EVALUATION OF THE *APHIDIUS COLEMANI-* *RHOPALOSIPHUM PADI* BANKER PLANT SYSTEM IN GREENHOUSE BIOLOGICAL CONTROL

By

TRACEY LEE PAYTON MILLER

Bachelor of Science in Horticulture
Oklahoma State University
Stillwater, Oklahoma
2004

Master of Science in Entomology
Oklahoma State University
Stillwater, Oklahoma
2007

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EVALUATION OF THE *APHIDIUS COLEMANI-*

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Dissertation Approved:

Dr. Eric J. Rebek

Dissertation Advisor

Dr. Steve Frank

Dr. Mike Schnelle

Dr. Kris Giles

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Banker plants are mobile habitats that provide alternate hosts or food for commercially available natural enemies. As a biological control strategy, banker plants offer a novel non-chemical approach to managing commonly encountered pests in the greenhouse. Most banker plants that target aphids consist of a graminaceous plant, a non-pest cereal grain aphid, and a parasitoid that attacks both the non-pest and pest aphids occurring on crop plants. Use of banker plants may provide more effective, long-term pest control than pesticide applications, but both can be combined. Banker plant systems have been used commercially in areas of the United States, Canada, Europe, and Asia. One of my goals was to ascertain if banker plants are a viable aphid pest management technique in the southwestern United States. The following study is an overview of the history of biological control in enclosed environments, the *Aphidius colemani-Rhopalosiphum padi* banker plant system in Oklahoma, pesticides compatible with *A. colemani* natural enemies, the costs and benefits of the *Aphidius colemani-Rhopalosiphum padi* system to manage aphid pests, and alternative species of grasses for potential use as banker plants.

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CHAPTER I

INTRODUCTION

Banker plants (a.k.a., open-rearing systems, biocontrol plants) offer sustained management of common arthropod pests and are often incorporated into greenhouse crop production (Kuo-Sell 1987, Van der Linden 1992, Jacobson and Croft 1998, Schoen 2000). Banker plant systems consist of arthropod natural enemies (i.e., predators or parasitoids), alternate prey, hosts, or food for the natural enemies, and banker plants that support these resources (Huang et al. 2011). Banker plants are placed throughout the greenhouse and provide reliable, long-term reproduction (Stacey 1977, Huang et al. 2011) and dispersal of natural enemies released for control of target pests (van Lenteren et al. 1997, Pratt and Croft 2000). Banker plants are considered a combination of augmentative and conservation biological control strategies (Parella et al. 1992, Frank 2010, Huang et al. 2011) as they provide an optimal habitat for natural enemies but do not require their frequent release. Specifically, natural enemies are released into the crop, and banker plants promote their survival, longevity, and reproduction by providing them with essential resources such as food or shelter (Arnó et al. 2000, Gurr et al. 2000, Huang et al. 2011). Ideally, banker plants are compact and mobile; thus, they do not need extensive production space and easily conform to current growing practices.

Additionally, banker plants can be moved closer to problem areas or removed from the greenhouse when pesticide sprays or other maintenance is necessary. Banker plants are replaced every few weeks or few months depending on the species (Frank 2010).

Objectives and Justification

In this study, I aimed to assess the effectiveness of banker plant systems in Oklahoma greenhouses and convey their use to growers; assess the effectiveness and economics of banker plants versus augmentative releases; evaluate pesticide compatibility with *Aphidius colemani* Viereck (Hymenoptera: Braconidae); identify alternative warm-season host plants of *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae); and, ultimately, be able to recommend a cohesive banker plant system for management of aphid pests in the southwestern U.S. This study will meet the following objectives:

1. Evaluate an established banker plant system in commercial greenhouses in Oklahoma.
2. Determine pesticide compatibility of commonly used insecticides and fungicides with the aphid parasitoid, *A. colemani.*
3. Compare warm-season Poaceae varieties with the effectiveness of wheat (*Triticum aestivum* (L.)) as a banker plant for rearing *R. padi* in greenhouses.

CHAPTER II

LITERATURE REVIEW

Biological Control in Greenhouses

The first documented, successful use of biological control in greenhouses was in 1927 targeting greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), with augmentative releases of *Encarsia* species (Hymenoptera: Aphelinidae) (Speyer 1927). However, the development and adoption of synthetic pesticides in the late 1940's led to a decline in use of biological control until pesticide resistance occurred in the 1960's and 1970's (van Lenteren 2007, Huang et al. 2011). In response to resistance management concerns, integrated pest management (IPM) strategies were considered, including biological, cultural, and mechanical controls. This ecologically based approach minimizes the risk of resistance as pesticides are used less frequently and replaced with preventive strategies and biologically based methods. Still, the adoption of IPM in greenhouses may lag for several reasons including availability of effective insecticides, fear of exporting pests, and reduced marketability of plants with visible damage (van Lenteren 2000). The use of banker plants in greenhouses is a fairly new concept in IPM, first described in the late 1970's in tomatoes

(*Solanum lycopersicum* (L.)) using *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae) and the pest-in-first strategy (see description below) with *T. vaporariorum* (Stacey 1977). As with other biological control strategies, banker plants can be used in the field but are ideal in controlled environments where higher profits can be generated per square foot of production space (van Lenteren 2000, Huang et al. 2011). Biological control may be easier in greenhouses compared to field-grown crops as many pests are excluded by the structure, fewer insect pests are encountered in greenhouses, and pest and natural enemy development is more predictable in known temperature ranges (van Lenteren 2000). Also, pests and natural enemies are readily monitored in enclosed environments, helping mitigate damage from costly pests (van Lenteren et al. 1997, van Lenteren 2000). Variations of banker plant systems can be used to control pests such as thrips (Ramakers and Voet 1995), whiteflies (Stacey 1977, Lambert et al. 2005), aphids (Hofsvang and Hågvar 1979, Wick 1992, Andorno and López 2014), spider mites (Van Rijn and Tanigoshi 1999, Pratt and Croft 2000), and leafminers (van Lenteren and Woets 1988). Banker plants and other IPM methods are frequently used in vegetables, but are being adapted for use in production of potted plants and cut flowers (Blumel and Hausdorf 1996, van Lenteren 2000, Vásquez et al. 2006, Van Driesche et al. 2008, Abraham et al. 2013). Banker plants may provide a food source such as pollen to conserve or attract natural enemies. Other banker plant systems may involve use of previously parasitized alternate hosts, an initial release of beneficial insects, or the pest-in-first approach. In the latter, the target pest is deliberately introduced prior to an infestation and acts as an alternate host for the natural enemy (Huang et al. 2011). The pest-in-first strategy can be successful when using parasitoids to control whiteflies in vegetables (Stacey 1977, Lambert et al. 2005) but may not be adopted by growers for fear of pest outbreaks.

Stacey (1977) documented control of *T. vaporariorum* in greenhouse tomatoes using pest-in-first tomato banker plants with whiteflies parasitized by *E. formosa*. In this study, no sooty mold was found in occurrence with the whitefly, and 8,000 parasitoids were produced over a nine-week period to suppress *T. vaporariorum*. In addition, Lambert (2005) successfully suppressed *T. vaporariorum* over five months in winter greenhouse tomatoes using *Dicyphus hesperus* (Knight) (Hemiptera: Miridae) on mullein banker plants with supplemental *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) egg releases.

Worldwide, an estimated 32,000 ha of greenhouse and interiorscapes are managed with biological control using 150 species of natural enemies (van Lenteren 2006, 2012). Traditionally, greenhouse growers have depended on pesticides and have not embraced biological control in their operations due to a zero-tolerance mentality for pests and associated damage in ornamentals and vegetables (van Lenteren and Woets 1988, van Lenteren 2000). However, biological control may be more readily adopted since pesticide use has lost favor because of lack of available chemistries (van Lenteren and Woets 1998), resistance management concerns (van Lenteren 2000, Desneux et al. 2007), required employee training and use of personal protective equipment (Kühne 1998), phytotoxicity or abscission in sensitive plants (van Lenteren and Woets 1998), potential health risks (van Lenteren and Woets 1998) and consumer demand for products with low pesticide residues and decreased environmental repercussions (Kühne 1998, van Lenteren 2000). Consumer backlash over the use of neonicotinoids and their negative effects on pollinator health has put pressure on greenhouse growers to label plants treated with neonicotinoids or use alternative pest management strategies in flowering ornamental plants (Rihn and Khachatryan 2016).

Biological control is an option for those greenhouse growers interested in low-impact pest management.

Banker plants provide an effective first step in pest management with little or no negative environmental effects and may be combined with other biological control agents or pesticides to solve many pest issues (Gentz et al. 2010, Prado et al. 2015). Biological control reduces the number of pesticide applications, decreases or eliminates the likelihood of pest resistance (Hågvar and Hofsvang 1994, van Lenteren et al. 1997, van Lenteren 2000, Goh et al. 2001, Heinz et al. 2004, Parker and Popenoe 2008), and is conducive to the survival and reproduction of beneficial insects (Gandhi et al. 2005, Desneux et al. 2007, Krischik et al. 2007, Rogers et al. 2007). Banker plant systems may provide cost savings to greenhouse growers (van Lenteren et al. 1997, van Lenteren 2000, Matteoni 2003, Van Driesche et al. 2008, Huang et al. 2011) as they may be less expensive than multiple releases of natural enemies and can be easier and less time consuming than pesticide applications (van Lenteren et al. 1997, Conte et al. 2000). Accounting for inflation, initial costs of *T. vaporariorum* control in greenhouse tomatoes using *D. hesperus* banker plants and supplemental *E. kuehniella* eggs costs U.S. $0.99 per m² per year and drops to U.S. $0.60 per m² per year after establishment of the natural enemies, while augmentative *E. formosa* controls without *D. hesperus* cost U.S. $1.08 per m² per year (Lambert et al. 2005). In addition, preliminary cost analyses show that implementing banker plants provides the greatest return on investment and costs approximately five times less per year (Payton Miller, unpublished data) than regular augmentative or ‘trickle’ releases of natural enemies (Jacobson and Croft 1998). Due to decreased effectiveness against common pests, repetitive insecticide applications can lead to increased treatment costs (van Lenteren et al. 1997, Foster et al.

2003, Davis and Radcliffe 2008, Frank 2010). Many chemical formulations may be ineffective against a pest, limiting options for growers to rotate pesticides (van Lenteren et al. 1997). Greenhouse growers, who combine multiple IPM practices successfully, should only need pesticides during pest outbreaks (van Lenteren 2000, Rebek et al. 2012). Integrated pest management programs can be tailored to fit specific greenhouse operations, target pests, and crops (van Lenteren 2000). In a survey of Oklahoma greenhouse producers, 56% did not use any biological control agents, but other IPM practices were embraced such as mechanical controls and pest monitoring with sticky traps (Payton Miller, unpublished data).

Major greenhouse pests such as aphids can be difficult to manage in a controlled environment as they can be unresponsive to diapause (van Lenteren 2000) and go unnoticed until population densities are high (Rabasse and Van Steenis 1999, Goh et al. 2001). Aphids are prevalent in temperate areas, cause issues in greenhouses globally, and serve as vectors for many plant viruses (van Lenteren et al. 1997). Van Driesche et al. (2008) showed aphids are the pest in most need of control in greenhouses, requiring a minimum of three insecticide treatments for suppression during a single crop cycle. Banker plants are used regularly for aphid management as the pest is less likely to develop resistance to a natural enemy than a conventional insecticide. Rabasse and Van Steenis (1999) illustrated that aphid populations increase quickly, making augmentative biological control problematic. Some pests must be present for releases of biological control agents, but if populations grow too fast, natural enemies are not able to maintain pest levels below treatment thresholds. In contrast, banker plants allow natural enemies to be introduced when pest population densities are low (Hofsvang and Hågvar 1979), providing a slow release of natural enemies over time and maintaining the pest below treatment levels (Wick 1992, Conte et al. 2000, van Lenteren

2000, Kim and Kim 2004). Similarly, greenhouse producers in Oklahoma responded that aphids are the most prevalent pest needing control and most growers manage aphids using conventional insecticides (Fig. 1, Payton Miller, unpublished data). Overall, banker plants reduce environmental concerns over pesticide application and disposal, and they provide the grower an opportunity to advertise earth-friendly, low-impact pest management to their customers (van Lenteren et al. 1997, van Lenteren 2000).

The Banker Plant Method

Banker plant systems are an innovative way to apply biological control in the field (Freuler et al. 2003) and greenhouse (Hågvar and Hofsvang 1994, Goh 1999, Kim and Kim 2004, Frank 2010, Andorno and López 2014). They are easily replaced when plant vigor is lost (7 to 14 days with winter wheat banker plants) and only a few plants may be needed in moderately sized greenhouses. Banker plants require some additional maintenance in sowing seed and maintaining alternate host colonies; however they do not usually require additional time to water as they are easily incorporated with the crop (Jacobson and Croft 1998). Protocols for commercially available banker plants advise as little as one, 15- to 25-cm pot of wheat (*Triticum aestivum* (L.)), barley (*Hordeum vulgare* (L.)), rye (*Secale cereale* (L.)), or oat (*Avena sativa* (L.)) banker plants infested with *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae) to provide parasitoids for 600 to 1,500 m^2 of greenhouse space (IPM Laboratories, Inc. 2013). The number of banker plants can be increased by grower preference or by timing with the crop and adding additional plants per week until crop sale or shipment. Efficacy of banker plant systems are difficult to quantify but tend to be measured in the

number of natural enemies present or a result of effective control in the crop (Huang et al. 2011).

Calendar-based augmentative releases of parasitoids may not be necessary when using banker plants (Hofsvang and Hågvar 1979, van Lenteren et al. 1997), however, augmentative release may allow more flexibility on targeting of pests in certain systems. This reduces costs to implement biological control and trades the effect of immediate releases for long-term pest control provided by several generations of predator or parasitoid progeny (Huang et al. 2011). Vásquez et al. (2006) showed continuous augmentative releases alone were almost five times more expensive than applying imidacloprid. In contrast, Stacey (1977) reported that only one augmentative release of *E. formosa* with a banker plant was needed to control greenhouse whitefly, *T. vaporariorum*, on greenhouse tomatoes. Despite the benefits, one obstacle to adoption of banker plant systems is the lack of knowledge concerning their function and incorporation into current greenhouse production systems (Parker and Popenoe 2008). Employment of banker plants in greenhouses may be effective on a case-by-case basis (Payton Miller, unpublished data).

Aphidius colemani (Viereck) and *Rhopalosiphum padi* Banker Plant System

Parasitoids in the Hymenopteran family, Aphelinidae, have been used since the 1920's for greenhouse biological control of whiteflies, armored scales, soft scales, and aphids (van Lenteren et al. 1997). *Aphidius colemani* (Viereck) (Hymenoptera: Braconidae) is a koinobiont endoparasitoid (Boivin et al. 2012) with four larval instars that attacks over 41 different aphid species (Starý 1975, Prado et al. 2015). This small (2 to 4 mm), solitary,

brown wasp has a short life span and high reproductive capacity (Ode et al. 2005, Stara et al. 2011). *Aphidius colemani* is native to northern India, but can be found in the Americas, Australia, Europe, and Hawaii (Starý 1975, Benelli et al. 2014). *Aphidius colemani* mates within minutes of emergence and may sting aphids to ingest their hemolymph (van Lenteren et al. 1997). Parasitoid performance is optimum at temperatures between 20° C to 27º C, with development time from egg to adult occurring in 11 to 13 days (van Lenteren et al. 1997, Ahmad et al. 2016). *Aphidius colemani* can survive at temperatures between 10° C to 30º C (Ahmad et al. 2016), but above this range development time would decrease as temperature increases.

The aphid host range of *A. colemani* may differ in tropical or temperate climates (Messing and Rabasse 1995). In greenhouse biological control, species strains are not mixed for pest management (van Lenteren et al. 1997). *Aphidius colemani* has been used successfully for decades as a biological control agent in controlled environments (Fernández and Nentwig 1997, Goh et al. 2001, Matteoni 2003, Van Driesche et al. 2008, Frank 2010, Prado et al. 2015), including greenhouse operations in Canada (Matteoni 2003), Germany (Kühne 1998), Japan (Nagasaka et al. 2010), Korea (Goh et al. 2001), the Netherlands (van Lenteren and Woets 1988), Norway, the United Kingdom, Czech Republic (Benelli et al. 2014), and the United States (Van Driesche et al. 2008).

The majority of natural enemies are purchased from commercial rearing companies (van Lenteren 2000), with quality standards set by the International Organization for Biological and Integrated Control-West Palaearctic Regional Section (IOBC-WPRS) (van Lenteren and Woets 1988). *Aphidius colemani* quality may vary by source and season, with unpredictable emergence rates, decreased parasitism efficiency, male-biased sex ratios,

reduced longevity, a shortage in shipped quantities of parasitoids as to what is advertised, differing affinity to aphid species based on rearing material, and mixed parasitoid species or hyperparasitoid presence (Fernández and Nentwig 1997, Van Lenteren 2000, Benelli et al. 2014). Contrary to mass-reared biological control agents, the use of banker plants offers a source of fresh natural enemies as adult parasitoids emerge and continually reproduce, offering control for generations (Hofsvang and Hågvar 1979, Matteoni 2003, Van Driesche et al. 2008). Banker plants provide food and shelter to parasitoids immediately upon emergence, ensuring an ideal reproductive environment for the wasp (Fernández and Nentwig 1997). Parasitoid progeny reared on banker plants may be female-biased and more effective for biological control over time (Prado and Frank 2014). Because traits of commercially produced parasitoids can be unreliable, banker plant systems help recoup some cost in low-quality shipments (Van Lenteren 2000). However, aphid colonies and banker plants should be regularly inspected for presence of hyperparasitoids that can decrease efficacy of the system (Fernández and Nentwig 1997).

A common, commercially available banker plant system targets pest aphids in controlled environments using the bird cherry-oat aphid (*R. padi*) as an alternate host for *A. colemani* (Goh 1999, Jandricic et al. 2014). *Rhopalosiphum padi* is a cereal grain pest maintained on wheat, rye, barley, oats, or other species in the Poaceae family (Conte et al. 2000, Pineda and Marcos-García 2008, Jandricic et al. 2014). This aphid is resistant to many wheat varieties which allows it to feed on and transmit Barley Yellow Dwarf Virus (D'Arcy and Domier 2000). The use of *R. padi* in banker plants is similar to the pest-in-first strategy in biological control (Huang et al. 2011). However, this species only feeds on monocots (Kieckhefer 1984), reducing the risk of an unintentional, secondary infestation in most

ornamental and vegetable greenhouse operations. However, *R. padi* could be a potential pest in greenhouses where ornamental grasses are grown. *Miscanthus* species, popular ornamental grasses, were undesirable banker plants for rearing *R. padi*, making the *A. colemani*-*R. padi* system potentially useful in mixed material greenhouses (Coulette et al. 2013).

Rhopalosiphum padi is used with *A. colemani* parasitoids in banker plant systems targeting *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) or *Aphis gossypii* (Glover) (Hemiptera: Aphididae), common agricultural pests (Grasswitz 1998). In choice tests, *A. colemani* prefers *M. persicae* over *R. padi* and yields larger offspring and greater offspring survival, increased offspring fecundity, and a female-biased population of parasitoids (Ode et al. 2005, Martinou and Wright 2007), especially when both aphids are present (Prado and Frank 2014). *Aphidius colemani* is effective in banker plant systems as it has a high potential to move from less preferred to highly preferred aphid hosts and maintain them below treatment thresholds (Ode et al. 2005, Zamani et al. 2006, Van Driesche et al. 2008). *Rhopalosiphum padi* may be a less desirable host for *A. colemani*, but an innate preference for the pest aphid may be beneficial to encourage foraging away from banker plants, leading to a more effective system (Prado and Frank 2014, Prado et al. 2015). However, *A. colemani* in the presence of both *R. padi* and *M. persicae* has shown better results than with just one aphid species present (Prado and Frank 2014)

Host preference among parasitoids appears to be a genetic or preconditioned trait (Messing and Rabasse 1995) based on host plant volatiles the wasps experience during development or emergence from host aphids (Van Emden et al. 2002, Douloumpaka and Van Emden 2003, Bilu et al. 2006, Fujinuma et al. 2010, Rehman and Powell 2010, Ameixa and Kindlmann 2012). *Aphidius colemani* will not parasitize foxglove aphid (*Aulacorthum solani*

(Kaltenbach) (Hemiptera: Aphididae)), chrysanthemum aphid (*Macrosiphoniella sanborni* (Gillette) (Hemiptera: Aphididae)), potato aphid (*Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae)), or banana aphid (*Pentalonia nigronervosa* (Coquerel) (Hemiptera: Aphididae)), potential pests in the field and greenhouse (Van Driesche et al. 2008, Benelli et al. 2014, Prado et al. 2015). Several companies rear other natural enemies to target a myriad of aphid pests (Table 1).

