ABSTRACT

PRADO, SARA GUI. Factors Affecting Biological Control of *Myzus persicae* using *Aphidius colemani*. (Under the direction of Steven D. Frank).

The purpose of the following studies is to increase adoption of biological control as a sustainable pest management strategy for green peach aphid (*Myzus persicae* Sulzer) (Hemiptera: Aphididae) management. As such, these studies were conducted with the purpose of reducing insecticide use and risks to workers, non-target organisms, and the environment. Aphids are among the most common and economically damaging pests of greenhouse crops as they cause aesthetic damage, reduce yield, and transmit lethal plant viruses. Therefore, growers rely on frequent insecticide applications to reduce aphid abundance and damage. However, aphid behavior and insecticide resistance make them increasingly difficult to manage.

Biological control can reduce pest abundance and damage to acceptable levels, however, growers have been slow to adopt biological control as part of their pest management program. The primary factors affecting adoption of biological control are grower concerns about efficacy, predictability, and cost. In the following studies, I investigate these three weaknesses of biological control in the hopes of identifying potential causes for the low efficacy and predictability problems, and a solution to the high cost problem. I first tackle the issue of efficacy and predictability by investigating how common horticultural practices, such as the use of plant growth regulators (PGRs), can negatively influence the outcome of biological control programs. Next, I assess the efficacy of a relatively new biological control method, a banker plant system, which can reduce the costs of biological control programs.
In all three studies, I use the parasitic wasp *Aphidius colemani* Viereck (Hymenoptera: Braconidae) as the biological control agent for *M. persicae*. *Myzus persicae* is an extremely important pest of greenhouse ornamental and vegetable crops, as it feeds on over 100 vegetable and ornamental plant species. *Aphidius colemani* is a solitary, koinobiont, endoparasitoid which is often used as a biological control agent for *M. persicae*. As many of the ornamental and agricultural crops *M. persicae* feed on are often treated with PGRs, the first and second articles investigate the effects of these PGRs on *A. colemani* efficacy. I began by investigating the effects of PGR-induced changes in plant architectural complexity on *A. colemani* foraging efficiency and pest suppression. I did so by comparing *M. persicae* abundance and suppression on PGR (paclobutrazol) treated and untreated plants when *A. colemani* was present and absent. I found that paclobutrazol alone reduced aphid abundance compared to untreated plants. However, when parasitoids were present paclobutrazol and associated changes in plant architecture reduced parasitism and increased aphid abundance compared to untreated plants. I determined that a likely mechanism for this result was that significantly more *M. persicae* fed in concealed locations on PGR-treated plants than on untreated plants. In the second article I investigated the effects of four of the most commonly used PRGs on *M. persicae* abundance and suppression, and *Aphidius colemani* fitness.

Unlike the first study, I did not find any significant effect of PGRs on *M. persicae* abundance and suppression, but I did find reduced mummy abundance in one PGR treatment. I also found significant negative effects of the four PGRs on at least one aspect of *A. colemani* life history. In the third article, I compared the effectiveness of a relatively new biological control system, a banker plant system, to the commonly used augmentative release of *A. colemani*. I did so by investigating the ecological theory behind parasitoid foraging efficiency in a two-
host system. I found that optimal foraging behavior by *A. colemani* resulted in apparent competition favoring the suppression of the pest aphid, *M. persicae*, and that using this aphid banker plant system can provide greater pest suppression than augmentative releases of *A. colemani*. 
DEDICATION

To my family and friends, and my long-lost Don Juan.
Sara Prado, born and raised in Montreal, Quebec, graduated from John Abbott College in Pure and Applied Sciences in May 2007. In September 2007, she began her Bachelor’s degree in Agricultural and Environmental Sciences with a concentration in Biodiversity and Conservation, at McGill University. She graduated in 2010 in the honor’s program. Throughout her bachelor’s degree, she spent her summers in the Amazon jungle of Peru, where her love for insects developed. In August of 2010, she began her Master’s degree with Dr. Steven Frank at North Carolina State University, where she was able to combine her passion for environmental conservation and insects. Her Master’s thesis research focused on biological control of greenhouse pests.

In September 2012, Sara will begin a three-year long research position at North Carolina State University in collaboration with USDA-NRCS, wherein she will work to promote sustainable agriculture in the Caribbean.
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CHAPTER 1: Plant architectural complexity reduces biological control of *Myzus persicae* by *Aphidius colemani*

**INTRODUCTION**

Horticultural practices that alter plant architecture and quality may have unexpected consequences on the efficacy of biocontrol programs. Plant growth regulators are non-nutrient, organic compounds used in ornamental plant production to modify plant growth and/or development (Basra 2000). Plant growth regulators can be used to reduce plant growth rate, improve coloring, increase branching and bushiness, or synchronize flowering times (Basra 2000). By changing plant chemistry, physiology, and architecture plant growth regulators may alter arthropod behavior and development. Although plant growth regulators are widely used in horticulture and agriculture, very little is known about their effects on herbivores, natural enemies, and their interactions.

Plant growth regulator chemistry could affect herbivore abundance directly or indirectly via their parasitoids. For example, high doses of chlormequat chloride adversely affect aphid reproduction (Singer and Smith 1976). Similarly, gibberellic acid significantly reduces melon fruit fly (*Bactrocera cucurbitae* Coquillett) fecundity and fertility (Kaur & Rup 2002). Thus, plant growth regulators could reduce pest herbivore population growth making biocontrol more effective. Alternatively, plant growth regulators may affect parasitoid fitness or abundance via the resources the chemically-altered plants provide for the parasitoid hosts. Uçkan et al. (2008) found that when herbivore hosts were fed high doses of giberellic acid, endoparasitoid emergence time increased and longevity decreased. Hence, the quality of the plant consumed by the herbivore host can negatively affect parasitoid fitness by
compromising the resources available during its development (Ode 2006). Unfortunately,

few studies have documented the effects of plant growth regulators on herbivores (Kaur and
Rup 2002, Robinson 1960, Singer and Smith 1976) and even less have determined their
effects on parasitoid fitness. Therefore, predicting the impact of plant growth regulator
induced changes in plant quality on biological control programs is difficult.

Plant architectural changes caused by plant growth regulators could also affect aphid
abundance through changes in parasitism. Increasing plant architectural complexity can
reduce parasitoid foraging efficiency and suppression of herbivores (Andow & Prokrym
1990). Traits that increase plant complexity and are relevant to parasitoid foraging efficiency
include the size, heterogeneity, and connectivity of plant structures (Gingras 2003; Cloyd &
Sadof 2000) and leaf texture (Lukianchuk & Smith 1997; Andow & Prokrym 1990). These
traits can reduce parasitoid foraging efficiency by increasing searching time or by otherwise
decreasing the odds of encountering prey (Price et al. 1980). For instance, the attack rate of
the citrus mealy bug parasitoid was negatively correlated with plant size, height, leaf number,
leaf surface area, and branch number (Cloyd & Sadof 2000). In addition, complex plants can
provide herbivores with concealed feeding locations, thus decreasing biological control

Understanding the ways in which plant architecture can affect pest suppression by parasitoids
will improve our ability to implement successful biocontrol programs.

*Myzus persicae* Sulzer (Hemiptera: Aphididae) is one of the most important pests of
greenhouse ornamental and vegetable crops (Heathcote 1962). *Myzus persicae* feeds on over
100 vegetable and ornamental plant species (Baker 1994), many of which are treated with
plant growth regulators during greenhouse production (Basra 2000). Biological control of *M. persicae* in greenhouse crops often entails releasing *Aphidius colemani* Viereck (Hymenoptera: Braconidae) (van Steenis 1995; Rabasse & van Steenis 1999), a solitary, koinobiont, endoparasitoid (Starý 1975). *Aphidius colemani* development is closely tied to its hosts’ development, making it vulnerable when its host feeds on toxic or low quality plant material (Kalule & Wright 2005). In addition, foraging efficiency and pest suppression by *A. colemani* and other parasitoids can be influenced by plant structure and aphid distribution (Stadler & Volkl 1991). Therefore we feel this herbivore-parasitoid system is an ecologically and economically relevant system in which to determine how plant growth regulators and parasitoids interact to affect aphid abundance. Specifically our objectives were to 1) Determine the effect of plant architecture on aphid feeding location; 2) Determine how paclobutrazol and *A. colemani* affect aphid abundance; and 3) Determine how plant architecture and *A. colemani* affect aphid distribution and parasitism on exposed and unexposed plant parts. To achieve our objectives, we compared *M. persicae* abundance and distribution on Black Pearl Pepper plants (*Capsicum annuum* ‘Black Pearl’) treated with paclobutrazol to untreated plants in the presence and absence of *A. colemani*. Our research is the first to examine the direct and indirect effects of plant growth regulators on pest abundance and should provide important management information to improve greenhouse plant production.
Methods

2.1. Study System:

For all experiments, A. colemani were purchased from Koppert Biological (Howell, MI). Upon receipt, the mummies were placed in a 61 x 61 cm cage where the parasitoids could emerge and mate. During that time, they were provided with a 25% sucrose-water solution. All female parasitoids were used less than 72 after emergence. We used M. persicae from a laboratory colony that was started from field-collected aphids. The aphids were reared on Black Pearl pepper plants (Capsicum annuum ‘Black Pearl’) in an incubator at 25°C and 70-80% RH.

Black Pearl pepper plants were obtained from Raker and Sons (Litchfield, MI) as plugs (128 plugs <7cm in height). Sixty plants were repotted into 15.2 cm-diameter pots filled with Fafard 2P soil mix (Agawam, MA) with 397g of Scotts Osmocote (N-P-K: 14-14-14) fertilizer (Marysville, OH) for every 0.08 m³ of soil. When plants were 2.5 weeks paclobutrazol (Bonzi®, Syngenta Crop Protection, Greensboro, NC) was applied as a drench to 30 plants with 1.5 mg a.i. given to each pot through a 118.3 ml solution. Plants were allowed to grow for another 13 days before the experiment began.

