USE OF MULTIPLE NATURAL ENEMIES FOR INOCULATIVE BIOLOGICAL CONTROL OF *BEMISIA TABACI* IN GREENHOUSE POINSETTIA PRODUCTION

A Dissertation

by

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ABSTRACT

Two natural enemies, *Eretmocerus eremicus* Rose & Zolnerowich (Hymenoptera: Aphelinidae) and *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae), that differ in their feeding niches were selected to determine whether the combination of natural enemies provides superior suppression of sweetpotato whiteflies, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), on poinsettias [*Euphorbia pulcherrima* Willd. ex Klotz. (Malpighiales: Euphorbiaceae)] compared to either natural enemy species alone. I started by surveying initial *B. tabaci* densities on poinsettia cuttings received by growers and retailer thresholds of finished poinsettias over two years. Initial *B. tabaci* densities were 0.1 nymphs per cutting received by growers and up to an average of 73 *B. tabaci* nymphs per finished poinsettia at any given retailer.

In caged greenhouse experiments, I investigated *B. tabaci* suppression by the combination of *E. eremicus* and *A. swirskii* compared to each natural enemy alone. Ultimately, the combination treatment suppressed *B. tabaci* population growth similarly to either natural enemy alone. In a separate set of caged greenhouse experiments, I challenged natural enemy (single species or combination) suppression of *B. tabaci* by modifying the natural enemy release schedule (one-week delay at weeks 4 and 8) and simulating *B. tabaci* immigration (at weeks 4 or 8). The combination of *E. eremicus* and *A. swirskii* maintained superior suppression of *B. tabaci* compared to *E. eremicus* alone. All combination natural enemy treatments ultimately resulted in *B. tabaci* densities that were below retailer thresholds on finished poinsettias.

Lastly, I compared the use of a seasonal inoculative biological control program using *E. eremicus* and *A. swirskii* to manage *B. tabaci* compared to conventional insecticide use in commercial poinsettia production at three grower facilities in east Texas. At all grower locations, *B. tabaci* densities were consistently similar or higher than the conventionally managed greenhouse; however, final *B. tabaci* densities were below retailer acceptable densities in all treatments. The cost of inputs for the biological control program was lower ($0.057) or higher ($0.178) than frequently reported insecticide input costs for 15.2-cm potted poinsettias ($0.09). My dissertation demonstrates effective and potentially economic use of multiple natural enemies for *B. tabaci* suppression in commercial poinsettia production for the first time.

DEDICATION

I dedicate this dissertation to my parents, Shahab and Nasrin Vafaie, who have and continue to be sources of absolute love, generosity, and encouragement. I know specializing in insects wasn't the kind of 'doctor' typical Persian parents have in mind, but my parents are anything but typical.

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This work was supervised by a dissertation committee consisting of Professors Kevin M. Heinz [advisor], Micky D. Eubanks, and David Kerns of the Department of Entomology and Professors H. Brent Pemberton and Mengmeng Gu of the Department of Horticultural Sciences.

The whitefly counts at the growers for Chapter 2 was conducted in collaboration with Dr. Francine Soares and Briton Grove. The whitefly counts at the retailers for Chapter 2 was conducted in collaboration with Chris Knight and Lua Eijsink. The release of natural enemies in Chapter 3 were conducted in collaboration with Dr. Francine Soares. The release and quantifying emergence of natural enemies for Chapter 4 were conducted in collaboration with David Newburn and Christine Pawlik. Whitefly counts and natural enemy releases in Chapter 5 were conducted in collaboration with David Newburn and Christine Pawlik.