Banker plant systems have been shown to be effective in several greenhouse production systems. For example, barley banker plants with *R. padi* and *A. colemani* parasitoids offered 73% to 90% control of *A. gossypii* and *M. persicae* on Marguerite daisies (*Argyranthemum frutescens* (L.)) and pansy (*Viola tricolor hortensis* (DC)) than non-treated controls over a seven-week period (Van Driesche et al. 2008). In addition, barley banker plants containing the greenbug (*Schizaphis graminum* (Rondani) (Hemiptera: Aphididae)) and *A. colemani* for *A. gossypii* management on melons resulted in good control, 0.2 to 5.0 aphids per leaf, after approximately two months, when introduced early in the crop cycle (Kim and Kim 2004). Furthermore, parasitism was greater and the number of live *A. gossypii* aphids was lower in red pepper (*Capsicum annuum* (L.)) and watermelon (*Citrullus lanatus* (Thunb.)) greenhouses with *A. colemani*-*R. padi* barley banker plants (1.3 to 2.4 aphids per 10 leaves, 73% to 92% parasitism) than in those without treatment (1,711 to 2,349 aphids per 10 leaves, 2% to 17% parasitism) after five weeks (Goh et al. 2001).

For control of other pests besides aphids, additional banker plant systems have been evaluated with various parasitoids and predators to target common pest species. Although some systems have alternate prey or hosts for beneficial insects that are not commercially available, they may be obtained through private sources, universities, or collected from the

field. Determine plant protection and quarantine policies in your state or country prior to acquiring insects that could be invasive species or agricultural pests.

Green Peach Aphid, *Myzus persicae*

Green peach aphids feed on over 800 species of plants, including ornamental plants (Van Driesche et al. 2008), vegetables (Hofsvang and Hågvar 1979, Freuler et al. 2003), fruits (Kim and Kim 2004), and weeds but may also be found attacking barley, rye, and winter wheat before feeding on potato (*Solanum tuberosum* (L.)) in northern climates (Davis and Radcliffe 2008). This aphid has a wide host range so biotype development is unlikely (Davis and Radcliffe 2008). However, *M. persicae* is a major pest in commercial greenhouses (Wick 1992, Van Driesche et al. 2008) as it is resistant to many classes of conventional pesticides (Goh et al. 2001, Foster et al. 2003, Davis and Radcliffe 2008).

Depending on weather, this polyphagous aphid overwinters outdoors as adults on one of many hosts, or as eggs on *Prunus* species. Green peach aphid infestations may require multiple treatments, a rotation of chemical modes of action, or there may be a lack of aphicides to control an outbreak. A lack of reliable controls makes *M. persicae* an excellent candidate for biological control programs, specifically banker plant systems.

Myzus persicae was shown to be controlled in Argentine arugula (*Eruca sativa* (Mill.)) and sweet pepper (*Capsicum annuum* (L.)) greenhouses over a two-month period, using the *A. colemani-R. padi* system on oat banker plants. In this experiment, banker plants provided the lowest pest aphid density, never reaching the critical spray threshold of 800

aphid nymphs per 16 leaves (Andorno and López 2014). In contrast, three of four non-treated controls exceeded the spray threshold (Andorno and López 2014).

Color variation in *M. persicae* can complicate IPM programs. Phenotypes of *M. persicae* may be dark green, light green, or red with varying feeding styles, reproductive rates, and susceptibility to *A. colemani*. Gillespie et al. (2009) found that dark green clones congregated along leaf veins or in growing tips of plants, while other clones were uniformly distributed on lower plant leaves. Furthermore, dark green clones were stung less by *A. colemani*, had a lower parasitism rate, a greater reproductive rate, and produced less mummies than light green or red clones. Light green clones had a slower population growth rate than red or dark green, but *A. colemani* stung more red aphids overall. Laboratory results show dark green clones lack modified acetylcholinesterase (MACE)-based insecticide resistance and esterase resistance observed in other phenotypes that correlates with varying degrees of susceptibility to parasitoids (Gillespie et al. 2009).

It is important to keep in mind that a parasitized aphid may continue to have offspring. *Aphidius colemani*, *A. gifuensis* (Ashmead) (Hymenoptera: Braconidae), and *Diaeretiella rapae* (M'Intosh) (Hymenoptera: Braconidae) were evaluated to determine the reproductive capacity of *M. persicae* post-parasitism (Mitsunaga et al. 2016). Once any of the parasitoids reached the second larval instar, reproduction of the aphid ceased due to consumption of the reproductive organs. Healthy *M. persicae* average a lifetime birth rate of 61.43 nymphs per aphid, but when parasitized by *A. colemani*, *A. gifuensis*, and *D. rapae*, this number decreased to 3.96, 6.40, and 6.48, respectively. Intrinsic rates of increase by *A. colemani* decreased the *M. persicae* population by 39.9% while the other two parasitoids decreased the population by a minimum of 24.4%. *Aphidius colemani* may be slightly more

effective than *D. rapae* or *A. gifuensis* during short-term applications, such as inoculative releases and overall may be a more effective parasitoid in control of green peach aphids (Mitsunaga et al. 2016).

Aphidius colemani has a preference for parasitizing third and fourth instar *M. persicae* even though larger aphids require more handling time and are able to fend off parasitoid attacks (Khatri et al. 2016). Parasitized third instar aphids accounted for the fastest development time of *A. colemani* offspring, but the parasitism rate was the same for all ages of aphids. Parasitism of second instar or older aphids allowed for maximum body size in *A. colemani*, but aphid age at parasitism had no effect on sex bias or emergence (Khatri et al. 2016).

Pesticide Compatibility with Banker Plants

Compatibility of compounds with natural enemies must be considered if biological control is to be implemented successfully, including banker plant systems. A comprehensive IPM program combines biological control with pesticide use and garners careful consideration of pesticide compatibility with natural enemies, including chemical modes of action, application rates and methods, timing of application, natural enemy life stage during application, and whether the biological control agent is a parasitoid or predator (Cloyd 2005, Rogers et al. 2007, Abraham et al. 2013, Prado et al. 2015). Commonly used greenhouse pesticides can have lethal and sub-lethal effects on predators and parasitoids (Rebek and Sadof 2003, Krischik et al. 2007, Rogers et al. 2007, Biondi et al. 2013, Joao Zotti et al. 2013, Thompson et al. 2014). Sub-lethal effects may include decreased host acceptance,

reduced natural enemy longevity, altered sex ratios, unsuccessful food acquisition, reduced fecundity, decreased emergence rates, or increased development time (Cloyd 2005, Prado et al. 2015). The IOBC-WPRS may select active ingredients that work in concert with IPM programs based on mortality and sub-lethal effects to natural enemies (van Lenteren and Woets 1988, Abraham et al. 2013). Lethal effects on *A. colemani* can be assessed as the wasp is commercially available and genetically homogeneous. Laboratory toxicity studies reveal maximum mortality when compared with field studies (Cloyd 2005), but even insecticides classified as harmless (<30% mortality in 48 h) or slightly harmful (30 to 79% mortality in 48 h) by the IOBC-WPRS still cause significant losses to *A. colemani* adults and their offspring (Prado et al. 2015). Pesticide applications at varying label rates can also show injurious effects on natural enemies, while the target pest may survive and resurge. Alternatively, pesticides that kill too many hosts limit the food available for natural enemies to survive and reproduce effectively (Cloyd 2005). This can be unacceptable when implementing biological control, so it is important to screen new compounds used in greenhouses for toxicity (Stara et al. 2011, Prado et al. 2015). Active ingredients that cause high mortality in natural enemies (Rebek and Sadof 2003, Krischik et al. 2007, Rogers et al. 2007) are not well-suited to biological control programs, including banker plant systems (Goh et al. 2001).

Compounds with high toxicity to *A. colemani* 24 to 48 h after exposure include abamectin, dimethoate, acetamiprid, spinosad, azadirachtin, and pyridaben (Bostanian and Akalach 2004, Cloyd 2005, Stara et al. 2011, Abraham et al. 2013). Van Driesche et al. (2008) showed a reduction in adult survival when pyriproxyfen and pymetrozine were used. Kim et al. (2006) demonstrated 97% or higher mortality in *A. colemani* 7 days post-

application with chlorpyrifos-methyl, diflubenzuron+chlorpyrifos, etofenprox+diazinon, and imidacloprid+chlorpyrifos, whereas insecticides to control thrips had no effect on mummy formation by the parasitoid (Kim et al. 2006).

Mummies and adults of *Eretmocerus mundus* (Mercet) (Hymenoptera: Aphelinidae), a parasitoid of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), were exposed to the maximum labeled rate of several insecticides (Fernández et al. 2015). Deltamethrin, flonicamid, and emamectin caused reduced adult longevity of emerged adults, and the long residual and harmful effects of spinosad and sulfoxaflor to *E. mundus* would not be ideal for use in IPM programs (Fernández et al. 2015). Metaflumizone and spirotetramat are chemical options compatible with biological control as the residual effects are reduced 5 to 7 days after application (Fernández et al. 2015).

Acaricides targeting pests like two-spotted spider mite, *Tetranychus urticae* (Koch) (Acari: Tetranychidae), may kill beneficial, predaceous mites like *Phytoseiulus persimilis* (Evans) (Acari: Phytoseiidae). Ditillo et al. (2016) screened several chemical classes for lethal and sub-lethal effects on *P. persimilis*. The organophosphate, dimethoate, caused high mortality and sub-lethal effects on *P. persimilis*. Compounds such as chlorantraniliprole, and the neonicotinoids dinotefuran, imidacloprid, and thiamethoxam, as well as moderate rates of pyrethroids, did exhibit sub-lethal effects but did not cause direct mortality of *P. persimilis*. Spinetoram, a derivative of spinosad, caused moderate mortality in *P. persimilis* (Ditillo et al. 2016).

Some biorational insecticides may cause sub-lethal effects on non-target insects (Cloyd 2005, Biondi et al. 2013). Biorational, or reduced-risk insecticides include

horticultural oils, insecticidal soaps, insect growth regulators, or beneficial fungi, and could negatively affect a broader range of insects than some conventional insecticides (Cloyd 2005). Horticultural oil and insecticidal soap sprayed directly on natural enemies can be detrimental, especially to parasitoids (Cloyd 2005). Insecticidal soap caused 100% mortality in adult parasitoids 24 h after treatment, but no effect was observed on immature stages or egg capacity in females (Tremblay et al. 2008). This study emphasizes the importance of correct timing of insecticidal soap applications, especially when releasing adult parasitoids and predators.

The use of imidacloprid has increased among growers for its systemic properties, low mammalian toxicity, long residual activity, and ease of application (Rogers et al. 2007, Scholer and Krischik 2014). Responses from a survey of Oklahoma greenhouse producers indicate that over one-third rely on imidacloprid to control phloem-feeding insect pests in controlled environments (Payton Miller, unpublished data). The use of imidacloprid has gained attention as it can be translocated to the floral organs of angiosperms, affecting beneficial Hymenoptera (Desneux et al. 2007, Krischik et al. 2007, Lawrence and Sheppard 2013, Scholer and Krischik 2014). Adult hymenopteran parasitoids may be at risk in production systems where neonicotinoids are used as the wasps could use flowering crops as food resources (Fujinuma et al. 2010, Goulson 2013). *Aphidius colemani* mortality increased when feeding on plants treated with imidacloprid via soil drenches, as the floral nectar exceeded the established LC_{50} for the parasitoid (Charles-Tollerup 2012). In addition, aphids may secrete systemic neonicotinoid products in their honeydew, potentially harming foraging parasitoids that feed on their excrement or hemolymph (Cloyd and Bethke 2010).

Foliar-applied fungicides may harm biological control agents in greenhouse operations (van Lenteren 2000). Five foliar fungicides screened against *P. persimilis* showed no lethal or sub-lethal effects, except mancozeb, that negatively affected fecundity of the predator (Ditillo et al. 2016). In Korea, however, fungicides used for powdery mildew showed no harmful effects on mummy formation in *A. colemani* (Kim et al. 2006). Fungicides used in combination with biological control had no negative effects on the leafminer parasitoid, *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) (Abraham et al. 2013). Regardless of the pesticides used, compatibility of compounds with natural enemies must be considered if biological control is to be implemented successfully.

Greenhouse Production and Variety Trials

Greenhouse growers maintain temperate environments all year, causing cool-season banker plant species to decline quickly. This is especially true in Oklahoma and other states in the southwestern U.S., where temperatures commonly exceed 32° C (Payton Miller, personal observation). While winter wheat and barley used for rearing *R. padi* currently provide the best banker plant material (Jandricic et al. 2014), these cool-season annual grasses must be replaced every 7 to 14 days, especially during summer months. Even mildew-resistant or other resistant grain varieties (Van Driesche et al. 2008) may not tolerate high summer temperatures. Other warm-season grasses may have potential as banker plants for *R. padi* in the southwestern U.S. While *R. padi* prefers to feed and reproduce on barley, it can also reproduce on sand lovegrass (*Eragrostis trichodes* (Nutt.)), sideoats grama

(*Bouteloua curtipendula* (Michx.)), buffalograss (*Buchloe dactyloides* (Nutt.)), switchgrass (*Panicum virgatum* (L.)), and indiangrass (*Sorgastrum nutans* (L.)) (Kieckhefer 1984).

Jandricic et al. (2014) conducted multi-generational studies of *R. padi* on wheat, barley, rye, and oats for use in the *A. colemani*-*R. padi* system. Results showed varying effects on aphid traits and parasitoid development; rye and oats were less suitable banker plants for *R. padi* and barley and wheat were most suitable. In addition, varieties within a species showed no direct bottom-up effects such as survival, mating, or fecundity, on *A. colemani* (Jandricic et al. 2014). However, mass-reared parasitoids tend to be more male-biased and vary in size. Male parasitoids, and those female parasitoids reared on unsuitable hosts, are typically smaller in size than healthy female adults. Thus, mixtures of wheat, barley, rye, and oats may hold promise when using parasitoids of varying uniformity, specifically because of the change in visual and volatile cues attractive to *A. colemani*.

McClure and Frank (2015) evaluated mixtures and monocultures of cereal grains as banker plants to see if species mixtures provided a greater diversity of *M. persicae* sizes and life stages for diversified quality of parasitoids. Species mixtures grew taller than monocultures, but did not provide improved biological control of *M. persicae* in any treatment. However, the use of banker plants did account for a more female-biased population of parasitoids when compared to augmentative releases. Additionally, rye banker plants sustained more live *R. padi* than other monocultures but not as many aphid mummies as wheat monocultures (McClure and Frank 2015).

Longer-lived species of banker plants have been evaluated for extended control of pests in hot greenhouses. Instead of using barley or rye and *R. padi* for *A. gossypii* control

(Higashida et al. 2016), *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae) predators were evaluated with the sorghum aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae), reared on sorghum (*Sorghum bicolor* (L.) (Higashida et al. 2017). Results showed that *A. aphidimyza* thrives best between 20º C and 30º C on the three aphid species, and the predator reared on *M. sacchari* had a shorter development time at 25º C and 30º C than when it was reared on *A. gossypii* or *R. padi* (Higashida et al. 2017). However, differences in development time between Japanese and European strains of *A. aphidimyza* were noted. *Aphidoletes aphidimyza* reared on *M. sacchari* had a similar lifetime fecundity than conspecifics reared on *R. padi*, but fecundity increased when reared on *A. gossypii* (Higashida et al. 2016, 2017). Thus, there appears to be potential for an *A. aphidimyza-M. sacchari* banker plant system.

Herbivore-induced plant volatiles could factor into the success of host plants used in banker plant systems. Mixtures of plant species have been evaluated to see if levels of diversity among and within species affects aphid size and use by parasitoids. In commercially reared *A. colemani* parasitoids, various sizes of female parasitoids may attack a variety of stages of aphids. Different species of banker plants have bottom-up effects on aphids (Jandricic et al. 2014) such as aphid size. Therefore, the species of banker plant may help optimize shipments of mass-reared parasitoids by providing aphids that will lead to the greatest parasitoid fecundity. Although monocultures are attractive to herbivores, diversity in plant mixtures may be attractive to foraging predators and parasitoids, having a dampening effect on fluctuating herbivore populations as well as decreased incidence of disease. Grettenberger and Tooker (2016) evaluated spring wheat plant mixtures versus monocultures as banker plants for the lady beetle, *Coleomegilla maculata* (De Geer) (Coleoptera:

Coccinellidae), reared on *R. padi*. Aphid biological control was not significantly different on mixtures or monocultures, but monocultures did account for a greater density of *R. padi* after one week. The lady beetle was significantly more attracted to and stayed longer in wheat mixtures. In contrast to Jandricic et al. 2014, only the 'Rollag' cultivar of *T. aestivum* repeatedly accounted for the least amount of aphids in mixtures than in monocultures at the plant level (Grettenberger and Tooker 2016).

Bottom-up effects by the host plant have been shown to alter success of biological control of the green peach aphid by parasitoids. For example, a study in China used the English grain aphid, *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae), on wheat as alternate hosts for the parasitoid, *Aphelinus asychis* (Walker) (Hymenoptera: Aphelinidae), for *M. persicae* control on greenhouse-grown pepper (*C. annuum*) and cabbage (*Brassica oleracea* (L.)) (Wang et al. 2016). The host plant of the pest aphid altered the effectiveness of the parasitoid as it had increased longevity, greater fecundity, and an increased female-biased sex ratio of progeny when reared from aphids on pepper compared to those on cabbage (Wang et al. 2016).

In Asia, *A. gifuensis* can be reared on graminaceous banker plants infested with greenbug (*S. graminum*) as an alternate host for control of *M. persicae* or *A. gossypii* (Sun et al. 2017). Greenbug infests oat, barley, and wheat and was screened for bottom-up effects on *A. gifuensis*. Of the three species, oats performed most poorly, having the least adult emergence overall, a less female-biased sex ratio, and decreased fecundity in female parasitoids. Development time of the parasitoid was not significantly different among the three species, however, *A. gifuensis* lives longer on *S. graminum* on cereal grains than on *M. persicae* on certain vegetable crops. Intrinsic rates of increase and life expectancy were also

higher on wheat and barley (Sun et al. 2017). Oats may be an unfavorable choice for banker plants in *A. gifuensis* and *A. colemani* systems.

In Argentina, *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Braconidae) was evaluated for use in a banker plant system in alfalfa (*Medicago sativa* (L.)) fields to control the cowpea aphid, *Aphis craccivora* (Koch) (Hemiptera: Aphididae) (Zumoffen et al. 2016). During field and border area sampling, over half of the parasitoids were *L. testaceipes* and 74% were collected from field margins using non-crop hosts. The oleander aphid, *Aphis nerii* (Fonscolombe) (Hemiptera: Aphididae), reared on *Araujia* species, was used as an alternate host by *L. testaceipes* in 52% of the border samples and the parasitoid accounted for 30% to 50% of the control of *A. craccivora* in the field. *Lysiphlebus testaceipes* is a generalist parasitoid, but when reared on *A. nerii* it had a shorter larval development time and a longer adult lifespan (Zumoffen et al. 2016). Using alfalfa banker plants for rearing cowpea aphid and *L. testaceipes* for oleander aphid control may be useful in ornamental greenhouses.

Characteristics of plant leaves, stems, or flowers may positively or negatively influence natural enemy survival and persistence in the greenhouse. Banker plants supply pollen as a food supplement for the predator, *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), for biological control of western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (Waite et al. 2014). *Orius insidiosus* is commonly reared on ornamental pepper (*C. annuum* 'Black Pearl'), however, Waite et al. (2014) found that 'Purple Flash' ornamental pepper accounted for the greatest long-term population growth of the predator. Pollen-producing plants are also used as banker plants to rear predaceous mites such as *P. persimilis* and *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) to control two-spotted spider mites (*T. urticae*). Bresch et al. (2015) screened

eight banker plants, and only two plant species, *Viburnum tinus* (L.) and *Vitis riparia* (Michx.) contained predatory mites and no pests.