2.2. Effect of plant architecture on aphid feeding locations

To determine how plant growth regulator-induced changes in plant architecture affect aphid concealment, we compared aphid distribution on treated and untreated pepper plants. We performed 18 replicates per treatment, in which 10 M. persicae of random instar were placed on the soil 1-2cm from the pepper plant’s stem. Aphids were allowed to climb up and establish feeding sites for one hour after which we searched the plants to determine if the
aphids were feeding in a ‘concealed’ or ‘exposed’ location. Aphids were categorized as ‘concealed’ if they were surrounded on 3 or more sides by plants structures, such as leaves or stems, less than 1 cm away. Other studies have found that herbivores hidden between plant structures are parasitized less successfully than herbivores that are more exposed (Gardner & Dixon 1985). A chi-squared test was performed to determine if the proportion of concealed aphids was different between treated and untreated plants, using R version 2.13.1.

2.3. Effect of paclobutrazol and A. colemani on aphid abundance

To determine how paclobutrazol affects aphid population growth and parasitism by A. colemani, we conducted a 2x2 factorial experiment that crossed two paclobutrazol treatments (‘untreated’ or ‘treated’) with two parasitoid treatments (‘absent’ or ‘present’). Every treatment combination was replicated 12 times for a total of 48 ‘Black Pearl’ pepper plants. The plants were placed on a greenhouse bench and randomly assigned to one of the four treatment combinations. Every pot was covered in a bag made of organdi fabric that was supported from within by 45 cm bamboo stakes and fastened around the base of the pot using a binder clip. On the first day, we infested each plant with 10 M. persicae of random instars from the laboratory colony. After 24 hours one mated female A. colemani was released into cages assigned to the parasitoid ‘present’ treatments.

One week after parasitoids were released, we inspected plants to record aphid and mummy abundance and distribution. All aphids and mummies were counted on the buds, the stem and the rest of the plant (Fig. 1). This process was repeated five times, every 72 hours, following the first data-collection day. After the final data collection, we measured the height of all plants from the base of the stem to the top of the plant. To determine plant biomass all
plants were cut at soil level, washed in soapy water to remove aphids and mummies, rinsed, placed in paper bags, and dried in an oven for 30 hours at 69 °C. Once dried, all plants were weighed to obtain their dry mass. In 10 of the plants (4 from treated, 6 from untreated) the parasitoid died before parasitizing any aphids so these were removed from the analysis.

A 2-way repeated measures ANOVA was used to determine how paclobutrazol, parasitoids, and their interaction affected total aphid abundance, mummy abundance, and percent parasitism. The total number of aphids were log(x+1) transformed. As data mummy abundance and percent parasitism could not be normalized, a nonparametric factorial repeated measures analysis was performed using the package nparLD (Noguchi et al. 2012) to determine how time, paclobutrazol, and their interaction affected total mummy abundance, and percent parasitism. A 2-way ANOVA was also used to determine how paclobutrazol, parasitoids, and their interaction affected final plant weight. T-tests were used to compare plant height before and after the experiment for treated and untreated plants. To determine whether plant biomass had an effect on aphid and mummy abundance, the final aphid abundances were divided by the final plant weight and analyzed using an ANOVA. All statistical analyses, except the nonparametric factorial repeated measures were performed in SAS (version 9.2). The nonparametric factorial repeated measures was performed using R version 2.13.1.

2.4. Effect of plant architecture on aphid distribution and suppression by A. colemani

To determine how paclobutrazol and A. colemani affect aphid distribution and parasitism, a repeated measures ANOVA was performed for both aphid abundance on stems and buds over time. All proportions including the percent parasitism were arc sin square root
transformed to correct non-normal distribution. All statistical analyses were performed in SAS (version 9.2).

**RESULTS**

3.1. Effect of plant architecture on aphid feeding locations

A chi-squared test showed that significantly more aphids were feeding in ‘concealed’ locations on the treated plants than on the untreated plants ($\chi^2 = 43.85; P < 0.0001$) (Fig. 2). Only 5% of the aphids on the untreated plants were concealed, while 37% of aphids were concealed on the more compact paclobutrazol treated plants.

3.2. Effect of paclobutrazol and A. colemani on aphid abundance

There was a significant interaction between parasitoid presence and paclobutrazol on aphid abundance, such that aphids were less abundant on paclobutrazol treated plants than untreated plants when no parasitoids were present but more abundant on treated plants when parasitoids were present ($F_{1,170} = 29.58; P < 0.0001$) (Fig. 3). Parasitoids reduced aphid abundance by 93% on untreated plants but only reduced aphid abundance by 52% on treated plants. There was also a significant interaction between parasitoid presence and time ($F_{4,170} = 2.6; P = 0.0378$) wherein aphid abundance declined over time when parasitoids were present but increased when they were absent. The main effects of time and parasitoids were also significant ($F_{4,170} = 37.90; P < 0.0001$; $F_{1,170} = 199.04; P < 0.0001$, respectively), however there was no significant main effect of paclobutrazol ($F_{1,170} = 1.44; P = 0.2311$). The interaction between time, parasitoid presence, and paclobutrazol was not significant ($F_{4,170} = 0.43; P = 0.7874$).
There were significant main effects of paclobutrazol and time on mummy abundance 
($F_{1.00,\infty}=4.525; P =0.0334; F_{2.13,\infty}=7.11; P <0.0001$; respectively) wherein mummy abundance was 66% lower on treated plants than on untreated plants. There was no significant interaction between paclobutrazol and time on mummy abundance ($F_{2.13,\infty}=0.73; P =0.4917$).

There was a significant main effect of paclobutrazol on percent parasitism ($F_{1,\infty}= 4.9787; P =0.025$) such that the percent parasitism on untreated plants was 2.5 times greater than on treated plants (Fig. 5). There was no significant main effect of time on percent parasitism nor was there a significant interaction between time and paclobutrazol ($F_{1.83,\infty}=1.8387; P =0.1625; F_{1.83,\infty}=0.9667; P =0.3740$, respectively).

The average height of the untreated plants (21.74±1.067) was significantly greater than that of the treated (11.07±0.38) plants at the beginning ($t_{20} = 9.01; P <0.0001$) and at the end (33.92±2.17; 12.71±0.58) of the experiment ($t_{20} = 9.21; P <0.0001$). Plant biomass was also significantly greater for the untreated plants (4.45± 0.56) than it was for the treated plants (1.69±0.21) ($F_{1.34}=19.91; P <0.001$). Parasitoid presence did not significantly affect plant biomass ($F_{1.34}=0.12; P =0.7363$) nor was there a significant interaction ($F_{1.34}=0.00; P =0.9698$).

The number of aphids per gram was significantly affected by parasitoid presence ($F_{1.34}=14.36; P =0.0006$) (Fig. 6). Paclobutrazol had a marginally significant effect on the number of aphids per gram ($F_{1.34}=3.81; P =0.0591$), wherein a greater number of aphids per gram was observed on treated plants than on untreated ones. There was no significant interaction between parasitoids and treatment on the number of aphids per gram ($F_{1.34}=0.00; P =0.9823$).
3.3. Effect of plant architecture on aphid distribution and suppression by A. colemani

There was a significant interaction between parasitoid presence and paclobutrazol on the proportion of aphids feeding on buds, an exposed location, such that parasitoids reduced the proportion of aphids feeding on the buds of untreated plants by 77% but it was only reduced by 34% on treated plants ($F_{1,169}=19.94; P<0.0001$) (Fig. 7). The main effects of parasitoid presence, paclobutrazol, and time were also significant ($F_{1,169}=48.02; P<0.0001; F_{1,169}=10.78; P=0.0012; F_{4,169}=2.63; P=0.0360$, respectively). The interaction between time and parasitoid presence, and between time and paclobutrazol did not have a significant effect on the proportion of aphids feeding on the buds ($F_{4,169}=1.06; P=0.3777, F_{4,169}=0.94; P=0.4443$, respectively). The three-way interaction between parasitoid presence, paclobutrazol and time was also not significant ($F_{4,169}=0.72; P=0.5804$).

There was a significant interaction between time, parasitoid presence, and paclobutrazol on the proportion of aphids feeding on the stem ($F_{4,169}=2.42; P=0.0504$) which is considered concealed in treated plants but exposed in untreated plants. Parasitoid presence significantly decreased the proportion of aphids feeding on the stems of untreated plants but not of treated plants and this effect became stronger over time (Fig. 8). There was a significant interaction between parasitoid presence and paclobutrazol on the proportion of aphids feeding on the stem ($F_{1,169}=17.92; P<0.0001$) and a significant interaction between parasitoid presence and time ($F_{4,169}=3.25; P=0.0135$). There was no significant interaction between time and treatment on the proportion of aphids feeding on the stems ($F_{4,169}=1.66; P=0.1615$). The main effects of effect of parasitoid presence, paclobutrazol and time were also...
significant ($F_{1,169}=18.29; P<0.0001; F_{1,169}=10.91; P=0.0012; F_{4,169}=3.05; P=0.0186$, respectively).

**Discussion**

This study assessed how paclobutrazol-induced changes in plant architecture affect *M. persicae* suppression by *A. colemani*. Previous studies have investigated the effect of plant architecture on pest suppression by natural enemies (Gardner & Dixon 1985; Randlkofer et al. 2010; Andow & Prokrym 1990). However, the ways in which such studies have been carried out vary greatly. Some compare the effects of plant architecture using artificial plants made from paper or plastic (Andow & Prokrym 1990; Lukianchuk & Smith 1997; Gingras et al. 2002). Others have used two or more cultivars with different architectural features (Cloyd & Sadof 2000; Gingras 2003) or manipulated plant structures (Gontijo et al. 2010; Randlkofer et al. 2010). Our novel approach was to use plant growth regulators to manipulate the architecture of a single pepper plant species. We found that plant growth regulators, which are frequently used in agriculture and horticulture, can significantly reduce aphid suppression by parasitoids. Our findings suggest that parasitoids cause aphids to move from exposed to concealed feeding locations which are more abundant on complex, paclobutrazol treated plants.