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NOMENCLATURE

No.	Number
SE	Standard Error
B. tabaci	*Bemisia tabaci*, sweetpotato whiteflies
E. eremicus	*Eretmocerus eremicus*
A. swirskii	*Amblyseius swirskii*
E. pulcherrima	*Euphorbia pulcherrima*

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1. INTRODUCTION

1.1. Poinsettias

Finished color poinsettias, *Euphorbia pulcherrima* Willd. ex Klotz. (Malpighiales: Euphorbiaceae), are a seasonal plant sold annually during Christmas in North America and Europe (Taylor et al. 2011) between November and December. The colorful bracts are an iconic symbol of several holidays that occur during that time. In 2018 alone, poinsettia wholesale value was $149 million of the $877 million potted flowering wholesale market (United States Department of Agriculture 2019) in the United States of America (USA). Poinsettias in the USA are grown from cuttings, which are increasingly sourced from stock plants grown in Mexico and Central America. Many poinsettia growers in the USA buy cuttings from propagators, rather than keep their own stock plants, because of improved economics, more suitable climate, and effective transport systems in Mexico and Central America (Ecke et al. 2004). Poinsettia cuttings are first received and stuck into material such as foam, rockwool, or peat moss between June – August (Hamrick 2003, Ecke et al. 2004). Cuttings are kept under low light levels and high humidity using misters until cuttings have rooted (7 – 14 days), at this time, light levels are gradually increased, humidity decreased, and they are ready to leave propagation (around 4 weeks) (Hamrick 2003, Ecke et al. 2004). After propagation, the rooted cuttings are potted into their marketable container size, spaced between 25 – 35.5 cm centers for 15.2-cm pots (Ecke et al. 2004). A combination of negative DIF (defined as the difference between daytime and night-time temperatures (Erwin et al. 1991)) and plant growth regulators are used to reduce stem elongation and poinsettias are pinched to provide the chassis for bract formation (Hamrick 2003, Ecke et al. 2004). Bract formation is controlled by day length (i.e. 12

hours uninterrupted night or longer), whereas bract number and quality are directly related to plant spacing, with tight spacing resulting in fewer bracts and tall straggly plants (Hamrick 2003, Ecke et al. 2004). Bract formation can be delayed when night temperature stays above 21°C for several nights (Hamrick 2003).

Aleyrodidae, commonly referred to as whiteflies, are a particularly problematic pest of poinsettias (Van Driesche et al. 2002, Ecke et al. 2004, Byrne et al. 2010). Below is a mini review focusing on integrated pest management strategies for one of the more frequent species of whitefly found on poinsettias in the USA, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), commonly known as the sweetpotato whitefly (McDonough et al. 1999, Van Driesche et al. 2002, Van Driesche and Lyon 2003, Vafaie, Pemberton, Gu, Kerns, et al. 2020c, 2020a).

1.2. Bemisia tabaci

Bemisia tabaci undergo four nymphal stages after emerging from an egg. The first stage, commonly referred to as crawlers, have legs for limited movement, and have greatly reduce legs and no movement after molting to second instar (Walker et al. 2010). Feeding continues through the second to fourth instar. During the latter part of the fourth instar, commonly referred to as the pupal stage, the nymph ceases feeding and metamorphoses to a winged adult (Walker et al. 2010). The entire life cycle from egg to adult of *B. tabaci* can take between 16.6 days and 65.1 days when held at a constant temperature of 30.0°C or 14.9°C, respectively (Butler et al. 1983). With total fecundity reaching an average of 263 eggs per whitefly female on poinsettias at 28°C (Enkegaard 1993), populations can quickly build if left unchecked.

Bemisia tabaci feed primarily on the highly soluble carbohydrates and free amino acids found in plant phloem (Pollard 1955, Crafts-Brandner 2002) from over 600 reported plant host species (Oliveira et al. 2001) which results in chlorotic spots caused by direct feeding damage. While feeding, *B. tabaci* excrete a sugary exudate called honeydew that can then serve as a substrate for the growth of a complex of dark-colored fungi commonly called sooty mold. These whitefly activities can cause leaf shedding and reduced plant growth rate (Pollard 1955). Even very low densities of *B. tabaci* on floral crops are considered unacceptable and can result in a stop-sale by state regulatory agencies or they can alter the aesthetic qualities of the crop to a level where they are considered unmarketable (Hoddle, Van Driesche, and Sanderson 1998); however, actual densities of *B. tabaci* found on poinsettias at retailers has yet to be quantified over multiple years and grower sources.

