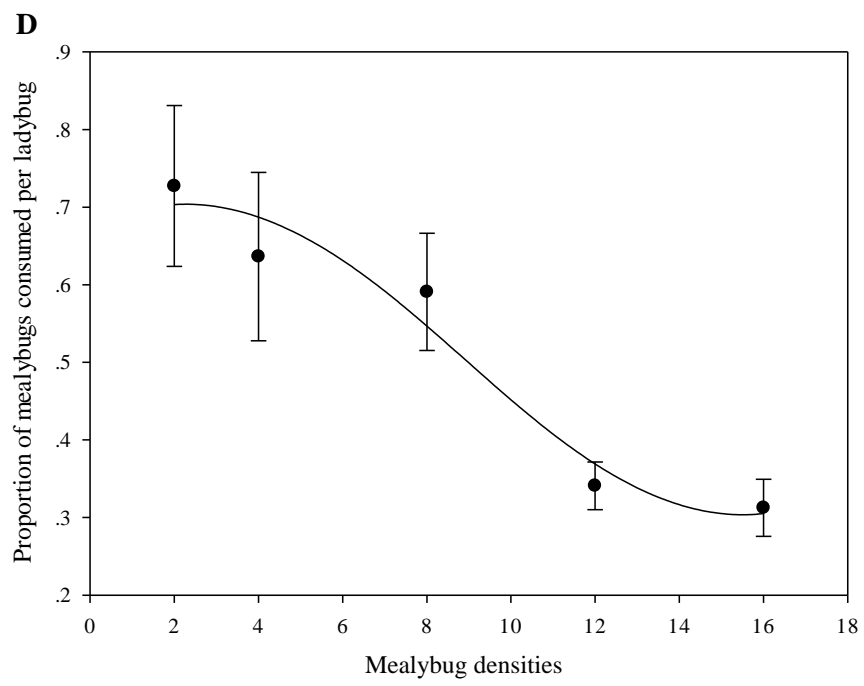
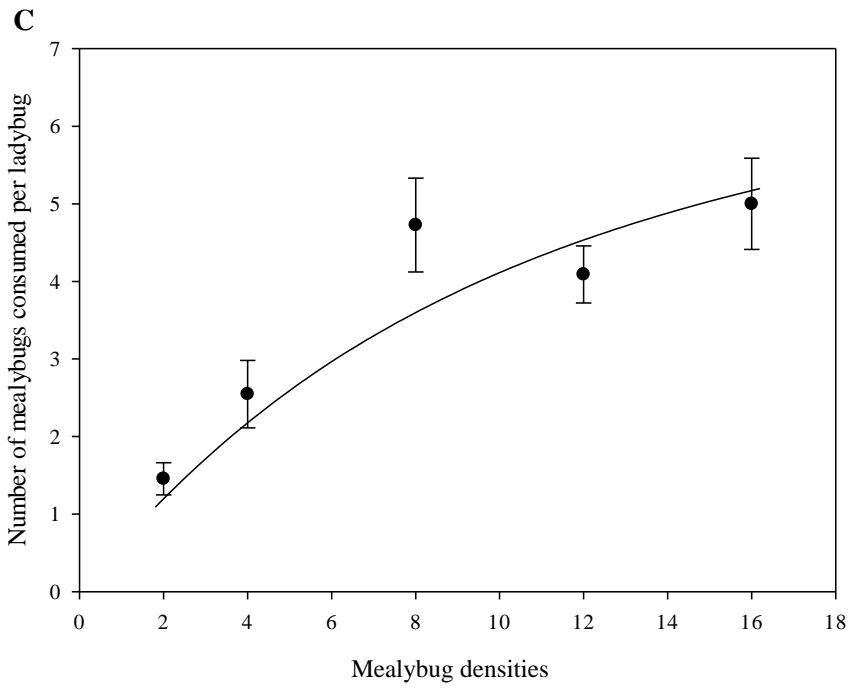
**Table 3.5.** Maximum likelihood estimates from logistic regression on the proportion of mealybugs consumed as a functional response of female *C. montrouzieiri*. Non-significant cubic terms were removed from the logistic regression models in all branch treatments and 5- and 10-leaved treatments.