We explicitly tested the combined effects of paclobutrazol and parasitoids on aphid abundance and found that paclobutrazol treated plants had about half as many aphids as untreated plants when parasitoids were absent. Despite this, parasitoids reduced aphid abundance to the lowest level on untreated plants indicating reduced parasitoid efficacy on paclobutrazol treated plants. We believe a primary mechanism for this is that aphids were
more likely to be concealed from parasitoids on paclobutrazol treated plants, which had a compact architecture with a high density of leaves and shoots. Results from our first experiment suggest that aphids on treated plants were more likely to feed in concealed locations than those on untreated plants. Although we did not test whether aphids categorized as ‘concealed’ were in fact parasitized less often, other studies have found that herbivore susceptibility to parasitism is reduced when feeding within tight plant structures (Gardner & Dixon 1985; Clark & Messina 1998). For example, Gardner and Dixon (1985) found that aphids feeding on wheat ears were parasitized at a lower rate than those feeding on the blades and hypothesized that aphids feeding in the tight spaces between the grains were less accessible to parasitoids. Likewise, boll weevil larvae concealed beneath wide cotton bracts are parasitized eight times less than those beneath narrow ‘frego’ type bracts that leave the larvae more exposed (McGovern & Cross 1976). As in these examples, in our caged experiment we observed significantly less parasitized aphids on the treated plants than on the untreated ones, suggesting A. colemani had reduced access to M. persicae.

In addition to feeding in concealed locations, aphids have a wide range of escape responses with which they can defend themselves from natural enemy attacks (Dixon 1958). Among these responses, is predator avoidance wherein an aphid can walk away from a threat (Dixon 1958). In our study, we observed a change in aphid distribution on plants when parasitoids were present. More specifically, we observed a significantly lower proportion of aphids feeding on the exposed buds of both treated and untreated plants and the exposed stems of untreated plants when parasitoids were present compared to when they were absent. We suspect that the threat of parasitism when foraging on exposed locations such as the buds
or stems caused aphids to move to more concealed locations. This is consistent with findings by Costamagna & Landis (2011), who also observed a shift in aphid within-plant distribution from high predation risk to low predation risk locations. Given the greater number of concealed feeding locations available on treated plants, we believe that more aphids were able to move from exposed to concealed locations on the treated plants than on the untreated plants. Thus, the combination of aphid escape behavior and the greater availability of refuges on treated plants reduced aphid suppression.

When no parasitoids were present aphid abundance was close to 2.5 times lower on treated than untreated plants. This is consistent with previous findings that plant growth regulators reduced herbivore reproduction or population growth (Honeyborne 1969; Coffelt et al. 1988) and may suggest that plant growth regulators reduced plant quality for aphids. We did not test plant quality directly however, as aphids are r-strategists (Yano 2006), meaning that their population growth is resource-limited (Gadgil & Solbrig 1972), we suggest that paclobutrazol reduced aphid abundance by reducing the carrying capacity of plants rather than by decreasing plant quality per se. Our finding that the number of aphids per gram did not differ between the treated and untreated plants when parasitoids were absent corroborates our hypothesis. Interestingly, there was no significant difference between the number of aphids per gram on the treated plants when parasitoids were present and the number of aphids per gram on the untreated plants when parasitoids were absent. The smaller plant size played an important role at reducing aphid abundance when parasitoids were absent, but the compact architecture of these smaller plants reduced A. colemani efficacy at suppressing M. persicae.
In this study parasitoid and aphid movement was restricted to one caged plant, so future studies should investigate the effects of plant growth regulators on pest suppression at larger scales. Nevertheless, we demonstrated that one of the most commonly used types of agricultural chemical, plant growth regulators, can reduce the efficacy of biological control by *A. colemani*. To compensate for this reduced efficacy, growers may need to increase the number or frequency of natural enemies released or integrate chemical and biological control (e.g. Tremblay et al. 2008) to achieve satisfactory pest suppression when plant growth regulators are used. Our study sheds light on plant growth regulators as one of many agricultural practices that could alter biotic interactions and make the efficacy of biological control unpredictable or context dependent.


Figure 1. Untreated plant (left) and treated plant (right). The stem (dashed line) is exposed for the untreated plant but protected for the treated plants. The buds (solid line) on both plants were considered exposed.

Figure 2. The proportion of aphids recovered from concealed locations on untreated pepper plants and plants treated with paclobutrazol after one hour of foraging.
**Figure 3.** Overall mean (± SE) number of aphids on caged pepper plants during a three week experiment in which plants were untreated or treated with paclobutrazol and had parasitoids absent or present within the cages. Means with different letters are significantly different at the P<0.05 level.

**Figure 4.** Overall mean (± SE) number of aphid mummies on caged pepper plants with parasitoids during a three week experiment in which plants were untreated or treated with paclobutrazol. Means with different letters are significantly different at the P<0.05 level.
Figure 5. Overall percent parasitism (mummies/aphids+mummies) (± SE) observed on caged pepper plants during a three week experiment in which plants were untreated or treated with paclobutrazol and had parasitoids within the cages. Means with different letters are significantly different at the $P<0.05$ level.

Figure 6. Overall proportion of aphids/gram of biomass (± SE) on caged pepper plants during a three week experiment in which plants were untreated or treated with paclobutrazol and had parasitoids absent or present within the cages. Means with different letters are significantly different at the $P<0.05$ level.
Figure 7. Overall proportion of aphids observed feeding on buds (± SE) of caged pepper plants during a three week experiment in which plants were untreated or treated with paclobutrazol and had parasitoids absent or present within the cages. Means with different letters are significantly different at the $P<0.05$ level.

Figure 8. Overall proportion of aphids observed feeding on stems (± SE) of caged pepper plants during a three week experiment in which plants were untreated or treated with paclobutrazol and had parasitoids absent or present within the cages.
CHAPTER 2: Tritrophic effects of plant growth regulators in an aphid-parasitoid System

INTRODUCTION

The efficacy of augmentation biological control is often unpredictable as it is influenced by many biotic and abiotic factors (Collier and Van Steenwyk, 2004; Frank, 2010). Although the effect of many ecological interactions on biological control efficacy has been well studied (Holt and Lawton, 1994; Martinou et al., 2010; Fill et al., 2012), we know little about the impact of common horticulture practices such as the use of plant growth regulators. Plant growth regulators (PGRs) are non-nutrient, organic compounds used in ornamental plant production to modify plant growth and development (Basra 2000). PGRs can be used to reduce plant growth rate, improve coloring, increase branching and bushiness, or synchronize flowering times (Latimer 2009). By changing plant chemistry, physiology, and architecture PGRs may alter arthropod behaviour and development (eg. Prado & Frank in review). Although PGRs are widely used in horticulture and agriculture, very little is understood about their effects on herbivores, natural enemies, and their interactions.

PGRs have the potential to reduce pest population growth by reducing fecundity, egg viability, and increasing development time which could improve pest management (Visscher, 1980; Coffelt et al., 1993; Kaur and Rup, 2002). For example, Coffelt et al. (1993) showed that high doses of paclobutrazol significantly slowed the development and decreased the survival of orange-striped oakworms. Several phloem feeding insects including aphids and lace bugs are also negatively affected by PGRs (Honeyborne, 1969; Coffelt and Schultz, 1988). Trimethylammonium chloride was found to reduce *Aphis fabae* Scopoli (Hemiptera:
Aphididae) fecundity and survival, and ethylene-bisnitrourethane to reduce its size (Honeyborne, 1969). The effects of PGRs on aphid size, development, and fitness could have important implications for pest management in greenhouse production where aphids are major pests and PGR use is widespread. Although numerous studies have shown that PGRs can compromise arthropod development, few studies have investigated the effects of these chemicals on natural enemies.

By directly affecting the quality of aphid hosts, PGRs could indirectly affect parasitoid abundance, fitness, or efficacy. As PGRs have been shown to reduce aphid size (Honeyborne 1969), parasitoids developing in these smaller hosts may also be reduced in size (Sequeira and Mackauer, 1992) resulting in reduced fecundity (Ellers et al. 1998; Eijs & van Alphen 1999; Sampaio et al. 2008). Parasitoids developing in small hosts also tend to have higher male sex ratio and mortality rates than those developing in large hosts (Jarosik et al., 2003). Additionally, small parasitoids tend to have fewer fat reserves (Ellers et al. 1998; Eijs & van Alphen 1999), thus reducing their travel distances (Ellers et al., 1998) and have their longevity when food is unavailable (Ellers et al. 1998; Eijs & van Alphen 1999). Along with altering parasitoid life history traits, PGRs can reduce parasitism by increasing plant complexity (Prado & Frank in review). Thus, the potential benefit of PGRs to reduce pest population growth could be nullified if negative effects on parasitoids disrupt biological control programs.

*Myzus persicae* Sulzer (Hemiptera: Aphididae) is one of the most important pests of greenhouse ornamental and vegetable crops (Capinera 2001). *Myzus persicae* feeds on over 100 vegetable and ornamental plant species (Baker 1994), many of which are treated with
plant growth regulators during greenhouse production. *Aphidius colemani* Viereck (Hymenoptera: Braconidae) is a solitary, koinobiont, endoparasitoid (Starý, 1975) used for biological control of economically important pest aphids including *M. persicae* (van Steenis 1995). As such, *A. colemani* development is closely tied to its hosts’ development, making it vulnerable to changes in host quality, when its host feeds on toxic or low quality plant material (Kalule and Wright, 2005). In a previous experiment we showed that the PGR paclobutrazol reduced aphid suppression by *A. colemani* by increasing plant complexity (Prado & Frank in review). In this study, we expand on our previous research to include four of the most commonly used PGRs to determine 1) how different PGRs interact with *A. colemani* to affect *M. persicae* abundance, and 2) how different PGRs affect *A. colemani* fitness and abundance. To achieve our objectives, we compared aphid populations on Black Pearl Pepper plants (*Capsicum annuum* ‘Black Pearl’) treated with one of four plant growth regulators to each other and to untreated plants in the presence and absence of *A. colemani*. Furthermore, we compared life history traits of parasitoids reared on in treated and untreated plants. This research will be the first to document the effects of multiple PGRs on an aphid parasitoid’s fitness and efficacy and should assist in improving biological control programs.