1.3. Scouting/Monitoring

The foundation of an effective integrated pest management program is systematic monitoring for the target pest. Monitoring *B. tabaci* is used to determine the timing of required management strategies to maintain marketable plants. *Bemisia tabaci* populations are highly aggregated within and between plants (Liu et al. 1993a) and have migratory forms that are unique in wing-shape, response to external cues, and distance of flight, with some individuals dispersing further than 5 km in the field (Byrne 1999). Due to the high aggregatory and dispersal behavior, and low retailer tolerance for *B. tabaci*, monitoring programs may greatly benefit from traps or lures for early detection of *B. tabaci* presence. To date, there are no effective pheromone traps commercially available for monitoring *B.*

tabaci; however, yellow sticky traps have been evaluated for usefulness in determining presence and abundance of *B. tabaci*.

Yellow stick traps to monitor *B. tabaci* has been standard practice for several decades (Berlinger 1980, Ohnesorge and Rapp 1986, Moerkens et al. 2019). Despite their common use, yellow sticky traps are not good indicators of *B. tabaci* densities in poinsettias (Pinto-Zevallos and Vänninen 2013b). This could be at least partially attributed to the relative attractiveness of the yellow visual cue compared to the surrounding commodities (Berlinger 1980) and environmental factors such as temperature and light intensity which can impact *B. tabaci* flight tendency (Pinto-Zevallos and Vänninen 2013b). Using yellow sticky traps as early indicators of *B. tabaci* populations can be increased with modifications, such as a small yellow circle on a black background (Kim and Lim 2011) or by adding two light emitting diodes (one on each side of the trap) (Chen et al. 2004, Chu et al. 2004); however, these augmentations are not yet widely commercially available. There's also a great lack of studies determining the optimal density of yellow sticky traps in different commodities for *B. tabaci*; with recommendations varying between one trap per 93 to 500 m^2 (McDonough et al. 1999, Pinto-Zevallos and Vänninen 2013b). In addition to trap density, trap height above the plant canopy can impact trap effectiveness, with 5 cm above the canopy height being optimal for trapping *B. tabaci* (Liu et al. 1994).

Directly monitoring poinsettias is still considered the most accurate method to determine *B. tabaci* densities in poinsettias. *Bemisia tabaci* are considered highly aggregated both within and between poinsettias (Liu et al. 1993a, 1993b, Burns et al. 1999). Despite *B. tabaci* having a clear vertical distribution throughout a given plant, with eggs and young nymphs on younger (frequently upper) leaves and pupae and exuviae on

older (frequently lower) leaves, counts of *B. tabaci* nymphs and pupae from leaves in the middle of the poinsettia canopy are considered reasonable predictors of whitefly immatures on the whole plant (Liu et al. 1993a). Several studies have tried to estimate *B. tabaci* densities on several commodities using various sampling techniques (Liu et al. 1993a, 1993b, Diehl et al. 1994, Tonhasca et al. 1994, Naranjo et al. 1996, Burns et al. 1999, Spinner et al. 2011, Lima et al. 2017); however, additional research to investigate reliable and practical sampling techniques for estimating *B. tabaci* in commercial poinsettia production is needed.

1.4. Cultural Control

Two cultural control strategies for *B. tabaci* management in poinsettias include host plant resistance and manipulation of fertilizer rates. Host plant resistance has been defined as "the collective heritable characteristics by which a plant species, race, clone, or individual may reduce the probability of successful utilization of that plant as a host by an insect species, race, biotype, or individual" (Beck 1964). On resistant plants, the pest population never reaches an economic injury level before the end of the growth season (Berlinger 1986). Variation in suitability to *B. tabaci* has been demonstrated in tomatoes (Heinz and Zalomi 1995) and poinsettias (Heinz and Parrella 1994b), with a lower oviposition rate on low trichome density commercial tomatoes compared to high trichome density, and increased efficacy of natural enemies in the lower trichome density poinsettia cultivar "Annette Hegg Brilliant Diamond". Keeping trichome densities constant, *B. tabaci* adults preferred to feed on cultivars with thin and light green leaves compared to dark and thick green leaf cultivars (Medina-Ortega 2011). Although breeding and growth of resistant

cultivars could provide one of the simplest and most convenient methods of insect pest control (Dent 2000), the marketplace may give higher priority to varieties with increased aesthetic and marketable qualities, such as size and number of bracts, color, and resilience to mechanical manipulation over *B. tabaci* resistance.