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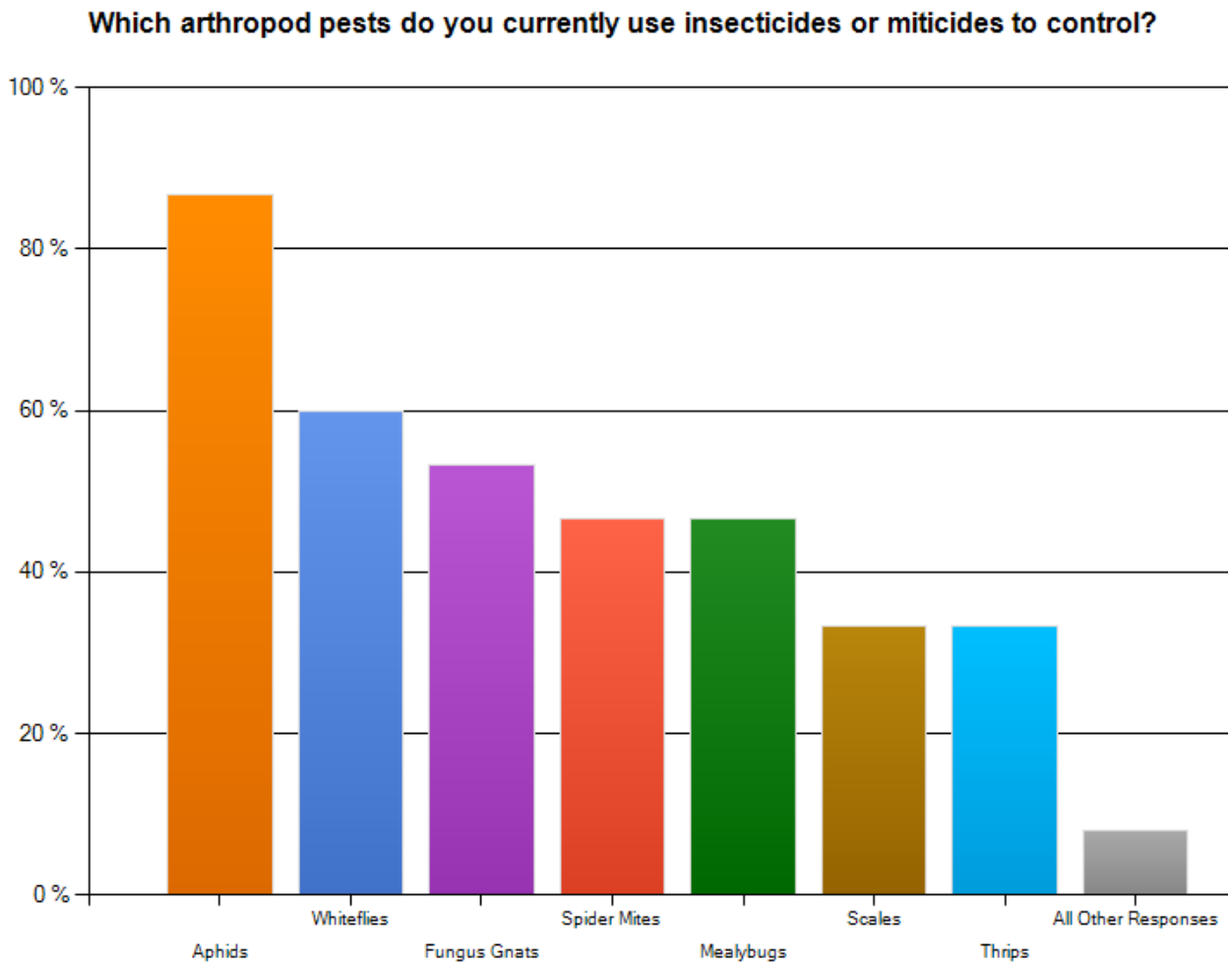

Figure 1. Response of 13 greenhouse growers on the common pests targeted with insecticides and miticides in Oklahoma greenhouses.

Table 1. Canadian and U.S. biological control suppliers.

Supplier	Location	Website
A-1 Unique Insect Control	California	www.a-1unique.com/
American Insectaries	California	www.americaninsectaries.com/
Applied Bio-Nomics	Canada	www.appliedbio-nomics.com/
Arbico Organics	Arizona	www.arbico-organics.com/
Associates Insectary	California	www.associatesinsectary.com/
Beneficial Insectary	Canada; California	www.insectary.com/
Bio Ag Services	California	www.bioagservicescorp.com/
BioBest	Canada	www.biobestgroup.com/
Bio-Controle Inc.	Canada	www.agrobiocontrole.ca/home.php
Biofac Crop Care	Texas	www.biofac.com/
Bioline AgroSciences	Canada; U.S.	www.biolineagrosciences.com/
BioLogic Company	Pennslyvania	www.biologicco.com/
Biotactics	California	www.benemite.com/
Bio-Works	California	www.bioworksinc.com/
BugLogical Control Systems	Arizona	www.buglogical.com/
Crop Defenders	Canada	www.cropdefenders.ca/
Entomology Solutions	Kentucky	www.idlewildbutterflyfarm.com/
Evergreen Growers Supply	Oregon	www.evergreengrowers.com/
Everwood Farm	Oregon	www.everwoodfarm.com/
Gardeners Supply Company	Vermont	www.gardeners.com/
Gardens Alive!	Indiana	www.gardensalive.com/
Green Methods	California	www.greenmethods.com
Hydro-Gardens	Colorado	www.hydro-gardens.com/
IPM Laboratories	New York	www.ipmlabs.com/
Koppert	Canada; U.S.	www.koppert.com/
Kunafin "The Insectary"	Texas	www.kunafin.com/
Natural Enemies	Oregon	www.naturalenemiesbiocontrol.com/
Natural Insect Control	Canada	www.naturalinsectcontrol.com/index.php
Natural Pest Control	California	www.natpestco.com/
Nature's Control	Oregon	www.naturescontrol.com/index.html
Peaceful Valley Farm Supply	California	www.groworganic.com/
Planet Natural	Montana	www.planetnatural.com/
Rincon-Vitova Insectaries	California	www.rinconvitova.com/
Sound Horticulture	Washington	www.soundhorticulture.com/
Territorial Seed Company	Oregon	www.territorialseed.com/
Tip Top Biocontrol	California	www.tiptopbiocontrol.com/

CHAPTER III

THE *APHIDIUS COLEMANI-RHOPALOSIPHUM PADI* BANKER PLANT SYSTEM IN OKLAHOMA GREENHOUSES

Abstract

The *Aphidius colemani* (Viereck) (Hymenoptera: Braconidae)-*Rhopalosiphum padi* (L.) (Hemiptera: Aphididae) banker plant system is a biological control technique used to control *Aphis gossypii* (Glover) (Hemiptera: Aphididae) and *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) in greenhouses in the U.S. and worldwide. In this study, I assessed the effectiveness of this banker plant system compared to augmentative releases of *A. colemani* against *M. persicae*. I set up one banker plant, one augmentative, and one control treatment at three greenhouse cooperator sites. I counted the number of aphids and mummies every week for seven weeks, then averaged the results to determine the amount of aphids and mummies per plant. Each set of treatments was repeated four times, in each season, to determine if the time of year resulted in different outcomes. I found that augmentative and banker plant treatments may not be significantly different regarding the proportion of parasitized aphids per plant, however, the banker plant

treatment had significantly lower mean number of aphids per plant in June and July 2016.

Overall, the banker plant treatment had fewer aphids per plant, especially in early spring and summer. As augmentative and banker plant treatments were implemented in late summer and fall the effectiveness of both declined. Mean maximum temperature and relative humidity were correlated with live aphids, total aphids, and mummified aphids in certain treatments, but results were inconsistent. Therefore, other factors must be influencing the seasonal effectiveness of augmentative and banker plant treatments in this study.

Introduction

Banker plant systems are an unconventional way to implement biological control in greenhouse production. The *A. colemani-R. padi* system consists of *A. colemani* as a parasitoid and *R. padi* as alternate host for the parasitoid when *M. persicae* or *A. gossypii* are not present. This allows long-term, sustainable production of parasitoids in the greenhouse without repetitive and expensive releases of parasitoids alone. When spraying is necessary for pests other than aphids, banker plants can be moved out of the greenhouse parasitoids from negative effects of pesticides. Banker plants that house *R. padi* typically consist of 15- to 25-cm pots or hanging baskets containing wheat (*Triticum aestivum* (L.)) or barley (*Hordeum vulgare* (L.)). As the plants begin to die, these grains are replaced with new plants and fresh *R. padi* are available to the parasitoids. This banker plant technique is an effective and inexpensive way to implement aphid pest control when conventional insecticides may not be effective or concerns for the effect of off-target arthropods are an issue (van Lenteren

2000). In addition, little training is needed to use banker plants and personal protective equipment is not required.

Despite the benefits, one of the main obstacles to considering banker plant systems is the lack of information on implementation and functionality in the southwestern U.S. The objective of this study was to determine whether banker plants work better than augmentative releases, if there is a time of year that works best with either approach, and if it is feasible for growers to use these methods for aphid control in the southwestern U.S. I predicted that banker plants would perform better than augmentative releases, regardless of season or greenhouse attributes. The results obtained from this study will be used to help growers implement effective biological control of aphids their greenhouses.

Materials and Methods

Methods for this objective were adapted from Hofsvang and Hågvar (1979), Blumel and Hausdorf (1996), Jacobson and Croft (1998), and Andorno and López (2014). The *A. colemani-R. padi* banker plant system was compared with stand-alone augmentative releases of *A. colemani* to determine the effectiveness of each strategy. Experiments took place at three greenhouse cooperator sites: Bear Creek Farms, Stillwater, Oklahoma; Oklahoma State University, Oklahoma City, Oklahoma; and Scissortail Farms, Tulsa, Oklahoma (Table 1). Each site consisted of three treatments: banker plants, augmentative releases, and a control. Overall, 10 augmentative, 10 banker plant, and 10 control replicates were evaluated throughout the study. Augmentative and banker plant treatments were assigned to separate greenhouses, or on either end of large houses with barriers between each treatment. Each

treatment contained 24 ornamental peppers (*Capsicum annuum* (L.)) 'Black Pearl' arranged in a square pattern, containing a center release point and four pots that were infested with ten *M. persicae* each (Fig. 1). This infestation level imitated the initial pest pressure a grower might experience, where aphid populations begin as patchy distributions densities low enough to go unnoticed. Ten days prior to setting up each trial, six 'Jagger' winter wheat plants were placed into screen cages and exposed to 32 parasitized aphids. Parasitoids emerged from these mummies in 24 to 48 hours, mated, and females parasitized *R. padi* on the wheat plants. Once mummies formed on these banker plants, approximately seven to ten days later, a single 'Jagger' winter wheat banker plant was placed in the center release point of the array of 24 ornamental pepper plants in each banker plant replicate, at each site. The augmentative treatment consisted of 32 *A. colemani* mummies placed in cardboard Bio-Boxes (Biobest USA, Romulus, MI), placed in the release point of the array of 24 ornamental pepper plants. Additional augmentative releases were made based on high non-parasitized aphid: mummy ratios. For example, if the number of mummies observed on crop plants were fewer than the number of aphids observed on crop plants, more parasitoids were added to the augmentative treatment release point. Banker plants were observed for *R. padi* aphids and mummies, and banker plants were replaced as needed based on plant health. A positive control consisted of 24 ornamental pepper plants treated with soil-applied imidacloprid (Mantra® 60 WSP, 0.16 g/L) and four pots infested with ten *M. persicae* each. Control plants were placed in four screen cages (15 x 22 holes per 2.5 cm^2) measuring 35.5 x 35.5 x 61 cm to prevent parasitism and predation by resident natural enemies. Caged plants were then placed in the same greenhouse section as banker plant treatments due to the lack of separate greenhouse space. Negative control plants were not used as all treatment sites were

commercial greenhouses and aphid pests could not be introduced without a means of control. All three treatments were repeated at each cooperator site (Table 1).

Aphid Colonies

Rhopalosiphum padi and *M. persicae* aphids were maintained under 12:12 artificial light with temperatures ranging from 21° C to 23° C, depending on the season. *Rhopalosiphum padi* were reared on 'Jagger' winter wheat and *M. persicae* on canola (*Brassica napus* (L.)) 'Wichita'. Aphid colonies were maintained in 35.5 x 35.5 x 61 cm screen cages on plants grown in 15-cm black plastic pots and hand watered as needed. Original *R. padi* and *M. persicae* colonies were obtained from field collections maintained at Oklahoma State University (OSU), Stillwater, OK and reared on winter wheat and canola, respectively. Additional colonies were obtained from laboratories at OSU as necessary to maintain alternate host and pest aphids. *Aphidius colemani* mummies were purchased one week prior to use and received 1 to 2 days before using in the greenhouses or for banker plant maintenance. Parasitoids were obtained from Biobest USA for all treatments.

One week prior to use as banker plants, winter wheat seeds were sown for experiments in 15-cm black plastic pots (10 g seed per pot) containing Sun-Gro Horticulture (Agawam, MA) Metro-Mix® 902 professional growing media. Seeds were fertilized with 5 g of 14-14-14 Classic Osmocote slow-release fertilizer (low to medium dose), placed in the greenhouse to germinate, and watered by automatic, overhead sprinklers at 5- to 12-minute intervals twice daily, depending on season and greenhouse temperatures. *Rhopalosiphum padi* aphids were placed on winter wheat plants one week after germination, according to

commercial protocol recommendations, when the plants are approximately 2.5 cm tall (IPM Laboratories, Inc., 2013).

'Black Pearl' ornamental peppers were purchased from growers that did not use insecticides on the plants. Peppers were purchased as 8- to 10-week-old rooted transplants in 6.4-cm pots or a 72-plug tray, then transplanted to 15-cm black plastic pots containing Sun-Gro Metro-Mix® 902 growing media and top-dressed with 5 g of 14-14-14 Osmocote slow-release fertilizer. One to two weeks after transplanting, four ornamental peppers from each treatment were infested with 10 *M. persicae* and placed in screen cages to be moved the following day to each cooperator site. Control plants were drenched with imidacloprid (Mantra® 60 WSP, 0.16 g/L) 2 to 4 hours prior to infestation with *M. persicae*. Experiments at each cooperator site were installed on the same day within three hours of each other at most sites. Experiment installations included moving and placing ornamental peppers, placing banker plants with parasitized *R. padi*, performing augmentative releases, placing control plants in screen cages, and placing environmental data loggers.

Eight ornamental pepper plants were used as sentinel plants for each banker plant and augmentative replicate at each cooperator site. Sentinel plants were used to determine parasitoid movement during a preliminary study conducted in March 2016, and also during Trial 1 (July 15, 2016) and Trial 4 (April 19, 2017). Each sentinel plant was infested with 10 *M. persicae*, and plants were placed 4.5 to 6.0 m from the release point (Fig. 1) in each banker plant and augmentative treatment. Sentinel plants were not placed further from the experimental plants as the greenhouse walls created space constraints at some sites. The sentinel plants were arranged in a large rectangle, with one plant in each direction: north, northeast, east, southeast, south, southwest, west, and northwest. Sentinel plants were

retrieved after 48 h from each site, placed in screen cages, and observed for 14 days for mummy formation.

Data Collection

The duration of a single trial was eight weeks, with week one being the initial installation of plants at each site (Table 1). During each replication, ornamental peppers in the augmentative and banker plant treatments and control were observed once per week for seven weeks. In each replicate, four random pepper plants, in addition to the four initially infested plants, were selected and the number of *M. persicae* were counted. These observations included counting aphids appearing parasitized by *A. colemani* and those that did not appear parasitized. Presence or absence of aphids and mummies were recorded for the remaining eighteen pepper plants. Winter wheat banker plants were also observed for presence of parasitized and non-parasitized *R. padi*, to ensure a source of parasitoids and alternate hosts. Replacement banker plants were prepared according to the protocol described in the previous section. Dead or dying banker plants with mummies were kept in the trial area for 1 or 2 weeks until emergence holes were visible. Additional augmentative releases of *A. colemani* were made if the number of non-parasitized *M. persicae* aphids greatly outnumbered those mummified.

Onset® HOBO U23 Pro v2 External Temperature and Relative Humidity Data Loggers (Bourne, MA), with a solar shield to protect the sensors, were placed next to each banker plant and augmentative replicate. Each logger measured relative humidity (RH) and temperature at 60-minute intervals. Trials comparing both treatments and the control were

repeated four times at the Oklahoma City and Stillwater sites and two times at the Tulsa site (Table 1).

Mean abundance of aphids and mummies per plant were calculated by treatment and site for each trial. In addition, differences in the abundance of aphids and mummies were analyzed separately from the four pepper plants initially infested with *M. persicae* and four randomly selected plants from the array. This analysis was done to compare differences in abundance both within and between treatments while accounting for spread of aphids among plants within each array. Only results that were significant were reported. An autoregressive covariance structure was used, square root transformations were performed, and a repeated measures analysis of variance was computed using PROC MIXED (SAS 9.4 Software, Cary, NC) at $P \leq 0.05$. Site was the random block variable while week as the repeated measure. Mean proportion of parasitism was calculated by treatment and site for each trial. Over all four trials, mean temperature, mean minimum temperature, mean maximum temperature, and mean relative humidity were determined for all sites each week. Weekly mean maximum temperature and weekly mean relative humidity for each trial was compared with mean abundance of aphids per plant and percent parasitism using PROC CORR (SAS 9.4 Software, Cary, NC) at $P \leq 0.05$. Imidacloprid-treated control plants contained no aphids or mummies for the duration of all four trials and were excluded from analysis.

Banker plant systems and augmentative releases were based on IPM Laboratories protocols. *Rhopalosiphum padi* aphids and *A. colemani* parasitoid costs were based on Biobest USA prices. Current prices of imidacloprid and dinotefuran and growing supplies were provided by American Plant Products and Services (Oklahoma City, OK). Medium and

high chemical concentrations, application amounts, and the number of pots that can be treated were obtained from pesticide product labels.

Results and Discussion

Over all four trials of this experiment, there were no statistically significant differences found between augmentative and banker plant treatments for *M. persicae* numbers per plant or *A. colemani* parasitism. (Figs. 2, 3). Seasonal variations were noticed between augmentative and banker plant treatments when each trial was analyzed separately. Ornamental pepper plants had the same amount of aphids per plant in banker plant and augmentative treatments, but time of year did play a role in aphid and mummy amounts between treatments. When the four initially aphid infested pepper plants were analyzed separately from the randomly selected pepper plants, we found little differences within banker plant or augmentative treatments in mean number of aphids or mean number of mummies. However, some differences were noted between the banker plant and the augmentative treatment. Only results that were significant are reported for this data.

In Trial 1 (June-July 2016), the number of aphids was significantly lower in the banker plant treatment than the augmentative treatment during Week 3 (P=0.05), Week 4 (P=0.034), and Week 5 (P=0.039) (Fig. 4). The mean number of aphids in the banker plant treatment increased to 121 aphids at Week 2 but decreased by over half at Week 3; populations remained low between 3 to 6 aphids per plant throughout the remaining four weeks of the experiment (Fig. 4). In addition, a greater proportion of aphids in the banker plant treatment were parasitized, nearing 60% parasitism overall (Fig. 5). In contrast, the

mean number of aphids in the augmentative treatment increased to over 378 aphids per plant in Week 3 and never fell below 139 aphids per plant (Fig. 4). When the four pepper plants initially infested with *M. persicae* were analyzed separately from four randomly selected plants from each array, we found little differences in mean numbers of aphids and mummies within banker plant or augmentative treatments. In addition, there were no differences between treatments and mean number of mummies in this replication. However, there were significant differences in the mean number of aphids between augmentative and banker plant treatments (Table 2).

In Trial 1, high aphid numbers observed in the augmentative treatment would be unacceptable to commercial greenhouse growers and an insecticide application would be necessary to prevent crop loss (Jacobson and Croft 1998). This trial was conducted June 9-July 21, 2016 and it provides impetus for early incorporation of banker plant treatments into the crop cycle when plants are small and aphid abundance is low (van Lenteren 2000).

During Trial 2 (August-September 2016), the augmentative treatment maintained mean aphid numbers lower than the banker plant treatment, a reduction of approximately 50 to 100 aphids per plant (Fig. 6). Similarly, Jacobson and Croft (1998) reported late summer "trickle" or augmentative treatments of *A. colemani* were more effective than banker plants for controlling *A. gossypii* in cucumber (*Cucumis sativus* (L.)). Mean percent parasitism was also higher in the augmentative treatment peaking at 72%, 39% higher than in the banker plant treatment (Fig. 7). However, when the four pepper plants initially infested with *M. persicae* were analyzed separated from four randomly selected plants from each array, the only differences found were in Week 1 within the augmentative treatment concerning mean number of mummies (P=0.014), and Week 6 within the banker plant treatment for mean

number of aphids (P=0.007). In both of these cases, random plants had more aphids and mummies than those initially infested. When initially infested plants and random plants were compared between treatments, differences were noted in the abundance of aphids and mummies (Tables 3, 4). Random plants selected from the augmentative treatment had a significantly more aphids than the banker plant treatment Week 1 (Table 3). However, significantly more aphids were found in the banker plant treatment compared to the augmentative treatments in the last two weeks for the study for both initially infested and random pepper plants (Table 3, 4). In addition, significantly more mean numbers of mummies were observed in the augmentative treatment in Week 1 through Week 7 in random plants and Week 2 through Week 6 in initially infested pepper plants (Table 4).