**Methods**

2.1. Study system

*Aphidius colemani* were purchased from Koppert Biological (Howell, MI). Upon receipt, the mummies were placed in a 61 x 61 cm cage where the parasitoids could emerge and mate. During that time, they were provided with a 25% sucrose-water solution. All female parasitoids were used less than 72 after emergence. We used *M. persicae* from a
laboratory colony that was started from field collected aphids. The aphids were reared on Black Pearl Pepper plants (*Capsicum annuum* ‘Black Pearl’) in an incubator at 25°C and 70-80% RH.

All Black Pearl pepper plants were obtained from cuttings. Source plants were cut 5-10 cm below the bud. The cut tips were then dipped into “Rhizopon AA Dry Powder Rooting Hormone #1” (Active ingredient: 0.1% 3-Indolebuteric acid) (*Earth City, MO*) and planted in 48 pot trays (56cm by 25.5cm tray) with sifted Fafard 2P mix (Agawam, MA) for germination. The cuttings were left to root in a misting area for 6 weeks before they were transplanted into 15.2 cm-diameter pots with Farfard 2P soil mix with 396.44g of Scotts Osmocote (N-P-K: 14-14-14) fertilizer (Marysville, OH) for every 0.08 m³ of soil. Each PGR was applied as a drench to 12 plants 2.5 weeks after transplanting the cuttings. 4 fluid ounces of solution were applied to each pot. Rates were as follows: 8 ppm of Bonzi® (a.i. paclobutrazol 0.4%), 2 ppm of Topflor® (a.i. flurprimidol 0.38%), 14 ppm of Abide® (a.i. ancymidol 0.0264%) and 2 ppm of Sumagic® (a.i. uniconazol 0.055%). Twelve plants were left untreated as controls. Plants were left to grow for another 10 days before the experiment began.

### 2.2. Effect of four PGRs on aphid abundance

To determine the effects of four plant growth regulators on aphid abundance and suppression, we conducted a 2x5 factorial experiment that crossed 5 plant growth regulator treatments (‘Abide’, ‘Bonzi’, ‘Control’, ‘Sumagic’ and ‘Topflor’) with two parasitoid treatments (‘absent’ or ‘present’). Every treatment combination was replicated 6 times for a total of 60 ‘Black Pearl’ pepper plants. Every pot was covered in a bag made of organdi
fabric that was supported from within by 45 cm bamboo stakes and fastened around the base of the pot using a binder clip. On the first day, we infested each plant with 15 *M. persicae* of random instars from the laboratory colony. After 4 hours two mated female *A. colemani* were released into cages assigned to the parasitoid ‘present’ treatments. One week after parasitoids were released, we recorded total aphid and mummy abundance on each pepper plant. This process was repeated five times, every 72 hours, following the first data-collection day.

2.3. Effect of four PGRs on *A. colemani* life history traits

On the last day of the experiment (day 19), mummies were picked off the plants from all the parasitoid ‘present’ treatments and placed in glass vials plugged with cotton. Parasitoids were reared out of mummies in the laboratory and preserved in 90% alcohol upon emergence. We examined each parasitoid under a dissection microscope with an ocular micrometer to determine their gender and measure the length of their left hind tibia. Parasitoid percent emergence was determined by dividing the total number of emerged parasitoids by the total number of mummies. In 5 of the plants (1 from Bonzi, 1 from the untreated, and 3 from the Topflor) the parasitoid died before parasitizing any aphids so they were included as replicates in the parasitoid ‘absent’ treatment.

2.3. Statistical analysis

As total aphid abundance and mummy abundance could not be normalized, a nonparametric factorial repeated measures analysis was performed using the package nparLD (Noguchi et al., 2012) to determine how time, PGRs, parasitoids, and their interaction affected their numbers. Differences in parasitoid percent emergence across PGRs was
determines using a Pearson’s Chi squared test. A Chi squared test was also used to calculate the differences in parasitoid sex ratio between PGR treatments. Lastly, a 2-way ANOVA was used to determine how PGRs and parasitoid sex affected parasitoid size. All analyses were performed using R version 2.14.2.

**Results**

3.1. **Effect of four PGRs on aphid abundance**

There was a significant interaction between parasitoid presence and time on aphid abundance, such that aphid abundance increased more slowly when parasitoids were present than when they were absent ($\text{F}_{1.94,\infty} = 9.74; P < 0.0001$) (Fig. 1). The main effects of time and parasitoids were also significant ($\text{F}_{1.94,\infty} = 188.34; P < 0.0001; \text{F}_{1,\infty} = 52.95; P < 0.0001$, respectively), however there was no significant main effect of PGR ($\text{F}_{3.50,\infty} = 1.38; P = 0.24$). The interaction between time, parasitoids and PGR was not significant ($\text{F}_{6.00,\infty} = 0.97; P = 0.44$) nor were the interactions between PGR and time or PGR and parasitoids ($\text{F}_{6.00,\infty} = 1.122; P = 0.35; \text{F}_{3.50,\infty} = 0.54; P = 0.68$, respectively).

There was a significant main effect of PGR on mummy abundance ($\text{F}_{3.47,\infty} = 3.71; P = 0.0077$) (Fig. 2) wherein mummy abundance was significantly lower on Bonzi-treated plants than for the other treatments. There was also a significant effect of time ($\text{F}_{2.19,\infty} = 13.66; P < 0.0001$) on mummy abundance. However, there was no significant interaction between PGR and time on mummy abundance ($\text{F}_{6.16,\infty} = 0.78; P = 0.59$).
3.2. Effect of four PGRs on A. colemani life history traits

A chi-squared test showed a significant effect of PGR on the frequency of emerged parasitoids ($\chi^2 = 123.99; P < 0.0001$) (Fig. 3). There was also a significant interaction between parasitoid sex and PGR on parasitoid size ($F_{3,192} = 2.83; P = 0.0392$), wherein female parasitoids emerging from Bonzi treated plants were significantly smaller, and male parasitoids emerging Topflor and Sumagic treated plants were significantly larger than females and males emerging from the control, respectively (Fig. 4). The main effects of sex and PGR were also significant ($F_{1,192} = 32.73; P < 0.0001; F_{3,192} = 10.05; P < 0.0001$, respectively). Parasitoid female ratio (female: total) was also significantly affected by PGR ($\chi^2 = 123.99; P < 0.0001$), resulting in highly male-biased populations on Bonzi and Sumagic treated plants and female-biased populations on Topflor and the untreated control plants (Fig. 5).

**Discussion**

In this study we assessed how four of the most commonly used PGRs affect *M. persicae* abundance and suppression, and *A. colemani* life history traits. Previous studies showed that PGRs can decrease herbivore reproduction rate (Visscher, 1980; Kaur and Rup, 2002), increase their development time (Coffelt and Schultz, 1988) and decrease their survival (Coffelt et al., 1993). Despite the generally negative effects of PGRs on herbivores, only one study has documented the effect of a PGR on parasitoid development and survival (Uçkan et al., 2008). Prado and Frank (*in review*) found negative effects of the PGR, paclobutrazol, on biological control of *M. persicae* but they did not consider physiological
effects on parasitoid life history traits as a mechanism for this reduced aphid suppression. Based on this previous work, our hypothesis was that PGRs would reduce aphid abundance and suppression by *A. colemani*. In addition, we predicted that PGR treated plants would have indirect negative effects on parasitoids via their aphid hosts. We did not find any significant effect of PGRs on aphid abundance or suppression but all PGRs tested had negative effects on at least one aspect of *A. colemani* life history.

In a similar experiment, Prado and Frank (*in review*) found that aphid abundance was 50% less on plants treated with paclobutrazol after 3 weeks, compared to untreated plants. In contrast to this and other studies (e.g. Visscher, 1980; Coffelt and Schultz, 1988; Kaur and Rup, 2002), none of the PGRs reduced aphid abundance in the present study. Prado and Frank (*in review*) also found that paclobutrazol significantly reduced aphid suppression by *A. colemani*. We found that parasitoids reduced aphid abundance by 69% overall, but we did not find any significant difference between aphid suppression on treated and untreated plants. Though we cannot fully explain what caused such a difference between this and our previous study, it is possible that the slightly lower dose of PGR combined with the already small number of generations reduced the expression of population-level effects. In addition, the extra female parasitoid added to each cage at the beginning of this experiment may have increased parasitism enough to overshadow the effects of PGRs on aphid suppression. Nonetheless, even with the added parasitoid, we found a significantly lower mummy abundance on the paclobutrazol treated plants than on the untreated plants. This was also found by Prado & Frank (*in review*). We consider the reduced mummy abundance in the paclobutrazol treatment to be an indicator of the potential negative effects PGRs can have on
natural enemy efficacy.

To understand the ways PGRs may affect natural enemy efficacy, we began by looking at the percentage of adult parasitoids successfully emerging from their hosts. We found a significantly lower percentage of adult parasitoids emerging from ancymidol, flurprimidol, and uniconazol treated plants than from paclobutrazol treated and untreated plants. While about 56% of adult parasitoids emerged from mummies on flurprimidol and uniconazol treated plants, 0% of the parasitoids successfully emerged in the ancymidol treatment. Several factors are known to affect *A. colemani* emergence, among which are temperature, desiccation, and declining parasitoid energy reserves (Colinet et al., 2006). As all mummies were placed in the same sized vials and in the same environmental chamber during emergence, we do not believe that temperature or humidity could have affected parasitoid emergence between treatments. Uçkan et al. (2008) suggested that changes in host hormones caused by the ingestion of the PGR, gibberellic acid, reduced the host survival and increased host developmental abnormalities, potentially affecting parasitoids. We do not know whether the PGRs in our study had a toxic effect on parasitoids, or reduced the nutritional quality of the plants or aphids but both of these have been shown to reduce parasitoid survivorship to adulthood (Slansky Jr, 1986; Thorpe and Barbosa, 1986; Holton et al., 2003). What is clear is that if the 0% emergence observed in the ancymidol treatment were to continue, the *A. colemani* population on these plants would eventually be reduced to zero, and along with it, aphid suppression. Though we did not observe reduced aphid suppression in this study, we suspect that prolonging the experiment for a few more parasitoid generations would have intensified the population-level effects of the PGRs on
both aphids and parasitoids, resulting in more distinct differences in aphid abundances.