Recent genetic engineering methods may preserve desirable marketable characteristics while introducing pest resistance genes. For example, poinsettias transformed with the tryptophan decarboxylase (TDC) gene had a measurable increase in tryptamine and resistance to *Botrytis cinerea* Persoon ex Fries (Helotiales: Sclerotiniaceae) in leaf disk assays (Sanford et al. 1999). Although similar techniques can be theoretically used to increase resistance to *B. tabaci* (Islam et al. 2014), current research falls short of successful propagation of transformed poinsettias (Perera 2009). Increased host-plant resistance through transgenics is a promising strategy for integration of integrated pest management that may see further development in the future (Suhag et al. 2020).

Increasing fertilization results in higher protein-nitrogen content in leaves (Bentz et al. 1995). Nitrogen is regarded as a key factor for performance of phloem-feeding insects, since dietary nitrogen in phloem is often a limiting factor to population growth (Dixon 1969, Mattson 1980, Weibull 1987, Medina-Ortega 2011). Increasing nitrogen fertilizer inputs result in increased *B. tabaci* population growth in several different crops (Bi et al. 2001, 2003, Idris et al. 2015, Islam et al. 2017, "Effect of Nitrogen Rates on the Whitefly (Bemisia tabaci) Population Infesting Chilli (Capsicum annum L.) | Request PDF" 2020). More specifically on poinsettias, leaf protein-nitrogen content correlated with number of *B. tabaci* found on poinsettias, number of eggs laid, number of crawlers produced from eggs, total leaf area, and dry weight of leaves (Bentz et al. 1995). Western flower thrips,

Frankliniella occidentalis Pergande (Thysanoptera: Thripidae), populations were reduced without any decrease in flowering or plant growth when fertilization rates were reduced by 50% in chrysanthemums (Chau and Heinz 2006) and 33% reduction in roses (Chow et al. 2012). Although similar work has not been conducted for *B. tabaci* on poinsettias, use of some types of sulfur-containing fertilizers in vegetables (Simmons and Abd-Rabou 2009) and fertilizers derived from oilseed extract of soybeans in poinsettias (England et al. 2011) resulted in reduced *B. tabaci* population growth compared to non-fertilized controls and conventional fertilizers.

1.5. Physical Barriers/Netting

Mechanical/physical control of *B. tabaci* includes insect screening and blocking of UV light. Insect pests can be excluded from an enclosed growing space using insect screening. Width of the insect thorax is a general criterion used to determine mesh size of insect netting, with the commonly accepted net opening size being 239 μm for *B. tabaci* (Bethke and Paine 1991a). Netting with openings of 200 by 700 μm have demonstrated ability to exclude *B. tabaci* effectively, but allow free entry of *Eretmocerus mundus* (Mercet) (Hymenoptera: Aphelinidae), a parasitic wasp of *B. tabaci* (Hanafi et al. 2007). However, influx of whiteflies increases in greenhouses with active ventilation (i.e. negative air pressure), which then requires smaller netting hole sizes, which decreases ventilation efficiency and increases fan power requirements (Berlinger et al. 2002). In warmer climates with high humidity any reduction in air movement (a frequent and unwanted consequence of screening vents) can result in high-humidity related plant pathogen problems (Walker et al. 2010). As an alternative to using fine mesh screening, Martin et al.

2014 demonstrated that treating netting with the active ingredient alpha-cypermethrin with larger pore size (900 μm pore diameter) was equivalent in excluding *B. tabaci* as non-treated small pore size net (400 μm pore diameter). For widespread adoption of treated-nets for greenhouse exclusion of *B. tabaci*, insecticides would need this specific purpose registered on the label or insecticide-impregnated nets would need to be on the market for this purpose. Additionally, the impact of retrofitting a 900 μm pore diameter net on the active ventilation system of existing greenhouses needs to be investigated, especially in hot and humid environments. When the regulatory and airflow challenges are overcome, netting can increase the success rate of inoculative biological control in poinsettia production by reducing high influxes of whiteflies.