Trial 2 was conducted August 17-September 20, 2016, and high, late-summer temperatures could explain a moderately high amount of aphids in both treatments while parasitism was approximately 60% beginning in Week 4 of the augmentative treatment. Also, the significance among random and initially infested mummies on plants between treatments could have been due to a greater number of aphids in augmentative treatments in general. Aphid populations will escalate in hot temperatures while *A. colemani* reproduction and efficacy may decrease at temperatures exceeding 30° C (Ahmad et al. 2016). Late-summer aphid control using augmentative releases of *A. colemani* may be more effective than a late application of banker plants in the southwestern U.S. However, use of banker plants early in the crop cycle when aphid numbers are still low (Bennison 1992) may negate the need for late-season aphid control altogether.

In Trial 3 (November-December 2016), aphid abundance fluctuated week to week and both the banker plant and augmentative treatments were similar with mean aphid

numbers ranging from 52 to 167 aphids per plant (Fig. 8). In this trial, percent parasitism remained at zero until Week 4 when parasitism reached 39% for the banker plant treatment and 56% for the augmentative treatment (Fig. 9). However, when initially infested pepper plants were analyzed separately from the random plants, significant differences in mean aphids and mummies were observed in Weeks 1, 6, and 7 within the augmentative treatment. In Week 1, the random plants had significantly less aphids than the initially infested plants (P=.0030). Later in the experiment, random plants had more aphids than those initially infested during Weeks 6 (P=.0039) and Week 7 (P=0.0119). Abundance of mummies was also significantly different in Week 6 within the augmentative treatment, with more mummies found on random plants (P=0.0221). In addition, between the augmentative treatment and the banker plant treatment, significant differences were detected between the initially infested pepper plants and random plants and mean numbers of aphids in Weeks 5, 6, and 7, and mean number of mummies in Week 2 and Week 7 (Table 5). However, mean aphid numbers were low for the first four weeks that could explain the low percent parasitism until Week 4 (Fig. 9).

Trial 3 took place November 3-December 15, 2016, and greenhouse temperatures ranged from 16° C to 27° C. Due to the season, short day lengths could have played a role in low *A. colemani* activity even though the parasitoid may be unaffected by photoperiod (Prado et al. 2015). Temperatures of 15° C did not affect parasitism of *M. persicae* by *A. gifuensis*, a braconid wasp related to *A. colemani*, but short day lengths did prolong development (Ohta and Ohtaishi 2006). A lag in development could contribute to less parasitism.

In Trial 4 (February-April 2017), mean aphid abundance remained lower throughout the experiment, ranging from 19 to 54 aphids per plant (Fig. 10). In addition, banker plants

showed a slight increase in mean percent aphid parasitism over augmentative releases, accounting for approximately 9% greater parasitism (Fig. 11). However, when initially infested pepper plants were analyzed separately from random plants there were no difference between treatments for mean aphids or mummies in any week. Within each treatment mean aphid abundance was significantly different in Week 4 with initially infested plants having significantly more aphids than those randomly selected (P=0.067, banker plants; P=0.0146, augmentative release).

Trial 4 occurred February 21-April 6, 2017. Therefore, mild temperatures in the greenhouse during this period could explain why aphid numbers remained low and percent parasitism was high compared to the other trials. Parasitism by *A. colemani* peaks between 10° C and 30° C (Zamani et al. 2006.) It is still preferred to employ biological control methods and banker plant systems early (Jacobson and Croft 1998) to prevent extreme aphid populations later in the growing season.

In conclusion, banker plant and augmentative treatments both appeared to work best in early spring trials, when temperatures are low and aphid growth is slower. Therefore, it is recommended that these methods of biological control be used early in the cropping cycle before aphid pest numbers build to levels where only pesticide application is feasible.

Relative Humidity and Temperature Correlation

Mean maximum temperature and mean relative humidity for each site was calculated by each week of experiments (Weeks 1 to 7) across all trials (Table 6). These environmental variables were correlated with the number of live aphids, mummies, total aphids (live aphids

+ mummies), and percent parasitism to access for relationships among these variables (Tables 7, 8). For the Stillwater augmentative treatment, mean maximum temperature by week was correlated with mean total aphids per plant, while relative humidity was positively correlated with mean number of live aphids and total aphids (Tables 7, 8). However, there was no relationship between parasitism and either of the environmental variables (Tables 7, 8). For the Oklahoma City augmentative treatment, the mean maximum temperature and relative humidity were positively correlated with percent parasitism, while mean maximum temperature and the abundance of mummies were positively correlated (Tables 7, 8). The Oklahoma City banker plant treatment showed a positive correlation between mean maximum temperature and mean number of total aphids as well as a positive correlation between relative humidity and both live and total aphids (Tables 7, 8). The Tulsa augmentative treatment showed no relationship between relative humidity and aphid abundance or parasitism (Table 8), but there was a positive relationship between temperature and both live and total aphids (Table 7). In contrast, the banker plant treatment at the Tulsa site showed a negative correlation between mean relative humidity and mean numbers of live aphids, mummies, and total aphids (Table 8). In addition, a negative correlation was found between temperature and both live and total aphids (Table 7).

The Stillwater banker plant treatment showed no correlations with either mean maximum temperature or mean relative humidity (Table 7, 8). This could be because the Stillwater banker plant site had the warmest temperatures of any site, with temperatures consistently over 31° C and favorable for *M. persicae* growth (Davis et al. 2006). However, because *Aphidius colemani* is native to India (Starý 1975), warm temperatures may still allow reproduction of the parasitoid (Prado and Frank 2014). Overall, the environmental factors

measured at all sites and in both treatments varied in relationship to aphid abundance. Additionally, percent parasitism was positively correlated with temperature and humidity only in the augmentative treatment at the Oklahoma City site. This could be because it was the coolest greenhouse overall, with mean maximum temperatures ranging from 25° C to 27° C and 73% to 76% mean relative humidity (Table 6). Temperature and relative humidity in these ranges may facilitate optimal reproduction of *A. colemani* (Prado and Frank 2014).

Variation in banker plant and augmentative treatment results among trials could be due to seasonal differences in temperatures. In some cases of extreme temperatures, aphid populations could increase and parasitoid populations may fail to provide adequate control. *Aphidius colemani* has a type II functional response, meaning parasitism will decrease with increasing aphid host density (Zamani et al. 2006). Also, this parasitoid has a greater searching efficiency than comparable *Aphidius* species in temperatures over 25° C (Zamani et al. 2006). Therefore, higher temperatures in the greenhouse should not drastically affect parasitism by *A. colemani* unless temperatures exceed 31° C (Goh et al. 2001). Greenhouse temperatures in Oklahoma readily exceed 30° C and parasitism is still observed (Table 6). Also, optimal growth of *M. persicae* is noted at 26.7° C with an upper limit of survival at 37.3° C (Davis et al. 2006). Based on this data, *A. colemani* may still be able to adequately survive and parasitize aphids in hot greenhouses.

Slightly fluctuating temperatures could also account for aphid and possibly parasitoid reproductive success, as most greenhouses will cool slightly in the evening hours. Constant temperatures at 35° C have been proven detrimental to *M. persicae* population growth (Davis et al. 2006). Therefore, temperatures above 30° C but not exceeding 35° C would still favor aphid reproduction (Davis et al. 2006) but possibly not parasitoid survival. Extreme high

fluctuating temperatures as the weather warms in the South further supports the use of biological control early in the season before temperatures complicate pest management. Hence, banker plants are best incorporated preventively rather than curatively in the greenhouse and at the beginning of the crop cycle before aphids are detected (Jacobson and Croft 1998) and while temperatures are mild. Using *A. colemani* alone, when greenhouse temperatures are high, may not result in successful establishment of the parasitoid for aphid pest control (Andorno et al. 2014).

Some site variation was noticed among the Oklahoma City, Stillwater, and Tulsa sites possibly due to greenhouse size, irrigation methods, cooling methods, use of shade cloth, or general purpose of the greenhouse, and the species of plants grown. In almost all trials, the pepper plants also became infested with *A. gossypii* indicating another aphid pest was already present in the greenhouses. In addition, pre-existing populations of *M. persicae* in cooperator greenhouses could have contributed to higher populations of aphids in this study. Fortunately, *A. colemani* parasitizes both aphids (Van Driesche et al. 2008, Jandricic et al. 2014). In addition, the presence of permanent plantings or interiorscapes at the cooperator sites could have harbored parasitoids between trials leading to better control when experiments were in place. This may have occurred in the augmentative treatment at the Oklahoma City site. The Tulsa greenhouses may have had less natural enemies in general, due to the use of occasional permethrin bombs when experiments were not in place (Garcia 2011). In addition, during Trial 2 the Tulsa site lost a roof covering and thus augmentative treatments were exposed to the elements as well as rainfall late in the sampling schedule. However, due to the tall ceiling height, it is believed that the parasitoids remained near their host source lower in the greenhouse environment.

Definite information regarding the number of banker plants needed in an area is necessary for successful use of the system (Parolin et al. 2010). Based on the sentinel plant data collected in this study, regardless of the variation in site and seasonal conditions, *A. colemani* readily parasitized aphids 4 m to 5 m away from the center release point of the augmentative and banker plant treatments (Table 9). Therefore, from these data a minimum of one banker plant per 70 m^2 of growing space can be recommended. This differs from the range (610m^2 to 1524m^2) suggested by IPM Laboratories, Inc. (Locke, NY). However, more data regarding placement in greenhouses is needed in the southwestern U.S.

Benefits of Banker Plants and Cost Analysis

Based on these results, biological control via augmentative release and banker plants provided unacceptable levels of aphid control. However, augmentative releases performed every two weeks are necessary to maintain effective control due to the short lifespan of the parasitoid (Stara et al. 2011). The annual cost of augmentative releases is approximately five times more than employing a banker plant system (Table 10). In a cost analysis, beginning with five banker plants and adding an additional five per week, the soil, wheat seed, *A. colemani* parasitoids (including quarterly replenishment costs), screen cages, and an initial supply of *R. padi* would cost the grower approximately $1481.59 USD per year for 1,524 m^2 of growing space based on protocols provided by IPM Laboratories, Inc. (Locke, NY) (Table 10). In addition, every additional banker plant would only cost $0.25 USD. In contrast, employing bi-weekly augmentative releases of 1,500 *A. colemani* wasps costs approximately $2,314.62 USD (Table 10). If more augmentative releases are needed, the cost can quickly

compound. Maintaining banker plant colonies requires need regular attention, but the labor required to implement these tools and time spent scouting are minimal compared to applying insecticides. However, banker plants are less expensive than imidacloprid soil drenches when considering labor costs to apply the chemical, barring chemigation. Where insecticides are included in irrigation, labor costs would be reduced in Table 10 and makes applying imidacloprid the least expensive option. Some aphid tolerance by the grower is necessary for banker plants to be successful (Andorno et al. 2014), whereas imidacloprid drenches would kill all available hosts for *A. colemani*, as seen in the control plants. In cases of foliar-applied insecticides, increased pesticide costs can be attributed to a need for repeat applications resulting from reduced effectiveness against common pests. In turn, pests may develop resistance to compounds after repeated treatments (van Lenteren and Woets 1988). Furthermore, few formulations may be effective against a pest, limiting rotational choices for chemical control (van Lenteren 2000). All of these implications can make using biological control, and especially banker plants, more attractive to growers (van Lenteren 2000). In addition, with banker plant use it may be necessary to educate consumers about the possible presence of aphid mummies on plants at the time of purchase. However, this unique opportunity could be marketed as "built-in" pest control and may bolster the appeal of biological control to the public. Public demand for sustainably produced plants could mitigate the use of conventional insecticides for pest control in greenhouses. Moreover, promoting the use of earth-friendly or reduced-risk practices may engender consumer loyalty.

When using biological controls, the proper disposal of remaining insecticide mixes is eliminated due to the reduced spray regimens required (van Lenteren et al. 1997). In addition, when implementing natural enemies there is less employee training required and no need for

licensing of spray technicians (van Lenteren 2000). Workers may enter the area and commence duties immediately after establishing banker plants or making augmentative releases, and there are no waiting periods or shipping intervals required. Furthermore, no supervision is required after the workers are educated about the basic function of the natural enemies being employed. Consumers may voice concerns for pollinator health (Rihn and Khachatryan 2016) and using natural enemies provides a unique opportunity to teach the public about biological control. Although considered a "softer" insecticide, insecticidal soaps can kill adult parasitoids (Tremblay et al. 2008) and other beneficial insects if contacted with the spray, and can also cause phytotoxicity in sensitive plants in warm greenhouses (van Lenteren and Woets 1998). Therefore, banker plants may be an option to consider even over reduced-risk insecticides.

In this experiment, I have shown that biological control using *A. colemani* is may be viable in Oklahoma using banker plants, as *M. persicae* aphid densities were reduced by *A. colemani* parasitism. However, results may vary due to greenhouse differences, season, and environmental conditions. The time of year may also dictate the method used. However, more banker plants may be added if control does not appear adequate. When using banker plants it is preferable to employ a "parasitoid-in-first" method, similar to the "standing army" approach of using predators (Janssen and Sabelis 2015). In this technique, banker plants are placed into the greenhouse at least one week prior to the crop cycle (Zamani et al. 2006) when temperatures are still mild and aphid development is slower. Subsequently, banker plants can provide control as soon as pests are present but not too abundant (Andorno et al. 2014). It is also recommended that banker plants be used in the greenhouse year-round to maintain a constant supply of parasitoids. Augmentative releases of *A. colemani* may be

more effective than banker plants if the grower prefers using biological control after the crop cycle begins. During extremely hot temperatures when parasitoids are less effective, spraying may be a less expensive option than augmentative releases. However, the use of banker plants preventively rather than curatively has been shown to be more effective than augmentative releases (Zamani et al. 2006, Andorno et al. 2014).

Use of parasitoids, especially in banker plant systems, is a useful tool for controlling aphids. Aphids can develop resistance to many insecticides, increasing the number of sprays necessary for control (van Lenteren 2000, Goh et al. 2001). Therefore, banker plants can be another option for control as aphids are much less likely to develop resistance to a natural enemy compared to a conventional insecticide. In addition, banker plants reduce environmental concerns with pesticide application and disposal, as well as offering the grower a unique opportunity to advertise earth-friendly, low-impact pest control methods when considering marketing of products (Kühne 1998).

Additional studies including the *A. colemani*-*R. padi* system could include evaluation of this system in conjunction with other predators, parasitoids, and banker plant species in the southwestern U.S. In addition, it is it important to evaluate alternative banker plant species that outlast or replace wheat and barley banker plants altogether and still or provide *R. padi* hosts to *A. colemani*.

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Table 1. Trial dates and sites for study.

Trial	Dates	Sites
1	June 9-July 21, 2016	Oklahoma City Stillwater Tulsa
2	August 27-September 20, 2016	Oklahoma City Stillwater Tulsa
3	November 3-December 15, 2016	Oklahoma City Stillwater
4	February 21-April 6, 2017	Oklahoma City Stillwater

Table 2. Mean number of *M. persicae* between augmentative and banker plant treatments in Trial 1. Separate analyses were performed for four pepper plants initially infested with aphids in week 1 and four randomly selected pepper plants within each array (PROC MIXED, $P \leq 0.05$).

		Randomly selected plants		Initially infested plants	
	Week	**Treatment**	**Mean number aphids (±S.E.)**	**Treatment**	**Mean number aphids (±S.E.)**
Trial 1	1	Augmentative Banker *P*	59.64 (±23.35) 55.33 (±20.67) 0.8659	Augmentative Banker *P*	41.50 (±7.74) 37.75 (±7.03) 0.8096
	2	Augmentative Banker *P*	223.83 (±48.76) 131.00 (±61.40) **0.0324**	Augmentative Banker *P*	167.33 (±39.60) 111.50 (±43.89) 0.1152
	3	Augmentative Banker *P*	390.00 (±88.33) 47.83 (±19.75) **0.0004**	Augmentative Banker *P*	369.92 (±83.83) 44.25 (±22.53) **0.0007**
	4	Augmentative Banker *P*	415.17 (±133.92) 6.92 (±5.30) **<0.0001**	Augmentative Banker *P*	320.75 (±82.52) 9.17 (±7.30) **<0.0001**
	5	Augmentative Banker *P*	323.50 (±95.65) 3.25 (±2.55) **<0.0001**	Augmentative Banker *P*	299.42 (±90.80) 2.00 (±1.16) **<0.0001**
	6	Augmentative Banker *P*	189.58 (±60.86) 6.33 (±3.67) **<0.0001**	Augmentative Banker *P*	238.58 (±75.65) 6.33 (±2.94) **<0.0001**
	7	Augmentative Banker *P*	143.50 (±41.21) 5.25 (±3.05) **<0.0001**	Augmentative Banker *P*	134.00 (±46.19) 4.58 (±2.72) **<0.0001**

Table 3. Mean number of *M. persicae* between augmentative and banker plant treatments in Trial 2. Separate analyses were performed for four pepper plants initially infested with aphids in week 1 and four randomly selected pepper plants within each array (PROC MIXED, $P \leq 0.05$).

		Randomly selected plants		**Initially infested plants**	
	Week	**Treatment**	**Mean number aphids (±S.E.)**	**Treatment**	**Mean number aphids (±S.E.)**
Trial 2	1	Augmentative Banker *P*	202.64 (±38.01) 139.83 (±56.53) **0.0157**	Augmentative Banker *P*	106.75 (±17.26) 87.17 (±23.75) 0.3600
	2	Augmentative Banker *P*	150.55 (±31.45) 262.75 (±104.01) 0.8981	Augmentative Banker *P*	143.64 (±23.19) 120.58 (±41.64) 0.3683
	3	Augmentative Banker *P*	199.75 (±48.94) 244.00 (±78.60) 0.9305	Augmentative Banker *P*	161.92 (±29.82) 161.08 (±43.60) 0.8527
	4	Augmentative Banker *P*	161.25 (±44.29) 224.91 (±92.59) 0.5196	Augmentative Banker *P*	121.00 (±27.51) 157.42 (±43.73) 0.5148
	5	Augmentative Banker *P*	139.42 (±45.52) 231.92 (±83.52) 0.1613	Augmentative Banker *P*	78.17 (±18.84) 145.75 (±33.43) 0.1013
	6	Augmentative Banker *P*	103.17 (±33.34) 311.08 (±75.88) **<0.0001**	Augmentative Banker *P*	86.33 (±23.74) 142.17 (±29.61) **0.0336**
	7	Augmentative Banker *P*	101.80 (±53.15) 148.08 (±21.08) **0.0219**	Augmentative Banker *P*	55.00 (±18.30) 193.08 (±24.00) **<0.0001**

Table 4. Mean number of aphid mummies between augmentative and banker plant treatments in Trial 2. Separate analyses were performed for four pepper plants initially infested with aphids in week 1 and four other randomly selected pepper plants within each array (PROC MIXED, $P \leq 0.05$).

		Randomly selected plants		**Initially infested plants**	
	Week	**Treatment**	**Mean number mummies (±S.E.)**	**Treatment**	**Mean number mummies (±S.E.)**
Trial 2	1	Augmentative Banker *P*	223.83 (±48.76) 131.00 (±61.40) **<0.0001**	Augmentative Banker *P*	15.00 (±3.73) 9.33 (±4.03) 0.2563
	2	Augmentative Banker *P*	158.27 (±45.08) 5.67 (±2.80) **<0.0001**	Augmentative Banker *P*	129.36 (±35.51) 26.67 (±12.04) **<0.0001**
	3	Augmentative Banker *P*	202.42 (±56.30) 15.25 (±4.70) **<0.0001**	Augmentative Banker *P*	180.92 (±35.45) 41.83 (±15.88) **0.0003**
	4	Augmentative Banker *P*	201.42 (±45.49) 68.82 (±32.32) **0.0007**	Augmentative Banker *P*	188.67 (±30.77) 65.75 (±21.88) **0.0008**
	5	Augmentative Banker *P*	168.33 (± 37.72) 28.08 (±8.23) **<0.0001**	Augmentative Banker *P*	161.67 (±30.32) 59.67 (±23.86) **0.0015**
	6	Augmentative Banker *P*	147.08 (±34.60) 63.33 (±31.31) **0.0125**	Augmentative Banker *P*	164.25 (±25.43) 53.42 (±24.58) **0.0009**
	7	Augmentative Banker *P*	129.50 (±32.31) 68.67 (±25.89) **0.0406**	Augmentative Banker *P*	109.67 (±11.10) 69.50 (±35.89) 0.291

Table 5. Mean number of aphid mummies between augmentative and banker plant treatments in Trial 3. Separate analyses were performed for four pepper plants initially infested with aphids in week 1 and four other randomly selected pepper plants within each array (PROC MIXED, $P \leq 0.05$).

