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Resource Provisioning as a Habitat Manipulation Tactic to Enhance the Aphid Parasitoid, *Aphidius colemani* Viereck (Hymenoptera: Braconidae: Aphidiinae), and the Plant-Mediated Effects of a Systemic Insecticide, Imidacloprid

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Dedications

In memory of Walter Caleb Hodge (1909 – 2002) for sharing the joy of learning and inspiring curiosity in a young child that has transformed into a life-long pursuit of knowledge

To my children who's generation will nurture the seeds of research on ecological engineering in agriculture systems and grow a bounty of knowledge about the design of farming systems for the benefit of all

ABSTRACT OF THE DISSERTATION

Resource Provisioning as a Habitat Manipulation Tactic to Enhance the Aphid Parasitoid, *Aphidius colemani* Viereck (Hymenoptera: Braconidae: Aphidiinae), and the Plant-Mediated Effects of a Systemic Insecticide, Imidacloprid

by

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Resource provisioning as a habitat manipulation tactic to control the melon aphid, *Aphis gossypii*, by the polyphagous aphid parasitoid, *Aphidius colemani*, was investigated in the ornamental, potted-plant nursery using the shrub *Photinia x fraseri* as a plant host. Floral food resources from an invasive, *Conium maculatum*, an ornamental, *P. x fraseri*, and a native, *Salvia apiana* considerably improved the longevity and fecundity of *A. colemani* in laboratory experiments. Additionally, floral nectar from *P. x fraseri* and honeydew from *A. gossypii* had a statistically similar effect on the longevity, fecundity, percent emergence, and sex ratio of *A. colemani* and enhanced the parasitoid more than extrafloral nectar from *Cucurbita pepo*. In common garden field studies, *A. colemani* remained for the entire seven days tested in the presences of resources while in the

absence of resources, the parasitoid was detected for only 3 days. Further field studies using floral food of *P. x fraseri*, honeydew of *A. gossypii*, and a flowers x aphids treatment were conducted to determine the effects on the abundance and movement of the parasitoid in a common garden. Significantly more parasitoids were initially associated with flowers x aphids treatment 24 h post release. This was followed by a switch to the aphids treatment 1 week later. Parasitoids were found in both the treatment plots and associated crop plots (no resources) suggesting that *A. colemani* moves in search of resources and may be switch-foraging to maximize fitness.

Imidacloprid, a systemic insecticide commonly used in nursery systems, was detected in the xylem, nectar, pollen, and leaves of *P. x fraseri* treated with a full or half label rate. The concentration of imidacloprid detected in the xylem and nectar was greater than the determined LC_{50}, LC_{75}, and LC_{90} of imidacloprid for *A. colemani*. Bioassay data suggested the survival of the parasitoid is negatively impacted by feeding on nectar from imidacloprid treated plants. No effect was observed when the parasitoid was exposed to leaves from imidacloprid treated plants. These findings suggest that soil-applied imidacloprid is moderately compatible with biological control due to toxicity occurring through contact with nectar from treated plants rather than direct exposure.

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Chapter 1. Introduction

Nursery production of ornamental plants in California relies heavily on chemical control to maintain pests at extremely low economic threshold levels throughout the growing season (Bethke and Cloyd 2009). The same production system is diverse and complex with species of mixed age classes representing herbaceous perennials, shrubs, and trees. Plant material is grown in above-ground containers for months to years before sale. It may be possible to utilize control strategies other than chemical control during the growth period and still achieve existing economic thresholds of a marketable crop.

Biological control has the potential to maintain pest populations below a threshold that sustains normal plant growth in ornamental nursery field crops. The effectiveness of biological control agents can be greatly enhanced by the implementation of habitat management strategies that provide refuge from disturbance along with requisite resources such as refuge from disturbance, food, and alternative prey, and hosts (Gurr et al. 2004a). The integration of biological control strategies into nursery production of ornamental plants will likely reduce the overall pesticide use and reduce the environmental and social effects of pesticide use (U.S. EPA 1988).

1. Conservation Biological Control: concept and history

Modern agriculture has been characterized as a component system with inputs, outputs, interactions, and processes (Oberle 1994). Linked to engineering, the systems approach to conceptualizing agriculture provides structure for understanding the function and maximizing the efficiency of modern agriculture. Agricultural practitioners strive to

define the relationships within and between components in order to more effectively manage the system. Pests, like the crop and soil, are one of the biotic components in an agricultural system that requires intensive management to protect yields against our greatest competitors.

Various control strategies are available for the management of pests. Chemical, cultural, and biological control strategies all aim to reduce pest population levels below an economic threshold (Stern et al. 1959b). Biological control is the only strategy that relies on populations of antagonistic organisms known as natural enemies to regulate pest populations (DeBach 1964). The unique feature of this population level phenomenon is that both the pest and natural enemy populations have the potential to be managed. There are two commonly accepted types of biological control, classical and new-association, and three methods of implementation (Van Driesche et al. 2008). Classical biological control is the importation and establishment of exotic natural enemies against exotic species (Van Driesche et al. 2008). New-association or neoclassical biological control targets native pest species with foreign natural enemies that have no evolutionary history with the native target (Van Driesche et al. 2008). Augmentative biological control involves the manipulation of existing natural enemy populations to improve their control of pest species either by inoculative releases that are expected to multiply and exert generationally control or by inundative or mass release that are expected to exert control only by the released individuals (Van Driesche et al. 2008). Conservation biological control (CBC) is the alteration of the environment to enhance the effectiveness

of existing natural enemy populations. While classical biological control introduces natural enemies to restore predator-prey trophic relationships, natural enemies must be present in the appropriate trophic relationships with the target pests before augmentation and/or conservation are possible. Classical and augmentative biological control are well documented control strategies (Caltagirone 1981, Van Driesche and Hoddle 2000, Van Lenteren and Bueno 2003) while CBC continues to gain interest as agricultural practitioners look for innovative ways to manage pest populations in agricultural systems.

Van den Bosch (1964) recognized the success of biological control programs depended on an amenable environment where natural enemies could function unabated. He suggested that the physical and biological properties of the environment could be modified spatially and temporally to protect and enhance natural enemies. His original definition of CBC outlined agricultural practices that modified the growing environment such as adjusting tillage times and restricting pesticide use. CBC is commonly understood and implemented by a) providing supplemental food resources, alternative prey, overwintering sites and refuge, b) reducing direct, nonlethal, and indirect mortality from pesticides, c) controlling secondary enemies, d) working with host plant attributes, and e) reducing negative cultural practices/tactics (van den Bosch and Telford 1964, Rabb et al. 1976, Mahr and Ridgeway 1993, Barbosa 1998, Gurr et al. 2000). Habitat management is one tactic within the CBC strategy that enriches the environment with resources needed by natural enemies for increased performance.

The first literature review of CBC noted one of the earliest known examples of CBC, approximately 900 AD, involved the use of the predaceous ant, *Oecophylla smaragdina* F. (Hymenoptera: Formicidae), against phytophagous insects of mandarin orange trees by Chinese growers (Sweetman 1958). Van den Bosch and Telford's (1964) significant review outlined tactics and techniques for application in agriculture. The next series of reviews also focused on potential CBC strategies but commented on the difficulties with implementing such strategies, concluding that these approaches were not practically valuable for integration into modern agriculture (Rabb et al. 1976, Coppel 1986, Gross 1987).

CBC remained unrealistic as a pest management strategy in modern agricultural systems until strip-harvesting of alfalfa in alfalfa-cotton systems was proposed (Stern et al. 1964, van den Bosch and Stern 1969, Stern et al. 1976). Large acreage blocks of alfalfa and cotton are planted in a mosaic pattern throughout California's Central Valley where lygus bug, *Lygus hesperus* Knight (Hemiptera: Miridae), is an economically important pest of cotton. Although pestiferous in cotton due to boll damage, lygus prefers alfalfa to cotton. Alfalfa is typically solid-cut harvested removing an entire field at one time. As the alfalfa dries down, lygus disperses to neighboring cotton in response to deteriorating vegetative resources. Strip-cut harvesting was recommended by researchers to suppress lygus movement into cotton. During experiments in alfalfa, alternate strips of 400 ft wide were harvested leaving behind uncut strips of lygus' preferred vegetative resource. Consequently lygus remained in the uncut alfalfa instead

of moving to cotton. Lygus populations in strip-harvested alfalfa were found to be less damaging to adjacent cotton than whole field cut alfalfa. Greater densities of natural enemies were suggested as the reason for the difference. Natural enemies were further conserved in the strip-harvested alfalfa-cotton system because fewer lygus in cotton translated to fewer pesticide applications.

Besides acting as a cultural control of lygus, strip-harvesting functioned to conserve natural enemies by providing resource-rich habitat, by serving as refuge, and by reducing pesticide use. The aphid parasitoid, *Aphidius smithii* Sharma and Subba Rao (Hymenoptera: Braconidae: Aphidiinae), was enhanced by the tempered microclimate of the uncut alfalfa (van den Bosch et al. 1967). Later Stern (1969) expanded these conservation principles and suggested interplanting cotton with alfalfa to retain lygus in its preferred host alfalfa. Strip cutting and interplanting have not been widely adopted by growers, although these practices are quite effective pest management strategies. Costs and operational problems were cited as reasons why the practices were not adopted (van den Bosch and Stern 1969).

Despite these concerns, this cultural practice continues to be of interest to researchers because of its success in controlling the target pest. Strip-harvesting of alfalfa was linked to lower lygus bug densities and lower aphid-to-predator ratios except in the spring (Cameron et al. 1983). The remaining uncut strips functioned as a beneficial refuge for brown lacewing, *Micromus tasmaniae* (Walker) (Neuroptera: Hemerobiidae), (Leathwick and Winterbourn 1984) and a range of coccinellid and

hemipteran predators (Hossain et al. 2001). Strip-harvesting in alfalfa still remains one of the most prominent examples of CBC. Future development of CBC strategies must focus on both the ecology of the predator-prey relationship and integration into modern agriculture.

Numerous general reviews of CBC have outlined definitions, principles, and practices (van den Bosch and Telford 1964, Rabb et al. 1976, DeBach and Rosen 1991, Mahr and Ridgeway 1993, Barbosa 1998). Few reviews have solely highlighted the enhancement of parasitoids in agricultural systems (Powell 1986, Altieri et al. 1993) while other reviews have focused on the conservation of natural enemies in relation to other aspects of the system. Assorted reviews have examined the associations between conservation and (1) noncrop plants (van Emden 1965, Altieri and Whitcomb 1979), (2) vegetational diversity (van Emden and Dabrowske 1994), (3) influence of spatial structure on dispersal dynamics (Wratten and Thomas 1990), (4) agroecosystem diversification (Sheehan 1986, Andow 1991), (5) plant attributes (Barbosa and Benrey 1998, Barbosa and Wratten 1998, Cortesero et al. 2000), (6) habitat management on the local and landscape scale (Altieri and Letourneau 1982, Bugg and Waddington 1994, Wratten and van Emden 1995, Pickett and Bugg 1998, Gurr et al. 2000, Landis et al. 2000, Tscharntke et al. 2007), and (7) habitat management in ecological engineering (Gurr et al. 2004a).

2. Agricultural and ecological systems: theory and practice

Agricultural systems are simplified analogues of complex natural ecological systems. Both agricultural and ecological systems have components such as soil, plants, and herbivores and processes such as nutrient cycling, host colonization, and predator-prey dynamics. Many theories have been proposed to explain the structure of biological communities based on various factors that influence community dynamics in ecological systems. Equilibrium theory, disturbance theory, and the diversity-stability hypothesis are just some of the predictive models that have been proposed for ecological systems and have been applied to agricultural systems. An examination and integration of these theories conveys a foundational understanding of the interactions and processes of biological communities in agricultural systems. We can employ the predictive power of community structure theory to elucidate strategies for the conservation of natural enemies and thereby promote the biological control of pest species.

2.1 Equilibrium and Disturbance Theories

Modern agricultural systems are intensely managed and rely heavily on inputs to produce a high-yielding, marketable crop for the least cost. For these reasons, disturbance is regularly introduced into the system in the form of tillage, fertilization, and pesticide application. Disturbance has been defined as any physical occurrence that removes individuals from a population disrupting ecosystem, community, or population structure (Pickett and White 1985). Biological processes such as digging by mammals in grasslands have also been defined as disturbances that kill, displace, or damage

individuals generating opportunities for colonizing individuals to become established (Sousa 1984). Disturbance regimes exhibit spatial and temporal variation and are any combination of their size, magnitude, frequency, predictability, and turnover rate (Sousa 1984). The successional development of the biological community after an abiotic or biotic disturbance is dependent on which organisms are lost and the extent of their elimination (Reice 1994).

Agricultural disturbance events often destroy biological communities. Frequent and severe disturbance regimes characteristic of modern agriculture are constantly resetting the system. The community structure that develops in such degraded systems is indicative of prevailing disturbance regimes. The application of pesticides as a pest management strategy is one of the most devastating agricultural disturbance events. Pesticide disturbance removes susceptible individuals from the community leaving behind organisms that physically and/or physiologically escape the disturbance. These conditions favor organisms with high reproductive rates that rapidly colonize and utilize remaining resources. Agricultural pests possess similar traits that establish their dominance in agricultural systems while their enemies are absent. Natural enemies are not quick to colonize and utilize resources because these requisite resources are not available. The lack of hosts after a pesticide disturbance event is the most obvious deficient resource. Agricultural practices that perpetuate disturbance regimes define a system where pests are favored and their natural enemies are hindered. Conservation

biological control strategies aim to provide resources needed by natural enemies and promote environmental conditions favorable to natural enemies and not to pests.

Disturbance theories have sought to explain community structure in natural systems and their predictions can be extrapolated for agricultural systems. Early theories suggested that environmental processes determined community structure such that colonization, reproduction, growth, and survival is environment dependent (Gleason 1926, Andrewartha and Birch 1954). Another paradigm dominated by equilibrium theory (Elton, 1927) arose later which suggested that community structure was a result of biotic interspecific interactions such as competition and predation. Systems were assumed to be at equilibrium when total species composition and relative abundances were stable through time. A system at equilibrium was expected to return to its equilibrium state following a disturbance event. The predictions of equilibrium models found many exceptions in natural systems (Peet et al. 1933, Sale 1977, Connell 1978, Reice 1985). Non-equilibrium theories of biological community structure emerged from these contradictory studies (Reice 1994). The most prominent of these non-equilibrium theories is the Intermediate Disturbance Hypothesis. This theory predicts that both minor and major disturbance events decrease species richness due to competition effects of superior and inferior competitors while the greatest species diversity is achieved from intermediate scales of disturbance (Connell 1978). Invasions following a severe disturbance event consist of very few species because only species producing colonizers within the dispersal range will be able to occupy the new resource space (Connell 1978).

Diversity is also restricted by the richness of the recolonization source pool. The biological community subjected to severe and frequent disturbance regimes will be composed of the hardiest of colonizing species that quickly reach maturity.

Pest and natural enemy community structure is shaped by the intense disturbance regimes operating in agricultural systems. Pests typically dominate the community and natural enemies are generally limited by the lack of resources needed to effectively control dominate pests. Efforts to conserve natural enemies must focus on the identification of disturbance factors, evaluation of the impact of disturbance factors on community structure, and methodologies to mitigate disturbance regimes in agricultural systems.

2.2 Diversity-Stability Hypothesis

Early ecologists developed ideas about the relationship between diversity and stability in ecological systems based on equilibrium theory. Most notably is the concept that complex food webs resist change better than simple ones due to interspecific interactions between trophic levels (Elton 1927). The diversity-stability hypothesis logically followed that the greater the diversity of a biological community, the greater the stability (Odum 1953, MacArthur 1955, Elton 1958, Hutchinson 1959, Pimentel 1961, Margalef 1968). Initial explanations of the diversity-stability hypothesis offered examples of simple habitats fostering insect outbreaks and diverse habitats resisting such outbreaks (MacArthur 1955, Elton 1958). Elton (1958) suggested that similar to ecological systems, simple agricultural systems like monocultures lack the complexity to

stabilize food web interactions and are therefore susceptible to pest outbreaks. Theory predictions implied that agricultural practitioners looking to control pest populations could simply diversify agricultural systems to achieve control. The problem with this approach is that the goal of pest management is not the stabilization but the suppression of pest populations below an economic threshold. Further investigations of the diversity-stability hypothesis confirmed that the hypothesis lacked logical strength and supporting data (Levins 1970, May 1973, van Emden 1974, Goodman 1975, Murdoch 1975). Stability was not obviously dictating pest population dynamics and so an alternative explanation was needed to account for the reduction of pest densities in diverse systems.

2.3 Resource Concentration Hypothesis and Natural Enemies Hypothesis

Modern theories accounting for the response of herbivores to different habitat diversities agree that herbivores will be less abundant in diverse habitats but propose different explanatory mechanisms. Root's (1973) critical paper presented the resource concentration hypothesis and the natural enemies hypothesis as possible explanations for the observed response. The resource concentration hypothesis states that herbivores are expected to be more abundant and less diverse in simple habitats such as monocultures because specialist herbivores more easily locate, remain, and reproduce in concentrated resources. Consequently, specialist herbivores condense the community's biomass into numerous individuals of only a few species. The natural enemies hypothesis maintains the same predictions as the resource concentration hypothesis but provides an alternative rationale. The herbivore community is expected to be more diverse in complex habitats

such as polycultures because generalist predators and parasitoids inhibit the potential dominant herbivore species from capitalizing on all the available biomass and competitively excluding other herbivore species. In this complex environment, the greater diversity of prey and microclimates are more regularly available in time and space allowing generalist predators and parasitoids to regularly persist. Specialized predators and parasitoids remain in the diverse habitats where refuge ensures a consistent supply of prey (Huffaker 1958, Pimental et al. 1963). Finally, both generalist and specialist natural enemies benefit from essential resources such as nectar and pollen that are more readily available in diverse habitats (van Emden 1963, 1965).

The resource concentration and natural enemies hypothesis have been the focus of much discussion regarding the factors driving the relationship between habitat diversity and herbivore community structure (Risch 1981, 1983, Baliddawa 1985, Sheehan 1986, Redfearn and Pimm 1987, Russell 1989, Andow 1991, Cromartie 1991). Initially the resource concentration hypothesis was thought to be a more powerful argument, but as more experimental evidence was evaluated, these two hypotheses were viewed as complementary (Russell 1989; Andow 1991; Cromartie 1991) (Russell 1989, Andow 1991, Cromartie 1991, Gurr et al. 2000) . Most reviewers admitted that their conclusions were limited by the lack of mechanistic data and called for more studies of the ecological mechanisms underlying these relationships (Risch 1983, Baliddawa 1985, Sheehan 1986). Additional studies were also suggested on the economics and practical application

of diversification to agricultural production (Andow 1991, Cromartie 1991, Gurr et al. 2000).

2.4 Habitat management in agriculture systems

Pest management strategies like conservation biological control have been developed based on the predictions of herbivore community structure hypotheses. The identification of diversity as a community structure determinant has led to the incorporation of diversity into cropping system design. Habitat management tactics diversify the cropping environment to provide resources fundamental to the success of natural enemies. Herbivore pest populations are consequently reduced by the enhanced natural enemy performance. In this way, plant protection is achieved by the predictions of both the resource concentration and natural enemies hypothesis. Diversity not only dilutes the cues that herbivores use to find their host plant disrupting colonization but results in an enhanced natural enemy complex.

The diversification of agricultural systems has great potential to reduce pestiferous herbivore populations although not all diversity has proven to be beneficial (Perrin 1977, Bugg et al. 1987, Kemp and Barrett 1989, Bugg 1992). The search is on for Way's "right kind of diversity" (Way 1966). We must identify the ecological factors and mechanisms influencing the herbivore community structure of diverse systems before we will be able to develop sound pest management strategies. Habitat management strategies have been implemented in agricultural systems to exploit the association

between diversity and the herbivore community, but we are just beginning to understand the different "kinds of diversity".

The ecological engineering approach to pest management aims to find the "right kind of diversity" by understanding the ecology of agriculture systems (Gurr et al. 2004a). The goal of engineering cropping systems using habitat management is to deter herbivores and encourage natural enemies. Provisioning food resources as a habitat management tactic has the potential to maximize natural enemy effectiveness in controlling pest populations. Food resources such as floral (nectar and pollen), extrafloral (nectaries, exudates, and fruits), and insect products (honeydew and host feeding) may provide nutrition benefits for natural enemies (Doten 1911, Syme 1975, 1977, Jervis et al. 1993). Some studies, mostly laboratory assays, have suggested that provisioning plant and insect-derived resources increases longevity and fecundity (Tylianakis et al. 2004, Hogervorst et al. 2007, Irvin and Hoddle 2007), searching activity (Budenberg et al. 1992, Grasswitz and Paine 1993), and field parasitism rate (Tylianakis et al. 2004, Berndt et al. 2006). A framework has been proposed for determining the effectiveness of habitat manipulation tactics based on trophic levels of food-web theory and an additional level, the farming system (Gurr et al. 2000). Suggested research questions target crops (trophic level 1), pests (trophic level 2), natural enemies (trophic level 3), predators/parasitoids of natural enemies (trophic level 4), and the farming system (Gurr et al. 2000). The compilation of the five levels of research questions into an investigative approach to the habitat manipulation tactic of provisioning food resources is

as follows: (1) establish attraction to food resources, (2) document enhancement of longevity and fecundity due to food resources, (3) demonstrate dispersal from field food resources, (4) show increased field parasitism rate, and (5) provide economic analysis of tactic implementation (Gurr et al. 2000).

3. Crop, pest, and natural enemy complex

3.1 The ornamental containerized nursery crop

The nursery industry in California is ranked 5th in commodity value accounting for $2.68 billion dollars in sales with $489 million dollars solely from containerized flowers (Tolomeo and Krug 2012). The production area in the ornamental containerized nursery is organized by blocks of plant species that together, represent a diverse and complex group of herbaceous perennials, shrubs, and trees of mixed age classes. *Photinia x fraseri* Dress is one woody shrub that is grown for its fire red leaf flush, compatibility with hedging, and usefulness as a screen plant.