Ultraviolet absorbing materials can decrease influxes of insect pests such as aphids, thrips, and whiteflies (Antignus et al. 1996, 1998, 2001, Costa and Robb 1999, Mutwiwa et al. 2005, Díaz et al. 2006, Mahmood et al. 2018). In two-choice experiments, approximately 90% of released *B. tabaci* (~500 – 600 whiteflies) moved into the UV transmitting plastic tunnels (0.5 m high x 0.5 m tall x 4.1 m long) compared to the UV absorbing plastic tunnels, both planted with *Brassica* sp. (Costa and Robb 1999). The same proportion of greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), were found to prefer UV transmitting plastic tunnels that had yellow sticky cards (no crop) compared to UV absorbing plastic tunnels (Mutwiwa et al. 2005). Tri-trophic consequences of UV blockage should be considered, as different species of plants react differently when exposed or protected from UV, which can in turn impact pest pressure and predator preference (Foggo et al. 2007). Dispersal of *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae), a parasitic wasp of *T. vaporariorum* and *B. tabaci*,

is unaffected when UV is blocked (Doukas and Payne 2007), making UV-absorbent materials a promising strategy to use with releases of natural enemies. Ultraviolet-absorbent materials should be considered in regions where influx of whiteflies results in the need for several curative spray applications. In such a case, UV absorbent materials could work to reduce the need for sprays and continue with releases of natural enemies.

1.6. Insecticidal Control

1.6.1. Dipping

Poinsettia cuttings coming from propagators have been considered an important source of whitefly populations (Buitenhuis et al. 2016). Dipping cuttings, also known as "immersion treatment", has been suggested as a preventative treatment to reduce the introduction of infested plant materials and to start with low populations of whiteflies, since management of *B. tabaci* is challenging if they establish early in the season. Dipping poinsettia cuttings in insecticidal soap (0.5%) + *Beauveria bassiana* (1.25g/L; BotaniGard® WP; BioWorks Inc., Victor, NY) or mineral oil (0.1% v/v) before potting can result in a 70% decrease in *B. tabaci* populations by week 8 of the crop and found no lethal effects on commonly used natural enemies of *B. tabaci* (Brownbridge et al. 2014, Buitenhuis et al. 2016). Dips of poinsettia cuttings in thiamethoxam in a production facility (15,000 poinsettias total) decreased *B. tabaci* populations to 0.02 whiteflies/plant twenty-three days after treatment compared to 0.33 whiteflies/plant in the untreated control (Krauter et al. 2017).

1.6.2. Foliar and Drench Applications

Long-term management of whiteflies has historically been challenging to achieve without frequent and regular application of insecticides (Sharaf 1986). Pesticide availability is reliable, provides relatively rapid pest suppression, and is often still considered the most economic management strategy compared to the use of biological control exclusively (Bethke and Cloyd 2009). The introduction of newer chemistries, such as insect growth regulators (i.e. buprofezin, pyriproxyfen, azadirachtin) and neonicotinoids (i.e. imidacloprid, dinotefuran, thiamethoxam) greatly improved the abilities of growers to manage their insecticide applications and associated control of whiteflies (Horowitz and Ishaaya 1996, Palumbo et al. 2001, Elbert et al. 2008).. Several insecticide rotations have been tested and demonstrated the ability to provide 95% suppression of *B. tabaci* (MEAM1 and MED mixed populations) compared to an untreated control (Mckenzie et al. 2014); however, the vast majority of the highly effective rotations (9 out of 11 rotations providing at least 95% suppression of *B. tabaci*) belong to the class of insecticides known as neonicotinoids. Excellent efficacy of neonicotinoids, systemic activity, and long-lasting control that they provide has made neonicotinoids very popular in crop protection (Elbert et al. 2008). Recent changes in retailer insecticide requirements, environmental concerns, and unintended side-effects of neonicotinoids have drastically reduced effective insecticide options.