		Randomly selected plants		**Initially infested plants**	
	Week	**Treatment**	**Mean number mummies (±S.E.)**	**Treatment**	**Mean number mummies (±S.E.)**
Trial 3	1	Augmentative	0.13 (±0.13)	Augmentative	0.00 (±0.00)
		Banker	0.13 (±0.13)	Banker	0.00 (±0.00)
		P	1.000	*P*	1.000
	2	Augmentative	1.63 (±1.35)	Augmentative	1.38 (±0.71)
		Banker	0.13 (±0.13)	Banker	0.00 (±0.00)
		P	0.1482	*P*	**0.0407**
	3	Augmentative	0.38 (±0.26)	Augmentative	1.38 (±1.02)
		Banker	0.00 (±0.00)	Banker	0.00 (±0.00)
		P	0.3091	*P*	0.0606
	4	Augmentative	3.25 (±0.65)	Augmentative	4.00 (±2.07)
		Banker	2.90 (±2.23)	Banker	0.63 (±0.42)
		P	0.2044	*P*	0.0807
	5	Augmentative	55.38 (±18.54)	Augmentative	29.25 (±15.35)
		Banker	6.13 (±1.73)	Banker	7.71 (±2.86)
		P	**0.0024**	*P*	0.1644
	6	Augmentative	116.63 (±18.13)	Augmentative	62.00 (±16.13)
		Banker	41.25 (±14.50)	Banker	28.29 (±11.51)
		P	**0.0007**	*P*	0.0683
	7	Augmentative	189.38 (±32.15)	Augmentative	138.00 (±43.55)
		Banker	87.63 (±42.53)	Banker	32.86 (±14.70)
		P	**0.0123**	*P*	**0.0275**

Table 6. Mean maximum greenhouse temperature and relative humidity by week and site.

Augmentative treatment			**Banker plant treatment**		
Week	Mean max. temp. (°C)	RH (%)	Week	Mean max. temp. (°C)	RH (%)
Stillwater			*Stillwater*		
1	28.03	70.81	1	31.87	69.01
2	31.17	78.10	2	35.17	73.14
3	29.56	75.44	3	38.41	66.86
4	28.05	74.73	4	37.62	69.39
5	26.93	77.08	5	35.09	69.77
6	27.94	81.03	6	36.72	69.26
7	27.64	85.56	7	38.32	73.11
Oklahoma City			*Oklahoma City*		
1	25.84	72.95	1	28.32	68.64
2	26.74	76.15	2	29.78	74.28
3	27.05	73.38	3	29.23	72.87
4	26.57	73.35	4	29.42	71.39
5	26.85	76.10	5	30.32	74.44
6	27.16	74.35	6	30.47	72.41
7	26.85	74.84	7	30.64	69.44
Tulsa			*Tulsa*		
1	26.75	70.85	1	27.61	77.16
2	30.91	72.43	2	30.87	77.59
3	32.53	75.86	3	32.06	79.79
4	32.42	77.55	4	31.07	79.82
5	30.43	75.48	5	30.30	75.39
6	30.54	73.40	6	30.74	75.74
7	32.37	71.20	7	31.80	80.13

Table 7. Correlation between mean maximum greenhouse temperature and per plant means of live aphids, mummified aphids, total number of aphids, and percent parasitism (PROC CORR Spearman, $P \leq 0.05$).

Mean maximum temperature correlations						
Site	**Treatment**		**Live aphids**	**Mummies**	**Total aphids**	**Percent parasitism**
Stillwater	*Augmentative*	*r*	0.40	-0.13	**0.43**	-0.35
		P	0.0605	0.5622	**0.0417**	0.0993
		n	23	23	**23**	23
Oklahoma City	*Augmentative*	*r*	-0.002	**0.41**	0.12	**0.49**
		P	0.9942	**0.0409**	0.5664	**0.0126**
		n	25	**25**	25	**25**
Tulsa	*Augmentative*	*r*	**0.78**	-0.39	**0.73**	-0.40
		P	**0.0030**	0.2081	**0.0065**	0.1993
		n	**12**	12	**12**	12
Stillwater	*Banker Plant*	*r*	-0.28	-0.03	-0.23	0.07
		P	0.1833	0.8765	0.2645	0.7322
		n	25	25	25	25
Oklahoma City	*Banker Plant*	*r*	-0.06	0.34	**0.41**	0.25
		P	0.7645	0.0982	**0.0422**	0.2264
		n	25	25	**25**	25
Tulsa	*Banker Plant*	*r*	**-0.62**	-0.31	**-0.55**	0.22
		P	**0.0235**	0.2974	**0.0500**	0.4706
		n	**13**	13	**13**	13

Table 8. Correlation between mean greenhouse relative humidity and per plant means of live aphids, mummified aphids, total number of aphids, and percent parasitism (PROC CORR Spearman, $P \leq 0.05$).

Relative humidity correlations						
Site	**Treatment**		**Live aphids**	**Mummies**	**Total aphids**	**Percent parasitism**
Stillwater	*Augmentative*	*r*	**0.55**	0.30	**0.71**	0.09
		P	**0.0060**	0.1614	**0.0001**	0.6973
		n	**28**	28	**28**	28
Oklahoma City	*Augmentative*	*r*	-0.15	0.39	0.05	**0.67**
		P	0.4719	0.0528	0.8251	**0.0003**
		n	28	28	28	**28**
Tulsa	*Augmentative*	*r*	0.14	0.55	0.34	0.27
		P	0.6646	0.0625	0.2756	0.3911
		n	14	14	14	14
Stillwater	*Banker Plant*	*r*	0.08	-0.14	0.04	-0.12
		P	0.6889	0.4965	0.8681	0.5840
		n	28	28	28	28
Oklahoma City	*Banker Plant*	*r*	**0.49**	0.03	**0.72**	-0.20
		P	**0.0131**	0.8738	**<0.0001**	0.3380
		n	**28**	28	**28**	28
Tulsa	*Banker Plant*	*r*	**-0.71**	**-0.60**	**-0.74**	-0.16
		P	**0.0067**	**0.0306**	**0.0040**	0.5905
		n	**14**	**14**	**14**	14

Table 9. Total number of *M. persicae* parasitized by *A. colemani* in 48 h on eight sentinel plants per treatment, from center release point (RP).

Site	Treatment	House size (m^2)	Mean distance from RP (m)	Mean number mummies
Oklahoma City	Augmentative	1,219	4.94	5.75
	Banker Plant	3,658	5.40	14.88
Stillwater	Augmentative	549	4.88	1.00
	Banker Plant	878	4.73	2.38
Tulsa	Augmentative	1,189	5.28	1.13
	Banker Plant	8,230	5.28	3.25

Table 10. Annual cost comparison of applications of two soil-applied neonicotinoids, banker plants, and *A. colemani* augmentative releases (based on 5,000 ft², IPM Laboratories, Biobest USA, American Plant Products, and product labels). Costs rounded to the nearest dollar.

Insecticides				**Biological Control**			
Imidacloprid	**Cost (US$)**	**Dinotefuran**	**Cost (US$)**	***A. colemani*-*R. padi* system**	**Cost (US$)**	***A. colemani* Releases**	**Cost (US$)**
5, 20 g bags in package	$126	1.36 kg container 19,200 15 cm pots treated per container	$455	1,000 *A.colemani* (once per 4 mos.) *R. padi* on barley	$265 $89	1,500 mummies	$88
100,000 pots treated per 7620 m²	$80	100,000 pots	$2,730	1 banker plant per 1524m²/wk: Wheat seed ($0.03/pot) Soil ($0.20)/pot 2 screen cages	$8 $52 $30	Every 2 wks.	$2,197
Labor ($10/h) 200 pots/h	$5,000	Labor ($10/h) 200 pots/h	$5,000	Labor ($10/h) 104 h/yr. min.	$1,040	Labor ($10/h) 3h/yr	$30
16 wk crop subtotal/yr	$5,206 $10,412	16 wk crop subtotal/yr	$8,185 $16,370				
Yearly Cost	**$20,824**	**Yearly Cost**	**$32,740**	**Yearly Cost**	**$1,484**	**Yearly Cost**	**$2,315**

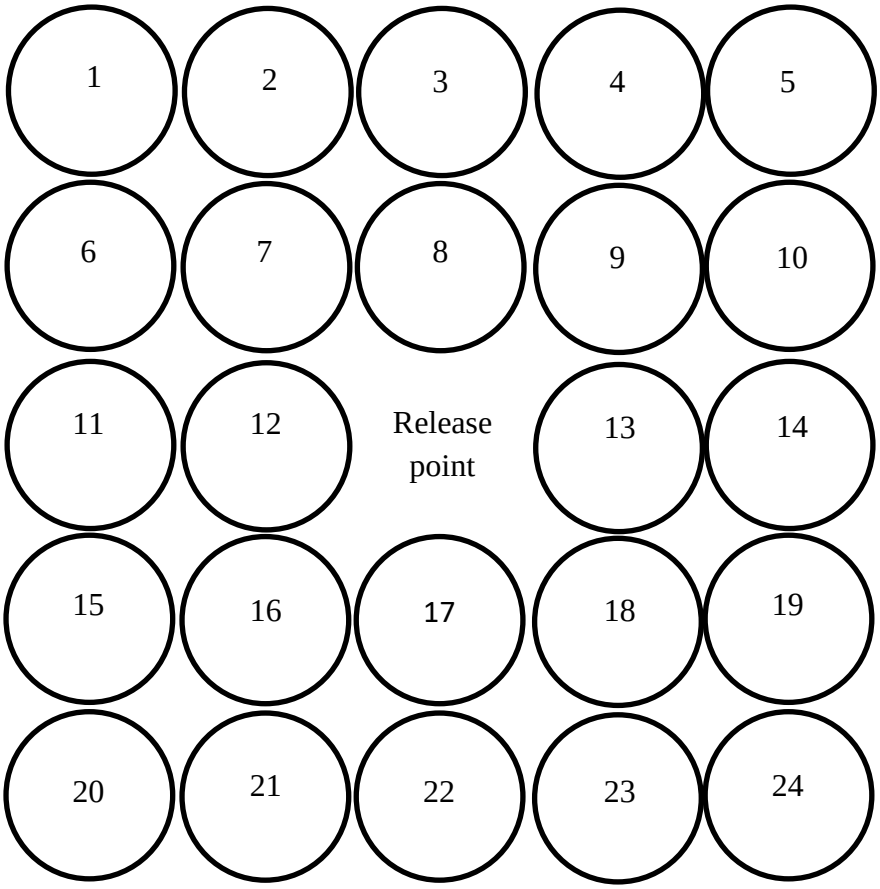

Figure 1. Ornamental pepper (*C. annuum* 'Black Pearl') plant array for banker plant and augmentative treatments at each cooperator site. Release point denotes where banker plants or aphid mummies were placed.

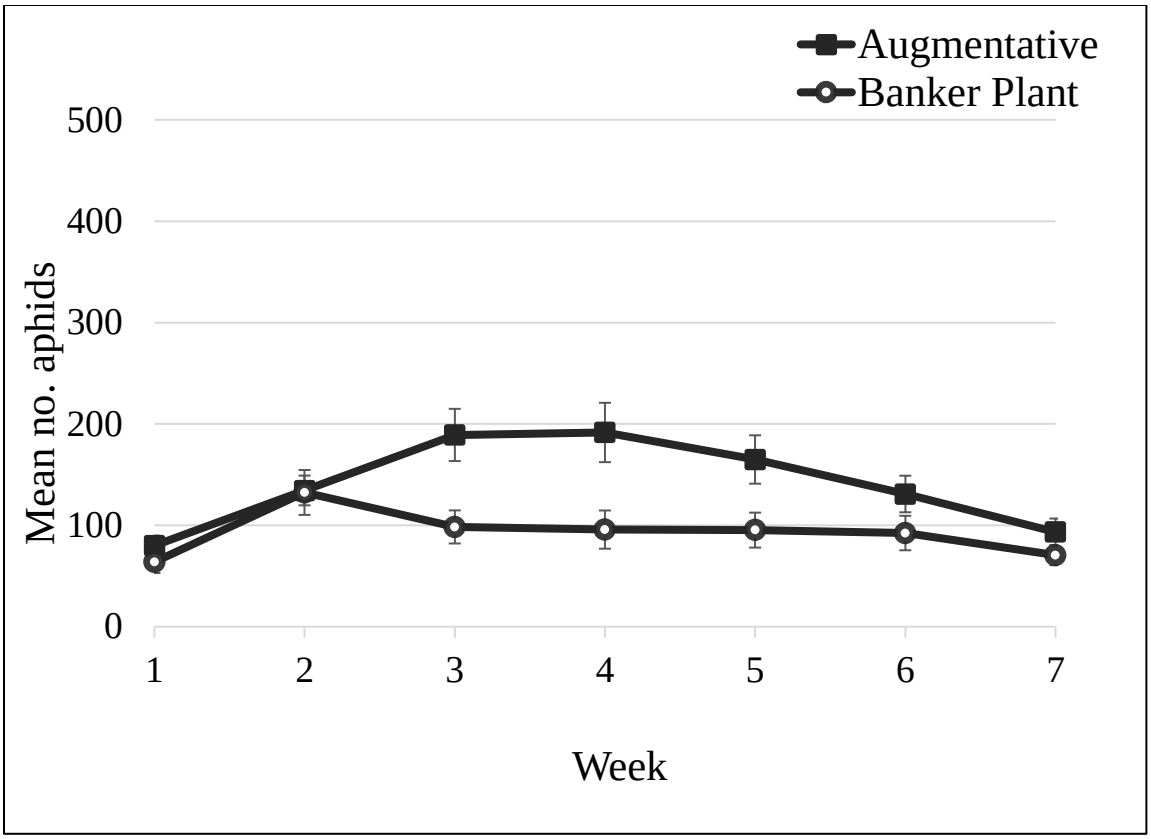

Figure 2. Mean (±S.E.) number of *M. persicae* per plant during all four trials. Significant differences between treatments were not detected for any week (PROC MIXED, $P \leq 0.05$).

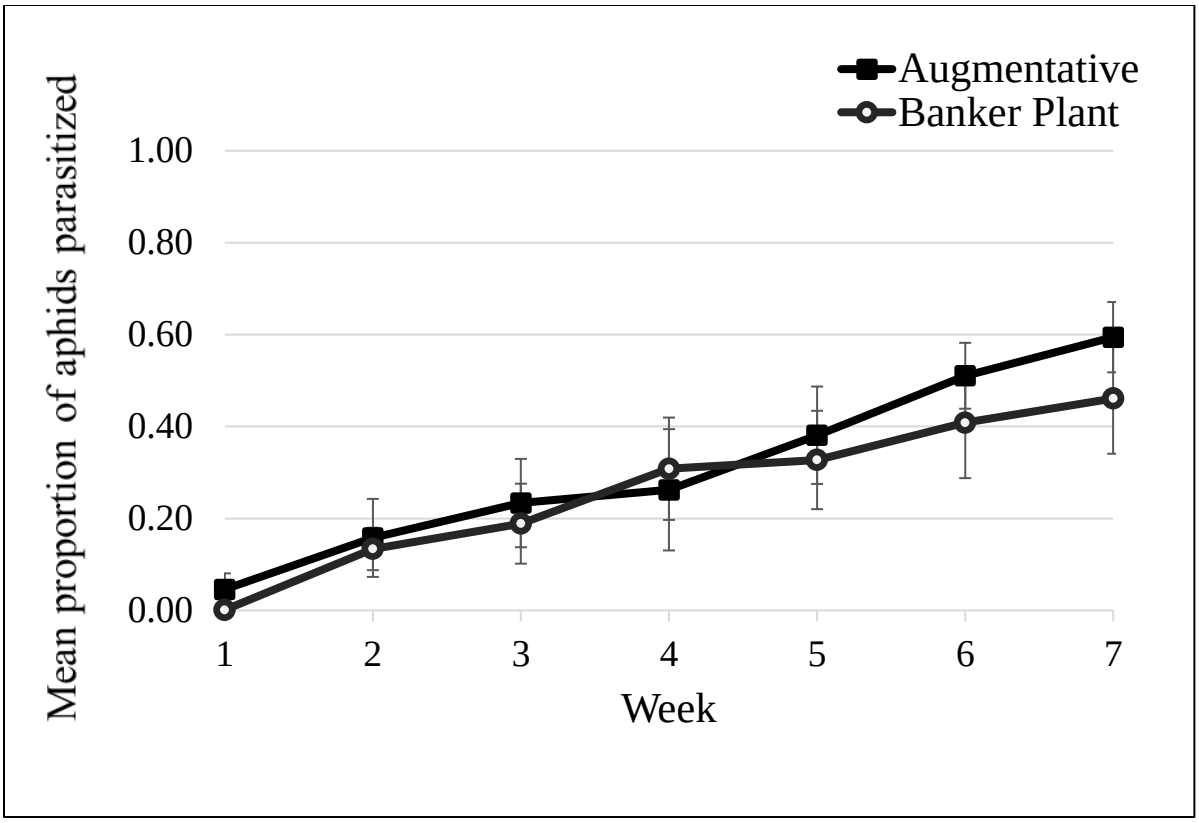

Figure 3. Mean (±S.E.) proportion of *M. persicae* parasitized by *A. colemani* during all four trials. Significant differences between treatments were not detected for any week (PROC MIXED, $P \leq 0.05$)

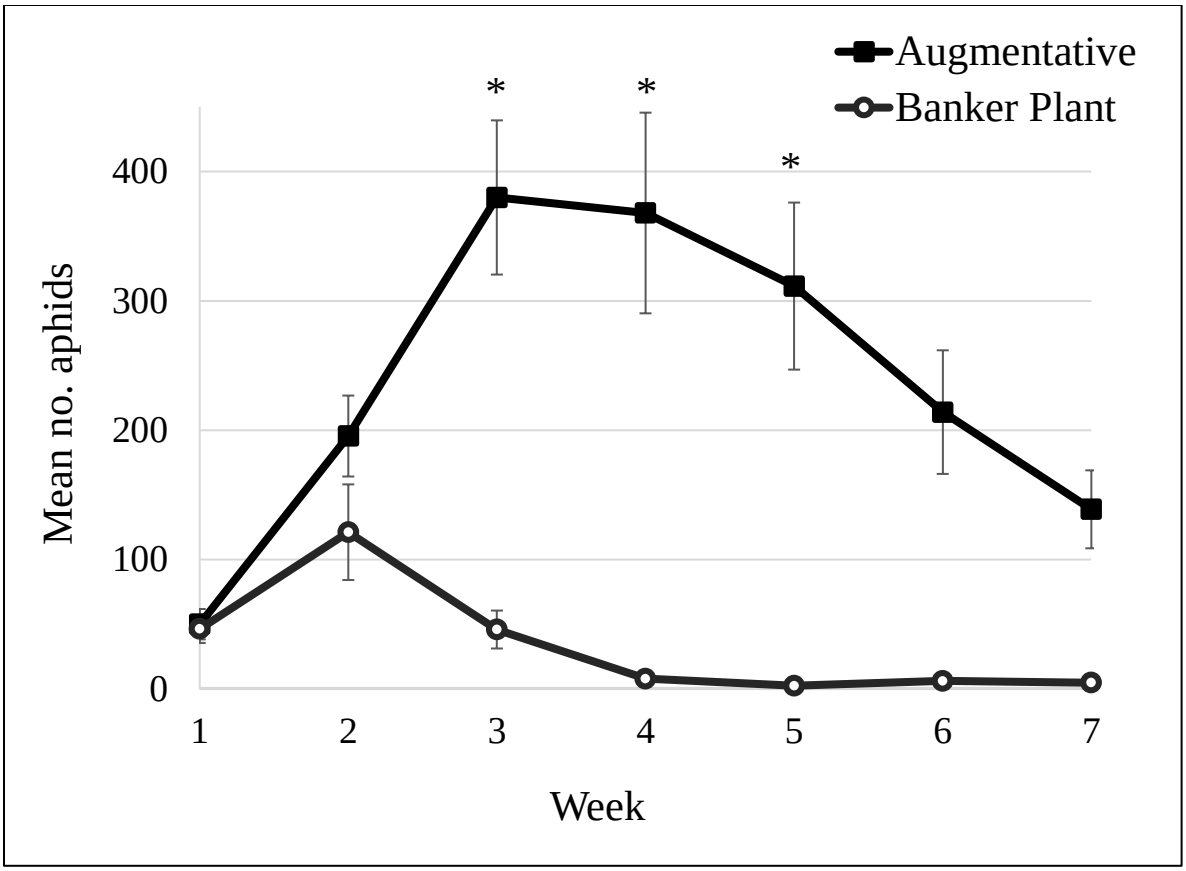

Figure 4. Mean (±S.E.) number of *M. persicae* per plant during Trial 1, June-July 2016. Significant differences were detected in Weeks 3, 4, and 5 indicated by an asterisk (PROC MIXED, $P \leq 0.05$).