P. x fraseri is a hybrid species (*Photinia glabra* (Thunb.) Maxim. and *P. serrulata* (Desf.) Kalkm.) in the Family Rosaceae originating in Asia that can grow from 3 – 4.5 m, requires sun or shade, and can tolerate most soil conditions including drought but prefers moist, well-drained soils (Bailey 1976, Brenzel 2007). The shrub has alternate, ovate leaves 6.5 – 9 cm long with serrated margins and an acute apex that emerge fire red and harden green (Bailey 1976). Inflorescences develop along with the red leaf flush, if not hedged, into showy, white corymbose panicles 12 – 15 cm wide with individual 8 mm white 5-petal flowers typical of the Rosaceae (Bailey 1976, Brenzel 2007). Pome fruits

form to 6 mm in diameter, mature red in the fall, and can hold through the following spring (Bailey 1976, Brenzel 2007). Propagation in the ornamental containerized nursery is by softwood stem tip cutting (Bailey 1976) and cultivation begins when liners are transplanted to containers. The fast growing habit of *P. x fraseri* necessitates pruning to shape the plant and to develop a hearty specimen that can overwinter and be sold within the year. Although pests of mature *P. x fraseri* are few, containerized nursery specimens can be susceptible to a leaf spot disease and aphids. The leaf spot fungus, *Entomosporium mespili*, is only a pest of *P. x fraseri* in humid climates similar to the Southern United States and is not considered a pest in California containerized nurseries (Baudoin 1986). However aphids are pestiferous throughout the growing regions as they feed on the signature red flush leaves (Dreistadt 2001).

3.2 Aphids as pests

Aphids (Hemiptera: Sternorrhyncha: Aphidoidea: Aphididae) are phytophagous, piercing-sucking insects that predominately feed on phloem (Minks and Harrewijn 1987). Their morphology is typically characterized by 4-6 antennal segments, either apterous or macropterous with tectiform wings, and a pair of cornicles or siphunculi on the posterior margin of the fifth abdominal segment (Minks and Harrewijn 1987). Although there are approximately 4700 described species worldwide (Remaudiere 1997), only 250 species have been associated with crop plants (Blackman and Eastop 2000) and feed on more than 243 plant species representing 62 families (Blackman and Eastop 2000). Even fewer, approximately 100 aphid species, are considered pestiferous with economic

significance in agricultural systems (Blackman and Eastop 2007). In addition to their broad host range, aphids are considered agricultural pests due to their high intrinsic rate of increase, effective reproductive strategies (parthenogenesis, telescoping generations, and viviparity), resistance to pesticides, role as virus vectors, and low aesthetic tolerance (Minks and Harrewijn 1987, Blackman and Eastop 2000).

3.2.1 The pest description and life cycle

The melon aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is considered one of the most economically destructive aphids in the United States (Slosser et al. 1989) and one of the top ten pests in California nurseries (Wilen et al. 2002). The host range of *A. gossypii* extends to more than 120 plant species representing 90 families (Ebert and Cartwright 1997) and is a common pest of many containerized nursery crops including *P. x fraseri*, chrysanthemum, gardenia, and hibiscus (Ebert and Cartwright 1997, Blackman and Eastop 2007).

A. gossypii is a wing-dimorphic species with apterous parthenogenetic females ranging from 1 to 2 mm long (Goff and Tissot 1932). The wingless form varies in color from light yellow to light green to dark green with the most common form being light green with mottled dark green (Goff and Tissot 1932). Small yellowish morphs are supposedly in response to overcrowding or plant stress (Stoetzel et al. 1996). The legs and antennae are pale, almost white-like with black tibiae and tarsi (Goff and Tissot 1932). The cornicles or siphunculi are also black (Goff and Tissot 1932). Antennal tubercles are weakly developed in both apterous and alate individuals (Dreistadt 2001).

The cauda of both apterous and alate individuals is pale with 2 or 3 pairs of setae (Stoetzel et al. 1996). Alate parthenogenetic females of *A. gossypii* are smaller reaching between 1.1 to 1.7 mm in length. Their head, including antennae, and thorax are black with a yellowish-greenish-brown abdomen darkening posterior (Goff and Tissot 1932). The wing veins are also dark with a brown hue (Stoetzel et al. 1996). The oviparous female and male are both greenish purple in hue (Stoetzel et al. 1996).

The life cycle of *A. gossypii* is temperature based and divided by latitude with northern populations switching between oviparity and viviparity (holocyclic life cycle, either autoecious or heteroecious) and southern populations relying solely on viviparous reproduction (anholocyclic life cycle) (Blackman 1987, Slosser et al. 1989, Zhang and Zhong 1990). In cooler climates, nymphs of *A. gossypii* emerge in the Spring from eggs that overwinter on the primary host. Individuals may remain on the primary host, mature, and begin viviparous reproduction or upon maturation, may produce alate individuals that move to a secondary host to initiate viviparous reproduction. These colonizers reproduce both apterous and alate individuals throughout the Summer with the alates continuing the dispersal process. Late in the summer season or early Fall, alate females move back to the primary host and produce oviparous females and males that mate resulting in the overwintering eggs. In warmer climates, *A. gossypii* females reproduce exclusively by parthenogenesis (viviparity) utilizing various hosts.

Development and reproduction is temperature dependent with optimal conditions ranging from 21 – 27 °C (Goff and Tissot 1932). At these temperatures, *A. gossypii* can

reach sexual maturation within one week and begin producing offspring at a rate of 4.3 per day for a total of 70 to 80 individuals (Goff and Tissot 1932). Based on these reproductive rates, multiple generations occur in one growing season (Ebert and Cartwright 1997). This ability to multiply quickly in high temperatures is unlike most aphid species.

3.2.2 Pest feeding and damage

Feeding and damage by *A. gossypii* is similar to other pestiferous aphids. Individuals feed on the underside of leaves piercing into the sugar-rich host phloem, preferring new growth (Ebert and Cartwright 1997). Feeding begins with probing that is initiated when the individual presses their labrum to the plant surface and their antenna lay back flush against their dorsum (Yuan and Ullman 1996). Probing and feeding may cause physical damage as in distortion of the leaves (Goff and Tissot 1932) and physiological damage as in reduced photosynthate production and virus transmission (Ebert and Cartwright 1997). Indirect damage to the fruit and leaves caused by the development of sooty mold on the honeydew secreted during feeding takes a week to 10 days to develop (Goff and Tissot 1932). In addition, virus transmission is considered the most important impact *A. gossypii* has on agricultural crops because viral infections cause greater losses than direct and indirect feeding damage combined (Ebert and Cartwright 1997). *A. gossypii* transmits over 50 viruses including Cucumber Mosaic Virus (Bromoviridae), Zucchini Yellow Moasic (Potyviridae), and Citrus Tristeza

(Closteroviridae) (Ebert and Cartwright 1997) but none have been shown as symptomatic in *P. x fraseri*.

3.3 Natural enemies of A. gossypii

Many predators and parasitic Hymenoptera have the potential to impact the population dynamics of *A. gossypii* (Slosser et al. 1989, Ebert and Cartwright 1997). Predators include lacewings (*Chrysoperla spp.* and *Chrysopa spp.*), ladybird beetles (*Hippodamnia spp.*, *Cycloneda spp.*, *Olla spp.*, and *Scymnus spp.*), syrphid flies (*Baccha spp.*, *Episyrphus spp.*, and *Syrphus spp.*), and hemipteran bugs (*Orius spp.*, *Geocoris spp.*, *Nabis spp.*, and *Zelus spp.*) (Goff and Tissot 1932, Slosser et al. 1989, Ebert and Cartwright 1997). Records of parasitic Hymentopera include *Lysephlebis spp.*, *Trioxys spp.*, *Aphelinus spp.*, and *Aphidius spp.*

3.3.1 The parasitoid description and life cycle

The solitary endoparasitoid, *Aphidius colemani* Viereck (Hymenoptera: Braconidae: Aphidiinae), is known to parasitize *A. gossypii* along with 41 other aphid host species (Stary 1975). The female parasitoid was originally described by Viereck (1912) as 2 mm in length, generally black or darkish colored with a yellowish prothorax and apical half of the abdomen (Viereck 1912). The abdomen appears striped with black sclerites and yellowish intersegmental membrane. The black propodeum in dorsal view has a distinctive diamond shaped areola joining to four other tergites (Viereck 1912) along with congruent opposite edges (Evans and Stange 1997). The petiole is moderately narrowed laterally (Evans and Stange 1997). The antennae consist of 14 flagellomeres

(Viereck 1912, Evans and Stange 1997). The forewing exhibits the characteristic darkened stigma with missing vein cu-a and 1A (Goulet and Huber 1993). In addition, the vein rm and recurrent vein form the discocubital cell (Evans and Stange 1997). The male parasitoid is very similar to the female except for 16-17 flagellomeres and an entirely black abdomen (Viereck 1912).

Laboratory data estimate the average longevity of adult female *A. colemani* to be 5.8 days at 20 °C and 4.4 days at 25 °C with a developmental period of 12.7 days at 20 °C and 10 days at 25 °C (Van Steenis 1993). Female *A. colemani* oviposit a single egg into nymphal *A. gossypii* preferring first and second instars (Perdikis et al. 2004). Their distinct oviposition behavior involves the female curling the abdomen forward 180 ° between her legs and injecting the ovipositor into the nymph like a needle, then quickly retracting it while still remaining in the folded posture. Superparasitism has been reported at 3.21% at 25 °C and 0.65% at 20 °C (Van Steenis 1993). Eggs eclose and the legless larvae develop by consuming the inside of the *A. gossypii* nymph. Pupation is marked by the formation of a brown, paper-like swollen nymph called the mummy. Adult *A. colemani* emerge through a crescent shaped cut in the mummy's shell. Females have been reported to oviposit 302 eggs at 20 °C with 14.1% immature mortality and 388 eggs at 25 °C with 27.8 % immature mortality (Van Steenis 1993). Another study reported females oviposit 420 eggs with 22.1% immature mortality at 22°C (de F. Torres et al. 2007). Variation has also been reported for the parasitoid's sex ratio, 0.65

(proportion female) (Elliot et al. 1994) and 0.58 (proportion female) (Harizanova and Ekbom 1997).

3.3.2 The parasitoid as a biological control agent

A. colemani is considered a cosmopolitan species originating in India (Stary 1975). The parasitoid and its host, *A. gossypii*, have a similar intrinsic rate of increase. (Van Steenis 1993). *A. colemani* also has the ability to discover and parasitize aphid populations at low densities (Van Steenis and Elkhawass 1995) thereby making it a potentially successful biological control agent. Unlike many polyphagous aphid parasitoids (Powell and Li 1983), *A. colemani* readily accepts alternative aphid hosts increasing its potential field presence (Elliot et al. 1994). This generalist parasitoid of aphids is adapted to Mediterranean climates (Stary 1975). Studies in greenhouses have reported *A. colemani* has been considered an effective but expensive aphid control option for chrysanthemums and cucumbers (Harizanova and Ekbom 1997, Jacobson and Croft. 1998, Vasquez et al. 2006) but the question of the parasitoid's field potential has not been critically addressed.

4. Chemical control of aphids

Chemical control is typically utilized to manage aphid populations in the containerized nurseries of California, drawing from many classes of insecticides including carbamates, organophosphates, and pyrethroids (Wilen et al. 2002). Increasing resistance to organophosphates and carbamates (Scopes and Ledieu 1980, Furk and Vedjhi 1990, Furk and Hines 1993, Herron et al. 2001) raise concerns for continued

effective population management of *A. gossypii* solely through chemical control (Vehrs and Parrella 1991). With the recent introduction of a new class of insecticides, the neonicotinoids have the potential to alter the IPM of aphids due to the compound's systemic activity, extended residual activity, and reduced application rates.

4.1 Chemical control of aphids by imidacloprid

The first commercially available neonicotinoid, imidacloprid, is a chloronicotynl (1-[(6-chloro-3-pyridyl)methyl]-*N*-nitro-2-imidazolidinimin) with systemic activity (Tomizawa and Casida 2003). The compound acts as an agonist at the nicotinic acetylcholine receptors (nAChRs) of insects inducing slow depolarizations of the neuron (Bai et al. 1991, Tomizawa and Casida 2003). Imidacloprid has activity against aphids among many other insects from the Blattodea, Diptera, Hemiptera, and Hymenoptera (Bai et al. 1991, Deglise et al. 2002, Tomizawa and Casida 2003). The principle target of imidacloprid is against numerous phloem and xylem feeding pests many of which are found in nursery production of container-grown plants, including the melon aphid, *A. gossypii*, common to multiple ornamental plants, among which is *P. x fraseri*. Although foliar applications are included on the label, it is the systemic activity from soil applications that may allow for improved integration of chemical and biological control.

5. Integrating chemical and biological control

5.1 Chemical control: impact on natural enemies

Natural enemies operating in agricultural systems are frequently exposed to pesticide disturbance because of the widespread use of pesticides. Exposure can be direct

as in contact with spray droplets or residues on the crop (Longley and Jepson 1996a, b), or feeding on contaminated nectar or honeydews. Indirect exposure can occur through the host during natural enemy development (Hsiech and Allen 1986, Longley 1999). Direct effects have the greatest impact on natural enemies because of their immediate mortality or latent sub-lethal effects (Johnson and Tabashnik 1999).

Sub-lethal effects are expected because parasitoids spend much of their time foraging for food and hosts on plants where their exposure to pesticide residues is high. The neurotoxic activity of pesticides in sub-lethal concentrations can alter the biology and behavior of parasitoids (De Jiu and Waage 1990, Umor et al. 1996). Biological characteristics such as longevity, fecundity and sex ratio can be distorted by exposure to sub-lethal levels of pesticides (Delpuech and Meyet 2003). Behaviors reported to be impaired by sub-lethal exposure include pheromonal communication (Delpuech et al. 1999), response to host karimone (De Jiu and Waage 1990), ability to find host (Longley and Jepson 1996b), oviposition behavior and patch-time allocation (Desneux et al. 2004b) , and food foraging patterns (Elzen 1989). Impairments to olfactory orientation and learning have been recently reported for the parasitoid, *Aphidius ervi* Haliday (Hymenoptera: Braconidae: Aphidiinae) (Desneux et al. 2003, Desneux et al. 2004a).

The success of biological control in agricultural systems depends on its integration with other control strategies. We must understand the impacts of pesticides on natural enemies to achieve success.

5.1.2 Chemical control by imidacloprid: contact and systemic effects

Although there are sufficient studies about the effects of imidacloprid on natural enemies (Villanueva-Jimenez and Hoy 1998, Oliver et al. 2006, Cloyd and Bethke 2011, Prabhaker et al. 2011), only a few studies have investigated these effects on *Aphidius spp.* These studies have focused on the systemic, contact, and sublethal activity of imidacloprid. Experiments with *A. ervi* found soil-applied imidacloprid had no impact on the instantaneous rate of population increase of the parasitoid as measured by a 24-hour exposure (Kramarz and Stark 2003). Sublethal imidacloprid spray treatments equivalent to half-field rate also had minimal impact on *A. ervi* survival as measured by 24-hour mortality (Araya et al. 2010). However, imidacloprid was considered harmful to *Aphidius gifuensis* Ashmead (Hymenoptera: Braconidae: Aphidiinae) with 71% of females dead after 24-hour exposure to field rate sprays (Kobori and Amano 2004).

Although soil-applied imidacloprid did not appear to negatively impact *A. ervi*, other studies have reported deleterious effects on natural enemies treated with systemic imidacloprid. Experiments on the coccinilid beetle, *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinellidae), found both the survivorship at day 30 and the pre-oviposition period were only negatively affected for sunflower, one of the three astereous plants treated with soil-applied imidacloprid (Smith and Krischik 1999). Survival of the encyrtid parasitoid, *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae), was significantly reduced by 2.5 times after a one day contact period with flowers from plants treated with soil-applied imidacloprid at label rates (Krischik et al. 2007). Similar results

were found for the green lacewing, *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), with survival reduced by 5 times after ten days but no effect after one day of exposure (Rogers et al. 2007). An additional study looked at the interaction with two parasitoids of eucalyptus pests, a braconid and an encyrtid parasitoid, using nectar collected from soil injected eucalyptus (Paine et al. 2011). This study found that concentrations of imidacloprid and its metabolites in nectar in the field were great enough to kill individuals of both species. Additionally survival and reproductive fitness of the encyrtid, *Avetianella lonoi* Siscaro (Hymenoptera: Encyrtidae), were significantly lower when fed nectar from eucalyptus trees treated with imidacloprid as compared with nectar from non-treated trees. A sufficient breadth of data exists in regards to the sub-lethal and lethal effects of contact pesticides on parasitoids but with only a few studies dealing with the effects of systemic pesticides like imidacloprid, we still lack knowledge of the lethal and/or sub-lethal effects on parasitoids feeding on nectar and honeydew sources.

6. Objectives Statement

Agricultural systems are highly disturbed environments that lack resources necessary for natural enemies to persist at optimal performance. Habitat management strategies have the potential to mitigate agricultural disturbance, provide requisite resources, and suppress pest populations without changing the basic agronomy of the crop. The objectives of this research are to investigate the impacts of pesticide disturbance by the systemic imidacloprid on *A. colemani* and to develop habitat management strategies that enhance the parasitoid. These investigations should also

reveal insights into resource use by *A. colemani* and the relationship between resource availability and natural enemy distribution and movement.

Chapter 2. Fitness effects of food resources on the polyphagous aphid parasitoid, *Aphidius colemani* Viereck (Hymenoptera: Braconidae: Aphidiinae)

1. Introduction

Over 100 aphid species are considered pervasive pests that are difficult to control due to their high intrinsic rate of increase, varied reproductive strategies (parthenogenesis, telescoping generations, and viviparity), resistance to pesticides, role as virus vectors, and low aesthetic tolerance as crop contaminants (Ebert and Cartwright 1997, Emden and Harrington 2007). The melon aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is one pestiferous species with a host range of more than 120 plant species representing 90 families (Ebert and Cartwright 1997, Emden and Harrington 2007). This aphid species is considered to be one of the most economically destructive aphids in the United States (Slosser et al. 1986), and California nursery growers have reported aphids as one of the top ten pests in terms of pesticide use (Wilen et al. 2002). The melon aphid is a common pest of many containerized nursery crops including *Photinia x fraseri* Dress (Rosaceae), chrysanthemum, gardenia, and hibiscus.

Melon aphid population management on containerized plants is typically reliant on insecticides. Organophosphates, pyrethroids, and carbamates are applied to achieve low aesthetic tolerance levels (Wilen et al. 2002). Increasing resistance to organophosphates and carbamates (Scopes and Ledieu 1980, Furk and Vedjhi 1990, Furk and Hines 1993, Herron et al. 2001) raises concerns for continued effective melon aphid population management solely through chemical control (Vehrs and Parrella 1991). A more integrated approach for melon aphid management in the containerized plant

industry could use biological control agents such as aphid-specific fungi and entomophagous natural enemies, in particular, parasitoids (Vehrs and Parrella 1991, van Lenteren 2000).

Although chemical control is typically used against aphids in nursery systems, biological control may have a role to play in the management of the melon aphid in IPM systems aiming to reduce resistance development. Appropriate selection of an insect biological control agent is essential if natural enemies are to be successfully used as part of an integrated management approach (Van Driesche and Bellows, 1996) . Ideally, the selected biological control agent should be compatible in as many biological and ecological characteristics, e.g. intrinsic rate of increase, climatic requirements, and habitat (Van Driesche and Bellows, 1996) . The solitary endoparasitoid, *Aphidius colemani* Viereck (Hymenoptera: Braconidae: Aphidiinae), is a cosmopolitan species parasitizing over 41 aphid host species, including the melon aphid (Stary 1975). *A. colemani* has a similar intrinsic rate of increase as *A. gosypii* (Van Steenis 1993) and has the ability to discover and parasitize low density aphid populations (Van Steenis and Elkhawass 1995). Unlike many polyphagous aphid parasitoids (Powell and Li 1983), *A. colemani* readily accepts alternative aphid hosts potentially increasing its abundance in the field (Elliot et al. 1994).

This generalist parasitoid of aphids is adapted to Mediterranean climates (Stary 1975) typical of California nursery systems and is theoretically compatible with existing climatic conditions. *A. colemani* has been considered an effective but expensive aphid

control option for chrysanthemums and cucumbers in greenhouses (Harizanova and Ekbom 1997, Jacobson and Croft. 1998, Vasquez et al. 2006), but the question of the parasitoid's ability to reduce pest populations in the field has not been critically addressed. In the California containerized nursery system, *A. colemani* is found in production areas and surrounding non-managed native vegetation as well as the invasive, weedy interface. This mixed-use agriscape (the area in and surrounding the agricultural environment) provides a unique context to examine the potential resource provisioning may have on improving the effectiveness of aphid biological control with *A. colemani* in outdoor nursery crops that are typically intensively managed with insecticides. Plants within and surrounding production fields may serve as resources for aphid parasitoids providing nutrition and habitat. Additionally, many ornamental crop plant species could be utilized as floral resource plants initially and then transitioned into sellable stock eliminating the need to sacrifice production area for growing resources specifically to enhance parasitoid populations. For example, *P. x fraseri* has the potential to serve as a resource plant, be cut-back, and then re-grown into sellable stock. This ornamental shrub is known for its compatibility with hedging and the subsequent desirable red leaf flush that is a necessary feature of marketable plants.