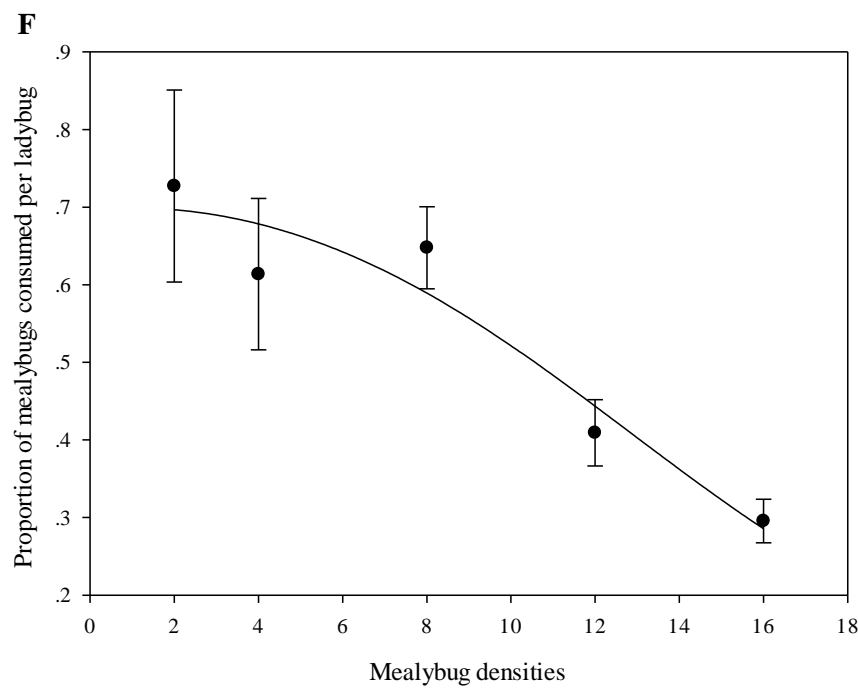
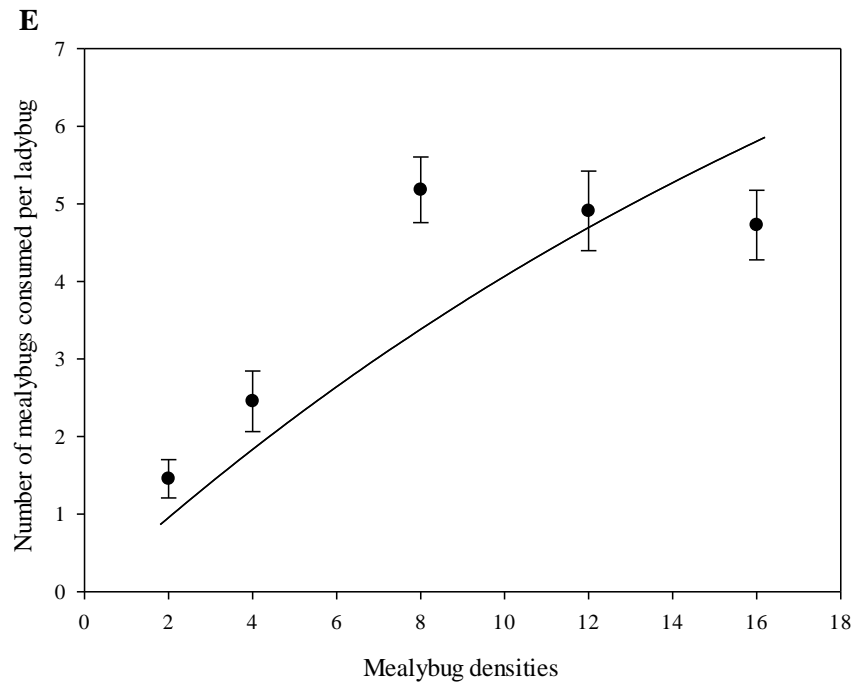
Parameter	Estimate $\pm$ SEM	$\chi^2$	df	P-Value
<b>2-branched pepper</b>				
$P_0$	0.3976 $\pm$ 0.4978	0.64	1	0.4245
$P_1$	0.0509 $\pm$ 0.1076	0.22	1	0.6362
$P_2$	-0.00915 $\pm$ 0.00520	3.10	1	0.0785
Likelihood ratio	-	70.54	52	0.0444
<b>4-branched pepper</b>				
$P_0$	1.8791 $\pm$ 0.5632	11.13	1	0.0008
$P_1$	-0.2650 $\pm$ 0.1180	5.04	1	0.0247
$P_2$	0.00590 $\pm$ 0.00562	1.10	1	0.2939
Likelihood ratio	-	80.40	52	0.0070
<b>6-branched pepper</b>				
$P_0$	0.9552 $\pm$ 0.5215	3.35	1	0.0670
$P_1$	-0.0373 $\pm$ 0.1116	0.11	1	0.7383
$P_2$	-0.00497 $\pm$ 0.00540	0.85	1	0.3574
Likelihood ratio	-	74.27	52	0.0230
<b>5-leaved pepper</b>				
$P_0$	2.0623 $\pm$ 0.5736	12.93	1	0.0003
$P_1$	-0.3063 $\pm$ 0.1192	6.60	1	0.0102
$P_2$	0.00841 $\pm$ 0.00564	2.22	1	0.1360
Likelihood ratio	-	56.61	52	0.3070
<b>10-leaved pepper</b>				
$P_0$	2.8079 $\pm$ 0.6326	19.70	1	<0.0001
$P_1$	-0.4509 $\pm$ 0.1279	12.42	1	0.0004
$P_2$	0.0150 $\pm$ 0.00594	6.41	1	0.0113
Likelihood ratio	-	87.62	52	0.0015
<b>15-leaved pepper</b>				
$P_0$	-0.6708 $\pm$ 0.6914	0.94	1	0.3319
$P_1$	0.8094 $\pm$ 0.2952	7.52	1	0.0061
$P_2$	-0.1238 $\pm$ 0.0354	12.20	1	0.0005
$P_3$	0.00440 $\pm$ 0.00124	12.52	1	0.0004
Likelihood ratio	-	111.35	51	0.0001