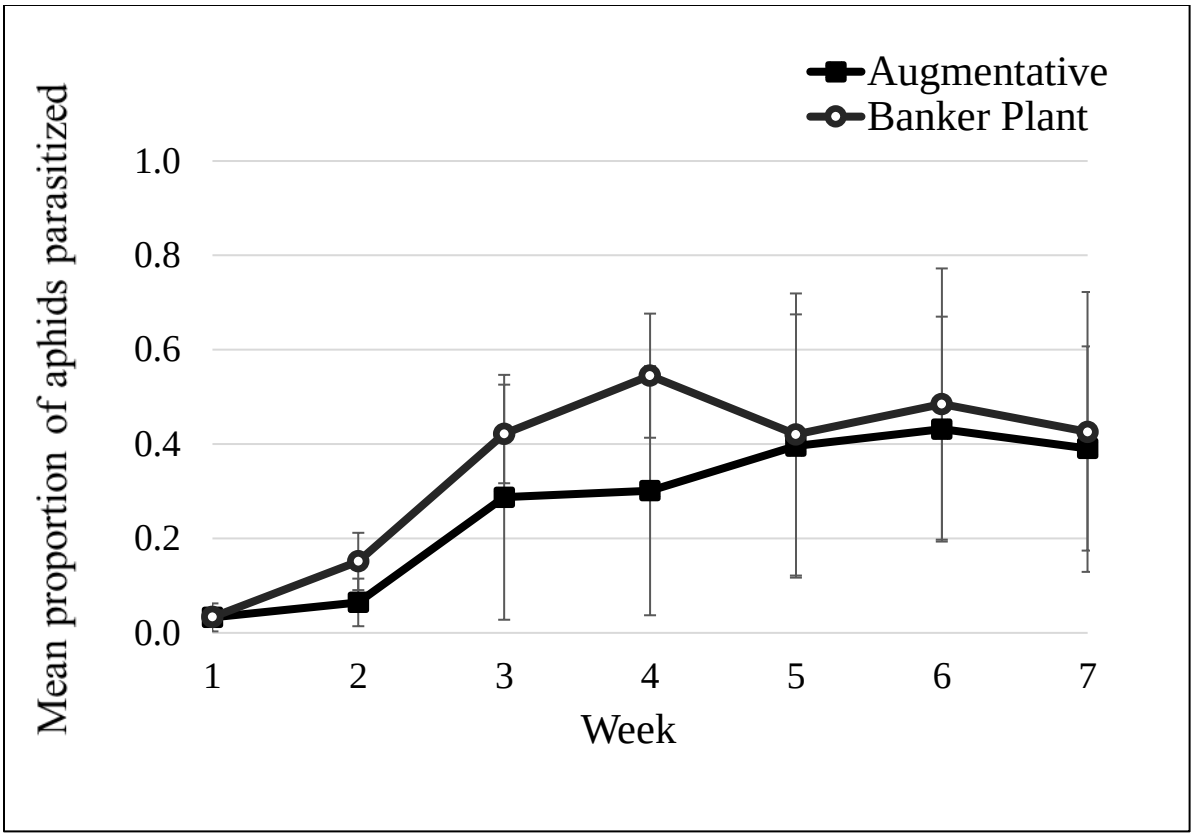

Figure 5. Mean (±S.E.) proportion of *M. persicae* parasitized by *A. colemani* during Trial 1, June-July 2016. Significant differences between treatments were not detected for any week (PROC MIXED, $P \leq 0.05$).

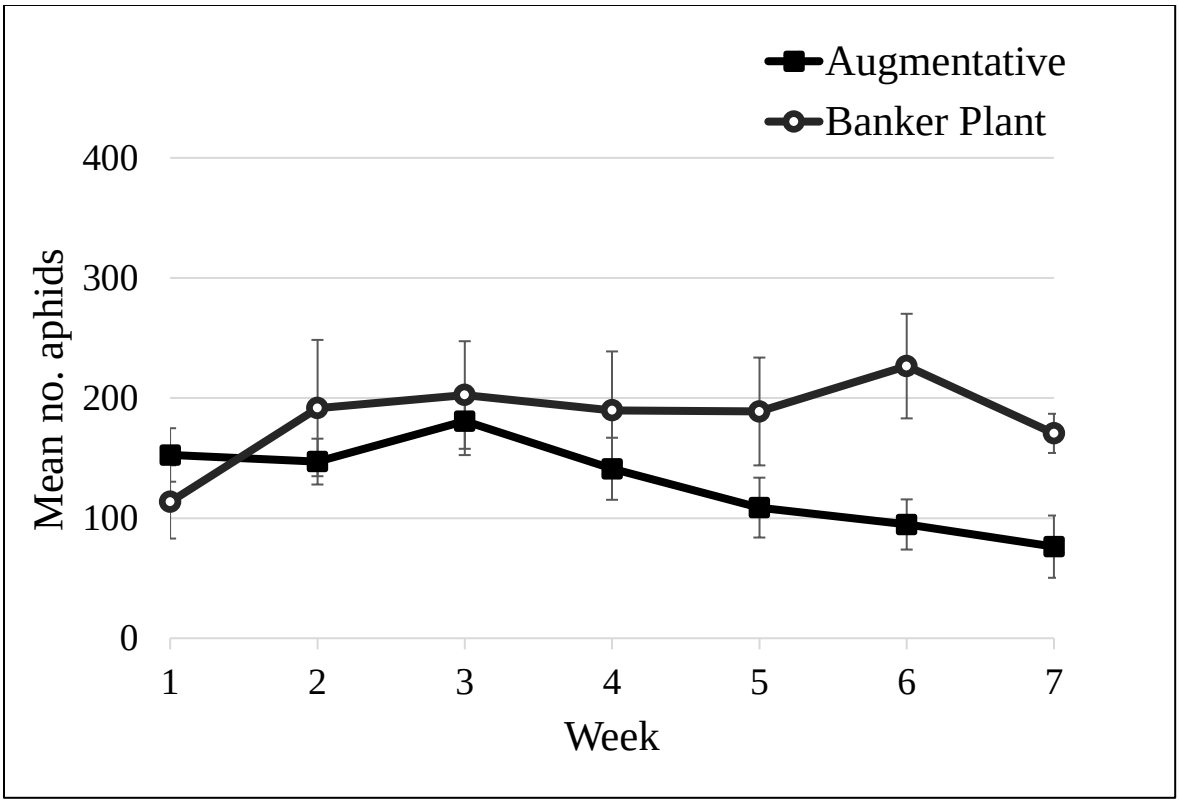

Figure 6. Mean (±S.E.) number of *M. persicae* per plant during Trial 2, August-September 2016. Significant differences between treatments were not detected for any week (PROC MIXED, $P \leq 0.05$).

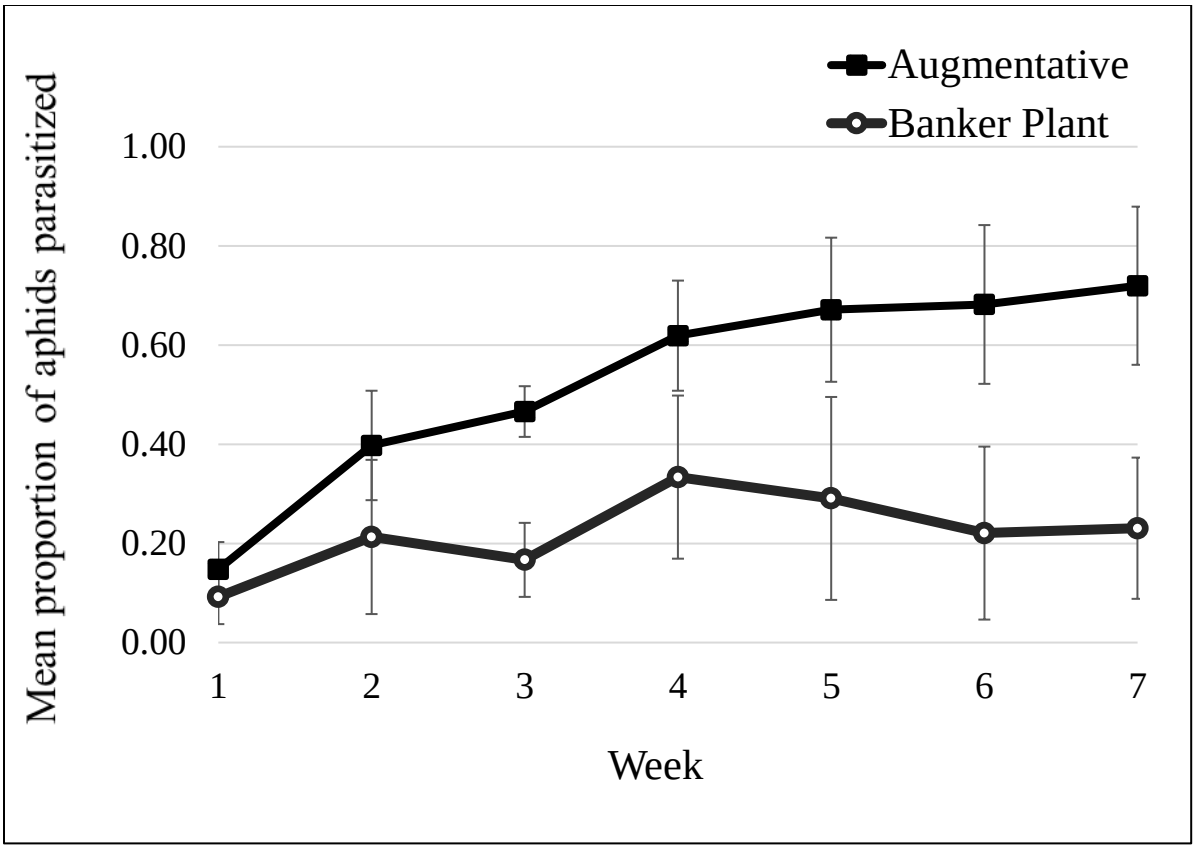

Figure 7. Mean (±S.E.) proportion of *M. persicae* parasitized by *A. colemani* during Trial 2, August-September 2016. Significant differences between treatments were not detected for any week (PROC MIXED, $P \leq 0.05$).

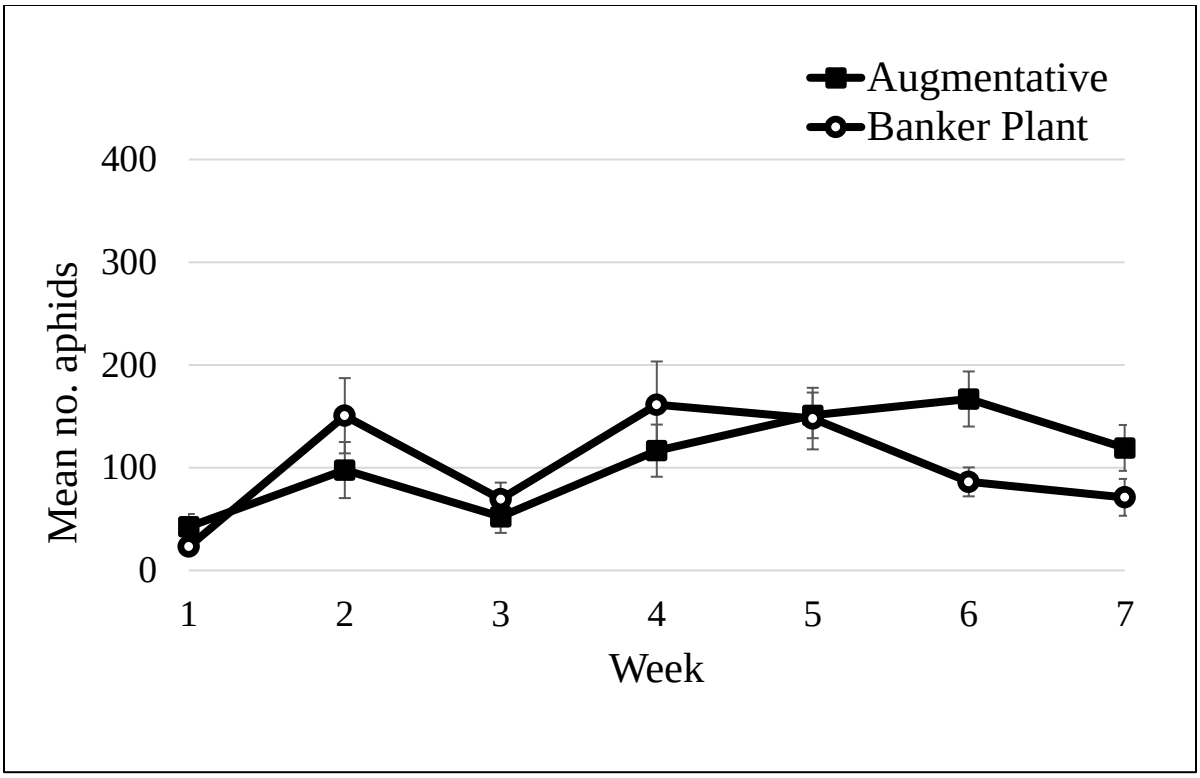

Figure 8. Mean (±S.E.) number of *M. persicae* per plant during Trial 3, November-December 2016. Significant differences between treatments were not detected for any week (PROC MIXED, $P \leq 0.05$).

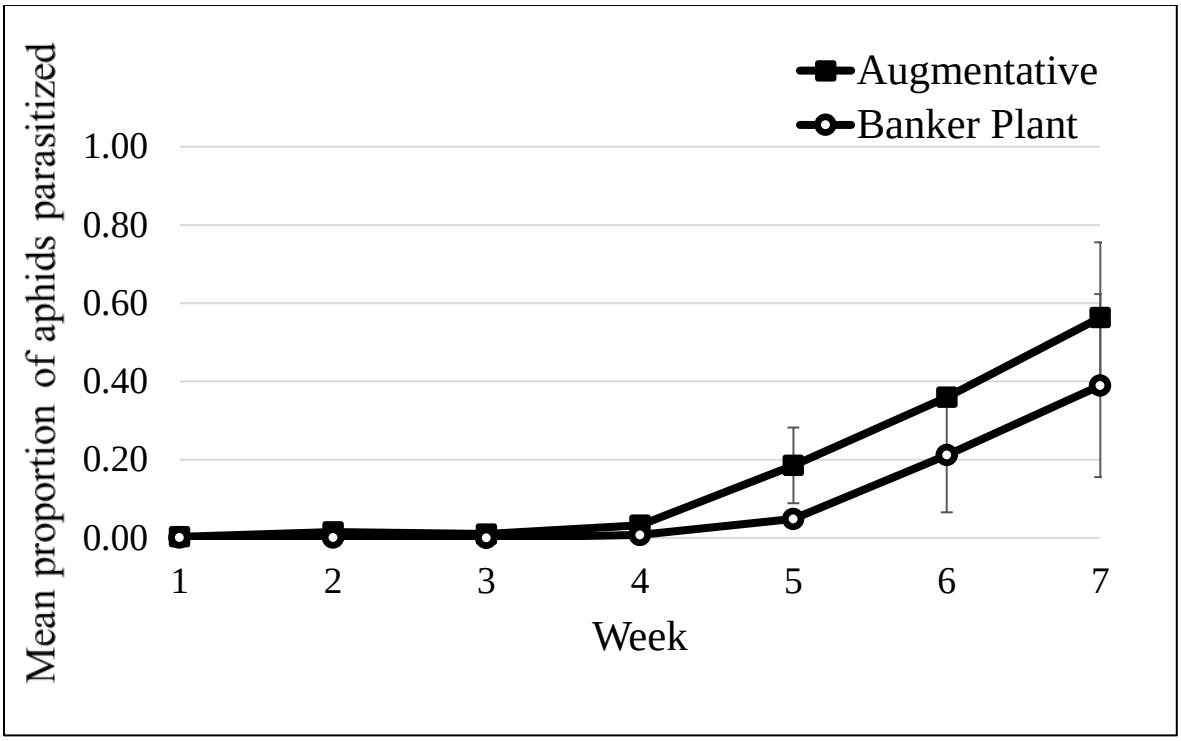

Figure 9. Mean (±S.E.) proportion of *M. persicae* parasitized by *A. colemani* during Trial 3, November-December 2016. Significant differences between treatments were not detected for any week (PROC MIXED, $P \leq 0.05$).

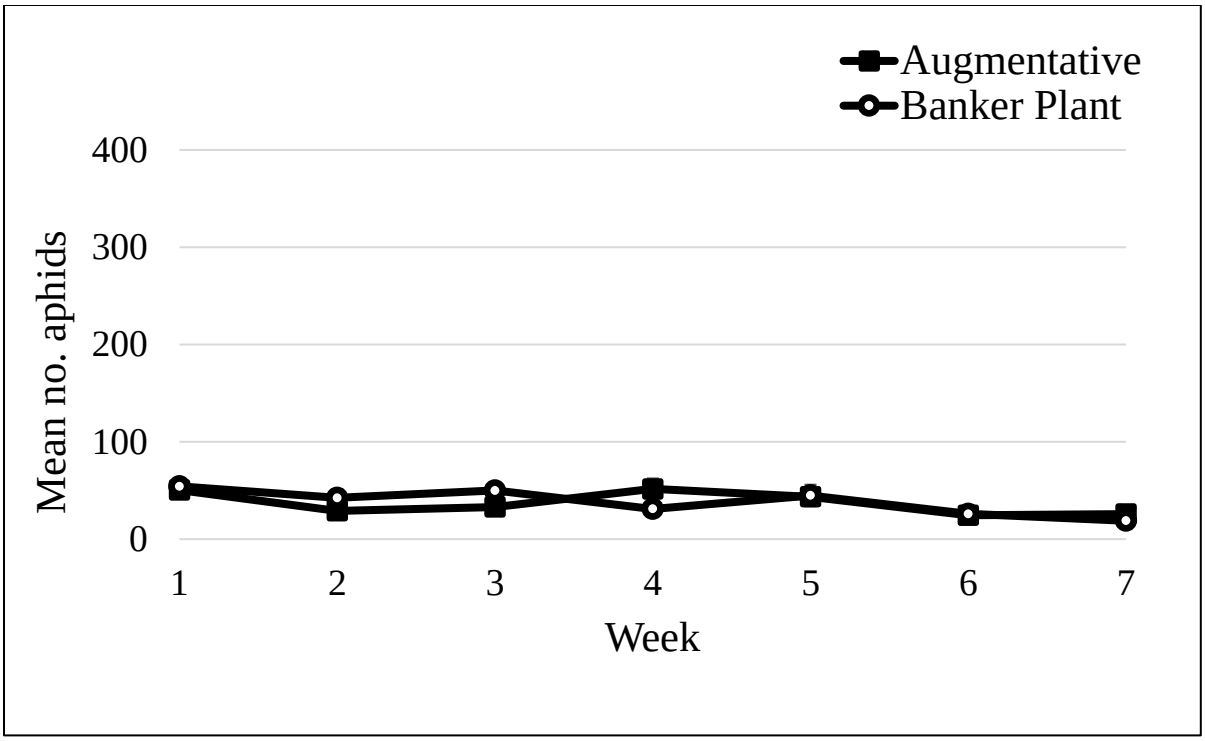

Figure 10. Mean (±S.E.) number of *M. persicae* per plant during Trial 4, late February-early April 2017. Significant differences between treatments were not detected for any week (PROC MIXED, $P \leq 0.05$).