The practice of modifying the physical and biological properties of the agricultural environment to protect and enhance natural enemies was first defined as conservation biological control by van den Bosch and Telford (1964), although the idea had been in use as early as 900 A.D. (Sweetman 1958). Enriching the agricultural

environment with food resources such as floral (nectar and pollen), extrafloral (nectaries, exudates, and fruits), and insect products (honeydew and host feeding) is one method that may advance biological control by promoting natural enemy longevity and achieve an effective integrated approach to aphid management. Floral and extrafloral resources along with insect products are a necessary source of nutrition for adult parasitoids (Doten 1911, Syme 1975, 1977). Some studies, especially laboratory assays, have shown that provisioning plant and insect-derived resources can increase longevity and fecundity (Tylianakis et al. 2004, Irvin and Hoddle 2007), parasitism rates (Tylianakis et al. 2004, Berndt et al. 2006), and searching activity (Budenberg et al. 1992, Grasswitz and Paine 1993). Additionally, plant-specific and aphid specific honeydews can influence parasitoid longevity (Hogervorst et al. 2007); however, laboratory studies indicate that not all plant and insect products enhance natural enemy fitness. Factors such as floral morphology (corolla length and shape) (Patt et al. 1997) and nectar components and composition ratios (Wäckers 2001) significantly affect parasitoid accessibility and acceptance.

In this study, we examined the fitness effects of naturally available and parasitoid accessible resources in the laboratory in order to evaluate the role resource provisioning may play in the aphid biological control of *A. gossypii* in California nursery systems. First, we evaluated invasive, native, and ornamental floral resources for their potential to enhance *A. colemani* longevity and fecundity. Second, we investigated the effects of floral and honeydew resources on the longevity, fecundity, and sex ratio of *A. colemani*

using resources collected from the containerized nursery plant, *P. x fraseri*, and its herbivore, *A. gossypii*.

2. Materials and Methods

2.1 Plant and insect colonies

All plant and insect colonies were maintained at the University of California, Riverside (UCR). Greenhouse grown colonies of *Cuburbito pepo* L. 'Raven' (Johnny's Selected Seeds, Albion, ME) and *Brassica juncea* (L.) Czern. 'Florida Broad Leaf' (Ferry-Morse Seed Company, Fulton, KY) were cultivated in 0.92 L pots (industry standard 4" pots, Farrand Enterprises, Chino, CA) filled with UC Soil Mix I and fertilized with *circa* 15 mL of Osmocote® 18-6-12 (The Scotts Miracle-Gro Company, Marysville, OH). Plants and insect colonies were grown in natural light conditions at 25 ± 2° C with 25 ± 5% RH. Three week old plants of *C. pepo* 'Raven' were infested with *A. gossypii.* Another aphid, *Myzus persicae* (Sulzer), was grown on 3 week old *B. juncea* plants to serve as additional host material for the parasitoid due to its polyphagous nature. After 1 week, the 2 aphids on their respective host material were transferred to the greenhouse colony of the parasitoid, *A. colemani*, caged in a BugDorm2 (BioQuip®, Rancho Dominguez, CA). Parasitoids were held in the above conditions until mummies were removed for experiments. *A. gossypii* and the parasitoid were originally field-collected and maintained in the greenhouse for 2 months prior to experiments. *M. persicae* was acquired from a laboratory colony held at UCR.

2.2 Floral resource evaluation & parasitism assessment

2.2.1 Resource presentation

Floral resources from two invasive weed species, three native plant species, and three ornamental species associated with Southern California ornamental nurseries were evaluated for their potential as a food resource for the parasitoid *A. colemani*. Species selection was based on seasonal availability representing 8 plant families. The three native species included *Encelia farinosa* A. Gray ex Torr. (Asteraceae), *Eriogonum fasciculatum* Benth. var. *foliolosum* (Nutt.) S. Stokes ex Abrams (Polygonaceae), and *Salvia apiana* Jeps. (Lamiaceae). The three ornamental species were *Lantana camara* L. (Verbenaceae), *Ligustrum japonicum* Thunb. (Oleaceae), and *P. x fraseri* (Rosaceae). The two invasive weed species were *Brassica nigra* (L.) Koch (Brassicaceae) and *Conium maculatum* L. (Apiaceae).

Inflorescences from mature, landscape individuals of each plant species in this experiment were bagged for 24 hours prior to removal. The white nylon sleeve bag (30.48 cm long with a 20.32 cm diameter) prevented other insects from removing resources. Inflorescences were cut, immediately placed in deionized water, and maintained in a sealed, ventilated container until the application of treatment. One inflorescence of *C. maculatum, E. fasciculatum, L. japonicum,* and *P. x fraseri* and two inflorescences of the remaining species were loaded into the floral bouquet vase. The number of inflorescences used was based upon equalizing the floral count. The bouquet vase consisted of a single capped 40 dram plastic vial filled with deionized water. The

inflorescences were held in place by inserting their stems through a standard hole-punch in the cap of the vial allowing the stem to pass into the deionized water. The thickness of the inflorescence stem(s) and the application of Parafilm M® Laboratory Wrapping Film across the opening of the capped vial prevented contact between the parasitoid and the deionized water. A total of 11 treatments were applied; 8 floral, 1 honey-water (1:1 by weight) as a positive control, and 1 water (deionized water) as a negative control, plus a blank control. The honey-water and water treatments consisted solely of a 2 µL droplet of the resource applied to the bouquet vase. The honey was obtained from the University of California, Riverside bee colonies.

Mated female parasitoid individuals less than 24 hours old were caged in 1.82 L plastic cylinders with top (1, 11.43 cm diameter circle) and side (2, 5.72 cm diameter circles) ventilation covered with hardware cloth. The bottom of the cage was closed with a plastic 0.95 mL food container top (Smart & Final, Commerce, CA). One female *A. colemani* was placed inside each cage at the beginning of the experiment and maintained inside the same cage as the same fresh treatments were applied every 24 hours. Individual females were caged with treatments for 23 hours before a 1 hour host-only exposure. Parasitoid longevity was recorded daily until the parasitoid died.

2.2.2 Host presentation

Hosts were delivered to caged parasitoids via a 2 – 4 day old *C. pepo* 'Raven' plant grown in a 0.14 L pot (industry standard 2" pots from Farrand Enterprises, Chino, CA) using UC Soil Mix I (Matkin and Chandler, 1957) grown in natural light conditions

at 25 ± 2° C with 25 ± 5% RH. All leaves were removed except for one true leaf that was infested with at least 100 first and second instars of *A. gossyppi* , the preferred life stages for parasitization (Perdikis et al. 2004). Exposed plants were removed after 24 hours and maintained under experimental conditions for 8 days before mummies were counted as a measurement of fecundity. Environmental conditions during experiments were 25 ± 1° C with 15 ± 10 % RH under inflorescent lighting with a L14:D10 photoperiod. The experiment was performed three times from April 2008 through May 2008 generating a range of 11 to 16 replicates per treatment. The number of treatments per experiment did not vary but escaped or lost replicates were not included in the analysis.

2.2.3 Statistical analysis

The data set was analyzed for treatment effects based on plant species using PROC GLM in SAS 9.3 (Inc. 2011). Prior to analysis, the water and blank control treatments were removed from the data set due to lack of variance. The dependent variables, longevity and fecundity, were square root transformed to achieve normality. An analysis of variance (ANOVA) ($p < 0.05$, model adequacy based on residual analysis) comparing treatment, date, and their interaction was performed to verify there was no date effect across treatments. Based on a non-significant date effect, all experimental dates were pooled prior to the statistical analysis presented here. An ANOVA model and Tukey-Kramer's test were constructed at a 0.05 significance level comparing plant species treatment at 9 levels (8 plant species listed above plus a honey-water positive control) along with a contrast analysis to investigate differences among the 3 ecological

classifications (native, ornamental, and invasive). Data presented in this paper have been back transformed to the original values.

2.3 Food resource use and A. colemani *fitness*

2.3.1 Collection and analyses of soluble carbohydrates

Two naturally occurring and parasitoid accessible resources, nectar and honeydew, were collected using the ornamental plant, *P. x fraseri*. Inflorescences of mature, landscape planted *P. x fraseri* were bagged 24 hours prior to nectar extraction using Drummond® Short-Length Microcaps® Micropipets (capillaries). The white nylon sleeve bag as described above prevented other insects from removing resources. Collections were taken during multiple dates in May 2007. Nectar was pooled from multiple inflorescences and multiple *P. x fraseri* individuals on each collection date. Prior to analysis and experimental use, all dates were combined. Honeydew collection was orchestrated using *A. gossypii* as a conduit through which *P. x fraseri* phloem was converted to honeydew. Parafilm M® Laboratory Wrapping Film was wrapped around *P. x fraseri* leaves infested with *A. gossypii* and sealed to create an open-ended tube that rested on the leaf's upper surface. After 24 hours, the parafilm was removed and laid open on the porcelain plate of a Fisher Scientifc® Desiccator with 2 cm of deionized water in the bottom for an additional 24 hours. Hydrated honeydew was wiped off the parafilm with a Fisherbrand® Spatula/Scraper. Collections occurred in February 2007 and honeydew was pooled from multiple leaves and multiple *P. x fraseri* individuals on each collection date. Prior to analysis and experimental use, all dates were combined.

Both nectar and honeydew samples were stored at -16˚ C for less than 1 year. Three resources, honey-water, nectar, and honeydew, were analyzed for soluble carbohydrates by the University of California Agricultural and Natural Resources Analytical Lab (Davis, CA) using the quantitative method described by (Johansen et al. 1996) as sugar composition may be an explanatory factor in resource suitability and fitness outcomes.

2.3.2 Resource and host presentation

The fitness of *A. colemani* was assessed using three parameters, longevity, fecundity, and offspring sex ratio, in response to three naturally occurring and parasitoid accessible resources; extrafloral nectar, nectar, and honeydew. Two additional treatments, honey-water as a positive control and water as a negative control, along with a blank control were also assessed for a total of 7 treatments.

Mated female parasitoid individuals less than 24 hours old were kept in the same cages described in section 2.2.1 and given access to a resource treatment for 23 hours followed by a 1-hour host exposure. Resource treatments consisted of a 2 µL droplet of resource applied to the cotyledon notch of a *C. pepo* 'Raven' plant, grown as previously described in section 2.2.2. All primary leaf growth was removed leaving only the cotyledons on each resource plant except for the extrafloral nectar treatment. The extrafloral nectar resource plant retained 1 true leaf containing extrafloral nectaries along with the cotyledons. This true leaf was the source of the resource. *A. gossypii* hosts were delivered to the experimental parasitoids as described in the previous floral resource experiment using *C. pepo*'Raven" plants.

Treatments were re-administered to the same parasitoid every 24 hours and longevity was recorded at that time. The exposed plants were removed from the cages that housed the parasitoids and maintained under experimental conditions for 8 days before mummies were removed, counted, and allowed to eclose. Emerged females and males were counted in order to determine sex ratio. Experimental environmental conditions were the same as noted for section 2.2. The experiment was performed 5 times from November 2007 through June 2008 generating a range of 12 to 35 replicates per treatment.

2.3.1 Statistical analysis

The data set was separately analyzed for treatment effects based on resource type using the GLM procedure in SAS 9.3 (Inc. 2011). Prior to analysis, the water and blank control treatments were removed from the data set due to lack of variance. The dependent variables, longevity, fecundity (mummies and emerged individuals), percent emergence, and sex ratio, were square root transformed to achieve normality. An analysis of variance (ANOVA) ($p < 0.05$, model adequacy based on residual analysis) comparing treatment, date, and their interaction was performed to verify there was no date effect across treatments. Based on a non-significant date effect, all experimental dates were pooled prior to the analysis. An ANOVA model comparing resource treatment at 4 levels (extrafloral nectar, nectar, honeydew, and honey-water) was constructed at a 0.05 significance level with model adequacy based on residual analysis

for each dependent variable. Treatment means were separated using the Tukey-Kramer's test. Data presented in this paper have been back transformed to the original values.

2.4 Hind tibia length measurements and statistical analysis

Many parasitoid fitness characteristics including fecundity (Opp and Luck 1986, Rosenheim and Rosen 1991) and longevity (Waage and Ming 1984) are positively correlated with tibia length. Measurements of hind tibia length were taken on *A. colemani* individuals post-experiment to determine if parasitoid body size was related to fitness measurements. There was potential for body size variation as 2 aphid species, *A. gossypii* and *M. persciae,* were used to rear the parasitoid. The right metathroacic tibia was measured using an ocular micrometer inserted into a dissecting microscope. Measurements were taken at 800X magnification with a 0.02 mm resolution. One ANOVA model using the data set from the floral resource study was constructed at a 0.05 significance level with model adequacy based on residual analysis to compare hind tibia length across 9 treatment levels (8 floral species and 1 honey-water). A second ANOVA using the food resource data set was performed at a 0.05 significance level with model adequacy based on residual analysis with food resource treatments at 4 levels: extrafloral nectar, nectar, honeydew, and honey-water.

3. Results

3.1 Floral resource effect on A. colemani *fitness*

3.1.1 Floral species effect on longevity and fecundity

A significant effect of floral species treatment was found for both fitness parameters, longevity ($F_{8,120}$= 7.26, $p < 0.0001$) and fecundity ($F_{8,120}$ = 5.70, $p < 0.0001$). Survival of *A. colemani* was at least 3.9 times numerically greater for 6 treatments, honey-water, *Salvia*, *Conium, Photinia, Lantana,* and *Ligustrum,* when compared with the blank control (Figure 2.1). The honey-water treatment exhibited the largest survival improvement of 5.9 times the blank control and was statistically different than *Eriogonum, Brassica, and Encelia* (Figure 2.1). The remaining 5 floral species treatments, *Salvia, Lantana, Ligustrum, Photinia, and Conium,* overlapped statistically with the honey-water treatment (Figure 2.1). The longevity for the 5 remaining floral species was largest for *Salvia* (5.1 days) and least for *Ligustrum* (4 days) (Figure 2.1). The fecundity of *A. colemani* was numerically greatest for the honey-water treatment at 114 individuals compared to 0 individuals for both the water treatment and blank control (Figure 2.2). *Encelia and Brassica* were the only floral species treatments that were statistically different than the honey-water positive control with average number of mummies to be 6.8 and 23.9 respectively (Figure 2.2). All other treatments, *Eriogonum, Salvia, Lantana, Ligustrum, Photinia, and Conium*, overlapped statistically with the average number of mummies ranging from 52.2 for *Eriogonum* and 90.2 for *Conium* (Figure 2.2).

3.1.2 Ecological classification effect on longevity and fecundity

The longevity of *A. colemani* provisioned resources from three ecological classification (native, ornamental, and invasive) was statistically different ($p < 0.05$) for

all contrasts except for ornamental vs. invasive (Table 2.1). The mean longevity for the honey-water positive control was the largest at 5.9 ± 0.63 days (Figure 2.1) while the smallest mean longevity was 2.9 ±0.33 days for the native plant type. The longevity for the ornamental classification was 4.22 ± 0.39 days and the longevity for the invasive plant type was 3.66 ± 0.42 days. Wasp longevity was numerically greater for all ecological classifications along with honey-water when compared to the water treatment and the blank control (Figure 2.1).

The effect of ecological classification on fecundity was similar to the effect on longevity with all contrasts except for ornamental vs. invasive being statistically different ($p < 0.05$) (Table 2). The mean fecundity for the honey-water positive control was largest (114.65 ± 18.76 mummies) and smallest for the native plant classification (42.09 ± 7.77 mummies). Fecundity was greater for the honey-water treatment along with all ecological plant classifications as compared to the water negative control and the blank control (Figure 2.2).

3.2 Resource effect on A. colemani *fitness*

There was a significant effect of resource treatment (extrafloral nectar, nectar, and honeydew) on *A. colemani* for four of the five fitness parameters; longevity ($F_{3,95} = 9.62$, $p < 0.0001$), number of offspring ($F_{3,95} = 4.84$, $p = 0.0035$), number of mummies ($F_{3,95} = 4.11$, $p = 0.0086$), and percent emergence ($F_{3,95} = 2.76$, $p = 0.0465$). There was no statistical effect of resource treatment for the fifth parameter, sex ratio ($F_{3,95} = 1.52$, $p = 0.2135$).

The longevity of *A. colemani* was improved by an average of 6.9 days for the honey-water treatment as compared with both the water treatment and blank control (Figure 2.3). The nectar treatment improved mean survival by 5.5 days while the honeydew treatment improvement was an average of 4.9 days more than both the water treatment and blank control (Figure 2.3). The extrafloral nectar treatment improved mean longevity by 2.2 days (Figure 2.3). Three treatments, honey-water, nectar, and honeydew, were statistically different from the extrafloral nectar treatment (Figure 2.3).

Fecundity as measured by the number of offspring was increased by an average of 120 individuals for the honey-water treatment as numerically compared to the 0 individuals of the water negative control and blank control (Figure 2.4). The mean fecundity for the water and blank control was not measurable as all individuals died prior to the host presentation. While the honey-water treatment exhibited the largest number of offspring, it was not deemed to be statistically different than the nectar (105 individuals) and honeydew (98 individuals) treatments (Figure 2.4). The remaining treatment, extrafloral nectar, was greater than both the water treatment and blank control but lower than the 3 other treatments (Figure 2.4).

Fecundity as quantified by the number of mummies exhibited similar statistical results as fecundity measured by offspring. The mean fecundity of *A. colemani* was greater for provisioned all 4 food resource treatments (honeydew, nectar, extrafloral nectar, and honey-water) as compared with both the water and blank control (Figure 2.4).

Three food resource treatments, honey-water, nectar, and honeydew, were significantly greater than the extrafloral nectar treatment (Figure 2.4).

The effect of food resource treatment on the percent emergence was less pronounced than the effects on longevity, fecundity as measured by offspring, and fecundity as measured by mummies. Percent emergence ranged from 0 % (both water and blank treatment) to 84.5 ± 8.5 % (nectar treatment). There was no statistical difference between the nectar, honey-water (82.1 ± 2.1 %), and honeydew (65.3 ± 7.5 %) treatments but all these treatments were statistically different than the extrafloral nectar treatment (55.7 ± 8.8 %).

3.4 Hind tibia lengths for verification of findings

There was no effect of floral resource treatment ($F_{4,70} = 0.55$, $p = 0.8323$) nor food resource treatment ($F_{4,81} = 1.06$, $p = 0.3793$) on hind tibia length. Fitness parameters studied in these experiments were not biased by parasitoid body size.

3.5 Soluble carbohydrates of food resources

All 3 sugar sources, honey-water, honeydew, and nectar, contained glucose, fructose, and sucrose (the only 3 sugars tested) (Table 2.3). Nectar, for the purposes of this study, contained the highest concentrations of all 3 sugars tested while honeydew contained the least (Table 2.3). This result is most likely an artifact of the collection method as the honeydew was hydrated. The ratio of sucrose to its breakdown products, glucose and fructose, was 0.02 for honey-water, 0.06 for honeydew, and 0.08 for nectar (Table 2.3).

4. Discussion

4.1 Choosing floral resource candidates that are accessible, available, and maximize parasitoid fitness

The fitness benefits of accessible and available floral resources in agricultural systems have been supported by many previous studies (Pickett and Bugg 1998, Landis et al. 2000) . In this study, the longevity and fecundity of *A. colemani* was markedly improved with the provision of floral resources from 5 of the 8 plant species tested. These 5 species, listed in order of decreasing fitness benefits, represent 5 different families, Apiaceae (*C. maculatum*), Rosaceae (*P. x fraseri*), Lamiaceae (*S. apiana*), Verbenaceae (*L. camara*), and Oleaceae (*L. japonicum*). Although floral morphology is a key indicator in plant family identification (Sivarajan 1991) and in parasitoid accessibility (Patt et al. 1997), specific plant species must be evaluated for not only parasitoid accessibility but fitness competence. The 4 most prominent floral resource plants shown to enhance natural enemy fitness in the laboratory include species from the Apiaceae, Brassicaceae, Hydorphyllaceae, and Polygonaceae (Fiedler et al. 2008). In this study, species representing the Brassicaceae and Polygonaceae were nutritionally inferior. Parasitoid accessibility and fitness competence along with availability in the agriscape are likely critical factors in choosing acceptable floral resource candidates.

The top three fitness-enhancing plants in this study, *C. maculatum*, *P. x fraseri*, and *S. apiana*, represented species from all three plant types, native, invasive, and ornamental, available in and adjacent to the Southern California ornamental nursery.

This agriscape and its inherent floral resources present biological control practitioners with the option to implement habitat manipulation techniques that provide more than pest population control. Choosing to provision native floral resources instead of the traditionally utilized non-native floral resources may increase biodiversity and restore disrupted ecosystems adjacent to agriscapes. The provisioning of accessible and available floral resources as a habitat manipulation technique may not only deliver pest population control but also deliver these additional ecosystem services (Fiedler et al. 2008).

C. maculatum, *P. x fraseri*, and *S. apiana* all enhanced the fitness of *A. colemani* and could be considered acceptable floral resource candidates for enhancing the control of *A. gossypii*. These three floral resources not only increased the longevity and fecundity of *A. colemani* but also may support additional ecosystem services that benefit both the agricultural environment and its surrounding ecosystems. Plant species that enhanced *A. colemani* may not enhance other natural enemies. Therefore, when evaluating candidates for floral resource provisioning, biological control practitioners should not only consider the traditionally utilized and easily cultivated non-native plant species but native plant species should also be considered. Finding native plant species that provide food resources for natural enemies may not only potentially improve pest population control but also their inclusion in the agriscape offers other ecosystem services like biodiversity conservation and ecological restoration.

4.2 Fitness enhancement through resource provisioning

Agriscapes are typically depauperate of resources necessary for parasitoids to exert any significant population control over their pest hosts (Gurr et al. 2004a). Provisioning supplemental resources including floral, extrafloral, and insect products has been one of the core efforts to conserve natural enemies and improve biological control in agriculture systems (Jonsson et al. 2008). Floral resources have been the primary focus in this effort while extrafloral resources and insect products like honeydew have been less studied (Jonsson et al. 2008). Supplying accessible floral resources not only attracts parasitoids to the agriscape (Leius 1960, Jervis et al. 1993), but also concentrates their presence which potentially increases control of pest populations (Leius 1967, Chaney 1998) while simultaneously boosting fitness parameters like longevity and fecundity (Landis et al. 2000, Heimpel and Jervis 2005). Suggestions about the mechanisms of honeydew's impact on biological control are similar to those for floral resources but empirical data across parasitoid families is lacking. The case supporting provision of extrafloral resources is also inadequate. Floral resources have been viewed as the superior sugar source (Wäckers 2000, 2001) and extrafloral resources have been demonstrated as suitable (Olson and Nechols 1995, Rose et al. 2006, Sivinski et al. 2006) and unsuitable (Elliott et al. 1987), while honeydew has been generalized as an inferior resource for subsidizing natural enemies (Wäckers et al. 2008). Alternatively, some soft scale honeydews may function as a higher quality resource (Irvin et al. 2007).