**Table 3.6.** Attack rates and handling times (mean  $\pm$  SEM) of *C. montrouzieri* when provided with varying densities of citrus mealybug on chili pepper plants of varying branch of leaf numbers. The attack rates and handling times were estimated with Roger's Type II or Hassell's Type III functional response equation. The range in parentheses is the 95% confidence interval of the estimate.

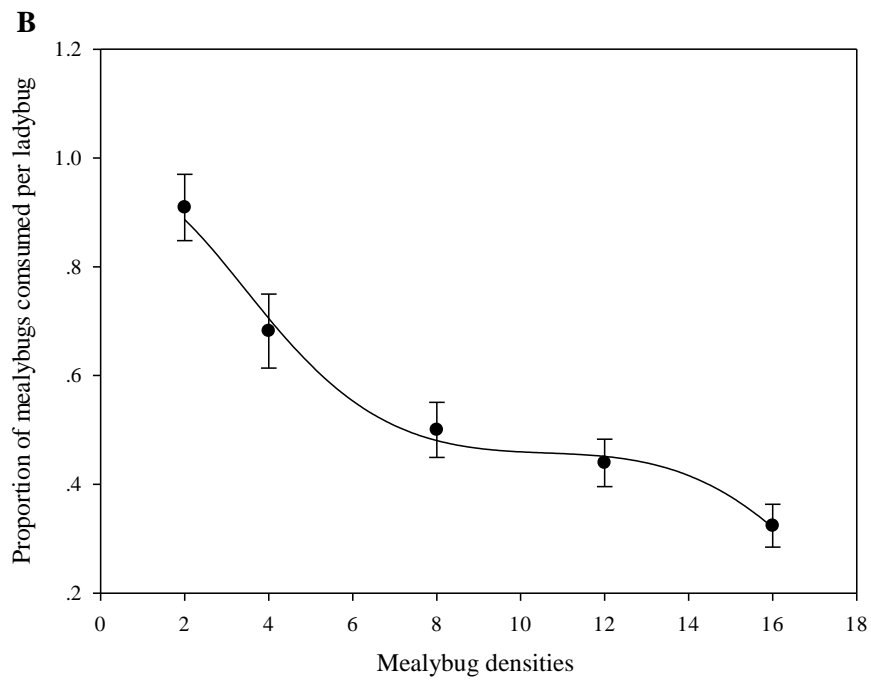
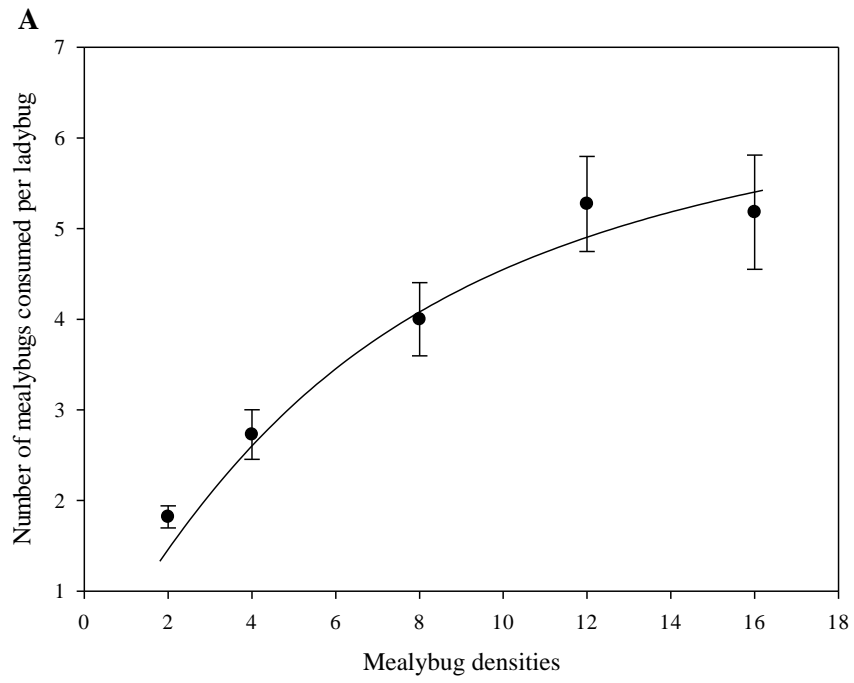
Branch number	$a$ (number of prey consumed/h)	$T_h$ (h)	$b$
2	0.083 $\pm$ 0.043 (-0.053, 0.218)	3.599 $\pm$ 0.603 (1.681, 5.518)	-
4	0.045 $\pm$ 0.033 (-0.061, 0.151)	2.952 $\pm$ 1.641 (-2.270, 8.174)	-
6	0.029 $\pm$ 0.048 (-0.124, 0.182)	1.428 $\pm$ 6.028 (-17.756, 20.613)	-
<b>Leaf number</b>			
5	0.069 $\pm$ 0.027 (-0.018, 0.155)	3.328 $\pm$ 0.563 (1.536, 5.120)	-
10	0.050 $\pm$ 0.023 (-0.023, 0.123)	2.544 $\pm$ 0.902 (-0.326, 5.413)	-
15	-	3.105 $\pm$ 1.395 (-1.333, 7.543)	3.84e <sup>-3</sup> $\pm$ 3.24e <sup>-3</sup> (-0.006, 0.014)