Although neonicotinoids are generally considered more targeted toward pests in their chemistry and application method and less toxic to mammals (Grafton-Cardwell et al. 2008), neonicotinoids have demonstrated varying negative impacts on non-target organisms, such as pollinators (Blacquière et al. 2012, Goulson 2013, Godfray et al. 2014,

Frank and Tooker 2020, Li et al. 2020) and natural enemies of *B. tabaci* (Fytrou et al. 2017, Drobnjakovic et al. 2019), and can cause increases in twospotted spider mite populations, *Tetranychus urticae* Koch (Acari: Tetranychidae) by altering plant defenses in cotton (*Gossypium hirsutum*), corn (*Zea mays*), and tomato (*Solanum lycopersicum*) (Szczepaniec et al. 2013). Several online petitions and news articles have called for the ban of neonicotinoids, resulting in major suppliers (i.e. Lowe's and Home Depot) calling for mandatory labeling of neonicotinoid-treated plants and phasing out of neonicotinoids pesticides as alternatives become commercially available (Lowe's 2014). In addition to social pressure to ban one of the most effective classes of insecticides for *B. tabaci* management on poinsettia, a biotype first detected in the USA in 2004, B. tabaci MED, has become an increasing concern due to its propensity to develop resistance to insect growth regulators and neonicotinoid insecticides (McKenzie et al. 2009, Dennehy et al. 2010, Xiao et al. 2012). Lastly, increasingly stringent regulations required by the Environmental Protection Agency (United States Environmental Protection Agency 2017) and enforced by state agricultural agencies for licensed pesticide applicators may call into question the sustainability of current insecticidal management programs for *B. tabaci* on poinsettias.

1.7. Biological Control

Seasonal Inoculation biological control has become a viable option for management of whiteflies in poinsettia production as an alternate to insecticidal control. Seasonal inoculative biological control is the periodical release of mass-reared natural enemies into short-term crops (6 – 12 months) (van Lenteren and Bueno 2003). Poinsettias are considered good candidates for biological control programs, because they are often grown

in monoculture (Ecke et al. 2004), are grown for several months of the year (Hamrick 2003), and have very few key arthropod pests, namely whiteflies, fungus gnats (Diptera: Sciaridae), and spider mites (Trombidiformes: Tetranychidae) (Ecke et al. 2004).

Seasonal inoculative biological control through use of beneficial insects in greenhouses has become an increasingly viable option for *B. tabaci* on poinsettias. Gerling et al. (2001) provide a comprehensive list of natural enemies of *B. tabaci*, but published research on natural enemies that have been tested on *B. tabaci* on poinsettias are limited to: *Delphastus pusillus* (Osborne and Landa) (Coleoptera: Coccinellidae) (Heinz and Parrella 1994a, 1994b), *Eretmocerus mundus* (Qiu et al. 2004, Ardeh et al. 2005), *E. eremicus* Rose & Zolnerowich (Hymenoptera: Aphelinidae) (Hoddle, Van Driesche, Sanderson, et al. 1998, Hoddle and Driesche 1999, Van Driesche et al. 1999), *Encarsia luteola* (Howard) (Hymenoptera: Aphelinidae) (Heinz and Parrella 1994a, 1994b), *En. transvena* (Timberlake) (Hymenoptera: Aphelinidae) (Heinz and Parrella 1994b), *En. pergandiella* (Howard) (Hymenoptera: Aphelinidae) (Heinz and Parrella 1994b), and *En. formosa* (Heinz and Parrella 1994b, Hoddle and Van Driesche 1996, 1999a). Despite the diversity of natural enemies studied for suppression of *B. tabaci* in lab culture, only two commercially available parasitic wasps of *B. tabaci* have been studied in poinsettia production in the U.S.A.: *En. formosa* and *E. eremicus* (Table 1.1).