Comparative analysis of the reproductive characteristics of *A.colemani* presented in this study examined 3 naturally available and parasitoid accessible sugar sources, floral, extrafloral, and honeydew along with honey-water. The longevity and fecundity of *A. colemani* were greatly improved when honey-water, nectar, and honeydew were provided as compared with water and the blank control but there appears to be little difference between these food resources. Extrafloral nectar was also different from the water treatment and blank control but was not comparable to honey-water, nectar, and honeydew as longevity and fecundity were less than half of the other resource treatments. The other key reproductive characters, sex ratio and percent emergence, were not affected by resource provisioning. These results do not support the hypothesis that honeydew is an inferior sugar source (Wackers et al., 2008).

Although honeydew provisioned in this study had the least amount of glucose, fructose, and sucrose as compared with nectar and honey-water, the resource was statistically equivalent in terms of the fitness parameters measured. An increasing number of studies are beginning to address the sugar profile of provisioned resources. A study of *Cotesia glomerata* (L.) (Hymenoptera: Braconidae) found that provisioning glucose (180160 mg/L), fructose (180160 mg/L), and sucrose (342300 mg/L) were equally successful at extending the parasitoid's lifespan as compared with water (Wäckers 2001). Hogervorst et al. (2007) compared sucrose (684600 mg/L) to various aphid honeydews from potato and wheat and reported no difference in the longevity of *Aphidius ervi* Haliday (Hymentoptera: Braconidae: Aphidiinae) when fed on potato

honeydew generated by *M. persicae*. The sugar profile of *M. persciae* potato honeydew consisted of 22081 mg/L glucose, 128132 mg/L fructose, and 96099 mg/L sucrose (Hogervorst et al. 2007). Honeydew from *A. gossypii* used in this experiment was more concentrated for glucose (1.68 times) and less concentrated for fructose (18.45 times) and for sucrose (35.33 times) than *M. persicae*'s potato honeydew. The combined glucose, fructose, and sucrose sugar concentration of *A. gossypii* honeydew was 3.85 times less than the single sugar sources of glucose and fructose fed to *C. glomerata* and 7.32 times less than the single sugar source sucrose. This simple comparison of three studies has demonstrated the potential variation in the sugar profiles of provisioned parasitoid food resources. Not only may this variation in sugar content and concentration explain differences in parasitoid fitness, but honeydews contain oligosaccharides that have been suggested to lower the nutritional quality (Wäckers 2000, 2001). As the discussion of specific sugars and nutritional quality of provisioned resources proliferates, attention to sugar profiles, concentrations, and component ratios may help explain underlying mechanisms that boost parasitoid fitness which may ultimately contribute to the control of a pest population.

Once a food resources' availability, accessibility, and quality is established in the laboratory, the next step is to conduct field studies assessing the parasitoid's dispersal from the resource and impact on the pest population (Gurr et al., 2000). The costs and benefits of each resource should be considered so the economics of the tactic can be established. In the context of agricultural systems, consideration should be given to

temporal and spatial fit with crop production schemes as well as non-target impacts causing secondary pest outbreaks. Although there are examples of provisioning floral resources as beneficial in enhancing the biological control in agricultural systems, other food resources like honeydew and food sprays (Hagen et al. 1971, Wade et al. 2008) may find a better fit in certain cropping systems.

The fitness of *A. colemani* was enhanced with the provision of food resources such as floral nectar and aphid honeydew under laboratory conditions. The expectation is that these positive effects on the parasitoid's reproductive activity can be translated into pest population control in the field. The ornamental nursery system offers an abundance of floral resource opportunities without the need to lose production space. Additionally surrounding areas of invasive and native vegetation could serve as both floral resources and honeydew food resources for *A. colemani*. In order to demonstrate the real influence of provisioning food resources to *A. colmeani* on its aphid host, field trials assessing the populations of both *A. colemani* and *A. gossypii* are necessary. Regard should also be given to the non-target impacts of provisioning and the economics of implementation of such tactics.

Table 2.1. Contrast analysis of the longevity of female *Aphidius colemani* feeding on three ecological classifications of floral resources (native, ornamental, and invasive) and 1 honey-water positive control

Contrast	df	F Value	$p > F$
honey-water vs. all other	1	12.42	0.0006
honey-water vs. native	1	17.63	< 0.0001
honey-water vs. ornamental	1	5.82	0.0174
honey-water vs. invasive	1	5.59	0.0197
native vs. ornamental	1	6.10	0.0149
native vs. invasive	1	4.94	0.0281
ornamental vs. invasive	1	< 0.01	0.9552

Table 2.2. Contrast analysis of the fecundity of female *Aphidius colemani* feeding on three ecological classifications of floral resources (native, ornamental, and invasive) and 1 honey-water positive control

Contrast	df	F Value	$p > F$
honey-water vs. all other	1	9.16	0.0030
honey-water vs. native	1	13.01	0.0005
honey-water vs. ornamental	1	4.18	0.0432
honey-water vs. invasive	1	5.20	0.0244
native vs. ornamental	1	4.68	0.0325
native vs. invasive	1	4.31	0.0400
ornamental vs. invasive	1	0.03	0.8603

Table 2.3. Liquid sugar concentration (mg/L) of honey-water and 2 naturally available and parasitoid accessible resources

Resource	Glucose	Fructose	Sucrose
honey-water	269000	151000	8150
honeydew	37100	6940	2720
nectar	312500	288500	47850

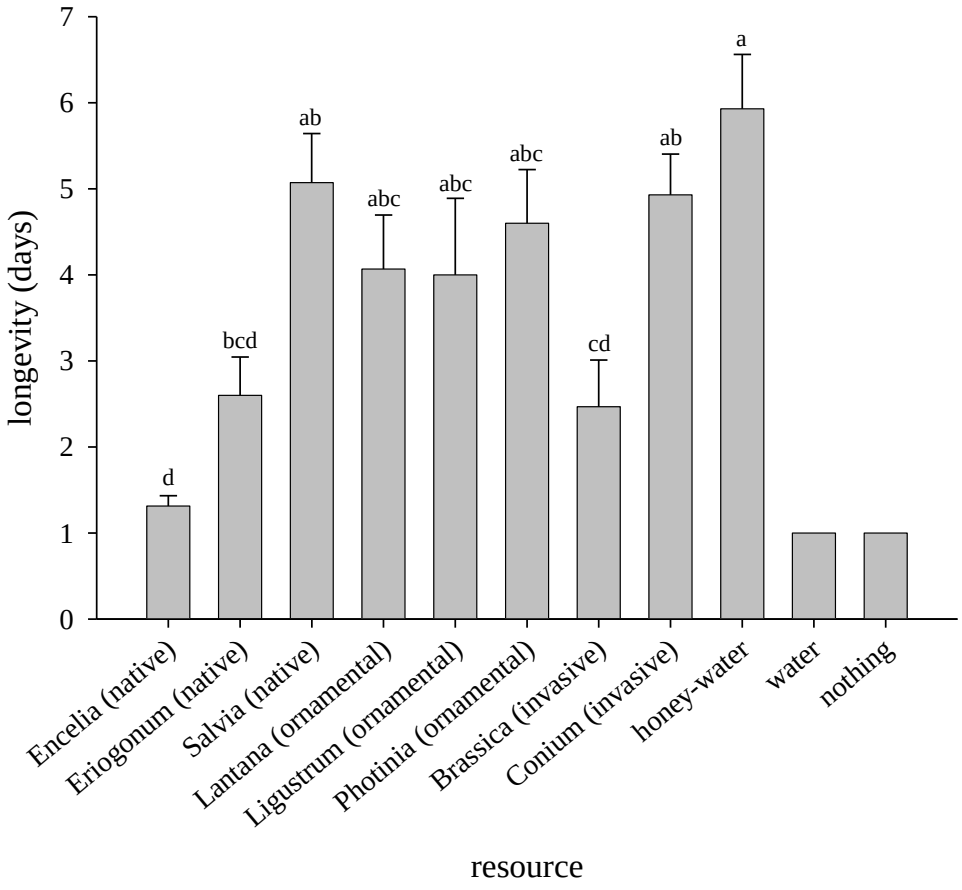

Figure 2.1. Survival of female *Aphidius colemani* on various food resources including floral resources consisting of three ecological types, water, honey-water, and a blank control. Significant differences ($p < 0.05$) between resource treatments are indicated by different letters. The water and nothing treatment were not included in the analysis.

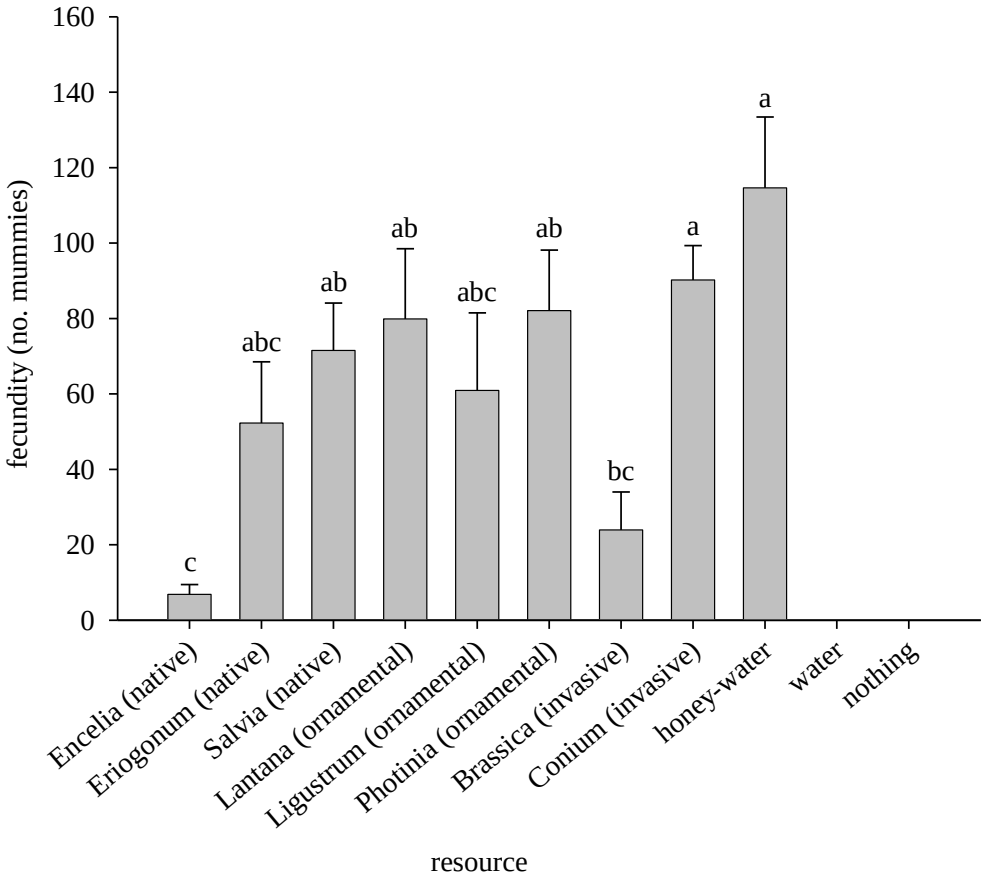

Figure 2.2. Fecundity of female *Aphidius colemani* on various floral food resources. Ecological classification indicated in parentheses. Significant differences ($p < 0.05$) between resource treatments are indicated by different letters. The water and nothing treatment were not included in the analysis.

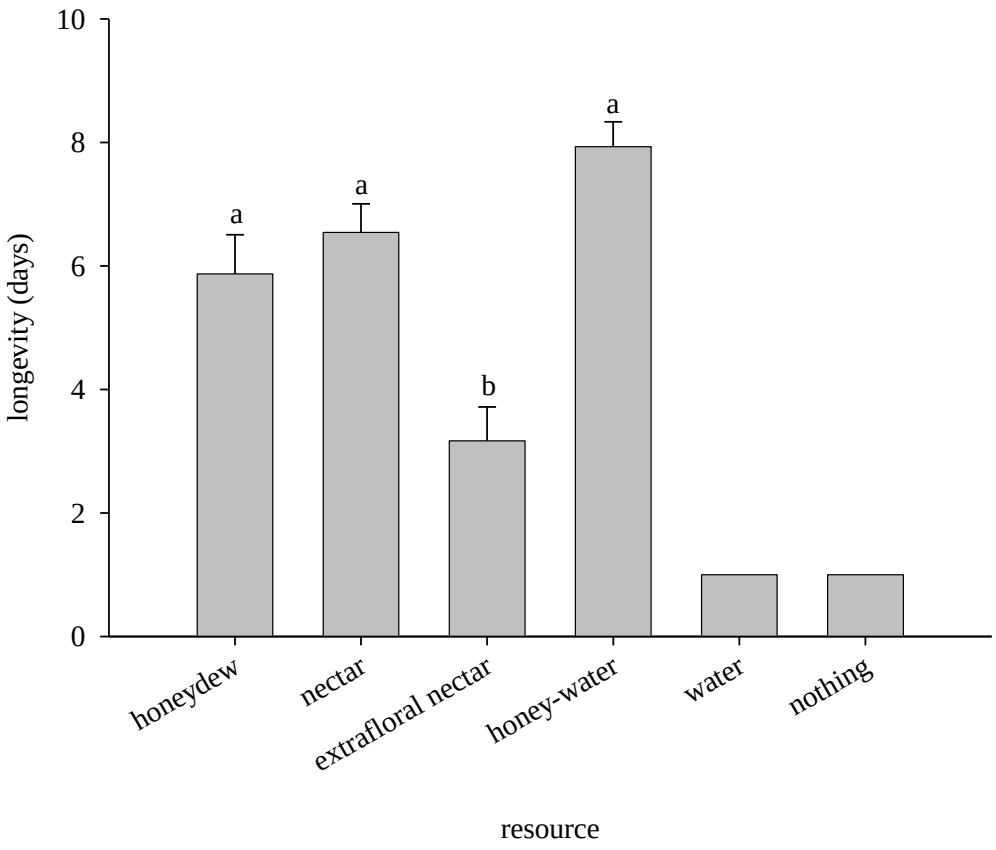

Figure 2.3. Survival of female *Aphidius colemani* on 3 naturally available and parasitoid accessible resources. Significant differences ($p < 0.05$) between resource treatments are indicated by different letters. The water and nothing treatment were not included in the analysis.

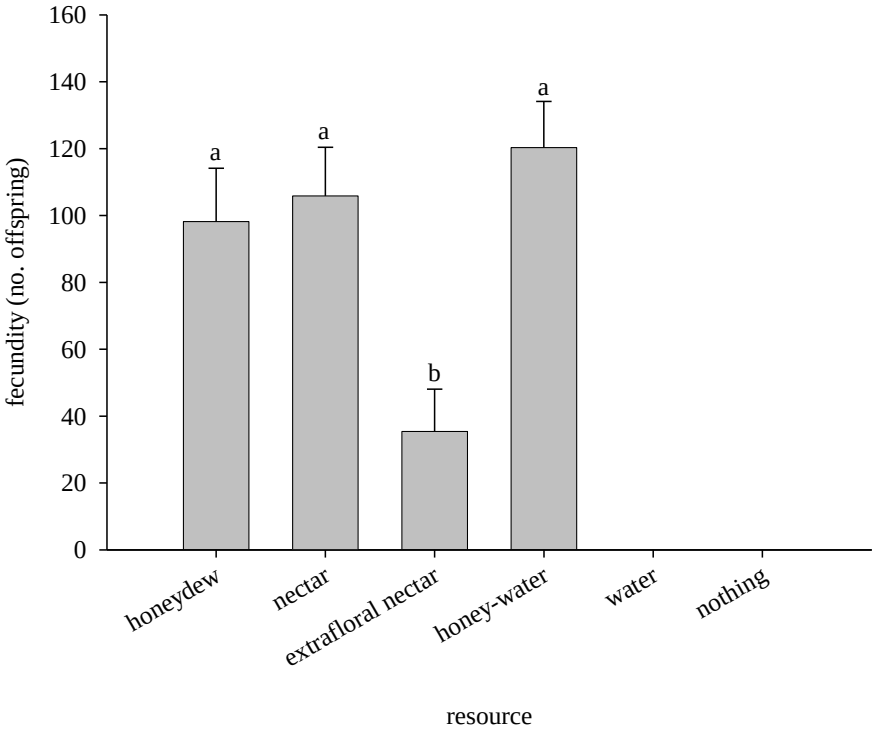

Figure 2.4. Fecundity of *Aphidius colemani* on 3 naturally available and parasitoid accessible resources. Significant differences ($p < 0.05$) between resource treatments are indicated by different letters. The water and nothing treatment were not included in the analysis.

Chapter 3. Field abundance, distribution, and dispersal of a polyphagous aphid parasitoid, *Aphidius colemani* (Hymenoptera: Braconidae: Aphidiinae), under habitat manipulation as a conservation biological control tactic.

1. Introduction

Conservation biological control is a pest management strategy that aims to protect and promote natural enemies in agricultural cropping systems through the implementation of various approaches that alter the environment to make it more suitable to natural enemies (Barbosa 1998, Eilenberg et al. 2001). Habitat manipulation is one such tactic whereby the agricultural landscape is modified to provide the resources natural enemies need to improve their capacity to suppress pest populations. Examples include providing supplemental food resources (nectar, pollen, and honeydew), alternative prey and host, and physical refuge (Gurr et al. 2004b). Resource provisioning is considered necessary to enhance biological control because cropping systems are designed to maximize yield and typically, this design construct is missing resources essential to natural enemies (Landis et al. 2000). Incorporating resources into agricultural systems for the benefit of natural enemies requires a different approach. An alternative design construct known as ecological engineering utilizes the principles of ecology to conceptualize and engineer agriculture systems to benefit natural enemies while maximizing yields (Gurr et al. 2004b).

Engineering cropping systems to provide food resources for natural enemies has the potential to maximize natural enemy effectiveness thereby providing better pest suppression (Gurr et al. 2004). Food resources such as floral (nectar and pollen),

extrafloral (nectaries, exudates, and fruits), and insect products (honeydew and host feeding), may provide nutrition benefits for natural enemies (Doten 1911, Syme 1975, 1977, Jervis et al. 1993). Some studies, mostly laboratory assays, have suggested that provisioning plant and insect-derived resources increases longevity and fecundity (Tylianakis et al. 2004, Hogervorst et al. 2007, Irvin and Hoddle 2007), searching activity (Budenberg et al. 1992, Grasswitz and Paine 1993), and field parasitism rate (Tylianakis et al. 2004, Berndt et al. 2006). A framework has been proposed for determining the effectiveness of habitat manipulation tactics based on trophic levels of food-web theory and an additional level, the farming system (Gurr et al. 2000). Suggested research questions target crops (trophic level 1), pests (trophic level 2), natural enemies (trophic level 3), predators/parasitoids of natural enemies (trophic level 4), and the farming system (Gurr et al. 2000). Based on this framework, research objectives for determining the benefits of food resource provisioning to natural enemies would be (1) establish attraction to food resources, (2) document enhancement of longevity and fecundity due to food resources, (3) demonstrate dispersal from field food resources, (4) show increased efficacy in the field, and (5) provide economic analysis of tactic implementation (Gurr et al. 2000).

Demonstrating natural enemy dispersal from a food source is a transitional step to field-exclusive studies. Understanding natural enemy resource use patterns in the context of dispersal data is key to the development of habitat manipulation tactics that work as intended. Population models have suggested that natural enemy dispersal is pivotal to

generational persistence and influential in exerting local mortality on targets (Strong 1988). Several factors may influence natural enemy movement and the realized distance moved including physiological state (Grasswitz and Paine 1993, Lee and Heimpel 2007), body size (Zhou et al. 1995, Ellers et al. 1998), plant structures like glandular trichomes (Cottrell and Yeargan 1999), and epicuticular wax (Gentry and Barbosa 2006), habitat structure (Schworer and Volkl 2001, Randlkofer et al. 2010), and landscape context (Thies et al. 2003, Petit et al. 2008). Natural enemy movement, immigration into and emigration from a crop, must be established along with the use of the crop-associated food resources in order to evaluate the resource as a potential habitat manipulation tactic (Gurr et al. 2004b).

Conservation biological control may be a viable pest management strategy in the ornamental, containerized nurseries of Southern California, because the agricultural landscape is rich with food resources. Ornamental production systems are abundant in floral resources and interface with disturbed and native habitats. The latter may provide additional and alternative food and host resources. An additional feature of the nursery cropping system is the motility associated with plants grown in containers allowing resource plants to be placed where needed. Ornamental shrub crops such as *Photinia x fraseri* Dress (Rosaceae) have the potential to function as a food resource plant and then be recycled back into marketable stock after a routine hedging and period of regrowth. The uniqueness of this highly mobile and resource rich cropping system lends itself to the development of habitat manipulation tactics in the ornamental nursery.

The melon aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is a pest of more than 120 plant species representing 90 families (Blackman and Eastop 2000, Emden and Harrington 2007) including *P. x fraseri*. This aphid species is considered to be one of the most economically destructive aphids in the United States (Slosser et al. 1986) and one of the top ten pests in California nurseries (Wilen et al. 2002). Although aphid control is typically achieved with insecticides such as carbamates, organophosphates, pyrethroids (Wilen et al. 2002), and more recently imidacloprid, it may be possible to utilize habitat manipulation tactics to promote biological control of aphid populations.