As only female parasitoids can parasitize aphids, the sex ratio of the emerged parasitoids can greatly affect aphid suppression (Hagen and van den Bosch, 1968). For instance, although the greatest number of parasitoids emerged from the paclobutrazol treated plants, only 6% of these parasitoids were female and able to contribute to biological control of *M. persicae*. In fact, so few females emerged from the paclobutrazol treatment that more females emerged from the flurprimidol treatment, even with the significantly lower percent emergence observed on these plants. The reduced female ratio observed for paclobutrazol and uniconazol reared parasitoids could be due to plant and/or host quality effects, or simply due to reduced mating. As female parasitoids need to mate to produce female offspring, it is possible that sex ratios were male-biased in these two treatments due to reduced mating (Hopper and Roush, 1993). Even with mating, a male-biased sex ratio may occur. Resource limitation during parasitoid larval development has been shown to cause female larval mortality, resulting in a higher male survivorship, and consequently a male-biased sex ratio (Jarosik et al., 2003). Aphid hosts may have been resource limited either because of direct toxicity effects (Uçkan et al., 2008) or because of the reduced nutritional value of the uniconazol and paclobutrazol treated plants (Fox et al., 1990, 1996; Rademacher, 2000).

While a high female ratio is important for effective biological control (Heimpel and Lundgren, 2000), not all females perform equally. In general, large parasitoids have higher fitness and have higher host searching efficiency than small parasitoids (Visser, 1994). Large parasitoids have more fat reserves than small parasitoids, allowing them to disperse farther and to survive longer when food is unavailable (Eijs & van Alphen 1999). Parasitoid size is
also positively correlated with egg number (Eijs & van Alphen 1999; Sampaio et al. 2008), meaning that smaller parasitoids may become egg-limited and consequently less effective biological control agents earlier in their life (Rosenheim and Rosen, 1991; Heimpel and Rosenheim, 1998). In our study, we found that female parasitoids reared on paclobutrazol treated plants were significantly smaller than parasitoids from the other treatments. As with female ratio, and parasitoid percent emergence, we can only hypothesize how parasitoid size was affected by the PGR. For instance, it is possible that parasitoid size was reduced via a reduced host size caused by toxic effects of paclobutrazol (Coffelt et al., 1993). It is also possible that direct PGR toxicity affected parasitoid development, reducing its body size (Couty et al., 2001).

Whatever the mechanism may be, of the four PGRs tested, the application paclobutrazol resulted in parasitoids with the lowest fitness. We believe that this reduced fitness is what led to the significantly lower mummy abundance in the paclobutrazol treatment. It is therefore possible that the reduced parasitism and aphid suppression in the paclobutrazol treatment in Prado & Frank (in review) were not only caused by increased plant architectural complexity but also due to the reduced parasitoid fitness. It is evident from this study that PGRs can have variable effects on parasitoid life history traits, however, further research is needed to uncover the mechanism through which these effects occur. Future studies should investigate the effects of PGRs on parasitoid life history traits in addition to their effects on plant and host quality. Furthermore, to determine longer-term effects on aphid abundance, we suggest extending the duration of the study to increase the number of parasitoid and aphid generations exposed to PGRs. We hope that this work
highlights the need for more studies on the direct and indirect effects of such a common horticultural practice on natural enemy fitness.
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**Figure 1.** Overall mean (± SE) number of aphids on caged pepper plants during a three week experiment in which plants were untreated or treated with one of four PGRs and had parasitoids absent or present within the cages. Though a non-parametric mixed-effects ANOVA was performed to determine the effects of parasitoids and PGRs on aphid abundance, the untransformed aphid abundances are presented in this graph. Different letters above horizontal bars indicate significant ($P<0.05$) main effect of parasitoids on aphid abundance.

**Figure 2.** Overall mean (± SE) number of aphid mummies on caged pepper plants with parasitoids during a three week experiment in which plants were untreated or treated with PGRs. Though a non-parametric mixed-effects ANOVA was performed to determine the effect of each treatment on mummy abundance, the untransformed mummy abundances are presented in this graph. Means with different letters are significantly different at the $P=0.05$ level.
Figure 3. Total number of parasitoids that emerged from mummies removed from pepper plants that were untreated or treated with PGRs. Bars with different letters are significantly different at the $P=0.05$ level. The gray dots indicate the total number of mummies that were removed from the pepper plants (right y-axis) from which the parasitoids emerged.

Figure 4. Overall mean (± SE) hind tibia length of parasitoids emerging from mummies removed from pepper plants that were untreated or treated with PGRs. No parasitoids emerged from Abide, and therefore no hind tibia length measurements could be taken. Means with different letters are significantly different at the $P=0.05$ level.
Figure 5. Overall ratio of female:total number of emerged parasitoids. Means with different letters are significantly different at the $P=0.05$ level. The gray dots indicate the total number of emerged parasitoids (right y-axis) from which the female ratio was calculated.
CHAPTER 3: Optimal foraging by *A. colemani* affects the outcome of apparent competition

**Introduction**

Apparent competition is an important mechanism structuring herbivore communities in natural and managed ecosystems (Holt and Lawton 1994; Morris et al. 2005). Apparent competition is the indirect negative interaction between two hosts mediated by a shared parasitoid. As a two-host system can support more parasitoids than a one-host system, the parasitoid population in the two-host system can grow larger and exhibit greater parasitism on each of the host species compared to parasitoids in a one-host system (Bonsall et al. 1997). It is possible that the higher parasitism will be evenly distributed across host populations, resulting in equally reduced host species populations, however, it is more likely that one host will be able to support a higher parasitoid population better than the other (e.g. Settle & Wilson 1990). The host species unable to sustain the high attack rate by that large population will eventually be excluded from the system (Holt and Lawton 1994).

Over two decades ago, Holt & Kotler (1987) predicted that the indirect interaction between two hosts should be strongly affected by natural enemy foraging behavior. More specifically, they suggested that natural enemies forage in accordance to optimal foraging theory (Holt and Kotler 1987) which predicts that female parasitoids should selectively oviposit into hosts that result in the greatest reproductive success (Charnov & Skinner, 1985). This suggests that in a one-parasitoid, two-host system, fitness of parasitoid offspring should play a role in host selection and consequently may determine which host will be excluded from the system. While much theoretical information is available on apparent competition
and optimal foraging (Hughes, 1979; Charnov & Stephens, 2012), to our knowledge, no studies other than Veech (2001) have investigated the link between natural enemy optimal foraging behavior and the outcome of apparent competition.

Apparent competition is a particularly important concept to understand when developing banker plants systems for conservation biological control (Frere et al. 2007; Huang et al. 2011). Banker plants are non-crop plants used to support natural enemy abundance and reproduction by providing them with an alternative host or food source (Frank 2010). Typically aphid banker plant systems consist of grain plants infested with bird cherry oat aphids, *Rhopalosiphum padi* Linnaeus (Hemiptera: Aphididae), which only feed on monocots and therefore are not a pest in most greenhouses (Frank 2010). *Rhopalosiphum padi* share the parasitoid *Aphidius colemani* Viereck (Hymenoptera: Braconidae) with important pests such as the green peach aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae). Therefore, by providing *A. colemani* with two hosts, the parasitoid population can grow larger than it would in a one-host system, and consequently can exhibit greater parasitism on both host species (Bonsall et al. 1997). In order for the banker plant system to work as an effective pest suppression system, the parasitoid must parasitize the pest host more than the non-pest host. This may occur if the parasitoids prefers the pest or if optimal foraging decisions by the parasitoids result in higher parasitism of the pest aphids than the non-pest aphids (Bonsall and Hassell 1998). However, the availability of the alternative non-pest hosts can also reduce parasitism of the pest host if preference is equal or greater for the
non-pest host than the pest-host, or if host-switching behavior relaxes parasitism on either host at any given time (Holt and Lawton 1994; Bonsall and Hassell 1998).

In this paper, we examine how parasitoid optimal foraging behavior affects the outcome of apparent competition. To test this, we used the common aphid banker plant system with *R. padi* and *M. persicae* as hosts for *A. colemani*. As Ode et al. (2005) and Bilu et al. (2006) suggested that *R. padi* is an inferior host for *A. colemani* compared to *M. persicae*, we hypothesize that optimal foraging by *A. colemani* will result in lower parasitism of *R. padi* and higher parasitism of *M. persicae*. In addition, although it has also been suggested that parasitoid’s exhibit strong preference for their natal host (Messing 1995; Storeck et al. 2000; Bilu et al. 2006), it does not appear as though this is the case for parasitoids reared on *R. padi*, as they do not preferentially return to their natal host system (Bilu et al. 2006). This further supports our hypothesis that parasitoids will exhibit higher parasitism on *M. persicae* then they will on *R. padi*. To test optimal foraging by *A. colemani*, we first determined how natal host and offspring fitness influences *A. colemani* host preference in small-scale Petri dish and caged experiments. Next, to determine how optimal foraging affects the outcome of apparent competition, we compared *M. persicae* suppression in our two-host banker plant system to a one-host, one-parasitoid system.

**Methods**

2.1 Effect of offspring fitness on *A. colemani* host preference

2.1.1. Study System

All *Aphidius colemani* used in this experiment originally came from Koppert Biological (Howell, MI). Three *A. colemani* sources were used: Koppert Biological,
subcultured *R. padi* on barley (*Hordeum vulgare* ‘Price’), or subcultured on *M. persicae* on Black Pearl pepper plants (*Capsicum annuum* ‘Black Pearl’). These three sources will be referred to as ‘store’, ‘barley’ and ‘pepper’, respectively. We reared pepper and store parasitoids for at least one month (~ 4 generations) prior to the initiation of the experiments. Separate parasitoid-free aphid colonies were also started. *Myzus persicae* and *R. padi* from colonies were started from field-collected aphids and maintained in the laboratory on pepper and barley plants, respectively. Aphid and parasitoid colonies were maintained in an incubator at 25°C and 70-80% RH with a 16:8 L:D.