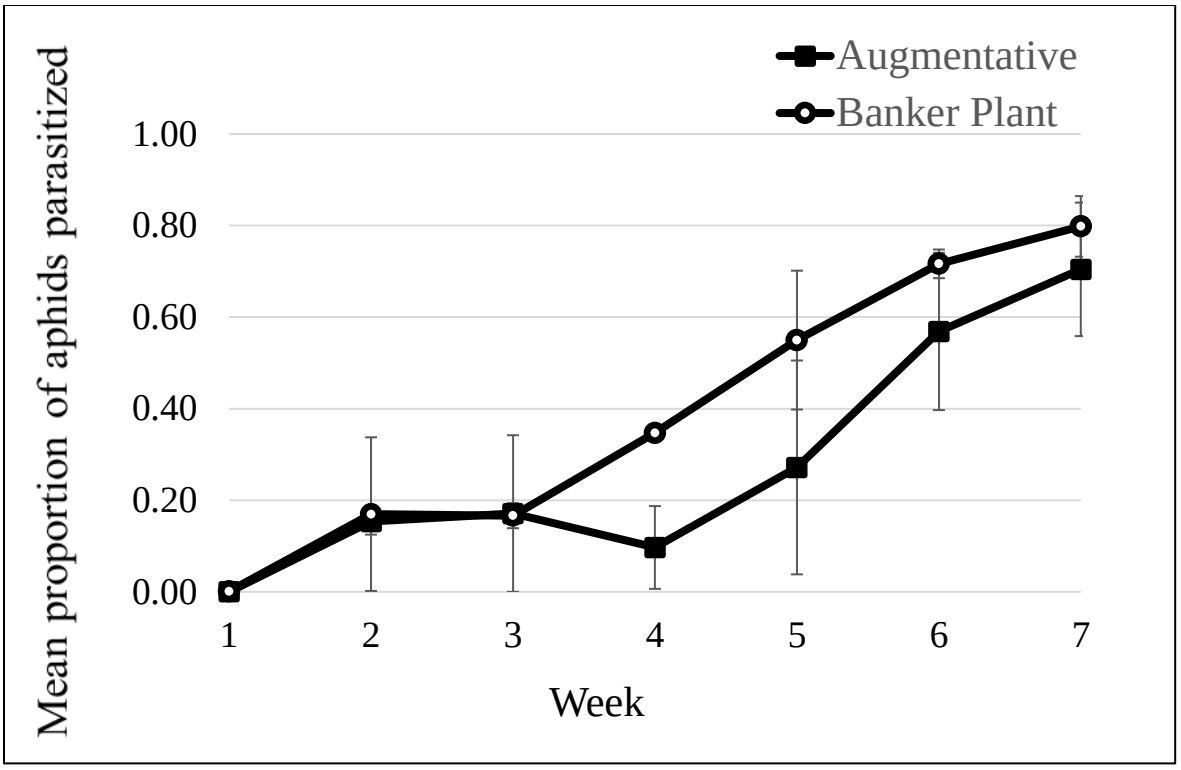

Figure 11. Mean (±S.E.) proportion of *M. persicae* parasitized by *A. colemani* during Trial 4, late February-early April 2017. Significant differences between treatments were not detected for any week (PROC MIXED, $P \leq 0.05$).

CHAPTER IV

PESTICIDE COMPATIBLITY WITH *APHIDIUS COLEMANI* PARASITOIDS FOR BIOLOGICAL CONTROL OF *MYZUS PERSICAE*

Abstract

When using parasitoids or predators for biological control, various arthropod pests may still become a concern and require pesticide applications. Therefore, it is important for growers to be familiar with pesticides that are toxic to natural enemies or at least have methods to prevent natural enemy mortality. In this experiment, I assessed mortality of *Aphidius colemani* Viereck (Hymenoptera: Braconidae) following exposure to contact insecticides and several fungicides commonly used in greenhouse and nursery production. In addition, I assessed the parasitism and mortality of *Myzus persicae* Sulzer (Hemiptera: Aphididae) when plants were treated with soil-applied systemic pesticides. I found that while most foliar-applied pesticides were considered only slightly or moderately harmful according to International Organization of Biological and Integrated Control-West Palaearctic Regional Section (IOBC-WPRS) standards, they may still

be harmful, accounting for 70% or higher mortality of *A. colemani*. In addition, systemic insecticides did not allow for any pest aphids to survive, eliminating hosts for *A. colemani* and limiting augmentative use of the parasitoid with these systemic controls.

Introduction

Aphidius colemani is a commercially available parasitoid used in augmentative and banker plant biological control applications. The parasitoid is used for management of the green peach aphid (*Myzus persicae*), the melon aphid (*Aphis gossypii* (Glover) (Hemiptera: Aphididae)), and the tobacco aphid (*Myzus nicotianae*) (Blackman) (Hemiptera: Aphididae)). For successful biological control, information is needed regarding the use of *A. colemani* in conjunction with pesticides used in the greenhouse to control for other types of aphid pests, mealybugs, spider mites, whiteflies, and fungi. In a recent survey of Oklahoma greenhouse producers, aphids are the most prevalent pest species and most growers control aphids using traditional insecticides (Payton Miller, unpublished data). However, whiteflies, thrips, mealybugs, and spider mites were also commonly encountered.

The purpose of this study was to determine insecticides and fungicides that are compatible with the use of *A. colemani* for biological control of aphids. With new pesticide formulations, screening compatible chemistries for use with biological control agents is imperative for effective pest management. The hypothesis was that fungicides would be fairly harmless and that neonicotinoids and pyrethroids would be harmful to *A. colemani*. Thirteen insecticides were screened for compatibility with the use of this parasitoid. Foliar-applied insecticide and fungicide residues, as well as common systemic insecticides, were

evaluated for *A. colemani* and *M. persicae* mortality, respectively. Pesticides were selected based on registered use in greenhouses and target pest, specifically aphids, mealybugs, thrips, and spider mites, with a focus on newly available chemical formulations (Table 1). Fungicides were selected based on foliar use and survey results from greenhouse growers (Payton Miller, unpublished data). The results of this study will be used to help growers incorporate insecticides and fungicides that can be used in conjunction with *A. colemani*, providing a more holistic Integrated Pest Management (IPM) approach.

Materials and Methods

Methods for this objective were adapted from Gandhi et al. (2005), Abraham et al. (2013), and Thompson et al. (2014). Thirteen insecticides and two fungicides were screened against adult *A. colemani*. Foliar-applied insecticide and fungicide residues were evaluated for *A. colemani* mortality over time. Soil-applied systemic insecticides were evaluated for parasitism of *M. persicae* by *A. colemani* over a two-week period.

Foliar-Applied Pesticides

A total of fourteen replications of each fungicide and insecticide treatment in this experiment were completed over four periods of time in Summer 2015 (Table 2). Each pesticide was tested at a moderate- and high-concentration based on label recommendations, for a total of 26 treatments per replication (Table 3). All pesticides were applied at a 148 ml concentration based on label information. Pesticide dilutions were prepared in one-pint glass

jars using deionized water (pH 6.4). Single *Capsicum annuum* 'Black Pearl' pepper leaves, approximately 2.5 cm wide x 4 cm long were removed from 30-cm tall plants contained in 15-cm plastic pots. Leaf petioles were inserted into a 10-µl pipette tip, hydrated, and wrapped in Parafilm® to maintain turgidity. A single pepper leaf was immersed in each jar containing the medium or high pesticide concentration or deionized water control for 10 seconds then allowed to air dry. Treated leaves with dried residues were placed in a 100-mm sterile plastic Petri dish containing a 2 x 2.5 cm square of moistened filter paper and honey streak for parasitoid feeding. *Aphidius colemani* wasps were cooled slightly for two minutes to slow activity, then five adult parasitoids aged 24 to 48 h were transferred to each Petri dish and covered. Each pesticide concentration plus a water control were tested concurrently and placed on a laboratory counter at 21° C to 24 °C with a photoperiod of 14:10 (L:D) h. Parasitoid mortality was observed at 6-, 12-, 24-, and 48-h intervals by assessing parasitoid activity inside the Petri dish. *Aphidius colemani* adults actively moving on leaf or dish surfaces were classified as live, those visibly deceased or lacking movement when disturbed were classified as dead. Parasitoids that were immobile except for twitching or other slow movements were classified as morbid.

Permethrin was used as a mortality check or reference insecticide. Results were classified using IOBC-WPRS classifications as harmless (<30% mortality within 48 h), slightly harmful (30-79% mortality within 48 h), moderately harmful (80-98% mortality within 48 h), and harmful (>99% mortality within 48 h). Medium or high concentrations were not significant between treatments of the same active ingredient; therefore, mortality data were pooled by compound. Contingency tables were constructed using pairwise

comparisons based on chi-square tests between active ingredients. Results were analyzed using PROC FREQ (SAS 9.4 Software, Cary, NC) at $P \leq 0.05$.

Systemic Pesticides

For evaluating systemic insecticide formulations, medium- and high-concentration drench applications were made to 15-cm pots containing ornamental pepper plants (Table 4). Each treatment was replicated 18 times over three time periods (March, June, and July, 2017). Medium and high concentrations of insecticides were prepared as per label directions using tap water. Each treatment was calculated to a 3.79 L dilution due to the low number of pots treated. Cyantraniliprole and imidacloprid labels listed only one concentration so only one treatment was evaluated for these two insecticides. Insecticide dilutions were prepared in plastic containers and used immediately. All *C. annuum* 'Black Pearl' plants in each replication were treated on the same day, with 89 to 118 fluid ounces of each diluted insecticide (Table 4). Then, each plant was infested with 10 *M. persicae* approximately four hours after pesticide applications. Control plants consisted of aphid-infested plants that were not treated with insecticides. Irrigation was withheld for the rest of the day to ensure insecticides were not washed out of the pot. For each replication, six randomized, 15-cm pots were placed in a 35.5 x 35.5 x 61 cm screen cage. However, control plants were isolated in a single screen cage so introduced parasitoids were not exposed to insecticide treated plants. Plants were moved to the greenhouse with average temperatures ranging between 25° C and 27° C, 66% to 71% relative humidity, and photoperiod of 14:10 (L:D) h. Eight adult *A. colemani* were added to each cage. Plants were hand watered as needed. *Myzus persicae* were observed for parasitism at 5 and 14 days. Mean number of aphids and mummies were

computed and results were analyzed using PROC UNIVARIATE (SAS 9.4 Software, Cary, NC) at $P \leq 0.05$.

Results and Discussion

Foliar-Applied Insecticide Compatibility

Medium- and high- insecticide concentrations on mortality of *A. colemani* were not statistically significant for most of the compounds tested. However, the spinetoram and sulfoxaflor combination appeared to have significantly higher mortality at high concentration (P=0.0214 at 6 h count, P=0.0008 at 12 h count). In addition, cyantraniliprole and dinotefuran both caused greater mortality than other insecticides at medium concentrations at 12 h (P=0.0329 and P=0.0207, respectively). The remaining compounds tested showed no significant differences in mortality of *A. colemani* between medium or high concentration, so the data were pooled for both concentration levels. Acetamiprid, dinotefuran, spinetoram and sulfoxaflor combination, permethrin, and tolfenpyrad accounted for the highest mortality, killing 86% to 100% of *A. colemani* after 48 h (Table 5). These compounds would not be recommended in conjunction with *A. colemani* use. Pymetrozine is considered safe to use with the aphid parasitoid *Diaeretiella rapae* (McIntosh) (Hymenoptera: Aphidiidae), especially in the mummy stage (Acheampong and Stark 2004). However, this compound caused over 60% mortality in *A. colemani* in 48 h, showing the need for natural enemy mortality studies. Pyriproxyfen and spirotetramat caused the lowest mortality to *A. colemani* after 48 h, 52.59% and 44.83%, respectively. Lower mortality in adult *A. colemani* with pyriproxyfen could be due to its mode of action as an insect growth regulator, although it is

harmful to immature parasitoids (Cloyd 2005). Mortality rates with spirotetramat were comparable to the water control where 38.39% mortality was observed (Table 5). Mortality in the water control could be due to the short lifespan of *A. colemani*, older adult parasitoids present in the shipment, or temperatures experienced by the insect during cooling, handling, or shipment. However, these factors would be present in every treatment tested therefore no adjustments were made in results. Spirotetramat has translaminar activity in the leaves of plants, therefore if sprayed as a contact insecticide it will likely exhibit some systemic properties (Brück et al. 2009). Therefore, spirotetramat may not be the best choice for use in biological control programs where pest health should be conserved for natural enemies. Based on these results, pymetrozine and pyriproxyfen caused the least amount of mortality in *A. colemani* adults and may be the most compatible conventional insecticides when trying to conserve these parasitoids in augmentative or banker plant systems. However, more research is needed on the inert ingredients in this compound and their effect on adult *A. colemani* (Acheampong and Stark 2004, Desneux et al. 2006).

The foliar-applied fungicides tested were considered slightly harmful by IOBC-WPRS guidelines causing 63% to 65% mortality in *A. colemani* after 48 h (Tables 4, 5). This level of mortality is comparable to the foliar-applied insecticides azadirachtin and pymetrozine (Tables 5, 6). Fungicides could cause mortality in *A.colemani* due to the inert ingredients or adjuvants included with the active ingredient in the formulation (Cloyd 2012). Adjuvants may improve the properties of the pesticide as a spreader, sticker, foam suppressor, wetting agent, emulsifier, penetrant, or to help in dispersion or in reducing drift (Acheampong and Stark 2004). However, these ingredients have been shown to have insecticidal properties in certain insects (Cloyd and Martin 2012, Mullin 2015). For example,

organosilicone surfactants used alone or as a synergist in pymetrozine causes reduced fertility in *D. rapae*, but the active ingredient alone is considered harmless to natural enemies (Acheampong and Stark 2004). In addition, many pesticide companies consider inert ingredients proprietary or trade secrets (Mullin et al. 2015) and may not be listed on the label or evaluated for activity against non-target arthropods (Mesnage and Antoniou 2018). Therefore, it is imperative to carefully apply fungicides when parasitoids are not active or banker plants have been removed from the area.

Contact toxicity based on IOBC-WPRS classification may not be the best method of determining toxicity of compounds to natural enemies. Even though this is the standard measure of mortality in the laboratory, slightly harmful (30 to 79% mortality in 48 h) insecticides may not be suitable when using *A. colemani* as too many parasitoids would be lost to adequately provide control (Table 6) (Prado et al. 2015). Because mortality was noted in the water control, some of the mortality observed may not have been caused by insecticide treatments alone. Because laboratory experiments consist of a "worst case" scenario, with insects being controlled and confined to an area, additional experimentation in the greenhouse is needed to evaluate the mortality of the examined compounds in the field (Desneux et al. 2007, Cloyd 2012). In addition, our experiments used fresh residues with parasitoids exposed constantly for 48 h. Further studies would be beneficial to ascertain the mortality of *A. colemani* with residues that are 6-, 12-, 24-, and 48-h old, as they may be less likely to cause high levels of mortality observed in this study.

Other pests will need control in the greenhouse if not using biological control agents. Therefore, spraying pesticides for control of other pests is the norm for most growers. In this case, banker plants would be preferred when treating plants with conventional insecticides as

they can be moved out of the greenhouse for several days after treatment then moved back inside once the residues on leaf tissues have dissipated. Compounds that may be more effective with banker plant and augmentative uses of parasitoids include reduced-risk insecticides such as insecticidal soap (Cloyd 2005), as well as foliar-applied pyriproxyfen and spirotetramat. However, care should be taken to avoid contact of any pesticide or fungicide with foraging parasitoids and banker plants should be removed from the greenhouse prior to application. Pesticides classified as slightly harmful still cause significant mortality to *A. colemani*, especially when using augmentative releases of parasitoids. Banker plants are small, mobile parasitoid habitats and can ensure the health of natural enemies is conserved even when pesticide applications are necessary.

An advantage of banker plants is the ability to spot treat heavy infestations of pests while still maintaining an acceptable level of biological control. One advantage of banker plants is mobility; they can be moved out of the greenhouse for several days if foliar spraying is necessary and returned when toxicity of residues has lessened. When treating the greenhouse it is recommended banker plants be removed from the growing area for at least 48-72 h to maintain the health of the parasitoids. When using conventional insecticides and biological control agents care must be taken to select effective insecticides that are also compatible with natural enemies. In this experiment none of the insecticides applied as a soil drench could be used effectively with *A. colemani* parasitoids as all hosts were eliminated. In addition, banker plant usage would not be compatible with these compounds as no aphids would be present for parasitism. Without continued parasitoid progeny biological control is not sustainable. Thus, these systemic insecticides would not be compatible with biological control of *M. persicae* using *A. colemani*.

Systemic Pesticide Compatibility

All concentrations of cyantraniliprole, dinotefuran, flupyradifurone, imidacloprid, and spirotetramat tested caused 100% mortality of *M. persicae* when evaluated 5 and 14 days after treatment (Table 7). Therefore, no parasitism by *A. colemani* was observed in any of the pesticide treatments due to death of aphid hosts. Only control plants contained enough *M. persicae* at 5 and 14 days after treatment to be available to parasitoids (Table 7).

In this study three neonicotinoid insecticides were examined (Table 1). Neonicotinoids are a popular choice for pest control in greenhouses as they are effective at killing sucking pests and yet exhibit low mammalian toxicity (Scholer and Krischik 2014). In addition, soil drench applications reduce the potential of inhalation of product or drift onto non-target plants. However, when choosing biological control with *A. colemani*, the use of soil-applied insecticide drenches may be prohibitive to the effectiveness of the parasitoid regardless of the insecticide class. For biological controls to be successful, some prey or hosts must be present for natural enemies to exploit (Cloyd 2005). When all pests are eliminated, biological control does not work. In this study, common, systemic, soil-applied insecticides would not be compatible with biological control using *A. colemani*. When aphids are eliminated no hosts are available for parasitism. In addition, feeding on hemolymph of treated aphids could still cause death of the parasitoid (Cloyd 2012). These systemic insecticides should be avoided when using *A. colemani* and other parasitoids for control of aphids.

Future work will be necessary to evaluate the effect of insecticides and fungicides on *A. colemani* as currently registered greenhouse products are reformulated or new compounds

are released (van Lenteren 2000). These experiments forced *A. colemani* to a constant exposure of compounds in Petri dish studies. Therefore, field studies evaluating contact insecticides are needed to ascertain realistic mortality rates of *A. colemani* in banker plant and augmentative release situations (Cloyd 2012). In addition, evaluation of inert ingredients on parasitoid mortality is needed to determine proper usage in biological control (Desneux et al. 2006). Experiments addressing sub-lethal effects of insect growth regulars on immature *A. colemani* should also be considered (Desneux et al. 2006).

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Table 1. Insecticides and fungicides tested for pesticide compatibility trials involving *Aphidius colemani*.

Active ingredient	Site of action	Group	Sub-group
Insecticides			
Acetamiprid	nAChR* modulator	4A	Neonicotinoid
Azadirachtin	Unknown	UN	Azadirachtin
Cyantraniliprole	Ryanodine receptor module	28	Diamides
Dinotefuran	nAChR* modulator	4A	Neonicotinoid
Flonicamid	Chordotonal organs	9C	Flonicamid
Flupyradifurone	nAChR* modulator	4D	Butenolides
Imidacloprid	nAChR* modulator	4A	Neonicotinoid
Permethrin	Sodium channel modulator	3A	Pyrethroids
Pymetrozine	Chordotonal organs	9B	Pymetrozine
Pyriproxyfen	Juvenile hormone mimic	7C	Pyriproxyfen
Spinetoram & sulfoxaflor	nAChR* modulators	5/4C	Spinosyns, sulfoximines
Spirotetramat	Acetyl CoA carboxylase inhibitor	23	Tetronic/Tetramic acid derivative
Tolfenpyrad	Electron transport inhibitor	21A	†METI insecticide
Fungicides			
Azoxystrobin	Quinone outside inhibitor	C3	Methoxy-acrylates
Myclobutanil	Demethylation inhibitor	G1	Triazole

*nAChR=Nicotinic acetylcholine receptor

†METI=Mitochondrial electron transport inhibitors

Table 2. Replication dates and times for each 6-, 12-, 24-, and 48-h assessment of foliar-applied insecticides and fungicides on mortality of *Aphidius colemani*.

Replication	Dates (2015)	6 h	12 h	24 h	48 h
1-3	May 29-31	5:00 PM	11:00 PM	11:00 AM	11:00 AM
4-6	June 5-7	4:30 PM	10:30 PM	10:30 AM	10:30 AM
7-10	June 26-28	5:00 PM	11:00 PM	11:00 AM	11:00 AM
11-14	July 3-5	4:30 PM	10:30 PM	10:30 AM	10:30 AM

Table 3. Dilutions for medium and high concentrations of foliar-applied insecticides and fungicides. Application rates follow label directions for each product.

Insecticides	**Med. rate (379 L)**	**High rate (379 L)**
Acetamiprid	488 ml	695 ml
Azadirachtin	355 ml	473 ml
Cyantraniliprole	237 ml	473 ml
Dinotefuran	170 g	227 g
Flonicamid	60 g	120 g
Permethrin	177 ml	237 ml
Pymetrozine	142 g	283 g
Pyriproxyfen	266 ml	355 ml
Spinetoram & Sulfoxaflor	67 g	100 g
Spirotetramat	59 ml	101 ml
Tolfenpyrad	769 ml	946 ml
Fungicides		
Azoxystrobin	57 g	114 g
Myclobutanil	237 ml	355 ml

Table 4. Dilutions for medium and high concentrations of soil-applied insecticides and total volume applied to each pot. Application rates follow label directions for each product.