The solitary endoparasitoid, *Aphidius colemani* Viereck (Hymenoptera: Braconidae: Aphidiinae), is a cosmopolitan species parasitizing over 41 aphid host species including the melon aphid (Stary 1975). *A. colemani* has a similar intrinsic rate of increase as *A. gossypii* (Van Steenis 1993) and has the ability to discover and parasitize aphids at low densities (Van Steenis and Elkhawass 1995). Unlike many polyphagous aphid parasitoids (Powell and Li 1983), *A. colemani* readily accepts alternative hosts increasing its potential field presence (Elliot et al. 1994). This generalist parasitoid of aphids is found throughout ornamental nursery production systems of Southern California as well as surrounding disturbed, urban, and native habitats.

A. colemani movement has been studied in greenhouses on chrysanthemums (Heinz 1998) where the parasitoid is considered an effective but expensive control option (Harizanova and Ekbom 1997, Jacobson and Croft. 1998, Vasquez et al. 2006). Parasitoid displacement distances and diffusion constants (the spread of dispersing

populations over time) were largest for individuals released on aphid infested plants (Heinz 1998). A mean displacement distance of 171.7 cm in 11 hours was reported for a 12.01 m^2 experiment area within a greenhouse (Heinz 1998). The dispersal distance of *A. colemani* was much greater, 16 m after 24 hours, in a field study using aphid infested kohlrabi trap plants placed in an 803.84 m^2 stubble field (Langhof et al. 2005). These movement studies provide a reference for *A. colemani* movement in the presence of aphids, a potential food resource, but do not address questions of movement from resources into associated crops where resources may not be present.

Floral and honeydew resources from the ornamental plant, *P. x fraseri*, and its herbivore, *A. gossypii*, enhance the longevity and fecundity of the aphid parasitoid, *A. colemani*, (Chapter 2). This study intends to take the next steps in evaluating the effectiveness of habitat manipulation in the ornamental nursery by (1) determining the abundance and movement of *A. colemani* in the presence and absence of resources and (2) assessing the field impact *A. colemani* may have on populations of *A. gossypii*.

2. Materials and Methods

A common garden of the ornamental plant, *P. x fraseri*, measuring 21 m x 29 m was established within a fallow block of land (63 m x 63 m) at the University of California, Riverside Agricultural Operations in April 2005. To the North, the area in which the plot was located was bordered by a grove of citrus trees and to the South, by a row of eucalyptus trees. Areas to the east and west were fallow for approximately 63 m. This configuration was intended to keep emigration by resident parasitoids low. The

growing ground was covered with a weed suppression fabric, Weed-Barrier® (De Witt Company, Sikeston, MO). The common garden was divided into 3 blocks each consisting of 4 plots (37.16 m^2) subdivided into 1 treatment sub-plot and 1 crop sub-plot. *P x. fraseri* was grown in all sub-plots except for the bare-ground control sub-plot (Figure 3.1). Plants were spaced 98 pots per plot. Plants were field grown from rooted cuttings (Monrovia Nursery, Azusa, CA) in 4.4 L pots (industry standard 1 gallon pots from Farrand Enterprises of Chino, CA) filled with 2:1 mixture of UC Soil Mix I (Matkin and Chandler 1957) and bark. Drip irrigation was applied regularly based on the evapotranspiration rate. Plants were fertilized with approximately 15 mL of Osmocote ® 18-6-12 (The Scotts Miracle-Gro Company of Marysville, OH) every 6 months. After the second year of growth, plants were hedged back to approximately 30 cm.

Meteorological data was retrieved from the University of California, Riverside Agricultural Operations station of the California Irrigation Management Information System to determine if weather was a factor in the movement, distribution, and/or dispersal of *A. colemani*. Weather determinants such as wind speed and direction have been shown to effect insect movement and dispersal (McManus 1988). In addition, temperature dependent development and survival is well documented among the insects (Gullan and Cranston 2010) with *A. colemani*'s optimal temperature estimated between 25 °C and 30 °C and the lower development threshold estimated by the Lactin 2 model to be 6.35 °C (Zamani et al. 2007). Wind speeds comparable to those recorded in this study, < 4.78 ms^{-1} (mean hourly), have shown little effect on minute hymenopteran

movement (Fournier and Boivin 2000{Corbett, 1996 #85) and therefore were not considered a factor in explaining the distribution and movement of *A. colemani* in response to resource availability. Temperature was also not regarded as an important explanatory factor despite deviation from the optimal temperature because developmental temperature extremes were not reached any time during the experiment.

2.1. Field dispersal in relation to resource availability

Field experiments were initiated to address questions about (1) the effect of trap height on detecting *A. colemani* and (2) the resident time of *A. colemani* in the presence and absence of resources. These data provided information about resource use by *A. colemani* and were necessary to devise the subsequent experiment (2.2).

Trap scaffolds consisting of a spray-painted black 5 gallon bucket filled with sand and 1 piece of 2.5 m rebar were placed in 6 different, equally spaced locations within the common garden containing *P. x fraseri*. Yellow sticky strip traps measuring 7.62 cm x 12.7 cm (BioQuip®, Ranch Dominguez, CA) were attached to the trap scaffold via the rebar using medium grip binder clips (Office Max®, Naperville, IL) at 0.5, 1, 1.5, 2, and 2.5 m from the ground. Both sides of the sticky traps were deployed and positioned facing east and west. Prior to the beginning of the experiment, background levels of *A. colemani* were zero. The experiment was initiated with the release of 2000 commercially available *A. colemani* parasitoids, emerged adults and mummies, (Rincon-Vitova Insectaries, Ventura, CA) from the center of the common garden plot upon arrival from

the supplier. The yellow sticky traps were replaced every 24 hours for 7 days. The number of *A. colemani* on sticky traps was recorded.

Two experiments, separated by time, were performed; one in the presence of *A. gossypii* and its honeydew, a food resource for *A. colemani*, and one in the absence. *A. gossypii* naturally colonized *P. x fraseri* plants in the common garden and were in patches throughout. The with-resource experiment consisting of *A. gossypii* feeding on *P. x fraseri* was completed three times during November 2007. The without-resources experiment, no *A. gossypii*, was executed three times during February 2008.

The two experiments were analyzed separately due to their temporal separation for the effects of date at 3 levels, height at 5 levels (0.5, 1.0, 1.5, 2.0, and 2.5 m), trap direction at 2 levels (east and west), and time at 7 levels (1, 2, 3, 4, 5, 6, and 7 days). The dependent variable, number of parasitoids, was rank transformed by all observations (RT-1) to an ordinal scale prior to analysis. An ANOVA on the ranks was performed on each data set separately using PROC GLM (SAS Institute 2011) . Based upon non-significant date and trap direction effects, these factors were removed from further analyses. Separate non-parametric repeated measures models using Friedman's test (Sokal and Rohlf 1981, Conover 1999) were constructed to determine the effects of height and time on the abundance of *A. colemani* using the with-resources and without-resources data sets. The analysis was run using PROC FREQ (SAS Institute 2011) with a cmh2 option to request row mean scores that are equivalent to ANOVA statistics (SAS Institute 2011). The dependent variable, number of parasitoids, was rank transformed by subject (RT-2)

to an ordinal scale as required by the analysis (Conover and Iman 1981). The null hypothesis for both models was the Chi-Square distribution of the ranked samples taken over time are the same for all heights. Original data are presented.

2.2. Distribution, movement and impact in relation to food resources

Resource treatments of flowers, aphids, and flowers plus aphids, were randomly assigned to each plot within the 3 blocks of the common garden (Figure 3.1). The control treatment consisted of bare ground in order to differentiate between treatments and crop. All the plots were split into a resource treatment sub-plot and a crop sub-plot with *P. x fraseri* serving as both the resource treatment plant and the crop plant. The crop sub-plot was included to determine if *A. colemani* would move from resource treatment sub-plots to crop sub-plots. Crop plants were trimmed to remove any new growth that supports *A. gossypii*. The resource sub-plot of the block 1 aligned with the resource sub-plot of block 2 and the crop sub-plot of the block 2 lined up with the crop sub-plot of the block 3 (Figure 3.1).

Prior to the experiment beginning, plant bloom and aphid immigration were monitored. Once the flower-only treatment plots reached 100 % of treatment plants in bloom, colony-reared aphids were applied to the aphid only treatment and flower plus aphid treatment such that 15 % of the plants in each plot were infested with 5 terminals of *A. gossypii*. Infestations were verified after 24 hours. The number of plants in bloom was limited to 50% of the plants in each plot for the flower plus aphid treatment. In

addition, another 15% of the treatment plants in each plot were aphid-infested and the remaining 35 % were without flowers or aphids.

Resource plants were marked with commonly available food product proteins, known to be detectable by enzyme-linked immunosorbent assay (Nordlee et al. 1988, Jones et al. 2006), in order to attempt to identify the movement of *A. colemani*. A 5% solution of the following proteins was sprayed to run-off using a Chapin® Home and Garden sprayer: (1) chicken egg albumin (egg whites) on flowers treatment plants, (2) bovine casein (cow's milk) on aphids treatment plants, (3) soy protein (soy milk) on control treatment plants, and (4) peanut butter proteins (peanut butter) on aphids x flowers treatment plants. The proteins were applied 24 hours before the beginning of the experiment and then again 2 weeks post experiment initiation. As this is a novel method for marking parasitoids, there were no data about the effects of the marking proteins on parasitoid behavior. Marking data are not presented as the proteins were not detected on the captured parasitoids.

The experiment began on 9 Apr 2008 when 1000 commercially available *A. colemani* (Rincon-Vitova Insectaries, Ventura, CA) were released into the center of the common garden upon their arrival from the supplier. One yellow sticky trap, the same type used in the previous experiment, was attached to the center plant of the treatment sub-plot and to the center plant of the associated crop sub-plot (2 per plot) for 24 hours. As there were no plants in the control, the sticky trap was attached to a 4.4 L pot. One sentinel aphid plant with new growth and 1 sentinel parasitoid plant with *A. gossypii*

feeding on new growth were placed equidistant in the crop sub-plot for 24 hours. The 2 yellow sticky strip traps and both sentinel plants were replaced every 7 days for 5 weeks. Sentinel aphid plants were assessed in the field for aphid colonization and sentinel parasitoid plants were held under natural light conditions at 27 ± 3° C with 25 ± 5% RH for 10 days before mummies were counted. The number of *A. colemani* individuals on the yellow sticky strip traps were counted post experiment.

A repeated measures model using PROC MIXED (SAS Institute 2011) was constructed with post-hoc comparisons using Duncan's Multiple Range at a 0.05 significance level to investigate the effects of treatment at 4 levels (flowers, aphids, flowers plus aphids, and bare-ground control), time at 5 levels, and the interaction between treatment and time on the abundance of parasitoids in the treatment sub-plot. Model adequacy was verified for this and all the following models. Data were transformed due to moderate negative skewness and lack of normality by computing the square root of a reflection equal to the difference between the largest score plus 1, and each score (Tabachnik and Fidell 2012). Additional post-hoc by-date ANOVA models using PROC GLM were generated to explore the effects of time and resource treatment on parasitoid abundance. Original data are presented here. In order to examine the movement of parasitoids from resources into the crop, a proportion was calculated to be the number of parasitoids in the crop sub-plot divided by the total number of parasitoids in both the treatment and crop sub-plots. A repeated measures model examining the effects of treatment at 4 levels (flowers, aphids, flowers plus aphids, and bare-ground

control), time at 5 levels, and the interaction between treatment and time on the proportion parasitoid in the crop was formulated using PROC MIXED (SAS Institute 2011).

3. Results

3.1. Effect of resource availability on field dispersal

3.1.1. Effect of height and time on dispersal in an environment with resources

Parasitoids were recovered at all heights (0.5, 1, 1.5, 2.0, and 2.5 m) for all 7 days of the experiments with resources present except for Day 3 at 2.5 m (Figure 3.2). In general, the number of parasitoids captured was low. The largest daily average catch was 1.35 individuals on Day 7 at 0.5 m (Figure 3.2). The largest per sticky card recovery was 12 individual parasitoids on Day 6 and Day 7 for both 0.5 m and 1 m trap heights along with Day 4 at 1.5 m.

There was a significant difference in the distribution of the ranked samples of parasitoids at different heights taken over time for the with-resources experiment (Friedman's $\chi^2 = 173.64$, $p < 0.0001$) (Figure 3.2). The greatest number of parasitoids was captured at 0.5 m on all dates (Figure 2). Overall, on all days, the higher the trap height, the smaller the average number of captured parasitoids except for Day 1 from 1.5 m to 2 m and Day 5 from 1 m to 1.5 m (Figure 3.2).

3.1.2. Effect of height and time on dispersal in a resource rich environment

In the experiments without resources present, parasitoids were only recovered through Day 4 (Figure 3.3). The only day with parasitoids at all heights (0.5, 1, 1.5, 2.0,

and 2.5 m) was Day 1 (Figure 3.3). Parasitoid individuals were detected at the 0.5 m height on Day 1 as well as the remaining 3 days (Figure 3.3). Overall, the number of parasitoids captured was low. The largest daily average catch was 0.60 individuals on Day 1 at the 0.5 m trap height (Figure 3.3). The largest per sticky card count was 4 individuals on Day 1 at the 1.0 m tarp height.

For the without-resources experiment, there was a difference in the distribution of the ranked samples of parasitoids at different heights taken over time (Friedman's χ^2 = 71.84, $p < 0.0001$) (Figure 3.3). The mean number of parasitoids at the 0.5 m trap height was always greater than the other 4 heights across dates (Figure 3.3).

3.2 Effect of food resources on the distribution, movement, and impact

A. colemani parasitoids were captured in both the treatment and crop sub-plots for the first 3 weeks of the 4 week experiment. The greatest average abundance of parasitoids in the treatment sub-plots (all treatment means greater than 20 parasitoids) was collected 24 hours post experiment initiation (Figure 3.4). Mean parasitoid abundance declined in all treatment sub-plots after 7 days and then fluctuated through the remaining 21 days. There was a significant effect of time ($F_{4,32} = 24.83$, $p < 0.001$) and the interaction between resource treatment and time date ($F_{12,32} = 2.73$, $p = 0.0115$). The effect of resource treatment was significant at the $\alpha = 0.11$ level ($F_{3,8} = 2.78$, $p = 0.1104$). Mean parasitoid abundance through time was significant for the first date, 24-hours post experiment initiation, while all other dates were the same (Figure 3.4). Comparing mean abundance of parasitoids at 24-hours, the flowers plus aphids treatment had the most

parasitoids followed by the aphids treatment ($F_{3,11} = 2.75$, $p = 0.1126$) (Figure 3.4). Seven days after the beginning of the experiment, the mean abundance of parasitoids in the aphids treatment was 3 times larger than the bare-ground control treatment and 6 times larger than the flowers plus aphids treatment ($F_{3,11} = 3.45$, $p = 0.0715$) (Figure 3.4). There were no significant differences between resource treatments on any of the dates for the last 3 weeks of the experiment (Figure 3.4).

The repeated measures model for the mean proportion of parasitoids in the crop sub-plot was only significant for the effect of time ($F_{4,32} = 4.63$, $p = 0.0046$) (Figure 3.5). There was no effect of treatment on the mean proportion of parasitoids in the crop ($p = 0.8345$) nor the interaction between treatment and time ($p = 0.1540$) (Figure 3.5). Resource treatment does not have an effect on movement from a resource into the crop.

There were no aphids recovered on the sentinel aphid plants and no parasitized *A. gossypii* on the sentinel parasitoid plants.

4. Discussion

Monitoring parasitoid abundance and movement in relation to resource availability is essential to understand parasitoid resource use patterns so habitats can be manipulated to reduce pest populations. The greatest number of parasitoids was captured at 0.5 m off the ground and this distance is equivalent to the height of the experimental plant, *P. x fraseri*. Although *A. colemani* was captured at all heights ranging up to 2.5 m above the ground, their presence in greater numbers at plant level may indicate parasitoids, searching for resources, move from plant to plant within a habitat.

Parasitoids may preferentially move at plant level to avoid the turbulence generally associated with the area above the plant boundary layer. This explanation has also been suggested for the flight patterns of an egg parasitoid, *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae), in California citrus orchards found most frequently at ground level where flight is assumed to be easiest to control (Hoddle and Boyd, unpublished data). Knowledge of where to track *A. colemani* may assist in the efforts to understand parasitoid movement patterns in relation to resource availability.

A. colemani is an obligate adult-feeding parasitoid who must procure resources to sustain adult life (Chapter 2). Adult-feeding, mated parasitoids move in search of resources, both food and hosts, such that their ability to survive and reproduce is maximized (Sirot and Bernstein 1996, Casas et al. 2003). Thus their realized fitness is dependent upon partitioning their efforts between host foraging and food foraging (Lewis et al. 1998). For non-host-feeding species, the decisions to seek out resources are complicated, because food and hosts may be in different locations (Lewis and Takasu 1990). For host-feeders and host-by-product feeders, this choice is simplified, because hosts and food reside together (Heimpel and Rosenheim 1995), thereby reducing the need for movement and its associated costs and risks. Once a host-feeding parasitoid encounters a single host, a choice is made to either move-on, reproduce in, or feed on that individual. Conversely, host by-product feeders like *A. colemani* do not have to choose between reproduction and feeding because an encounter with a single host is an opportunity to fulfill both fitness requirements (Chapter 2).

Based on an understanding of parasitoid foraging and dispersal, we would expect resource availability to effect the movement of *A. colemani* as adult-feeding parasitoids must procure food and hosts to ensure survival and reproduction. *A. colemani* individuals remained in a resource rich habitat filled with hosts and food for the length of the field dispersal experiment, 7 days. In the absence of resources and at a different time of year, parasitoids were detected for only 4 days. Previous laboratory experiments included in this dissertation suggested the mean longevity of *A. colemani* was less than 7 days in the presence of various floral and host by-product food resources but no more than 1 day in the absence of food resources (Chapter 2). These field results possibly indicate the laboratory studies underestimated the longevity of *A. colemani* just as other laboratory experiments have (de F. Torres et al. 2007). This is further supported by the fact that background abundance of *A. colemani* at the beginning of this field study was 0. Despite this, *A. colemani* may benefit from the provisioning of food resources in terms of survival and retention in a resource rich environment if this effect translates into increased reproduction. This is the core of Root's natural enemies hypothesis (1973), provisioning resources lacking in agriculture systems encourages predators and parasitoids that inhibit potential pests.

The full extent at which *A. colemani* may benefit from food and/or hosts has yet to be determined based on a single year field study and furthermore, the impact this has on *A. gossypii* populations is also unknown. An experiment attempting to estimate the impact resources have on the control of *A. gossypii* was unsuccessful, possibly due to a

short host exposure period. Sentinel parasitoid plants were placed in the field for 24 hours in order to prevent 100 percent parasitization but this time may not have been long enough to successfully detect reproductive activity (data not presented).

Field resource use experiments may provide critical data about the distribution and movement of biological control agents. This important information coupled with parasitism rates would be useful for determining the degree at which provisioning resources translates into control of target pest populations (Gurr et al. 2000). Of the 3 resources assessed, the largest abundance of parasitoids was initially found in the combination resource treatment consisting of flowers x aphids. This distribution of individuals was partially expected based on the assumption that resource availability effects the movement of *A. colemani* such that fitness is maximized and the cost of movement is minimized.

Individual *A. colemani* were expected to associate equally with treatments where both food and host resources are found in the same location, e.g., aphids treatment and flowers x aphids treatment. This expectation is based on the assumption that both food resources, aphids and flowers, are equivalent (Chapter 2). However, a growing number of studies have suggested that floral food like nectar has some nutritive advantage over host by-product food like honeydew (Wäckers 2000, Wäckers et al. 2008). Although this idea is rooted in feeding studies of non-aphidiine parasitoids (Wäckers 2001, Luo et al. 2010), anomalies emerge with a review of the literature. An analysis of published data compared longevity ratios (non-honeydew sugars /honeydew) for parasitoids of

honeydew excreting hosts versus parasitoids of non-honeydew hosts and reported greater longevity ratios for parasitoids of honeydew excreting hosts (Wäckers et al. 2008). Since these ratios were not statistically different based on the conservative Wilcoxon's rank sums test, the hypothesis that parasitoids of honeydew excreting hosts are more adapted to honeydew sugars than parasitoids of non-honeydew hosts lacked support. Alternatively, soft scale (Irvin and Hoddle 2007) and aphid honeydew (England and Evans 1997, Fuchsberg et al. 2007) have both improved the longevity of parasitoids of non-honeydew excreting hosts and the longevity data was comparable to non-honeydew sugar sources. Finally, naïve *Aphidius ervi* Haliday (Hymenoptera: Braconidae: Aphidiine) showed no preference for honeydew or nectar when given a choice and only a slight preference for nectar once experienced with honeydew (marginally significant) (Vollhardt et al. 2010). These anomalies highlight the variation of honeydew suitability and question the generalization that honeydew is an inferior food resource.

While the initial distribution of *A. colemani* was greatest in the aphids x flowers treatment, the 7-day distribution was concentrated in the aphids treatment. These different results may be explained by the laboratory results presented in this dissertation indicating no difference in the longevity and fecundity of *A. colemani* fed on floral nectar and honeydew (Chapter 2). It may be possible that if given a choice, *A. colemani* would prefer the more nutritional floral nectar. In addition, the conventional method of provisioning floral nectar may benefit from the presence of aphid hosts and their by-products in a system where aphidiine parasitoids need a nutritional boost in order to

control pestiferous aphid populations. This habitat manipulation tactic combining food and host resources may be successful if the provisioned floral food is different from the crop and the provisioned aphid species is unable to feed on the crop.