Black Pearl pepper plants were obtained from Raker and Sons (Litchfield, MI) as plugs (128 plugs <7cm in height). They were repotted into 15.2 cm-diameter pots filled with Fafard 2P soil mix (Agawam, MA) with 396.44g of Scotts Osmocote (N-P-K: 14-14-14) fertilizer (Marysville, OH) for every 0.08 m³ of soil. All pepper plants were 3-4 months old. Barley plants were started by planting 14g of barley seeds into 15.2 cm-diameter pots filled with the same Fafard 2P soil mixed with Scotts Osmocote as used for the pepper plants, and left to growth for 3 weeks before the start of the experiment.

2.1.2 Effect of natal host on parasitoid host preference

To determine if *A. colemani* natal host affects host preference, we conducted a choice assay by presenting *A. colemani* with *M. persicae* on pepper and *R. padi* on barley, and monitoring probing behavior. Before the experiment began, mummies were removed from barley and pepper plants and placed into separate 61 x 61 cm cages containing a 25% sucrose-water solution. The same procedure was taken upon receipt of store mummies from Koppert Biological. Parasitoids were left in the cages to emerge, eat, and mate for 48 hours.
We began by placing 1.5 cm long segments of Black Pearl pepper and barley leaves 12 cm apart in a 14.5 cm diameter Petri dish. Two *M. persicae* and two *R. padi* were then transferred onto the pepper and barley leaves, respectively. We left the aphids in the Petri dish for one hour before starting the experiment. During that hour, female *A. colemani* were aspirated from their respective cages. After one hour, one of either pepper, barley or store parasitoids was placed in the center of the Petri dish and observed for 30 minutes to record which species of aphid was first probed (‘*R. padi*’, ‘*M. persicae*’). This experiment was replicated 30 times for each parasitoid source. Thirteen store, twenty pepper and sixteen barley parasitoids did not probe an aphid within 30 minutes and were removed from the preference analysis leaving 17, 10, and 14 replicates respectively. However, all 30 replicates were used to determine the proportion of probing *A. colemani* from each source.

A Pearson’s chi-squared test was then used to determine the effect of parasitoid natal host (store, barley, pepper) on the frequency of *A. colemani* selecting *M. persicae* over *R. padi*. A second chi-squared test was used to determine the effect of natal host on the proportion of probing parasitoids.

### 2.1.3. Effect of offspring fitness on parasitoid host preference

To determine the effect of offspring fitness on *A. colemani* host choice, we presented *A. colemani* from all three sources (store, barley pepper) with *M. persicae* on pepper and *R. padi* on barley plants, allowed parasitism to occur, and then measured life history traits of the emerging offspring. Before the experiment began, mummies were removed from barley and pepper plants and placed into separate 61 x 61 cm cages containing a 25% sucrose-water
solution. The same procedure was taken upon receipt of mummies from Koppert Biological. Parasitoids were left in the cages to emerge, eat and mate for <72 hours.

Our experimental arena consisted of twenty-four 61 x 61 cm cages built using PVC pipes and organdi fabric. We placed one barley and one pepper plant 34 cm apart in each cage. One day before the start of the experiment, 20 *M. persicae* and 20 *R. padi* were placed on the pepper and barley plants, respectively. The next day, female *A. colemani* were aspirated from their respective cages and transported in their individual, labeled, aspirator vials to the greenhouse. Each aspirator vial containing one female parasitoid was placed in between the two plants in the cage and opened so that the parasitoid could fly out. Parasitoids were allowed to move around the cage for four hours after which they were removed to end the experiment.

The plants were left in their cages for 7 days, at which time we removed the plants and counted all mummies. The mummies were then removed by lifting the mummies off the leaves using a small paintbrush, and then placed into vials labeled according to their source and chosen host. Each vial was stopped with a cotton ball to prevent parasitoids from flying out. Parasitoids were reared out of mummies in the laboratory and preserved in 90% alcohol upon emergence. We examined each parasitoid under a dissection microscope with an ocular micrometer to determine their gender and measure the length of their left hind tibia. Parasitoid percent emergence was determined by dividing the total number of emerged parasitoids by the total number of mummies. This experiment was replicated 40 times for ‘barley’ and ‘store’ sourced parasitoids, and 51 times for ‘pepper’ sourced parasitoids. 35 of the ‘pepper’, 15 of the ‘barley’, and 8 of the ‘store’ sourced parasitoids did not parasitize any
aphids and therefore were removed from the preference analysis. However, all replicates were used to determine the frequency of parasitizing *A. colemani* from each source.

A Pearson’s chi-squared test was then used to determine the effect of parasitoid source (store, barley, pepper) on the frequency of *A. colemani* parasitizing *M. persicae* over *R. padi*. A second chi-squared test was used to determine the effect of *A. colemani* source on the frequency of parasitizing *A. colemani* out of all the replicates. A Chi-squared test was also performed to determine the effect of host choice and source on the overall frequency of adult parasitoids successfully emerging from their hosts. A two-way ANOVA was used to determine the effect of parasitoid source and chosen host on the mean number of emerging females per host species. Chi-squared tests were performed to determine if sex ratio (female:total), and percent emergence were affected by parasitoid source and host choice. The mean number of emerging females was log + 1 transformed to obtain normality. Another three-way ANOVA was used to determine the effect of source, chosen host, and sex on parasitoid size.

### 2.2. Effect of parasitoid optimal foraging on the outcome of apparent competition

#### 2.2.1. Study system

All *A. colemani* used in this experiment originally came from Koppert Biological (Howell, MI). *Aphidius colemani* were used either directly from Koppert Biological or from a subculture of *A. colemani* reared on *R. padi* on barley (*Hordeum vulgare* ‘Price’). These two sources will be referred to as ‘store’ and ‘barley’, respectively. The barley parasitoids had reared on *R. padi* for over 4 months (~16 generations) before the experiment began. We used *M. persicae* and *R. padi* from laboratory colonies that were started from field-collected
aphids. All insects were reared on their respective host plant/aphid in incubators at 25°C and 70-80% RH with a 16:8 L:D.

All Black Pearl pepper plants (*Capsicum anuum* ‘Black Pearl’) were obtained from cuttings. Source plants were cut 5-10 cm below the bud. The cut tips were then dipped into “Rhizopon AA Dry Powder Rooting Hormone #1” (Active ingredient: 0.1% 3-Indolebuteric acid) (Earth City, MO) and potted into a sifted Fafard 2P mix for germination. All cuttings were planted in 48 pot trays (56cm by 25.5cm tray). The cuttings were left to root for 6 weeks before they were transplanted into 6-inch pots with Farfard 2P soil mix with 396.44g of Scotts Osmocote (N-P-K: 14-14-14) fertilizer for every 0.08 m³ of soil. All Black Pearl pepper plants were between 2-3 months-old. Barley plants were started by planting one tablespoon of barley seeds into 15.2 cm-diameter pots filled with the same Fafard 2P soil mixed with Scotts Osmocote. One-week old barley plants were moved into the incubator containing *R. padi* and *A. colemani*. All barley plants were 2-weeks old at the start of the experiments.

Two greenhouses were set-up in the same way as Vásquez et al. (2006). Part of each greenhouse was partitioned into three bays (2.08m by 6.1m) using Pro19 grade agrifabric (Agrofabric, Alpharetta, GA), which allowed air circulation through the bays but no movement of aphids or parasitoids. Two bays were used for each replicate, wherein one bay was used for the two-host banker plant system and the other bay was used for the one-host, one-parasitoid system. Two days before the start of this experiment, two Black Pearl pepper plants were infested with 30 *M. persicae*. These infested plants (‘focal plants’) were kept in an incubator at 25°C and 70-80% RH with a 16:8 L:D until the experiment began. On the
first day of the experiment, 28 pepper plants, including one focal plant, were placed in each bay (Fig. 1). In the 2-host banker plant bay, we placed two barley plants with 11-42 *R. padi* and 15 *A. colemani* mummies per plant at each end of the greenhouse bench in the 2-host banker plant bay. In the one-host, one-parasitoid bay, two Petri dishes containing 15 store mummies were placed at each end of the greenhouse bench. Each of the 28 pepper plants were numbered in order to facilitate counting of aphids and monitoring of their movement within each bay.

Also on the first day of the experiment, but in the laboratory, we placed 30 barley mummies and 30 store mummies into separate vials with cotton balls as stoppers. The mummies were removed from the barley plants by lifting the mummies off the leaves using a small paintbrush. Barley and store parasitoids were reared out of the mummies in each vial and preserved in 90% alcohol upon emergence. We examined each parasitoid under a dissection microscope with an ocular micrometer to determine their gender and measure the length of their left hind tibia. Parasitoid percent emergence was determined by dividing the total number of emerged adult parasitoids by the total number of mummies. This allowed us to determine what the parasitoids’ pre-parasitism life-history traits were.

On the 7th, 14th, 21st and 28th day of the experiment all aphids and mummies were counted on the pepper and barley plants in each bay. At the end of the experiment, mummies were removed from the pepper plants by lifting the mummies off the leaves using a small paintbrush, and then placed in vials stopped with cotton balls. Pepper parasitoids from each bay (two-host vs. one-host) were reared out of their mummies and, upon emergence were preserved in 90% ethanol. As with the barley and store parasitoids at the beginning of the
experiment, we determined parasitoid gender, hind left tibia length, and percent emergence. This allowed us to determine what the parasitoids’ post-parasitism life-history traits were. Also at the end of the experiment, we counted the total number of aphids and mummies on each barley plant to determine mean percent parasitism and relative abundance of parasitoids (mummies/aphid).