Insecticides	Med. rate (379 L)	High rate (379 L)	ml per 15 cm pot
Cyantraniliprole	---	355 ml	118
Dinotefuran	510 g	680 g	118
Flupyradifurone	621 ml	828 ml	89
Imidacloprid	---	62.5 g	118
Spirotetramat	50 ml	101 ml	118

Table 5. *Aphidius colemani* mortality at 6-, 12-, 24-, and 48-hour intervals following application of foliar-applied insecticides and fungicides. Mortality rates with different superscript letters indicate significant differences (PROC FREQ, $P \leq 0.05$).

	Mean percent mortality (±S.E.)			
Active ingredient	**6 h**	**12 h**	**24 h**	**48 h**
Acetamiprid	32.58^{c} (±0.24)	48.80^{c} (±0.26)	64.23^{d} (±0.30)	86.21^{c} (±0.21)
Azadirachtin	8.53^{d} (±0.13)	15.08^{ef} (±0.15)	26.89^{fg} (±0.22)	66.07^{d} (±0.30)
Cyantraniliprole	23.53^{c} (±0.17)	34.33^{d} (±0.19)	46.62^{e} (±0.22)	74.19^{d} (±0.23)
Dinotefuran	55.73^{b} (±0.25)	72.00^{b} (±0.27)	86.67^{c} (±0.22)	96.52^{ab} (±0.22)
Flonicamid	9.76^{d} (±0.14)	14.88^{ef} (±0.16)	37.50^{ef} (±0.23)	75.00^{d} (±0.32)
Permethrin	71.65^{a} (±0.25)	89.68^{a} (±0.22)	99.21^{a} (±0.14)	100^{a} (±0.14)
Pymetrozine	9.02^{d} (±0.15)	10.00^{f} (±0.14)	18.18^{gh} (±0.21)	65.22^{de} (±0.31)
Pyriproxyfen	13.97^{d} (±0.15)	14.81^{ef} (±0.15)	18.90^{gh} (±0.17)	52.59^{ef} (±0.30)
Spinetoram & sulfoxaflor	73.02^{a} (±0.23)	85.48^{a} (±0.22)	93.50^{b} (±0.15)	100^{a} (±0.14)
Spirotetramat	9.02^{d} (±0.14)	11.54^{ef} (±0.15)	16.80^{gh} (±0.16)	44.83^{fg} (±0.27)
Tolfenpyrad	48.82^{b} (±0.30)	60.00^{c} (±0.27)	73.28^{a} (±0.29)	93.86^{bc} (±0.22)
Azoxystrobin	14.18^{d} (±0.20)	20.15^{e} (±0.21)	26.36^{fg} (±0.23)	63.72^{de} (±0.30)
Myclobutanil	9.63^{d} (±0.13)	9.77^{f} (±0.13)	17.97^{gh} (±0.17)	65.55^{d} (±0.26)
Water Check	9.68^{f} (±0.11)	9.76^{f} (±0.11)	12.40^{h} (±0.13)	38.39^{g} (±0.29)

Table 6. Mortality of *A. colemani* 48 hours following application of foliar-applied insecticides and fungicides. Mortality percentages with different superscript letters indicate significant differences (PROC FREQ, $P \leq 0.05$).

Active ingredient	Trade name	Group	Percent mortality at 48 h (±SE)	Mortality rating (IOBC)
Insecticides				
Acetamiprid	TriStar 8.5 SL	4A	86.21^{c} (±0.21)	Moderately Harmful
Azadirachtin	Azatin XL Plus	UN	66.07^{d} (±0.30)	Slightly Harmful
Cyantraniliprole	Mainspring	28	74.19^{d} (±0.23)	Slightly Harmful
Dinotefuran	Safari 20 SG	4A	96.52^{ab} (±0.22)	Moderately Harmful
Flonicamid	Aria	9C	75.00^{d} (±0.32)	Slightly Harmful
Permethrin	Astro	3A	100^{a} (±0.14)	Harmful
Pymetrozine	Endeavor	9B	65.22^{de} (±0.31)	Slightly Harmful
Pyriproxyfen	Fulcrum	7C	52.59^{ef} (±0.30)	Slightly Harmful
Spinetoram & Sulfoxaflor	Xxpire WG	5/4C	100^{a} (±0.14)	Harmful
Spirotetramat	Kontos	23	44.83^{fg} (±0.27)	Slightly Harmful
Tolfenpyrad	Hachi-Hachi	21A	93.86^{bc} (±0.22)	Moderately Harmful
Fungicides				
Azoxystrobin	Heritage	C3	63.72^{de} (±0.30)	Slightly Harmful
Myclobutanil	Eagle 20 EW	G1	65.55^{d} (±0.26)	Slightly Harmful
Water Check			38.39^{g} (±0.29)	Slightly Harmful

Table 7. Mortality of *M. persicae* 5 and 14 days after application of soil-applied insecticides (PROC UNIVARIATE, $P \leq 0.05$).

	5 d after treatment		14 d after treatment	
Insecticide	**Total aphids**	**Total mummies**	**Total aphids**	**Total mummies**
Cyantraniliprole	0	0	0	0
Dinotefuran				
Medium	0	0	0	0
High	0	0	0	0
Imidacloprid	0	0	0	0
Flupyradifurone	0	0	0	0
Medium	0	0	0	0
High	0	0	0	0
Spirotetramat				
Medium	2	0	0	0
High	2	0	0	0
Control				
Rep 1	186*	0	1000*	5
Rep 2	45*	0	250	1
Rep 3	401*	0	3000*	9

*significantly different from zero at $P<0.05$.

CHAPTER V

VARIETY TRIAL OF POACEAE SPECIES FOR BANKER PLANT POTENTIAL IN SOUTHWESTERN GREENHOUSES

Abstract

Banker plant systems for control of aphid pests in greenhouses incorporate cool-season cereal grains as banker plants. In the southwestern U.S. and areas where greenhouses experience high temperatures, banker plants may die more quickly requiring them to be replaced frequently. This can add to the labor involved in implementing banker plants for biological control. In this study, I assessed the potential of several types of warm-season grasses to support *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae) as hosts for *Aphidius colemani* (Viereck) (Hymenoptera: Braconidae) parasitoids. The purpose of this study is to ascertain if other plant species can be recommended as banker plants that do not require replacement every 7 to 14 days. In this experiment, I screened five warm-season grasses previously reported to sustain some level of bird cherry-oat aphids: corn, sorghum, spring wheat, switchgrass, and winter wheat. Each species was infested with *R. padi* then observed twice weekly for

population changes and overall plant health and longevity. I found that corn had the shortest longevity and supported few *R. padi*. Winter wheat and spring wheat also had a shorter lifespan, but supported several hundred *R. padi* aphids. Switchgrass grew tall and supported few *R. padi*. Sorghum lasted the longest and supported thousands of *R. padi* aphids after two months, making it an option for a banker plant.

Introduction

To best understand how to employ biological control using banker plants it is imperative to consider the effects occurring at the first trophic level (Parolin et al. 2010). The first trophic level includes traits in plant materials and can have various effects on pests and natural enemies, such as fecundity, egg load, fitness or efficacy (Prado and Frank 2013, Jandricic et al. 2014). In many banker plant systems for aphid control, cool-season grasses are used to provide food for a non-pest aphid as an alternate host for a parasitoid. These grasses typically include wheat, *Triticum aestivum* (L.), or barley, *Hordeum vulgare* (L.), and are fed on by bird cherry-oat aphids (*R. padi*) (Jandricic et al. 2014). A drawback to using these grasses in greenhouse operations is they have a short window of viability before succumbing to aphid pest pressure (Jacobson and Croft 1998), lasting approximately one to two weeks in warm temperatures. Because *R. padi* transmits barley yellow dwarf virus, even plants with some resistance to the virus may become chlorotic in as little as three or four days (Payton Miller, unpublished data.) In addition, humid greenhouse temperatures increase the incidence of fungal growth on these species (Bennison 1992). In essence, banker plants are effective because they allow a greater number of parasitoids to be reared on alternate aphid

hosts than with the pest aphid alone (Jandricic et al. 2014). Therefore, there is a need for a well-adapted, longer-lived banker plant species to provide *R. padi* to sustain parasitoids. Some grass species may allow for reproduction and survival of *R. padi* at various stages of growth (Kieckhefer 1984) and should be evaluated against wheat or barley, as they are the most effective options (Jandricic et al. 2014).

The purpose of this study was to evaluate other Poaceae species as banker plant options for use in greenhouses in the southwestern U.S. In this experiment, I screened six warm-season grasses for use with *R. padi*. The expectation was that switchgrass or spring wheat would be more robust and outlive winter wheat while supporting *R. padi*. The results from this study will be used to help growers keep banker plants longer in the greenhouse, reducing the cost of labor to implement the *R. padi-A. colemani* banker plant system.

Materials and Methods

Methods for this objective were adapted from Jandricic et al. (2014) and McClure and Frank (2015). The following Poaceae species were evaluated as possible alternatives for use as a greenhouse banker plant: dwarf deer corn (*Zea mays*) (L.), sorghum (*Sorghum bicolor*) (L.) Moench 'Martin Milo', spring wheat (*T. aestivum*) 'Glenn', winter wheat (*T. aestivum*) 'Jagger', and switchgrass (*Panicum virgatum*) (L.) 'Alamo' (Table 1). 'Jagger' winter wheat was used as the banker plant in my previous studies, as wheat is a recommended banker plant for the *R. padi-A. colemani* system (Jandricic et al. 2014), and *R. padi* aphids received from Oklahoma State University were reared on this variety. 'Jagger' winter wheat is a variety that has been bred for resistance against several diseases (Sears et al. 1997) and may be longer

lived in humid greenhouses than other varieties of winter wheat. Corn and sorghum varieties were selected based on dwarf plant characteristics to not impede overhead boom sprayers or other commercial greenhouse equipment. Seeds were sown in 15-cm black plastic pots (Table 1) containing Sun-Gro Horticulture (Agawam, MA) Metro-Mix® 902 professional growing media. Amount of seeds to sow per pot was determined by plant growth habit and size of seed. Seeds were fertilized after sowing with 5 g of 14-14-14 Classic Osmocote slow-release fertilizer (low to medium dose), placed in the greenhouse to germinate. Seeds were watered by automatic, overhead sprinklers at 5- to 12-minute intervals twice daily depending on season and greenhouse temperatures. Switchgrass seeds took longer to germinate and were started approximately one month prior to infesting with *R. padi*. Corn, sorghum, and spring and winter wheats were started approximately one week to ten days before being infested with *R. padi*. Once seedlings were at least 7 cm tall, 3 to 6 pots of each plant species were infested with 12 aphids then placed in 35.5 x 35.5 x 61 cm screen cages. Corn was infested at 30 cm as it grew rapidly within only one week of germination. Each species was kept in separate cages. Plants were watered by hand as needed. The number of *R. padi* on each species of grass was counted twice weekly, at three- and four- day intervals, for a total of 67 days. In addition, grass plants were observed for any changes in plant health as well as overall longevity in high temperatures. Because this study began in January 2018, summer conditions were replicated in the greenhouse by providing supplemental lighting from 5 p.m. to 7 p.m. and increasing temperatures to 32° C day and 21° C night. Onset® HOBO U23 Pro v2 External Temperature and Relative Humidity Data Loggers (Bourne, MA) with a solar shield to protect the sensors were placed next to the experiment in the greenhouse. The logger measured relative humidity (RH) and temperature at 60-minute intervals. The mean

number of aphids per plant species was calculated by each observation and an analysis of variance was computed using PROC MIXED (SAS 9.4 Software, Cary, NC) at $P \leq 0.05$.

Results and Discussion

In this study, corn was the shortest lived species (Table 2). Corn grew tall and supported few *R. padi* aphids, having the most aphids in the experiment immediately after infesting and close to zero aphids present on the plants by Day 19. Contrary to this experiment, corn was successfully used with *R. padi* as a banker plant for three months to control *A. gossypii* in England (Jacobson and Croft 1998). Variations in environmental conditions worldwide reaffirms the need for evaluation of banker plant species regionally.

Spring wheat and winter wheat were shorter in size and were also shorter lived than some other species, lasting approximately 33 days (Table 2). However, both types of wheat were able to support almost 200 *R. padi* aphids by Day 22, but declined rapidly afterward (Table 2, Fig. 1). At this point, both wheat varieties were chlorotic, lodging, and the number of live aphids had declined drastically. In addition, at Day 12 winter wheat began exhibiting fungal growth and spring wheat starting drooping.

Switchgrass steadily maintained a low level of *R. padi* for the longest period of time, approximately 67 days, before observations ended. However, this species was still in good health and showing no signs of damage. By the end of the experiment switchgrass grew to a mean height measuring over 91 cm (Table 2).

Sorghum maintained the greatest number of aphids, steadily increasing over the course of the experiment. However, this species did lag behind both types of wheat supporting only 85 aphids at Day 33 (Fig. 1). Still, the potential of sorghum as a banker plant is positive as this species lasted 67 days and exhibited little chlorosis, curling of leaves, or other health problems. However, more time may be required to get the plants to support a large number of aphids as sorghum took about two weeks to support almost 200 *R. padi*. In contrast, that many aphids were supported by Day 19 and 22 for winter wheat and spring wheat, respectively (Table 2). However, sorghum supported exponentially more aphids as time went on, long past both wheat varieties (Fig. 1).

Based on this experiment, corn is not a viable option as a banker plant in systems using *R. padi* as an alternate host. This is in contrast to a study where corn plants were used as a banker plant to successfully control *A. gossypii* and lasted over three months (Jacobson and Croft 1998). In addition, switchgrass may not support enough aphids to be an option for a banker plant, even though it maintained good health throughout the experiment. Winter or spring wheat is an option for about two weeks, supporting enough aphids to sustain *A. colemani*. However, these varieties would still need frequent replacement in warm greenhouses. Wheat also experiences fungal problems in humid conditions. These factors could make wheat a less desirable option for use as a banker plant as it would require more frequent replacement (Huang et al. 2011). Sorghum lasted about 2.5 times longer than both types of wheat and can potentially support eight times more *R. padi*. For sorghum to be used as a banker plant in the greenhouse, growers would need to start seeds one month prior to establishing them in the greenhouse. Based on the results of this experiment, sorghum seedlings should be infested with aphids once they reach a height of 2 to 3 cm. Subsequently,

A. colemani can be introduced once *R. padi* is established. Sorghum would make an attractive banker plant choice as plants would not need to be replaced as often as wheat, and, theoretically, one plant could support enough parasitoids to provide protection for thousands of square feet of growing space.

Future research concerning evaluation of species of banker plants for use with the *A. colemani*-*R. padi* system should include evaluating sorghum and possibly switchgrass for parasitism by *A. colemani*. In addition, banker plant traits at the first trophic level should be evaluated for effects on pest and alternate aphid hosts that could result in altered health of parasitoids (Prado and Frank 2013).

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Table 1. Species of Poaceae assessed for use with the *Rhopalosiphum padi-Aphidius colemani* system.

Species	Cultivar	Seed (g) per 15 cm pot	Size at infestation
Corn	Dwarf	0.77	30 cm
Sorghum	Martin Milo	1.40	7 cm
Spring Wheat	Glenn	5.00	8 cm
Switchgrass	Alamo	0.86	8 cm
Winter Wheat	Jagger	5.00	8 cm

Table 2. Mean number of *R. padi*, day of maximum aphid abundance, plant longevity, and maximum plant height for different species of Poaceae evaluated.

Species	***n***	**Mean number *R. padi* (± S.E.)**	**Maximum *R. padi* (days)**	**Plant longevity (days)**	**Mean height (cm)**
Corn	6	4.00 (±0.97)	4	19	76.2
Sorghum	3	1,711.33 (±299.15)	67	67	61.0
Spring Wheat	6	187.83 (±43.77)	22	33	25.4
Switchgrass	3	50.00 (±17.16)	55	67	91.4
Winter Wheat	6	194.00 (±10.39)	19	33	25.4

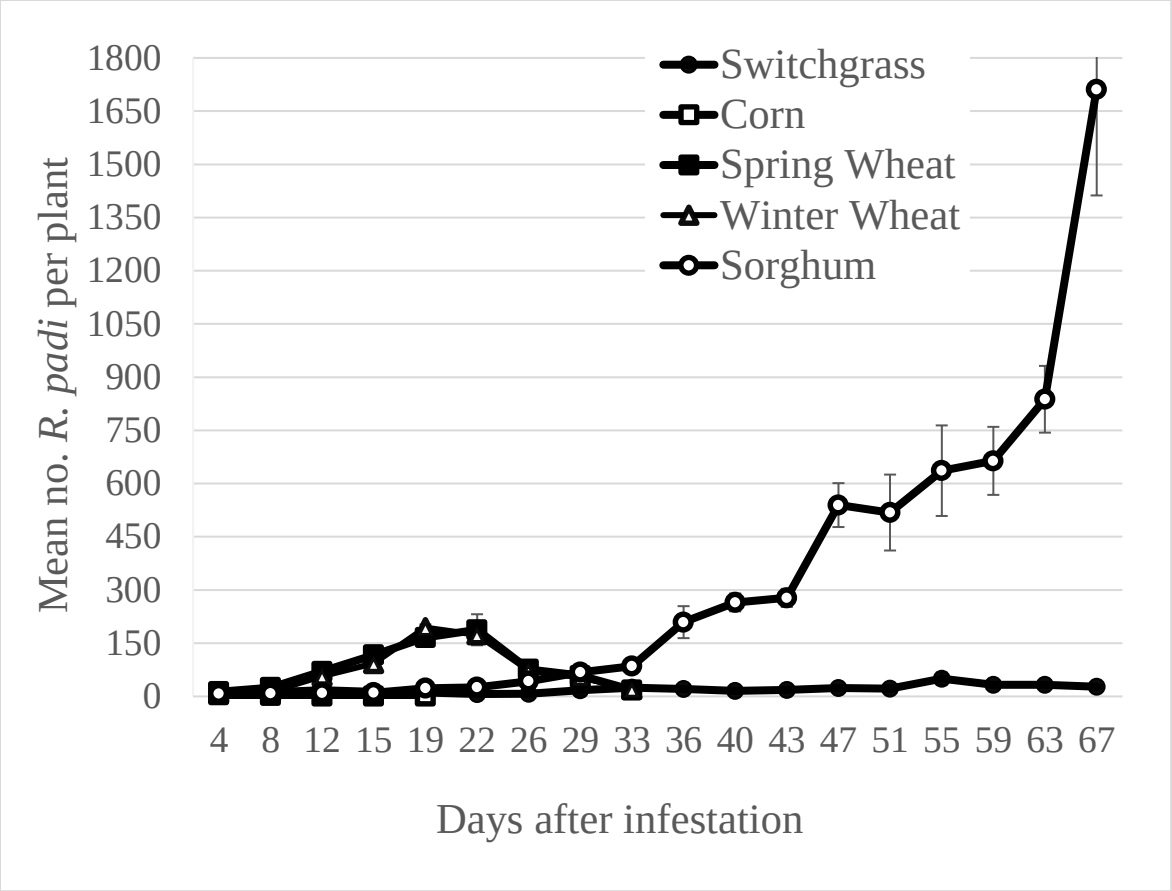

Figure 1: Mean number of *R. padi* measured on corn, spring wheat, sorghum, switchgrass, and winter wheat.

VITA

Tracey Lee Payton Miller

Candidate for the Degree of

Doctor of Philosophy

Thesis: EVALUATION OF THE *APHIDIUS COLEMANI-RHOPALOSIPHUM PADI* BANKER PLANT SYSTEM IN GREENHOUSE BIOLOGICAL CONTROL

Major Field: Entomology

Biographical:

Education:

Completed the requirements for the Doctor of Philosophy in Entomology at Oklahoma State University, Stillwater, Oklahoma in May 2018.

Completed the requirements for the Master of Science in Entomology at Oklahoma State University, Stillwater, Oklahoma in 2007.

Completed the requirements for the Bachelor of Science in Horticulture at Oklahoma State University, Stillwater, Oklahoma in 2004.

Experience: Commercial greenhouse production, insecticide toxicity studies, greenhouse experimental trials, plant variety trials, aphid rearing, biological control, organic insecticides, conventional insecticides, greenhouse pests, Aphididae, Braconidae.

Professional Memberships: Entomological Society of America, Nearctic Regional Section of the International Organization of Biological and Integrated Control, Oklahoma Nursery and Landscape Association, Southern Region American Society of Horticultural Science, Society of Southwestern Entomologists.