Parasitoids may indicate the benefit of a resource by preferentially associating with it. Many studies have demonstrated that parasitoids associate with floral resources (Jervis et al. 1993, Tooker and Hanks 2000, Hogg et al.). The question remains if this aggregation impedes the movement of parasitoids into neighboring crops and ultimately, the colonization of pest populations. The movement of *A. colemani* was estimated using the mean proportion of *A. colemani* in the crop. Although there were no statistical differences between resource treatments, *A. colemani* did move into the crop and nearly half of the total parasitoids were collected in the crop associate with each of the 3 resources. A mark-capture study with *Diadegma semiclausum* (Hellen) (Hymenoptera: Ichneumonidae), the larval parasitoid of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), found the parasitoid moved into the brassica crop from the brasscia flowering refuge. Additionally, male and female *D. semiclausum* exhibited a random spatial pattern (Schellhorn et al. 2008). Movement out of a food resource and into an adjacent crop is consistent with movement patterns that maximize a parasitoid's fitness. Once longevity has been ensured, host foraging would most likely be initiated. The movement patterns exhibited by *A. colemani* and *D. semilausum* add support to the idea that choices to either host forage or food forage are based on maximizing fitness.

The findings from the distribution and movement study should be considered with caution as the experiment was only performed for 1 season and the statistical significance level of $\alpha = 0.11$ is less than the standard significance level. Despite these concerns, the resource use patterns of *A. colemani* may further the theory that in a resource rich environment, parasitoids are most likely switch-foraging between hosts and food in order to maximize their fitness. Given this relationship, habitat manipulation techniques that provision accessible and available food resources in proximity to the crop would initially move parasitoids to the edible resource. Following satiation, parasitoids would most likely switch forage and move to find nearby hosts in the crop.

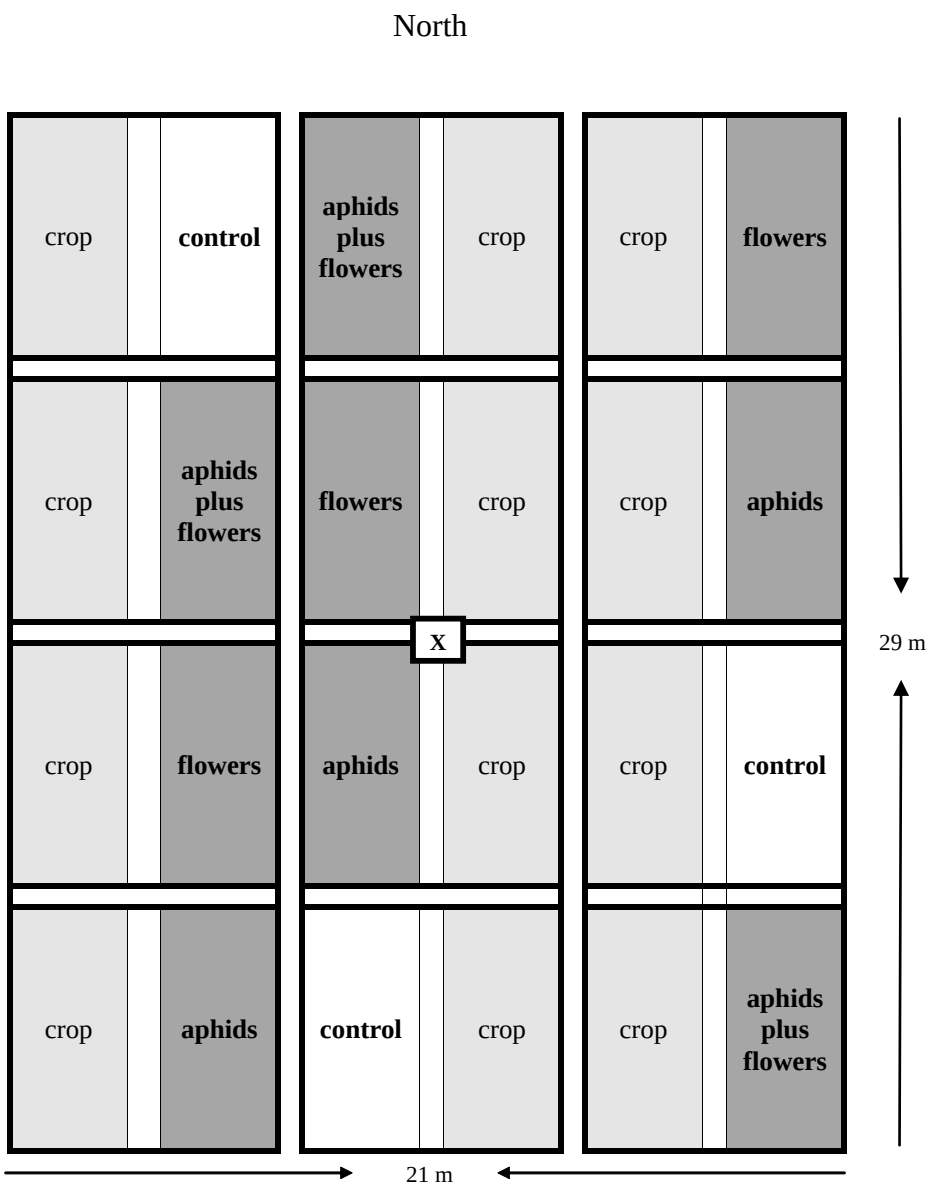

Figure 3.1. Diagram of experimental layout. "X" indicates release point of *A. colemani* individuals.

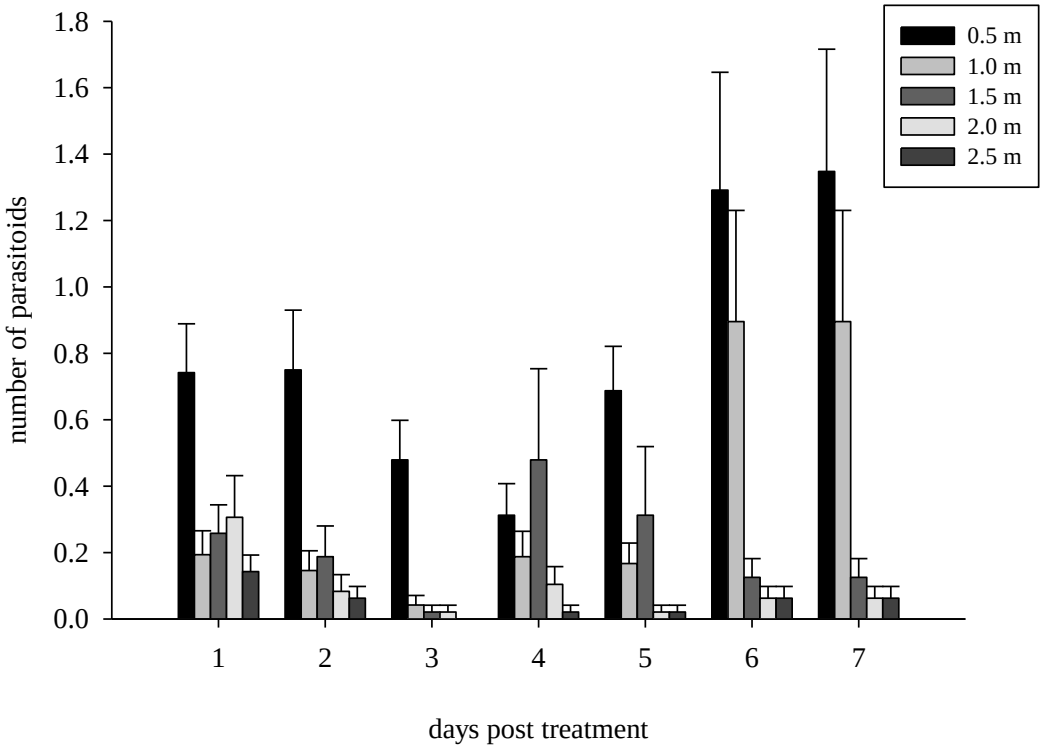

Figure 3.2. Mean number of *A. colemani* parasitoids captured at 5 different heights during a 7-day period during November 2007 in the presence of aphid resources.

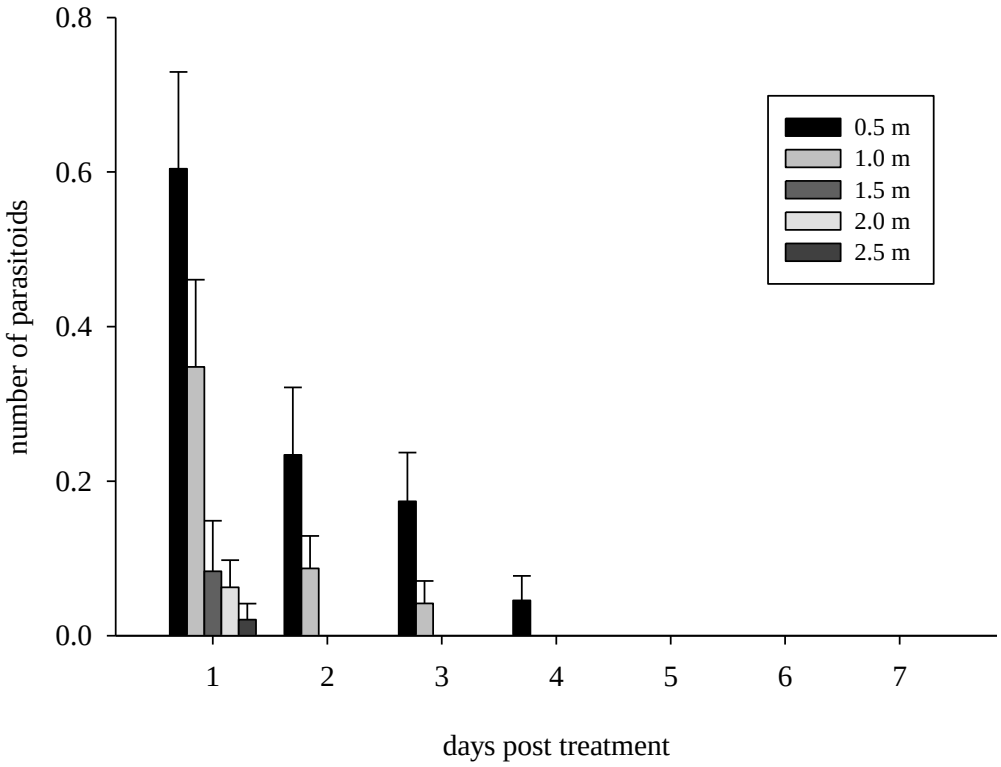

Figure 3.3. Mean number of *A. colemani* parasitoids captured at 5 different heights during a 7-day period during February 2008 in the absence of aphid resources.

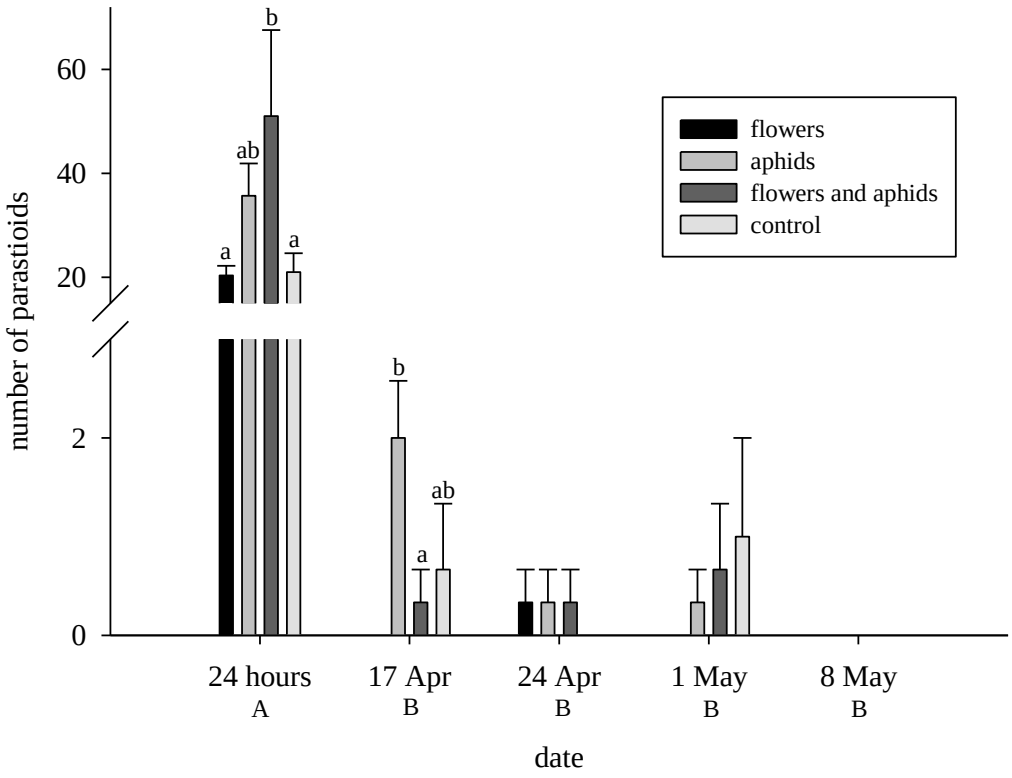

Figure 3.4. Mean number of *A. colemani* captured in 3 resource treatments and the control during a 4-week period. Uppercase letters represent significant differences ($p = 0.1126$) for the effect of date. Significant differences among resource treatments by-date are noted in lowercase letters ($p = 0.0715$).

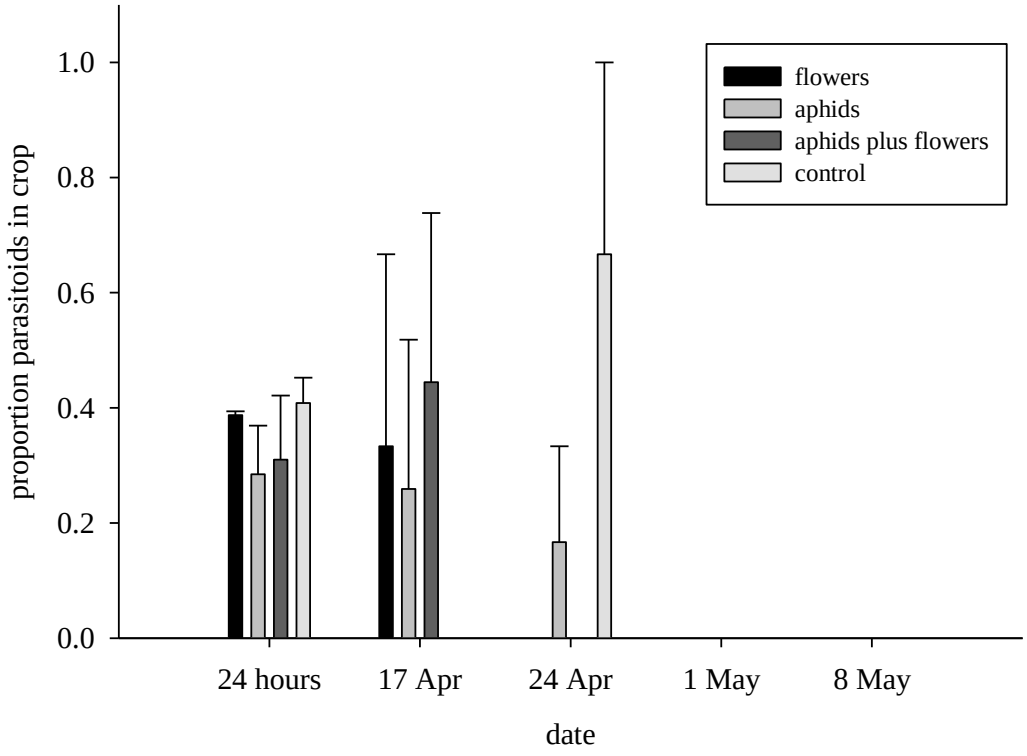

Figure 3.5. Mean proportion of *A. colemani* captured in the crop associated with 3 resource treatments and the control during a 4-week period. Different letters represent significant differences ($p < 0.05$) for the effect of date.

Chapter 4. Plant mediated effects of the systemic insecticide, imidacloprid, on the survival of the polyphagous aphid parasitoid, *Aphidius colemani* (Hymenoptera: Braconidae: Aphidiinae)

1. Introduction

The integrated concept as first introduced by Stern et al. (1959a) revolutionized the management of pests by suggesting that chemical and biological control methods could be compatible. Questions of integration asked if these control methods or tactics could be unified under a single strategy and advanced the development of a new idea, Integrated Pest Management (IPM). Since the concept origination (Stern et. al, 1959), our understanding of IPM has melded into a universally recognized strategy that utilizes a myriad of tactics to not only achieve population control below the economic injury level, but to consider the comprehensive risks involved with the implementation of IPM (NCIPM 1994).

The integration question of the original IPM innovators remains relevant today as novel pest suppression tactics are integrated into agricultural pest management strategies. The release of imidacloprid, the first commercially available neonicotinoid insecticide, raised integration questions about the compatibility of chemical and biological control based on the compounds' novel application method. Soil-applied imidacloprid was initially considered to be less toxic to natural enemies as there was presumed to be no direct contact with the compound (Mizell and Sconyers 1992). A closer look at the routes of exposure for imidacloprid and its potential compatibility with biological control

are warranted in a system involving an aphid, one of its many host plants, and one of its parasitoids.

Aphids are ubiquitous, agricultural pests due to their high intrinsic rate of increase, effective reproductive strategies (parthenogenesis, telescoping generations, and viviparity), resistance to pesticides, role as virus vectors, and low aesthetic tolerance (Emden and Harrington 2007). There are more than 100 pestiferous species that feed on more than 243 plant species representing 62 families (Blackman and Eastop 2000, Emden and Harrington 2007). Aphid population management is typically achieved by chemical control utilizing compounds from many classes of insecticides (e.g., carbamates, organophosphates, and pyrethroids) (Wilen et al. 2002). Recent introductions of novel chemistries, such as the neonicotinoids, have the potential to change the practice of aphid IPM due to these compounds' systemic activity, extended residual activity, and reduced application rates.

Imidacloprid (1-[(6-chloro-3-pyridyl)methyl]-*N*-nitro-2-imidazolidinimine) is a chloronicotynl acting as an agonist at the nicotinic acetylcholine receptors (nAChRs) of insects (Bai et al. 1991, Tomizawa and Casida 2003) with more toxicity to insects than mammals due to differential receptor binding (Tomizawa and Casida 1999). Although foliar applications are included on the label, it is the systemic activity from soil applications that reduces potential contact with natural enemies and therefore, may allow for improved integration of chemical and biological control. Imidacloprid is active against numerous phloem and xylem feeding pests found in nursery production of

container-grown plants, including the melon aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), common to many ornamental plants, including *Photinia x fraseri* Dress (Rosaceae). The solitary endoparasitoid of aphids, *Aphidius colemani* Viereck (Hymenoptera: Braconidae: Aphidiinae), with cosmopolitan distribution (Stary 1975), is found throughout the containerized nurseries of Southern California and the surrounding native and urban vegetation. This proximity to the imidacloprid-rich nursery environment raises new questions of the integration of imidacloprid and *A. colemani* in the IPM of aphids.

Although there are sufficient studies about the effects of imidacloprid on natural enemies (Villanueva-Jimenez and Hoy 1998, Oliver et al. 2006, Cloyd and Bethke 2011, Prabhaker et al. 2011), only a few studies have investigated these effects on *Aphidius* sp. These studies have focused on the systemic, contact, and sublethal activity of imidacloprid. Experiments with *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae: Aphidiinae) found soil-applied imidacloprid had no impact on the instantaneous rate of population increase of the parasitoid as measured by a 24-hour exposure (Kramarz and Stark 2003). Sublethal imidacloprid spray treatments equivalent to half-field rate also had minimal impact on *A. ervi* survival as measured by 24-hour mortality (Araya et al. 2010). However, imidacloprid was considered harmful to *Aphidius gifuensis* Ashmead (Hymenoptera: Braconidae: Aphidiinae) with 71% of females dead after 24-hour exposure to field rate sprays (Kobori and Amano 2004).

Although soil-applied imidacloprid did not appear to negatively impact *A. ervi*,

other studies have reported deleterious effects on natural enemies treated with systemic imidacloprid. Experiments on the coccinilid beetle, *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinellidae), found both survivorship at day 30 and pre-oviposition period were only negatively affected for when the insect was raised on sunflower (Smith and Krischik 1999). Survival of the encyrtid parasitoid, *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae), was significantly reduced by 2.5 times after a one day contact period with flowers from plants treated with soil-applied imidacloprid at label rates (Krischik et al. 2007). Similar results were found for the green lacewing, *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae), with survival reduced by 5 times after ten days but no effect after one day of exposure (Rogers et al. 2007). An additional study looked at the interaction with two parasitoids of eucalyptus pests, a braconid and an encyrtid parasitoid, using nectar collected from trees treated with soil injected imidacloprid (Paine et al. 2011). This study found that concentrations of imidacloprid and its metabolites in nectar in the field were great enough to kill individuals of both species. Additionally, survival and reproductive fitness of the encyrtid, *Avetianella lonoi* Siscaro (Hymenoptera: Encyrtidae), were significantly lower when fed nectar from eucalyptus trees treated with imidacloprid as compared with nectar from non-treated trees.

This study aims to ask questions of integration by determining the compatibility of chemical and biological control through examining the effects of imidacloprid on the polyphagous aphid parasitoid, *A. colemani*, following the treatment of the containerized

nursery plant, *P. x fraseri*. First, we investigated the routes of exposure for imidacloprid to *A. colemani* by detecting the concentration of imidacloprid in the xylem, nectar, pollen, and leaves of *P. x fraseri.* Second, we determined LC_{50} of imidacloprid for *A. colemani.* Third, we determined the effect of imidacloprid in nectar and in leaves on the survival of *A. colemani*.

2. Materials and Methods

2.1 Insect and plant colonies

All plant and insect colonies were maintained at the University of California, Riverside. Greenhouse grown colonies of *Cuburbito pepo* L. 'Raven' (Johnny's Selected Seeds, Albion, ME) and *Brassica juncea* (L.) Czern. 'Florida Broad Leaf' (Ferry-Morse Seed Company, Fulton, KY) were cultivated in 0.92 L pots (industry standard 4" pots from Farrand Enterprises, Chino, CA) filled with UC Soil Mix I (Matkin and Chandler 1957) and fertilized with approximately 15 mL of Osmocote® 18-6-12 (The Scotts Miracle-Gro Company, Marysville, OH). Plants grew in ambient light conditions in a greenhouse at 25 ± 2° C with 25 ± 5% RH. After 3 weeks, *C. pepo* 'Raven' plants were infested with the aphid, *A. gossypii*. A second aphid, *Myzus persicae* (Sulzer), was reared on 3 week old *B. juncea* plants to serve as additional host material for the polyphagous parasitoid in order to simulate field conditions. After 1 week, the 2 aphid species on their respective host material were transferred to the greenhouse colony of the parasitoid, *A.colemani*. Parasitoids were held in the same greenhouse conditions mentioned above until mummies were removed for experiments.