This experiment was replicated four times, however one replicate in each treatment had zero parasitism throughout the experiment, and therefore was removed from the analysis.

2.2.2. Statistical Analysis

Four mixed effects ANOVA were used to determine how time and treatment (two-host vs. one-host) interacted to affect aphid abundance, mummy abundance, the number of mummies per aphid and percent parasitism. To obtain normality aphid abundance was log transformed, mummy abundance was log + 1 transformed, and the number of mummies per aphid and percent parasitism were arc sin square root transformed. To determine the mean percent parasitism of barley parasitoids in the two-host system, we averaged the total number of mummies/mummies+aphids, and to determine the relative number of parasitoids to aphids on barley, we used mummies/aphids.

A one way ANOVA was used to determine if parasitoid size varied with sex and treatment and source (barley, store), pre and post parasitism. Due to the small sample size, a Mann-Whitney U-test was used to determine if parasitoid percent emergence was affected by treatment and source. Lastly, a chi squared test was performed to determine if female:total ratio varied between treatments and source, at the beginning and at the end of the experiment.
Results

3.1. Effect of offspring fitness on A. colemani host preference

3.1.1. Effect of natal host on parasitoid host preference

A chi-squared test showed a significant effect of parasitoid source on the frequency of *M. persicae* probed over *R. padi* ($\chi^2 = 7.12; P = 0.028$), wherein a significantly greater amount of *M. persicae* were probed by pepper parasitoids than by barley and store parasitoids (Fig. 2). A chi-squared test also showed that frequency of probing parasitoids was significantly greater for store parasitoids than for pepper parasitoids ($\chi^2 = 5.40; P = 0.020$), but did not significantly differ between the store and barley parasitoids, nor between barley and pepper parasitoids ($\chi^2 = 0.63; P = 0.429; \chi^2 = 1.70; P = 0.193$; respectively) (Fig. 2).

3.1.2. Effect of offspring fitness on parasitoid host preference

As in the previous experiment, a chi-squared test showed a significant effect of parasitoid source on the frequency of *M. persicae* parasitized over *R. padi* ($\chi^2 = 38.85; P < 0.0001$), wherein a significantly greater amount of *M. persicae* were parasitized by pepper parasitoids than barley and store parasitoids (Fig. 3). Barley and store parasitoids showed equal preference for either host ($\chi^2 = 0.01; P = 0.93$). Proportionally more store parasitoids parasitized aphids than both barley and pepper parasitoids ($\chi^2 = 7.58; P = 0.006; \chi^2 = 7.03; P = 0.008$; respectively). No significant difference was found between the proportion of parasitizing barley and pepper parasitoids ($\chi^2 = 0.01; P = 0.934$) (Table 1).

The frequency of emerging adults from store and barley reared parasitoids did not significantly differ between host ($\chi^2 = 0.04; P = 0.85; \chi^2 = 0.0005; P = 0.982$; respectively).
On the other hand, pepper parasitoids had 31% greater emergence when emerging from *M. persicae* than from *R. padi*, indicating that *R. padi* is a less suitable host for pepper parasitoids than *M. persicae* (Table 1). The mean number of emerged females was not significantly affected by sources or host (*F* = 2.24; *P* = 0.115, *F* = 0.15; *P* = 0.702, respectively) (Table 1). There was also no significant interaction between host and source on the mean number of emerged female parasitoids (*F* = 0.79; *P* = 0.456). Parasitoid female ratio (female: total) was not significantly different across sources for each host (*R. padi*: *χ₂²* = 1.92; *P* = 0.382; *M. persicae*: *χ₂²* = 1.18; *P* = 0.553) (Table 1).

There was a significant interaction between parasitoid source and sex on parasitoid size (*F* = 3.02; *P* = 0.050), however the interaction between sex, host and source was not significant (*F* = 0.27; *P* = 0.764) nor were the interactions between sex and host or host and source (*F* = 0.88; *P* = 0.350; *F* = 0.87; *P* = 0.418, respectively). The main effects of sex and host were significant (*F* = 8.94; *P* = 0.003, *F* = 91.47; *P* < 0.0001, respectively), however there was no significant main effect of source (*F* = 2.69; *P* = 0.069).

### 3.2 Effect of parasitoid optimal foraging on the outcome of apparent competition

There was a significant interaction between time and treatment (two-host vs. one-host) on aphid abundance on the pepper plants (*F* = 4.31; *P* = 0.052), wherein aphid abundance in the one-host, one-parasitoid system was at least double the aphid abundance in the two-host banker plant system from days 14-28 (Fig. 4A). There were no significant main effects of time and treatment on aphid abundance (*F* = 2.77; *P* = 0.113, *F* = 3.21; *P* = 0.090, respectively). Mummy abundance on the pepper plants was also significantly affected
by time, \( (F_{1,18}=11.92; \ P = 0.003) \) (Fig. 4A), but was not significantly affected by treatment nor the interaction between treatment and time \( (F_{1,18}=0.33; \ P = 0.571, \ F_{1,18}=0.48; \ P = 0.497, \) respectively). Percent parasitism was significantly affected by treatment \( (F_{1,18}=4.22; \ P = 0.054) \), wherein a significantly higher percent parasitism was found in the two-host treatment than in the one-host treatment. The main effect of time also significantly affect percent parasitism \( (F_{1,18}=8.66; \ P = 0.009) \), but no significant interaction between time and percent parasitism was found \( (F_{1,18}=2.70; \ P = 0.117) \) (Fig. 4C). Lastly, the number of mummies per aphid was significantly affected by treatment \( (F_{1,18}=4.68; \ P = 0.044) \), wherein there were significantly more mummies per aphid in the two-host treatment than in the one-host treatment. There was also a significant main effect of time on the number of mummies per aphid, but no significant interaction between time and treatment \( (F_{1,18}=7.19; \ P = 0.015, \ F_{1,18}=2.48; \ P = 0.132, \) respectively) (Fig. 4D).

There was a significant interaction between sex and host on parasitoid size \( (F_{1,365}=4.47, \ P = 0.004) \) (Fig. 5) wherein female store and barley parasitoids were significantly smaller than pepper parasitoids from the two-host banker plant system and the one-host, one-parasitoid system. This suggests that pepper-reared female parasitoids have higher fitness than store and barley female parasitoids. Males reared on pepper plants in the one-host, one-parasitoid did not differ in size from the store and the barley parasitoids. There were also significant main effects of sex and host on parasitoid size \( (F_{1,365}= 8.43, \ P = 0.004; \ F_{1,365}= 108.896, \ P< 0.0001, \) respectively).
Mann-Whitney U-tests showed no significant difference between the percent emergence of mummies collected from barley and pepper (two-host) \((W=5, P = 0.857)\) nor was there a difference in percent emergence between mummies collected from barley and pepper (one-host) \((W=2.5, P = 0.481)\) (Fig. 6). No significant difference in percent emergence was found between mummies collected from pepper (two-host) and pepper (one-host) \((W=4, P = 0.8)\), nor was there one between mummies collected from store and pepper (two-host) \((W=8, P = 0.476)\). There was a significant difference between percent emergence from mummies from store and the pepper (one-host) \((W=8, P = 0.030)\), wherein a significantly higher percentage of adult parasitoids successfully emerged from pepper than from store mummies. There was also a significant difference in the percent emergence from mummies from barley and store \((W=16, P = 0.020)\), wherein a significantly higher percentage of adult parasitoids successfully emerged barley than from store mummies.

Parasitoid female ratio (female: total) significantly differed between pepper parasitoids in the two-host banker plant system and those in the one-host, one-parasitoid system \((\chi^2 =4.72; P =0.030)\), resulting in 37% less females emerging from pepper in the one-host, one-parasitoid system and pepper in the two-host banker plant system (Fig. 7). Parasitoid female ratio also significantly differed between the barley and store parasitoids \((\chi^2 =4.522; P =0.033)\), barley and pepper (one-host) parasitoids \((\chi^2 =11.48; P =0.001)\) and barley and pepper (two-host) parasitoids \((\chi^2 =4.72; P =0.030)\). No significant differences were found between percent emergence of store and \((\chi^2 =1.0954; P =0.295)\), nor between store and banker plant parasitoids \((\chi^2 =0.40; P =0.528)\).
Discussion

In a two host one parasitoid system, apparent competition theory predicts that the host species that is unable to reproduce fast enough to support the large parasitoid population developing on these two hosts, and the high attack rate by this large parasitoid population will eventually be excluded from the system (Holt and Lawton 1994). Holt & Kotler (1987) models predict that optimal foraging by a natural enemy results in apparent competition favoring the survival of the least optimal host. In our system, optimal foraging behavior led female *A. colemani* to prefer *M. persicae* over *R. padi*, resulting in apparent competition favoring survival of the least preferred host, *R. padi*.

Optimal foraging theory predicts that female parasitoids should preferentially oviposit in hosts that would produce offspring with the highest fitness (Cook and Hubbard 1977; Charnov and Skinner 1985). We used offspring survival (emergence) as a proxy for fitness as we found no significant source by host effect on offspring size and sex ratio. We found that offspring survival influences *A. colemani* oviposition behavior. More specifically, our results indicate that when *A. colemani* experiences reduced offspring survival on one host, as was the case for pepper parasitoids (reared on *M. persicae* on Black Pearl pepper) ovipositing in *R. padi*, it exhibits a strong preference for the other host, *M. persicae*. On the other hand, when no negative effects are experienced by offspring reared on either host, as was the case for store (from Koppert Biological – unknown host) and barley parasitoids (reared on *R. padi* on barley), *A. colemani* exhibits roughly equal preference for both host. Unlike other studies (Messing 1995; Storeck et al. 2000), we found no evidence that *A. colemani* preferentially parasitizes its natal host.
Although optimal foraging behavior by *A. colemani* is indicative of host preference, this alone does not determine which host will be excluded from the system. Therefore, in order to test how optimal foraging affects the outcome of apparent competition, we used store and barley parasitoids, in the same way they are used in biological control for *M. persicae*. According to apparent competition theory, we would expect to find a larger parasitoids population in the two-host system, than in the one host system, and based on the results of our preference experiment, we expected to see 70% of this larger parasitoids population parasitizing *M. persicae* in the two-host system, and 100% of a smaller parasitoid population parasitizing *M. persicae* in a one-host, no-choice system. Our findings went in accordance with our hypothesis, as we found significantly less *M. persicae* in the two-host system than in the one-host system.