Encarsia formosa is a thelytokous endoparasitoid, producing only female offspring, whereas commercially available *E. eremicus* is arrhenotokous (Javad et al. 2005) and vary in male:female sex ratio in the literature, from 1:1 (Simmons and Minkenberg, Oscar 1994, Van Driesche et al. 1999a, Soler and van Lenteren 2004) to 1:1.69 (male:female) (Bellamy and Byrne 2001) from commercial insectaries that mass produce them. In Petri dish

studies, *E. eremicus* demonstrated a clear preference to probe second instar and host feed on first instar *B. tabaci* on sweet potato (Headrick et al. 1995). In a 24-hr assay comparison between *E. eremicus* and *En. formosa* (Beltsville strain) on managing *B. tabaci* on poinsettia stock plants in greenhouses, *E. eremicus* found *B. tabaci* patches faster, "was observed on a higher percentage of patches, killed more nymphs on greater number of plants, and was observed foraging on patches in higher numbers than *E. formosa* Beltsville strain" (Hoddle, Van Driesche, Elkinton, et al. 1998, Hoddle and Van Driesche 1999a). On colored poinsettias, *E. eremicus* at a release rate of 2.9 – 3.7 females per plant per week were able to maintain *B. tabaci* below grower acceptable limits of 0.66 - 1.49 live *B. tabaci* nymphs and pupae per leaf, whereas *E. formosa*, released at a rate of 1.9 – 2.4 females per plant per week were unable to maintain *B. tabaci* below economic threshold levels (Hoddle and Van Driesche 1999a). Weekly releases of *En. formosa* at a rate of 4 – 7 females per plant per week were also considered unable to manage *B. tabaci* alone (Hoddle and Van Driesche 1996), suggesting that *E. formosa* is a less effective natural enemy against *B. tabaci* compared to *E. eremicus*. The use of *E. eremicus* at the above levels was effective, but is hard to justify when it costs 27 – 30 times more than conventional insecticide use (Table 1.1) (Hoddle and Van Driesche 1999a, Van Driesche et al. 1999a). However in the face of highly resistant pest populations and loss or restricted use of effective pesticides, adoption of biological control can increase rapidly (Parrella et al. 1992, Murphy et al. 2011).

In open-fields, *E. eremicus* failed to manage *B. tabaci* on cucumber due to dispersion at low densities or influxes of immigrating whiteflies (Bellamy et al. 2004).

Table 1.1. Summary of research investigating augmentative biological control of *B. tabaci* in poinsettia production.

Study	GH size m^2 (No. poinsettias)	Location (U.S.A.)	Natural Enemies	Input Cost ($ USD)		
				Conventional	Biological	Conv:Bio. ratio
(Van Driesche and Lyon 2003)	2219 (15408)	Massachusetts	*E. eremicus*	0.14 / plant	0.1 - 0.14 / plant	0.71
(Van Driesche et al. 2002)	123 (2340)	Massachusetts	*E. eremicus*	0.14 / plant	0.27 / plant	2
(Van Driesche, Hoddle, Lyon, and Sanderson 2001)	275 (1250)	Massachusetts	*E. eremicus*	0.14 / plant	0.38 - 1.18 / plant	2. 7 - 8.4
(Stevens et al. 2000)	856 (3152)	New England	E. formosa	1.54 / m^2 or 0.27 / plant	7.33 / m^2 or 1.27 / plant	3
(Hoddle and Van Driesche 1999a)	307 (3200)	Massachusetts	*E. eremicus* or *E. formosa*	1.11 / m^2 or 0.09 / plant	15.19 / m^2 or 2.70 / plant	3
(Van Driesche et al. 1999a)	420 (3500)	Massachusetts	*E. eremicus*	0.08 / plant	2.14 / plant	27
(Hoddle and van Driesche 1999)	419 (2500)	Massachusetts	*E.eremicus and E. formosa*	0.09 / plant	2.78 / plant	44
(Hoddle, Van Driesche, Elkinton, et al. 1998)	419 (2240)	Massachusetts	*E. eremicus* or E. formosa	N/A	N/A	N/A
(Hoddle et al. 1999)	20 (90)	New York	*E. eremicus*	N/A	N/A	N/A
(Hoddle et al. 1997a)	20 (90)	Massachusetts	*E. formosa*	N/A	N/A	N/A
(Hoddle and Van Driesche 1996)	170 (576)	Massachusetts	*E. formosa*	0.09 / plant	1.02 / plant	9.5

Maximum greenhouse (GH) size and number (No.) of poinsettias per experimental unit, location of trial, natural enemies used, and input costs (if calculated) of conventional insecticidal control, biological control, and ratio for cost of conventional:biological control.