A greenhouse colony of 3-year old *P. x fraseri* were grown from rooted cuttings obtained from Monrovia Nursery, Azusa, CA. Plants were cultivated in 4.4 L pots (industry standard 1 gallon pots from Farrand Enterprises, Chino, CA) filled with 2:1 mixture of UC Soil Mix I (Matkin and Chandler 1957) and bark (6.35 mm - 9.525 mm). Plants were fertilized on a 9 month cycle with approximately 15 mL of Osmocote® 18-6-12 (The Scotts Miracle-Gro Company, Marysville, OH). No new fertilizer was added to plants upon treatment as described below. Plants grew in ambient light conditions in a greenhouse at 27 ± 3° C with 25 ± 5% RH.

2.2. Insecticide treatment

Individual plants were selected from the *P. x fraseri* colony upon bud break, and all individuals in that budding cohort were randomly assigned to one treatment (full rate, half rate, or control). Imidacloprid plants were treated with a Merit 2F (Bayer CropScience, Monheim am Rhein, Germany) soil drench at the full label rate of 3 mL (0.84 g imidacloprid) per foot of shrub height or half label rate of 1.75 mL (0.42 g imidacloprid) per foot of shrub height. Control plants were treated with water in the same manner. Start dates varied throughout April and May of 2007. Once treated, plants were maintained in the greenhouse under natural light conditions at 27 ± 3° C with 25 ± 5% RH. Treatment plants received circa 800 mL water every other day until bloom when water was applied every day to insure optimum plant turgidity.

2.3. Investigation of plant-mediated exposure routes for imidacloprid to A. colemani

The routes of exposure for imidacloprid to *A. colemani* were investigated by measuring the concentration of imidacloprid in various plant fluids and tissues of *P. x fraseri*. Samples of xylem sap and nectar along with pollen and leaf tissue were collected from individual *P. x fraseri* plants and held in an ultracold freezer at -70^{o} C until processing. Plant collections were taken on 3, 5, 7, 10, 12, 14, 18, and 32 days post treatment. Xylem sap was extracted using the pressure bomb method introduced by (Castle et al. 2005). Microcapillary tubes (Drummond® Short-Length Microcaps® Micropipets) were used to draw off nectar from all accessible nectaries. Nectar was pooled from multiple inflorescences within each plant on a given day. Pollen was harvested from 3 different flowers within the same inflorescence for a total of 15 anthers per sample. A sub-sample of 10 anthers was used for the imidacloprid analysis. Leaf samples consisted of two types; old and new leaves. Old was defined as tough leaves that were dark green, thick, and waxy. New leaves were defined as tender leaves that were red and thin. Old leaves were collected from any location on the plant while new leaves were collected from the third oldest leaf in a shoot terminal.

To prepare the leaves for the imidacloprid analysis, each leaf was sub-sampled twice using a cork borer #4 (8 mm diameter). The 2 leaf discs were homogenized into a 10% methanol solution in a 1.5 mL Eppendorf ® tube by first chopping with a micro spatula followed by grinding with a Kontes pellet pestle®. The homogenate was shaken with an orbital shaker for 24 hours prior to analysis. A total of 15 *P. x fraseri* plants were

assessed with 5 replicates per treatment. The number of replicates was limited by the required balanced design of the repeated measures model.

Imidaclorpid concentrations were determined using the ELISA method described by Bryne et al., (2005) and Paine et al., (2011) using the QuantiPlate kit for imidacloprid (EnviroLogix, Portland, ME) with a sensitivity range of 0.2 - 6 µg imidacloprid L^{-1}. Samples were diluted from 10 to 25000 times (dilutions above 10000 were used only for the xylem and nectar from the full-label rate treatment) in order to meet the detectable range of the assay and to remove any matrix effects that cause false positives. Purification of imidacloprid using thin layer chromatography (TLC) was completed as described by Paine et al, (2011) to verify if the ELISA results were solely based on imidacloprid concentration (Nauen et al. 1998, Nauen et al. 1999) and not contaminated by the metabolites of imidacloprid that can also react with the antibodies of the kit (Byrne et al. 2005). The purified imidacloprid was assayed using the ELISA method. Proportion imidacloprid was calculated by dividing the ng imidacloprid (assuming imidacloprid-only detection in ELISA of plant fluids and tissues) by ng imidacloprid recovered from TLC purification and subsequent ELISA quantification.

The data set was separately analyzed by plant tissue (xylem sap, nectar, pollen, and leaf) for treatment effects at 2 levels (full label rate and half label rate) and time effects at 8 levels (3, 5, 7, 10, 12, 14, 18, and 32 days post treatment) based on imidacloprid concentration. Additionally, the xylem and nectar data were examined in order to determine if the imidacloprid concentration was different between xylem sap, a

source fluid, and nectar, a sink fluid. The effects of plant fluid at 2 levels (xylem sap and nectar), of treatment at 2 levels (full label rate and half label rate), of time at 8 levels (3, 5, 7, 10, 12, 14, 18, and 32 days post treatment), and of the interaction between treatment and time on imidacloprid concentration were analyzed. A total of 5 ANOVA repeated measures models, one for each plant tissue and one for the comparison of xylem and nectar, were constructed in SAS 9.3's PROC MIXED at a 0.05 significance level with model adequacy verification (Inc. 2011). Additional post-hoc ANOVA models were generated for each of the plant fluids or tissues with significant main effects to differentiate between insecticide treatments (full rate and half rate) and date post treatment (3, 5, 7, 10, 12, 14, 18, 32 days). Post-hoc by-date ANOVA models (one-way) compared treatment means on each date using Tukey's HSD method ($p < 0.05$). The by-rate ANOVA models (one-way) compared dates for each treatment using Tukey's HSD method ($p < 0.05$). All post-hoc models were constructed in SAS 9.3's PROC GLM at a 0.05 significance level with model adequacy verification (Inc. 2011). Type I error rate in the post-hoc models was controlled for using Šidák's procedure. The additional age factor present in the leaf data set required the post-hoc analysis to be a 2-way ANOVA model (by-date and by-rate) in order to determine differences between leaf age. Data presented in this paper have been back transformed to the original values.

2.4. Bioassays

2.4.1. Probit analysis for lethal concentration of imidacloprid

The lethal concentration of imidacloprid against *A. colemani* was determined

using a serious of dilutions of imidacloprid in honey-water (50:50). Concentrations (ppb) tested were 0, 100, 200, 300, 400, and 500. Ten female parasitoids, less than 24 hours old, were exposed to a 3 µL droplet of one concentration that was placed on the bottom of a 40 dram plastic vial vented with a 2.5 cm diameter mesh-covered hole on top. Mortality was recorded after 24 hours. 250 individuals were tested per imidacloprid concentration. Environmental conditions during the experiment were 25 ± 1° C with 15 ± 10 % RH under inflorescent lighting with a L14:D10 photoperiod.

Probit analysis (Finney 1971) was used to determine the lethal concentration at which 50 percent of the individuals within the sampled population would die (LC_{50}). Abbot's correction for control mortality (Abbott 1925) was applied to the data prior to analysis using PROC PROBIT in SAS 9.3. (Inc. 2011).

2.4.2. Plant-mediated toxicity

Plant-mediated effects of imidacloprid on the parasitoid, *A. colemani*, were tested using the leaves and nectar of the plant, *P. x fraseri*. Old leaves of *P. x fraseri* plants treated as described in the above insecticide treatment section were loaded into modified Munger cells (Munger 1942, Morse et al. 1986) made of Plexiglas. Old leaves were removed 7 days post treatment. One leaf was placed into each cell. Two additional binder clips were used to secure the cells instead of the 2 rubber bands in the original design. One unmated female *A. colemani*, less than 24 hours old, was enclosed in each cell for 24 hours at which time survival was recorded. Treatments included imidacloprid full label rate (0.84 g imidacloprid pot $^{-1}$), imidacloprid half label rate (0.42 g

imidacloprid pot^{-1}), and blank control. The experiment was performed on various dates during April and May 2007 based on treatment dates of *P. x fraseri*. Environmental conditions during this experiment were 25 ± 1° C with 15 ± 10 % RH under inflorescent lighting with a L14:D10 photoperiod. A total of 12 replicates per treatment were tested before the experiment was terminated due to no effect of treatment.

Nectar collected from *P. x fraseri* plants in the aforementioned experiment (2.3) was presented to *A. colemani* caged in 40 dram plastic vials with mesh-covered 2.5 cm diameter vent holes on top. Ten female parasitoids less than 24 hours old were given a 3 µL droplet of nectar from imidacloprid treated plants, applied to the bottom of the cage. Survival was recorded after 24 hours. Treatments included nectar with 6 different imidacloprid concentrations (0, 217, 1550, 20250, 25400, and 56230 ppb) along with a honeywater control treatment. The experiment was performed on 6 August 2009 generating 3 replicates per treatment. The number of replicates was restricted by the amount of nectar remaining after imidacloprid quantification by ELISA (2.3). Environmental conditions during the experiment were 25 ± 1° C with 15 ± 10 % RH under inflorescent lighting with a L14:D10 photoperiod.

The nectar data set was analyzed for the effect of treatment, imidacloprid concentration at 6 levels (0, 217, 1550, 20250, 25400, and 56230 ppb) with 3 replicates per level, on the survival of *A. colemani*. A one-way ANOVA model was constructed at a 0.05 type I error rate with model adequacy verification. Due to a significant treatment effect, Tukey's method (HSD) was used to differentiate between treatment means.

Analyses were conducted using PROC GLM in SAS 9.3 (Inc. 2011).

3. Results

3.1. Plant-mediated exposure routes for imidacloprid

Imidacloprid was detected in all plant tissues at both the full label rate (0.84 g imidacloprid pot^{-1}) and half label rate (0.42 g imidacloprid pot^{-1} rate) beginning at 3 days post treatment (Tables 4.1, 4.2, 4.3, and 4.4). The amount of imidacloprid generally increased with time for all plant tissues except for pollen. The imidacloprid concentration measured in pollen did not appear to follow any pattern (Table 4.2). The exceptions to the gradual increase were xylem on day 10 at the half label rate (Table 4.1), old leaf tissue on day 12 at the label rate (Table 4.3), and new leaf tissue on day 14 and 18 at the label rate (Table 4.3). All of these measurements were slight decreases in imidacloprid concentration from the previous sample day.

A sub-sample of nectar, pollen, and leaf tissues were purified using thin layer chromatography to verify the ELISA results were based solely on imidacloprid. The proportion of imidacloprid recovered suggested that the concentration detected by the ELISA is imidacloprid (Figure 4.1) although metabolites may also have been present.

3.1.2 Imidacloprid uptake in xylem sap

A significant effect of imidacloprid treatment ($F_{2,12}$= 219.76, $p < 0.0001$), time ($F_{7,84}$ = 44.94, $p < 0.0001$), and the interaction between treatment and time ($F_{14,84}$ = 40.03, $p < 0.0001$) was found for the repeated measures xylem model (Table 4.1). There was no significant difference between treatments for days 3, 5, 7, 10, and 12 (Table 4.1). On day

14 the mean imidacloprid concentration in the full label rate treatment was ten times greater than the half label rate (Table 4.1). Mean imidacloprid concentration on days 18 and 32 was 20-fold higher in the full label rate treatment as compared with the half label rate (Table 4.1). The concentration of imidacloprid in the xylem sap of plants treated with either full label rate or half label rate does not become statistically different until 14 days post treatment (Table 4.1).

The increase in mean imidacloprid concentration in the xylem sap over time was more pronounced for the full rate treatment than the half rate treatment (Table 4.1). The average concentration of imidacloprid in the xylem sap of plants in the half rate treatment on day 32 was 19-fold higher than day 3 while the full rate treatment plants exhibited a 168-fold difference between day 32 and 3 (Table 4.1). This difference in the overall change in mean imidacloprid concentration for xylem sap may explain the interaction between treatment and time.

The relationship between the amount of imidacloprid applied to the soil and the amount of uptake in the xylem was not direct. Doubling the application rate did not double the uptake amount on days 5, 7, 10, 12, 14, 18, and 32 but rather increased the mean concentration imidacloprid between 4-fold and 20-fold (Table 4.1).

3.1.3 Imidacloprid in nectar

There was a significant effect of imidacloprid treatment ($F_{2,12}$= 197.59, $p <$ 0.0001), time ($F_{7,84}$ = 36.65, $p < 0.0001$), and the interaction between treatment and time ($F_{14,84}$ = 24.66, $p < 0.0001$) for nectar (Table 4.2). There was no significant difference of

mean imidacloprid concentration in nectar on days 3, 5, and 7 (Table 4.2). Differences in the mean concentration of imidacloprid between treatment rates were less than detected for xylem. The average imidacloprid concentration for the full rate was significantly greater than the half rate on days 10, 12, 14, 18 and 32 with increases from 6-fold (day 32) to 16-fold (day 12) (Table 4.2). Translocation of imidacloprid through the phloem to the nectaries was detectable on day 3, 5, and 7 but day 10 is the first day the average imidacloprid concentration in the nectar was statistically different between treatment rates. The change of mean imidacloprid concentration found in nectar through time was different for the full rate and half rate treatments increasing 80-fold and 70-fold, respectively from day 3 to day 32 (Table 4.2). This difference between mean imidacloprid concentration over time for the two treatments may explain the significant interaction effect.

3.1.3 Imidacloprid in pollen tissue

The repeated measures model for pollen was only significant for imidacloprid treatment ($F_{2,12} = 4.25$, $p = 0.0403$) (Table 4.3). Time and the interaction between treatment and time were not significant. The mean concentration of imidacloprid in pollen was no different between full label rate and the half label rate for all dates (Table 4.3).

3.1.4 Imidacloprid in leaf tissue

A significant effect of leaf age ($F_{1,14} = 8.14$, $p = 0.0128$), imidacloprid treatment ($F_{2,14} = 145.19$, $p = < 0.0001$), time ($F_{7,84} = 15.99$, $p = < 0.0001$), and the interaction

between time and treatment ($F_{14,84} = 12.12$, $p = < 0.0001$) was found for the repeated measures leaf tissue model (Table 4.4). There were no significant differences between old and new leaves for the half label rate treatment on all dates (Table 4.4). Day 32 was the only day that the mean imidacloprid concentration in the old leaves was statistically greater than the new leaves for the full label rate treatment (Table 4). The mean imidacloprid concentration in both leaf ages on all days was greatest in the label rate treatment as compared to the half label rate ($p < 0.05$) (Table 4.4).

The mean concentration of imidacloprid in both the new and old leaves increased with time for both the full label rate and half label rate treatments (Table 4.4). Mean imidacloprid concentrations in new leaves from plants treated with the full label rate were statistically different over time exhibiting a 50-fold increase in imidacloprid concentration from day 3 to day 32 (Table 4.4). In contrast, the mean imidacloprid concentration in new leaves from plants treated with the half label rate did not statistically differ through time despite a 256-fold increase (Table 4.4). The change in mean imidacloprid concentration after 32 days in new leaves was 5-fold greater for the half label rate as compared to the full label rate (Table 4.4), a deviation from the relationship between the two label rates found in xylem and nectar. The full label rate for both xylem sap and nectar exhibited a larger mean imidacloprid increase over the 32 day experiment than the half label rate (Tables 4.1, 4.2, and 4.4). Similarly the mean imidacloprid concentration in the old leaves of the half label rate treatment increased more through time than the full label rate treatment, 101-fold as compared with 64-fold

(Table 4.4). The interaction between treatment and time may be explained by the difference in the overall change, from day 3 to day 32, in mean imidacloprid concentration between the full label rate and half rate for new leaves (5-fold difference) and old leaves (1.5-fold difference) (Table 4.4).

3.1.5 Comparison of imidacloprid in xylem sap and nectar

In the analysis of plant fluids, there was a significant effect of imidacloprid treatment ($F_{1,8} = 101.01$, $p < 0.0001$) and time ($F_{7,63} = 21.81$, $p < 0.0001$), but not the interaction between treatment and time ($F_{7,63} = 0.330$, $p = 0.9393$). Mean concentration of imidacloprid in xylem sap and nectar are not different as indicated by the non-significant effect of plant fluid ($F_{1,9} = 2.46$, $p = 0.1510$).

3.2. Bioassays

3.2.1. Effect of imidacloprid toxicity on A. colemani

A Probit analysis of a range of imidacloprid concentrations in honey-water fed to female *A. colemani* estimated the LC_{50} of imidacloprid to be 326.69 ppb (sd = 2.59, χ^2 = 238.2, df = 1, p <0.0001, y = -6.0910x + 2.42). The LC_{75} of imidacloprid was estimated to be 620.21 ± 2.59 ppb and the LC_{90} to be 1104 ± 2.59 ppb. This concentration was found in the xylem sap and nectar of *P. x fraseri* treated with imidacloprid at the full label and half label rate. The only exception for the xylem data was on day 3 for the half label rate (265.08 ppb) and the only exception for the nectar data was for the same date and treatment rate (259.38 ppb) (Tables 4.1 and 4.2).

3.2. 2. Plant-mediated effects of imidacloprid on the survival of A. colmeani

Female *A. colemani* were exposed to leaves and nectar from plants treated with imidacloprid at full and half label rates. Old leaves treated with imidacloprid had no effect on the survival of *A. colemani*. All female wasps caged with old leaves from the full label rate, half label rate, and blank control treatments survived for 24 hours. There was an effect of imidacloprid treatment ($F_{6,14} = 85.78$, $p < 0.0001$) on the survival of female *A. colemani* fed nectars containing a range of imidacloprid concentrations derived from plants treated with imidacloprid (Figure 4.2). As imidacloprid concentration in nectar increased, survival decreased (Figure 4.2). The survival of *A. colemani* was significantly greater when given either the honey-water or nectar-only treatment as compared with all the other treatments of nectar from imidacloprid treated plants (Figure 4.2). Survival of *A. colemani* was 32 ± 3.30 % for the 217 ppb imidacloprid treatment (Figure 4.2). The Probit analysis predicted a greater survival of 65% for an imidacloprid concentration of 226.51 ± 2.59 ppb. There was a 14 ± 3.59 % survival for the 1550 ppb treatment (Figure 4.2) while the Probit analysis predicted only 5 % survival for an imidacloprid concentration of 1560 ± 2.59 ppb.

4. Discussion

The neonicotinoid, imidacloprid, was detected in the xylem, leaves, nectar, and pollen of the treated *P. x fraseri.* The presence of soil-applied imidacloprid in the xylem is consistent with other crops such as avocado (Byrne, 2010), citrus (Castle et. al, 2005), and grapes (Byrne et. al, 2005). Imidacloprid from soil-applications has also been identified in the leaves of field-grown avocados (Byrne et al.), avocado nursery trees

(Byrne et al. 2007), and container poinsettias (Byrne et al.). In addition, imidacloprid in nectar has been found in buckwheat (Krischik et al. 2007), eucalyptus (Paine et al. 2011), and summer squash (Stoner and Eitzer 2012). Although there were measurable quantities of imidacloprid in the pollen of *P. x fraseri*, the levels were not statistically distinguishable from the controls. This result is unlike imidacloprid found in the pollen of treated summer squash (Stoner and Eitzer 2012). This inability to separate the treatments and the control may be due to the quantity of pollen material assayed. If the concentration of imidacloprid is low in *P. x fraseri* pollen, assaying larger quantities may register more accurate values. In addition, increasing the number of samples could also reduce the variation, increasing precision, and support statistical separation.

The amount of imidacloprid recovered in the various plant tissues from my studies and others was somewhat variable. Several factors could modify a plant's imidacloprid profile and may explain the variability, including the rate and timing of imidacloprid application, days post treatment, growth medium, plant type and size along with plant tissue type and age. For example, concentrations of imidacloprid in the leaves of first flush avocado and mature poinsettia in containers was greater than in both the new and old leaves of young avocado trees planted in groves despite more active ingredient (A.I.) being applied per grove tree (Byrne et al. 2010b, Byrne et al. 2010a). The difference in the amount of A.I. applied was 7.5 times greater per grove tree as compared to single nursery avocado tree and 192 times greater as compared to containerized poinsettia (Byrne et al. 2010b, Byrne et al. 2010a). In another example,

imidacloprid recovered in the old leaves of *P. x fraseri* was 2 times less than in the leaves of poinsettia after 1 week while the application rate was 40 times greater. In contrast, after 4 weeks the concentration of imidacloprid in the old leaves of *P. x fraseri* had increased to 20 times greater than what was found in poinsettia at the same time. It may be possible that with more time the detected levels of imidacloprid in leaf tissue would have reflected the comparative difference in treatment application. The variation in imidacloprid concentration found in treated plant material warns against generalizations about the relationship between the A.I. applied and recovered.

While most previous work investigating the quantity of soil-applied imidacloprid in plants focused on one or two plant tissues, our investigation aimed to characterize the imidacloprid present throughout a plant, the plant's imidacloprid profile, and relate that to the potential risk presented to a foraging parasitoid wasp. Imidacloprid detected in the xylem sap, nectar, and leaves of *P. x fraseri* generally increased from 3 to 32 days post treatment as evidenced by the post-hoc models. Although other studies have reported dates of peak concentration (Byrne et al. 2005, Castle et al. 2005, Byrne et al. 2010b), the blooming period of *P. x fraseri* constrained our efforts to find this value as sampling floral resource was the focus. The imidacloprid application rate directly related to the amount of imidacloprid recovered in all plant fluids and tissues except for pollen. Finally, leaf age was not a factor appearing to influence the imidacloprid profile.