According to Holt and Lawton (1994) exclusion of a host can occur if $0 < r < aP$, wherein $r$ is the species intrinsic rate of increase, $a$ the natural enemy’s attack rate on that species, and $P$ the size of the natural enemy population. Comparing both *M. persicae* populations, we found a significantly higher attack rate of *M. persicae* (percent parasitism) and relative abundance of parasitoids (mummies/*M. persicae*) on pepper plants in the two-host system than in the one-host system (Table 2). In addition, although we found a much higher percent parasitism and relative abundance of parasitoids on *R. padi* than *M. persicae*, our preference experiment shows that ~56% of the parasitoids emerging from *R. padi* will move on to parasitize *M. persicae*, causing the total parasitoid population to increase (Table 2). Therefore, as predicted by apparent competition theory, the availability of two hosts
allowed the parasitoid population to grow larger and attack more *M. persicae* than the smaller parasitoid population in the one-host system.

Our analysis of life history traits pre- and post-parasitism, showed that both store and barley parasitoids were inferior to pepper parasitoids in at least one aspect of life history, confirming that optimal foraging was taking place. In the two-host banker plant experiment, we found that (pre-parasitism) barley parasitoids were inferior to pepper parasitoids (post-parasitism) in terms of their size. As size is generally positively correlated with host searching efficiency (Visser 1994), flight distance (Eijs & van Alphen 1999), and egg number (Sampaio et al. 2008), we believe larger parasitoids have higher fitness than smaller ones, and that this behavior is consistent with optimal foraging. On the other hand, we observed a significantly higher female sex ratio on the barley parasitoids than we did on the pepper parasitoids in the two-host system. Several factors could explain this higher female sex ratio. For instance, Hamilton (1967) demonstrated that when a population is small, as it was on the barley plants, more females are present in the population, in order to decrease the odds of mating with siblings. As the population size increases, so should the number of males (Hamilton 1967), thus producing more or less even sex ratios as we observed at the end of our experiment. A male-biased sex ratio may also occur on resource limited plants (Fox et al. 1990). Pepper plants may have been more resource limited than barley plants due to the higher number of aphids (max. 51/plant vs. 536/plant, respectively) feeding on them (Awmack and Leather 2002). The same proportion of adult parasitoids successfully emerged from both pepper and barley mummies.

We also found inferior life history traits for store parasitoids than for pepper
parasitoids in the one-host systems. Life history analysis of store and pepper parasitoids showed store parasitoids had significantly lower offspring survival and were significantly smaller in size than pepper parasitoids. However, as was observed for the pepper parasitoids in the two-host system, pepper parasitoids in the one-host system had significantly lower female:total ratio than did the store parasitoids. This reduced female ratio could be due to the reduced plant quality of the heavily infested pepper plants (max. 2010 aphids/plant) (Fox et al. 1990), or simply due to reduced pepper parasitoid mating (Hopper and Roush 1993).

We cannot eliminate the possibility that store and barley parasitoids initial life history traits may have led to the differences in aphid abundance across treatments. However, we do not believe this to be the case, as we found no significant difference in mummy abundance across treatment, indicating that parasitism was the same in both treatments. Furthermore, we suspect that the store parasitoids were in fact at a starting advantage due to their close to equal sex ratio, and their early, synchronized emergence (personal observation; Fernández & Nentwig, 1997), which likely allowed for easy mate-finding and mating within the first days of the experiment. In addition, based on our observations in the caged preference experiment, a greater proportion of store (80%) than barley (65%) parasitoids parasitized aphids. Thus, even with the significantly lower offspring survival of store parasitoids, only two extra barley parasitoids would have parasitized aphids (12 store vs. 14 barley) at the start of the experiments.

This study demonstrates the relationship between parasitoid preference, offspring fitness and apparent competition. To our knowledge, this is the first study to investigate the link between parasitoid optimal foraging behavior and the outcome of apparent competition,
and the application of these theories in a biological control system. Knowing that optimal foraging by a natural enemy can significantly affect pest suppression can help researchers understand what factors may be affecting the efficacy of a biological control program. We hope that this study can help growers and researchers design more effective biological control systems when multiple hosts or prey items are available.
References


APPENDICES
Table 1. *A. colemani* host preference and source by host effects on life history traits. Means with different letters next to them are significantly different at the $P \leq 0.05$ level.

<table>
<thead>
<tr>
<th>Source</th>
<th>Store parasitoids</th>
<th>Barley parasitoids</th>
<th>Pepper parasitoids</th>
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<tbody>
<tr>
<td></td>
<td><em>M. persicae</em></td>
<td><em>R. padi</em></td>
<td><em>M. persicae</em></td>
</tr>
<tr>
<td>Chosen Host</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Petri</td>
<td>60% <strong>B</strong></td>
<td>40%</td>
<td>36% <strong>B</strong></td>
</tr>
<tr>
<td>Greenhouse</td>
<td>53.4% <strong>B</strong></td>
<td>46.6%</td>
<td>56.2% <strong>B</strong></td>
</tr>
<tr>
<td>Percent Emergence</td>
<td>71% <strong>A</strong></td>
<td>69.8% <strong>A</strong></td>
<td>71.6% <strong>A</strong></td>
</tr>
<tr>
<td>Female:total emerged</td>
<td>32:78 <strong>A</strong></td>
<td>29:67 <strong>A</strong></td>
<td>58:101 <strong>A</strong></td>
</tr>
<tr>
<td>Mean females/plant</td>
<td>3.2±1.4 <strong>A</strong></td>
<td>1.45±0.4 <strong>A</strong></td>
<td>3.63±0.8 <strong>A</strong></td>
</tr>
<tr>
<td>Size (mm)</td>
<td>No host by source interaction</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Results of the apparent competition experiment demonstrated in terms of Holt and Lawton (1994) exclusion criterion.

0<r<aP (Holt & Lawton 1994)

<table>
<thead>
<tr>
<th>Pepper (2-host) (90% pepper parasitoids stay on pepper)</th>
<th>Pepper (1-host)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>r</em>= population growth of <em>M. persicae</em></td>
<td><em>r</em>= population growth of <em>M. persicae</em></td>
</tr>
<tr>
<td><em>a</em>=12.15% parasitism</td>
<td><em>a</em>=2.57% parasitism</td>
</tr>
<tr>
<td><em>P</em>= relative abundance : 0.16 mummies/aphid</td>
<td><em>P</em>= relative abundance : 0.03 mummies/aphid</td>
</tr>
</tbody>
</table>

| Barley (50% barley parasitoids move to pepper)          | |
|--------------------------------------------------------| |
| *r*= population growth of *R. padi*                    | |
| *a*=62.05% parasitism                                  | |
| *P*= relative abundance : 3.37 mummies/aphid          | |
Figure 1. Greenhouse set-up for both the augmentative release and banker plant treatments. The gray circles indicate where 15 mummies (in Petri dishes for the augmentation release or on the banker plants) were positioned. The black circle is where the infested plant (with 30 *M. persicae*) was placed.

Figure 2. The effect of parasitoid source on parasitoid host choice in a Petri dish experiment. Parasitoids were given the choice to either parasitize *M. persicae* on Black Pearl pepper plants or *R. padi* on barley, over a 30 minute period. The black dots indicate the proportion of *A. colemani* that probed an aphid out of the 30 replicates for each treatment. Bars (all caps) and dots (lower case) with different letters are significantly different at the \( P<0.05 \) level.
Figure 3. The effect of parasitoid source and parasitoid host choice on the number of mummies in a caged experiment. The black dots indicate the proportion of A. colemani that parasitized at least one aphid out of the 40 replicates for store and barley parasitoids and the 52 replicates for pepper parasitoids. Bars (all caps) and dots (lower case) with different letters are significantly different at the P<0.05 level.
Figure 4. A) Overall mean (± SE) number of *M. persicae* present over the course of the 28-day in a one-host (*M. persicae*), one-parasitoid treatment and in a two-host (*R. padi* and *M. persicae*), one parasitoid treatment. A significant interaction of treatment*time was found to affect *M. persicae* abundance.

B) Mean (± SE) number of *M. persicae* mummies over the course of 28 days, in a one-host (*M. persicae*), one-parasitoid treatment and in a two-host (*R. padi* and *M. persicae*), one parasitoid treatment. There was no significant difference between mummy abundance in each treatment.

C) Mean (± SE) percent parasitism of *M. persicae* (mummies/mummies+aphids) over 28 days, in a one-host (*M. persicae*), one-parasitoid treatment and in a two-host (*R. padi* and *M. persicae*), one parasitoid treatment. There was a significant difference between percent parasitism in the one-host and two-host treatments.

D) Mean (± SE) relative abundance of *A. colemani* (mummies/aphids) over the course of the 28 days, in a one-host (*M. persicae*), one-parasitoid treatment and in a two-host (*R. padi* and *M. persicae*), one parasitoid treatment. There was a significant difference between *A. colemani* relative abundance in the one-host and two-host treatments.
Figure 5. Initial (barley and store) and final (pepper 1-host, pepper 2-host) parasitoid sizes were measured to determine host effects. Means with different letters are significantly different at the P<0.05 level.

Figure 6. Initial (barley and store) and final (pepper 1-host, pepper 2-host) parasitoid percent emergence were determined. Means with different letters are significantly different at the P<0.05 level.
Figure 7. Initial (barley and store) and final (pepper 1-host, pepper 2-host) parasitoid female:total ratio were determined. Means with different letters are significantly different at the $P<0.05$ level.

Figure 8. Schematic representation, based on parasitoid preference results, of parasitoid movement in the one-host and two-host treatments.