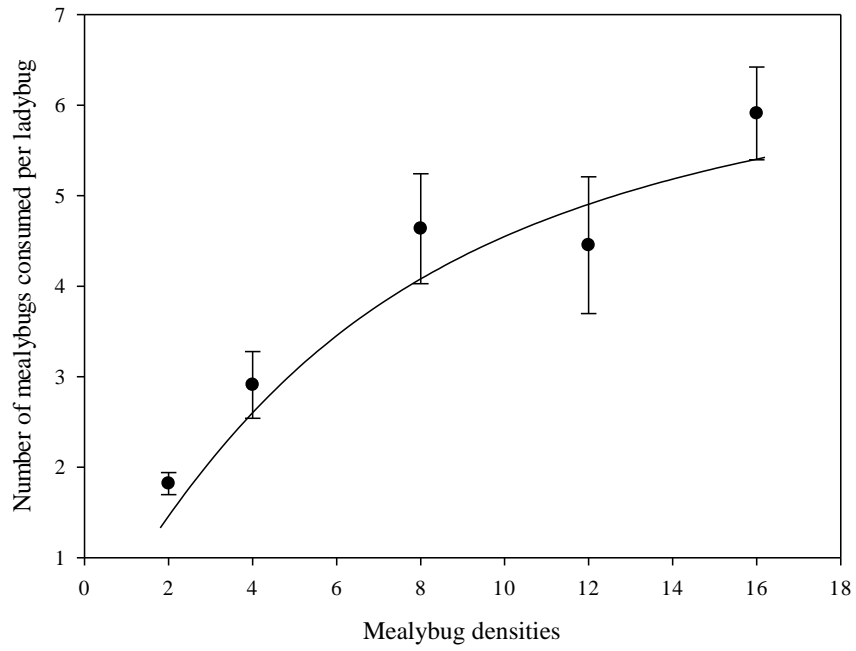
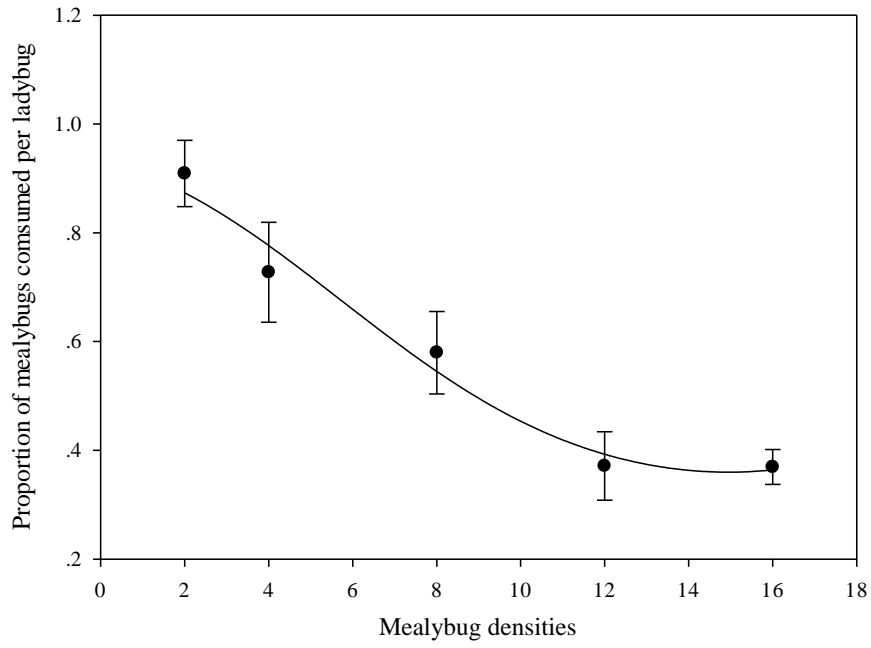