In addition to generating a whole plant imidacloprid profile, we wondered if systemic compounds like imidacloprid would accumulate in larger concentrations in the

sink-tissues of a plant. A comparison of *P. x fraseri*'s xylem tissue, the conduit through which imidacloprid is translocated from the roots to the above-ground tissues, and nectar, generated in a sink-tissue, revealed that in general, source and sink tissues contain a similar amount of imidacloprid. Although there were 3 date and rate combinations out of a possible 16 where the concentration of imidacloprid was significantly greater in nectar than in xylem sap, these data are not compelling enough to claim imidacloprid is typically found in larger concentrations in sink tissue. However, this finding does suggest that sampling the xylem, an easier procedure than sampling the nectar, may provide a reasonable estimation of the imidacloprid concentration in nectar.

Nectar from *P. x fraseri* is most likely the sole route of exposure for the parasitoid, *A. colemani,* to soil-applied imidacloprid. Xylem tissue is not accessible to the parasitoid as it is wrapped by phloem tissue and the plant's cortex (Taiz and Zeiger 2002). The bioassay data from the foliage suggests there is no mortality from surface contact with *P. x fraseri*; the exposure route is apparently limited. Finally the amount of imidacloprid present in pollen was not distinguishable among the 2 treatments. Despite this, it may still be possible that pollen is an additional route of exposure for imidacloprid to *A. colemani* and needs further investigation.

The concentrations of soil-applied imidacloprid measured in the nectar of *P. x fraseri* were all greater than the LC_{50} determined by Probit analysis except for one measurement, 3 days post treatment at half label rate. Similarly the quantity of imidacloprid recovered in the nectar was greater than the LC_{75} and LC_{90} for all dates and

rates examined apart from the same exception for the LC_{50}. Furthermore the survival data based on nectar from *P. x fraseri* treated with soil-applied imidacloprid offers additional support to the Probit analysis. Recent research has also implicated imidacloprid in reducing the growth rate of the bumble bee, *Bombus terrestris* (Linn.) (Hymenoptera: Apidae) (Whitehorn, et. al, 2012), and concurs with our findings along with others (Krischik, V.A. et. al, 2007; Paine et. al, 2011) that imidacloprid negatively impacts the survival of hymenopteran species.

The imidacloprid profile of *P. x fraseri* in conjunction with the Probit analysis and survival data for *A. colemani* indicates that imidacloprid is present in nectar in toxic concentrations that impact a majority of parasitoids. The actual concentration of imidacloprid in nectar from field grown plants will most likely be determined by factors influencing the plant's imidacloprid profile but field effects are still unknown. Parasitoids such as *A. colemani* that encounter and feed on available and accessible flowers from plants treated with soil-applied imidacloprid may greatly reduce their survival.

Table 4.1 Imidacloprid concentration (ppb) in the xylem sap of *Photinia x fraseri* at 2 application rates of imidacloprid

	treatment mean ± SEM		[d]between treatments	
[a]dpt	[b]0.42 g imidacloprid pot^{-1}	[c]0.84g imidacloprid pot^{-1}	$F_{1,8}$	*p*
3	265.082 ± 70.38a	656.4 ± 432.38a	0.85	0.384
5	715.96 ± 255.94a	2694.53 ± 1343.57a	2.09	0.186
7	1378.88 ± 459.06ab	5060.32 ± 1660.15a	4.57	0.065
10	1625.15 ± 586.63ab	16541.05 ± 5811.71a	6.52	0.34
12	2307.82 ± 767.19ab	23378.9 ± 9488.06a	4.90	0.058
14	2748.66 ± 805.27ab	31124.81 ± 7270.05ab*	15.05	0.005
18	3287.53 ± 751.56ab	68701.71 ± 9527.86b*	46.84	<0.001
32	5183.18 ± 886.65b	110398.34 ± 3097.89c*	1066.18	<0.001

Mean imidacloprid concentration for all control treatments was 0 ± 0 ppb, not included in analysis
Means with the same letter within columns are not significantly different, $\alpha = 0.002$
[a] Days post treatment
[b] Within treatment analysis across dates, F = 6.17, df = 7, 32; $p < 0.001$
[c] Within treatment analysis across dates, F = 42.07, df = 7, 32; $p < 0.001$
[d] Asterisk indicates significant difference between treatments, $\alpha = 0.05$

Table 4.2. Imidacloprid concentration (ppb) in the nectar of *Photinia x fraseri* at 2 application rates of imidacloprid

	treatment mean ± SEM		[d]between treatments	
[a]dpt	[b]0.42 g imidacloprid pot^{-1}	[c]0.84 imidacloprid pot^{-1}	$F_{1,8}$	*p*
3	259.37 ± 67.45a	1408.8 ± 972.83a	1.39	0.272
5	1281.51 ± 772.81a	4421.72 ± 1354.78a	4.05	0.079
7	3372.44 ± 1946.18a	8951± 3000.70a	3.09	0.117
10	1325.99 ± 201.78 a	20880 ± 7304.15ab*	7.16	0.028
12	2039.78 ± 324.22 a	34390 ± 11316.20abc*	8.17	0.021
14	4374.45 ± 661.79a	61705 ± 10931bc*	27.22	<0.001
18	9779.68 ± 2558.90ab	70514.8 ± 7442.32cd*	56.56	<0.001
32	18056.86 ± 3829.60b	114480 ± 7618.4d*	127.88	<0.001

Mean imidacloprid concentration for all control treatments was 0 ± 0 ppb, not included in analysis
Means with the same letter within columns are not significantly different, $\alpha = 0.002$
[a] Days post treatment
[b] Within treatment analysis across dates, F = 11.55, df = 7, 32; $p < 0.001$
[c] Within treatment analysis across dates, F = 29.67, df = 7, 32; $p < 0.001$
[d] Asterisk indicates significant difference between treatments, $\alpha = 0.05$

Table 4.3. Imidacloprid concentration (ng anther^{-1}) in the pollen tissue of *Photinia x fraseri* at 2 application rates of imidacloprid

	treatment mean ± SEM		[d]between treatments	
[a]dpt	[b]0.42 g imidacloprid pot^{-1}	[c]0.84 g imidacloprid pot^{-1}	$F_{1,8}$	*p*
3	0.075 ± 0.07	0.01 ± 3.88E-03	1.19	0.307
5	0.042 ± 0.0	0	4.02	0.08
7	0.098 ± 0.06	3.51E-03 ± 2.31E-03	1.45	0.262
10	6.31E-03 ± 3.87E-03	2.82E-03 ± 2.82E-03	0.53	0.487
12	0.017 ± 0.02	0	1.14	0.317
14	3.64E-03 ± 3.08E-03	0.03 ± 0.02	1.46	0.236
18	0.07 ± 0.07	0.11 ± 0.05	2.57	0.148
32	0.07 ± 0.04	0.067 ± 0.03	<0.01	0.99

Mean imidacloprid concentration for all control treatments was 0 ± 0 ng anther^{-1}, not included in analysis
Means with the same letter within columns are not significantly different, $\alpha = 0.002$
[a] Days post treatment
[b] Within treatment analysis across dates, F = 1.00, df = 7, 32; $p = 0.447$
[c] Within treatment analysis across dates, F = 1.79, df = 7, 32; $p = 0.124$

Table 4.4. Imidacloprid concentration (ng cm^{-2}) in the new and old leaf tissue of *Photinia x fraseri* at 2 application rates of imidacloprid.

	treatment mean ± SEM			
	0.42 g imidacloprid pot^{-1}		0.84 imidacloprid pot^{-1}	
	leaf age		leaf age	
[a]dpt	[b]new	[c]old	[d]new	[e]old
3	13.25 ± 4.32a	44.18 ± 24.77a	532.86 ± 164.25a	653.38 ± 203.41a
5	31.10 ± 11.12a	97.21 ± 72.46a	1158.30 ± 410.65a	1248.09 ± 420.97a
7	59.92 ± 32.35a	108.25 ± 86.45a	4141.33 ± 1117.07ab	3794.10 ± 943.74ab
10	131.22 ± 66.89a	166.07 ± 92.23a	12421.26 ± 3759.24ab	16405.19 ± 6814.41abc
12	235.30 ± 120.52a	667.31 ± 245.96ab	16443.11 ± 3741.80ab	14243.84 ± 4390.74abc
14	821.10 ± 407.54a	1163.78 ± 608.53ab	14649.10 ± 3461.07ab	24233.41 ± 6148.85abc
18	2381.64 ± 1241.53a	2681.42 ± 1193.28ab	14713.55 ± 4668.77ab	34585.79 ± 9410.88bc
32	3401.14 ± 1249.78a	4464.79 ± 1490.97b	26669.70 ± 4241.15b	42032.69 ± 4978.27c*

Mean imidacloprid concentration for all control treatments was 0 ± 0 ng cm^{-2}, not included in analysis

Means with the same letter within columns are not significantly different, $\alpha = 0.002$

[a] Days post treatment

[b] Within treatment analysis across dates, F = 4.73, df = 7, 32; $p = 0.001$

[b] Within treatment analysis across dates, F = 5.42, df = 7, 32; $p < 0.001$

[d] Within treatment analysis across dates, F = 5.80, df = 7, 32; $p < 0.001$

[b] Within treatment analysis across dates, F = 10.58, df = 7, 32; $p < 0.001$

Asterisk indicates significant difference between leaf age set within treatment, $\alpha = 0.05$, F =10.81, df =1,8 ; $p = 0.011$

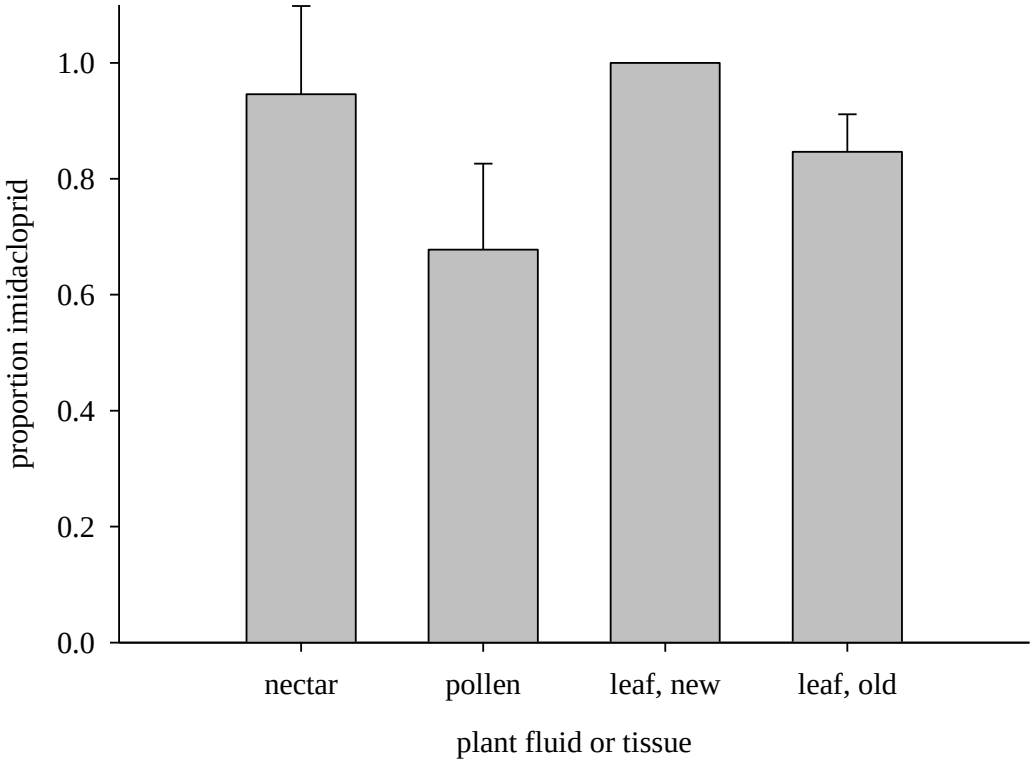

Figure 4.1. Comparison of imidacloprid concentration (mean ± SEM) detected by the ELISA method and the actual amount of purified imidacloprid in nectar, pollen, and leaf tissues. The proportion imidacloprid recovered would be 1.0 if no metabolites were present. Sampling dates represented include day 7 through day 32.

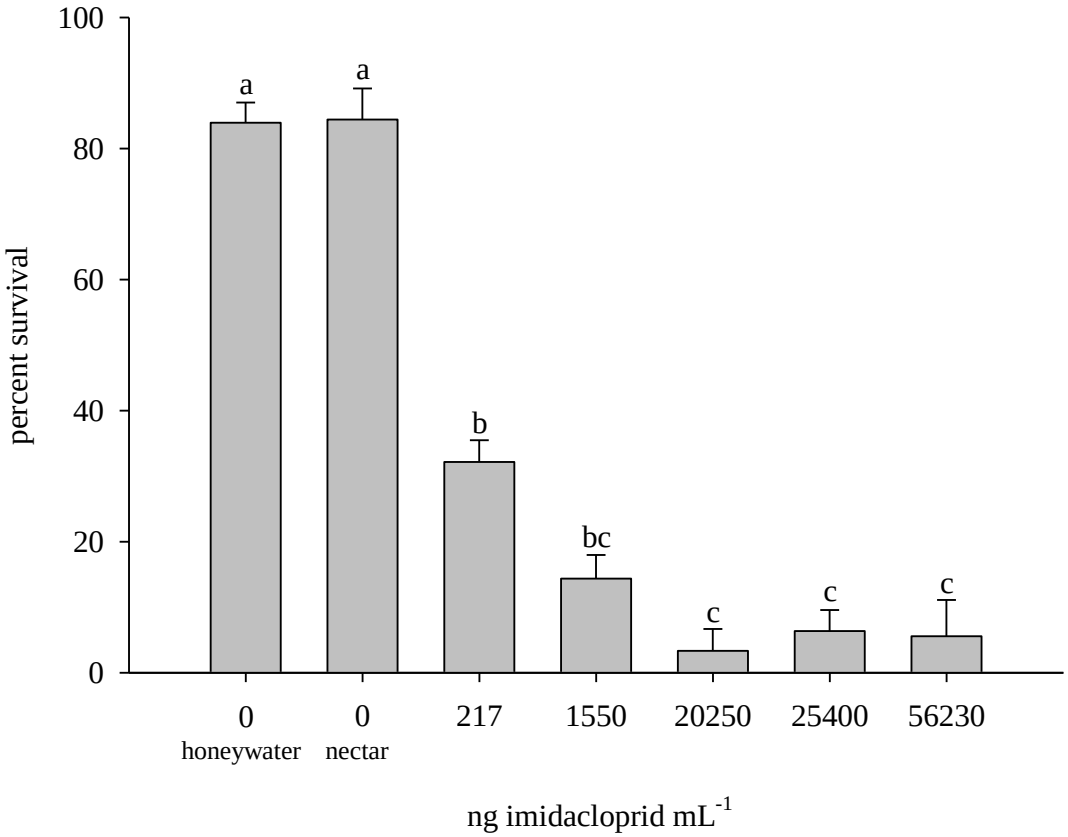

Figure 4.2. Mean survival ± SEM of *Aphidius colemani* after 24 hours treated with nectar from imidacloprid treated plants, nectar from control plants, and honey-water. Imidacloprid concentrations tested represent the range found in nectar treated with label rates of imidacloprid. Significant differences ($p < 0.05$) between imidacloprid concentrations are indicated by different letters.

Chapter 5. Conclusion

Conservation biological control is a pest management strategy that aims to protect and promote natural enemies in agricultural cropping systems through the implementation of various approaches that alter the environment (Barbosa 1998, Eilenberg et al. 2001). Habitat manipulation is one such tactic whereby the agricultural landscape is modified to provide the resources natural enemies need to improve their capacity to suppress pest populations. Conservation biological control may be a viable pest management strategy in the ornamental, containerized nurseries of Southern California, because the agricultural landscape is rich with food resources essential to natural enemies. Ornamental production systems are abundant in floral resources. The growing sites may be adjacent to disturbed and native habitats that can provide additional or alternative food and host resources. A unique feature of the nursery cropping system is the motility associated with plants grown in containers allowing resource plants to be placed where needed. Ornamental shrub crops such as *Photinia x fraseri* Dress (Rosaceae) have the potential to function as a food resource plant and then be recycled back into marketable stock after a routine hedging and period of regrowth. The uniqueness of this highly mobile and resource rich nursery cropping system lends itself to the development of habitat manipulation tactics.

The melon aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is a pest of *P. x fraseri* and more than 120 other plant species representing 90 families (Blackman and Eastop 2000, Emden and Harrington 2007). This aphid species is considered to be one of

the most economically destructive aphids in the United States (Slosser et al. 1986) and one of the top ten pests in California nurseries (Wilen et al. 2002). Although aphid control is typically achieved with insecticides such as carbamates, organophosphates, pyrethroids (Wilen et al. 2002), and more recently imidacloprid, it may be possible to utilize habitat manipulation tactics to promote the biological control of aphid populations.

The solitary endoparasitoid, *Aphidius colemani* Viereck (Hymenoptera: Braconidae: Aphidiinae), is a cosmopolitan species parasitizing over 41 aphid host species including the melon aphid (Stary 1975). *A. colemani* has a similar intrinsic rate of increase as *A. gossypii* (Van Steenis 1993) and has the ability to discover and parasitize aphids at low densities (Van Steenis and Elkhawass 1995). This generalist parasitoid of aphids is found throughout ornamental nursery production systems of Southern California as well as surrounding disturbed, urban, and native habitats.

1. Resource provisioning as a habitat manipulation tactic

The initial objective of this research was to develop a habitat manipulation tactic to control populations of *A. gossypii* by enhancing the performance of *A. colemani* through provisioning food resources. There floral candidates, *Conium maculatum* L. (Apiaceae), *P. x fraseri*, and *Salvia apiana* Jeps. (Lamiaceae), were identified as potential floral resource plants based on the enhanced longevity and fecundity of *A. colemani*. Additional laboratory studies evaluated the performance of *A. colemani* fed on naturally available and accessible resources: floral nectar from *P. x fraseri*, honeydew from *A. gossypii*, and extrafloral nectar from *Cuburbito pepo* L. 'Raven'. Floral nectar and

honeydew had a statistically similar effect on the longevity, fecundity, percent emergence, and sex ratio of *A. colemani* and enhanced the parasitoid more than extrafloral nectar.

Floral resources from *P. x fraseri* and honeydew resources from *A. gossypii* were used in a field study investigating the effects of food resources on the abundance and movement of *A. colemani*. After 24 hours, significantly more parasitoids initially associated with the combination flowers x honeydew treatment followed by a switch to the aphids treatment 1 week later. There was no significant difference in the abundance of *A. colemani* during the remaining 3 weeks of the study. Parasitoid abundance in the crop plots associated with food resource treatments was measured to assess the movement of *A. colemani* into a no-resource crop. Although there was no effect of food resource treatment on the movement of the parasitoid into the crop, approximately 40% of the total parasitoids found in the treatment plot and associated crop plot were found solely in the crop plot. These data suggest that *A. colemani* moves in search of resources and may be practicing switch-foraging. Failed studies examining the movement of *A. colemani* using protein markers and assessing the impact on *A. gossypii* made it difficult to develop a habitat manipulation tactic for the control of *A. gossypii* by *A. colemani*. Despite these problems, there still may be a possibility for implementing habitat manipulation in the ornamental nursery. Research questions addressing the effect of resource provisioning on improving biological control and the logistics of implementing such tactic may be better addressed in the nursery.

2. The plant-mediated effects of imidacloprid

Imidacloprid (1-[(6-chloro-3-pyridyl)methyl]-*N*-nitro-2-imidazolidinimine) is a chloronicotynl insecticide with systemic activity against aphids among other insects. Although foliar applications are included on the label, it is the systemic activity from soil applications that may reduce contact with natural enemies and allow for the integration of chemical and biological control. Imidacloprid is active against numerous phloem and xylem feeding pests found in nursery production of container-grown plants, including the melon aphid, *A. gossypii*, common to many ornamental plants, including *P. x fraseri*. The presence of natural enemies in an imidacloprid-rich nursery environment raises questions about the possibility for compatibility between biological control of aphids by *A. colemani* and chemical control by imidacloprid.

The final objective of this research was to determine the plant-mediate effects of imidacloprid on *A. colemani*. Experiments to assess the routes of exposure of imidacloprid to the parasitoid found imidacloprid in the xylem, nectar, pollen, and leaves of *P. x fraseri* when treated with full label rate and half label rate. The concentration of imidacloprid in the plant fluids and tissues increased over time but the peak concentration was not determined. The flowering cycle for *P. x fraseri* limited the collection period to 32 days post treatment. In addition, the concentration of imidacloprid in the full label rate treatment was significantly greater than the half label rate. Xylem sap and nectar were the only comparable samples as the concentration of imidacloprid in pollen was ng per anther and the imidacloprid in leaves was ng per cm^2. The concentration of

imidacloprid in xylem and nectar were not significantly different. This finding suggests that sampling the xylem, an easier procedure than sampling the nectar, may provide a reasonable estimation of the imidacloprid concentration in nectar.

The concentration of imidacloprid detected in the xylem sap and nectar of *P. x fraseri* was greater than the LC_{50}, LC_{75}, and LC_{90} of imidacloprid for *A. colemani* on all dates and rates except for Day 3 of the half label rate. The survival of *A. colemani* fed with nectar from imidacloprid treated plants was reduced as the concentration of imidacloprid in nectar increased while the survival of the parasitoid was not affected by leaves from imidacloprid treated plants. Recent research has also implicated imidacloprid in reducing the growth rate of the bumble bee, *Bombus terrestris* (Linn.) (Hymenoptera: Apidae) (Whitehorn, et. al, 2012), and concurs with our findings along with others (Krischik, V.A. et. al, 2007; Paine et. al, 2011) that imidacloprid negatively impacts the survival of hymenopteran species. In order to assess the potential field effects of imidacloprid on natural enemies and other hymenopterans, additional studies investigating landscape level effects in cropping systems are needed.

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