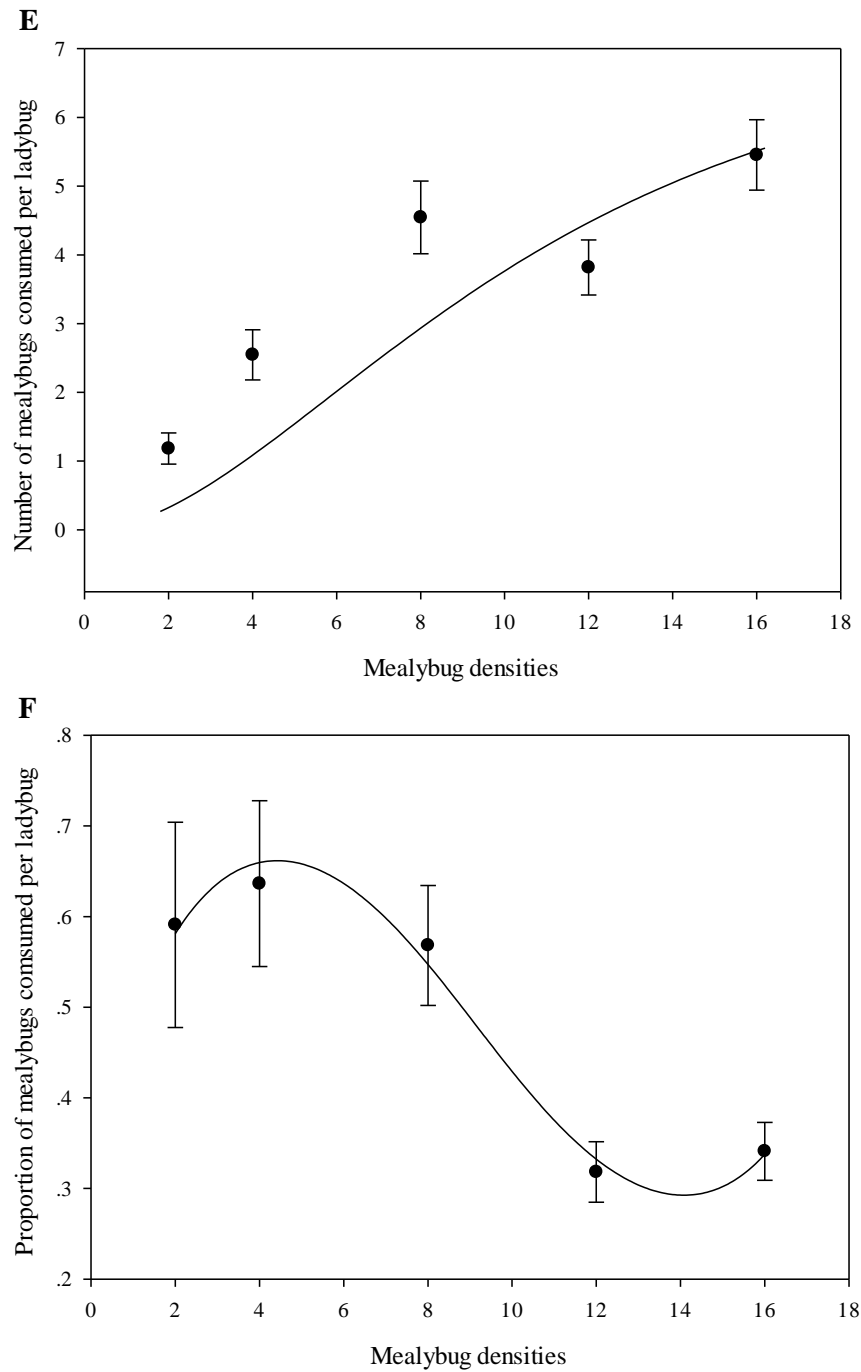




**Figure 3.1.** Mean ( $\pm$ SEM) number (A, C, E) and proportion (B, D, F) of citrus mealybugs consumed by each *C. montrouzieri* at prey density of 2, 4, 8, 12 or 16 per plant with 2, 4 and 6 branches. The best fitted Type II functional response and logistic regression model for *C. montrouzieri* consumption on 2, 4 and 6-branched plants are presented as solid lines.

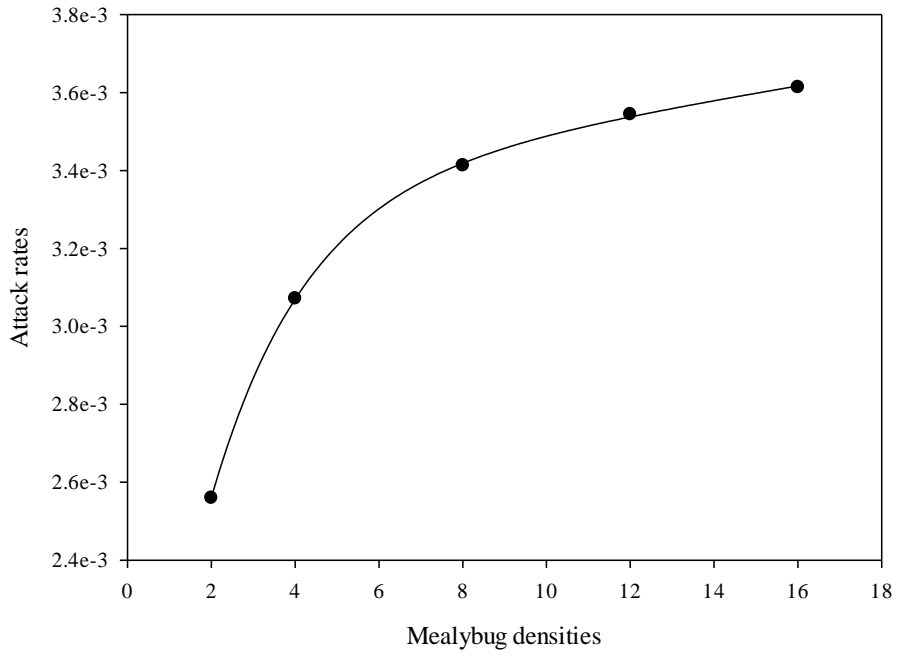


**C****D**



**Figure 3.2.** Mean ( $\pm$ SEM) number (A, C, E) and proportion (B, D, F) of citrus mealybugs consumed by each *C. montrouzieri* at prey density of 2, 4, 8, 12 or 16 per plant with 5, 10 and 15 leaves. The best fitted Type II or III functional response and logistic regression model for *C. montrouzieri* consumption on 5, 10 and 15-leaved plants are presented as solid lines.





**Figure 3.3.** Attack rates of *C. montrouzieri* at different prey densities on host plants with 15 leaves.

## CHAPTER FOUR

### DISCUSSION AND CONCLUSIONS

In this study I showed that *C. montrouzieri* exhibited Type II functional response on plants with 2, 4 and 6 branches and on those with 5 and 10 leaves. The functional response of *C. montrouzieri* on plants with 15 leaves was Type III. This observation is in contradiction to other previous studies on the functional response of *C. montrouzieri* when foraging for various mealybug species. Under laboratory conditions, adult female *C. montrouzieri* exhibited Type III functional response to varying density of citrus mealybugs (Atif et al. 2011), and Type II functional response to citrus mealybug (De Bortoli et al. 2014), *Maconellicoccus hirsutus* (Green) (Torres and Marcono 2015) and *Phenacoccus solenopsis* Tinsley (Saljoqi et al. 2015). Care should be taken in comparing the estimated attack rates and handling times reported in this study and previous studies due to the differences in experimental conditions, environmental conditions, species of prey, hunger levels of *C. montrouzieri* and the host plants. Based on the results of previous studies, the maximum consumption rate of adult citrus mealybugs by adult females of *C. montrouzieri* released in petri dishes was 8 – 11 mealybugs/arena (Atif et al. 2011, De Bortoli et al. 2014), which was about 2-fold greater than the plateau of consumption rate in our study (3-5 mealybugs/plant).

The differences in the results between this study and previous studies may be due to the structural complexity of host plants used in our study. A Type II functional response for adult females of *C. montrouzieri* has often been demonstrated in petri

dishes under lab conditions (De Bortoli et al. 2014, Saljoqi et al. 2015, Torres and Marcono 2015). The petri dish is a simple foraging area (small area and no spatial structure) for an active and fast-moving *C. montrouzieri*. The surface area of a 9 cm-diameter petri dish was about 169.56 cm<sup>2</sup>, whereas the minimum average total surface area in my study was 415.3 cm<sup>2</sup> on 6-branched plants, and the largest total surface area was 462.7 cm<sup>2</sup> on plants with 15 leaves. The total surface areas in my study were about 2.5 - 2.7 times larger than the surface area of a petri dish, and so too the predator's searching area. As a result, the increasing searching area and spatial complexity (e.g., more branches and leaves) on my plants would have reduced the attack rates of *C. montrouzieri* in this study.

Slight differences in attack rates on plants of different structural complexity are likely the results of varying branch and leaf numbers. The attack rates of *C. montrouzieri* were higher on plants with fewer branches, whereas the searching efficacy of *C. montrouzieri* was higher on plants with lower structural complexity. The plants with more branches provided more refuges to the prey and more obstacles to the predator, leading to decreasing attack rates (Hauzy et al. 2010). Other studies also suggested that there might be an inverse relationship between the attack rates and number of branches. Legrand and Barbosa (2003) reported that *C. septempunctata* killed significantly more prey over 24 hours on plants with increasing morphological complexity (smaller leaves and more branches). Gontiji et al. (2012) found that the predator mite *P. persimilis* had a trend to search for prey sooner on the smaller leaves of plants with 6 leaves than on the larger leaves of plants with 2 leaves. Our research

demonstrated that although the total stem and branch length, stem surface area, leaf surface area and total surface area (sum of stem and leaf surface areas) were similar, the average length and number of initial prey of each branch were different. For example, a 2-branched plant with a prey density of 16 mealybugs/plants had about 8 mealybugs/leaf, which was about 2.7 times larger than a plant with 6 branches, Therefore, *C.*

*montrouzieri* on plants with 2 branches spent more time searching and consuming prey on leaves. In addition, the average lengths of the 2 branches were longer than the average of 6 branches. As a result, *C. montrouzieri* on plants with 2 branches spent more time traversing on a single branch before reaching the next leaf than they would on plants with 6 branches. Another reason why predators spend more time eating could be due to the preference of a predator to forage on infested leaves (Gontijo et al. 2012). For example, in 15-leaved plants with 12 mealybugs evenly distributed on all leaves, the proportion of leaves that were infested was 80%, whereas the infested leaves made up 100% of the canopy of the 10-leaved plants. As a result, the chance of encountering a prey was higher on plants with fewer leaves, leading to more time used for prey consumption when the predators find prey more easily and quickly.

In the leaf experiment, the attack rates were higher when the plants had fewer leaves, whereas *C. montrouzieri* was more efficient on plants with lower structural complexity. With higher plant structural complexity, each leaf had fewer prey (assuming even distribution of prey on each plant). As a result, *C. montrouzieri* was more successful in searching and consuming prey on a plant with fewer leaves.

*Cryptolaemus montrouzieri* spent more time searching on plants with 5 leaves than on

plants with 10 leaves, because an average leaf on the 5-leaved plants was about two times larger than an average leaf on plants with 10 leaves. Hauzy et al. (2010) found that the predatory mite, *Pergamasus crassipes* L., in a habitat with greater spatial complexity had lower attack rates and even changed the functional response from Type II to Type III. In our study, *C. montrouzieri* exhibited Type III functional response on plants with 15 leaves, and Type II functional response on plants less complex in structure. Since Type III functional response occurs more often in the field (Schenk and Bacher 2002), the plant structural complexity created in 15-leaved treatment might be similar to a natural condition. The handling time of 15-leaved treatment was higher than 10-leaved treatment maybe due to the same reason, that *C. montrouzieri* spent more time finding prey on the plants with more complex structures.

This study was the first detailed analysis of how the numbers of branches and leaves influence functional response of *C. montrouzieri*. The results might allow us to make better recommendations for the use of *C. montrouzieri* in a biological control program against mealybugs. Fewer *C. montrouzieri* will be needed to control citrus mealybugs on crops with lower structural complexity, both in the fields and the greenhouses. To control low citrus mealybug density or on plants with a large number of branches and leaves, more *C. montrouzieri* may be released to achieve successful control.

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