Failure to perform in open fields can be problematic for *E. eremicus* in regions with warmer climate, where greenhouses are often open and allow for influxes of whiteflies. The lack of a prolonged cold season and continuous production of whitefly susceptible host plants in warmer climates also makes biological control more challenging (Lindquist and Short 2004) and results in decreased reliability of control. For biological control of *B. tabaci* on poinsettias to be taken seriously in warmer climates, the challenge of high whitefly starting populations and whitefly influx throughout the growing season will need to be resolved.

Whether introducing a complex of species increases the efficacy and reliability of biological control compared to the release of a single species has been considered a controversial topic (Myers et al. 1989). Where intraguild predation exists between the released biological control agents, models consistently predict a disruption in biological control (Rosenheim et al. 1995); however, empirical evidence has demonstrated that competition between natural enemies can still result in successful suppression of prey species (Heinz and Nelson 1996, Bográn et al. 2002). Out of 25 projects that had successful pest control using multiple natural enemy species, a single species was responsible for the successful control in 14 of the projects, whereas 2 – 4 species were responsible for successful control of the remaining 11 (Denoth et al. 2002). Bográn et al. (2002) found that three parasitoid species that compete for the same species and even life stages (2nd and 3rd instar *B. tabaci* on cotton) altered behavior to reduce competition when released together and as a result, found competitive interactions among parasitoids did not affect host population suppression. These studies suggest that the controversy is less about the number of natural enemy species released, but rather getting the right agent (Myers et

al. 1989); a perspective that has been reiterated by Heinz and Nelson (1996) when they suggested that species composition is of greater consequence than the number of natural enemy species.

Amblyseius swirskii controls several major ornamental and vegetable pests, including the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), whiteflies, *B. tabaci* and *T. vaporariorum*, and the broad mite, *Polyphagotarsonemus latus* (Banks) (Trombidiformes: Tarsonemidae) (Calvo et al. 2015). *Amblyseius swirskii* was originally described from the East Mediterranean coast, in Bet Dagan, Israel (Athias-Henriot 1962) and was first discovered as a predator while investigating the cause of continuous disappearance of *B. tabaci* eggs and larvae in a lab culture (Teich 1966). On cucumber leaf discs, *A. swirskii* fed primarily on eggs and 1st instar *B. tabaci* (Nomikou et al. 2004). The warmer climate of *A. swirskii*'s original habitat makes it an efficacious predator on *B. tabaci* in warmer climates, such as summers in the Netherland's sweet pepper glasshouse production (Bolckmans et al. 2005), where maximum daily temperatures were between 28 – 30ºC, with peaks up to 40ºC, and greenhouse cucumber production in Spain (Calvo et al. 2011), where maximum and minimum temperatures were 38.2ºC and 21.6ºC, respectively. *Amblyseuis swirskii* is able to survive on pollen (Goleva and Zebitz 2013) and remain on the crop throughout the growing season (sweet peppers) even at low *B. tabaci* densities (Bolckmans et al. 2005). Cattail pollen is the commercially available pollen for *A. swirskii*, because it is a good dietary resource and easy to collect in large quantities. Cattail pollen is spread at a rate that is virtually unnoticeable on the plant to the naked eye. These characteristics make *A. swirskii* a promising agent for control of *B. tabaci* in poinsettias in warmer climates.

Despite *A. swirskii*'s ability to suppress *B. tabaci* in some cases (Gerling et al. 2001, Nomikou et al. 2002, Berndt et al. 2007, Calvo et al. 2011, 2012, Buitenhuis et al. 2015), the ability of this predator to disperse within a greenhouse is limited when plant canopies are not interconnected (Buitenhuis et al. 2010). Release of *A. swirskii* may be best suited for early release in poinsettias, when poinsettia leaves are touching, to reduce starting *B. tabaci* populations and establish in the crop before poinsettias are spaced out. Early introduction of *A. swirskii*, however, can be unsuccessful if cuttings have residues from pesticides sprayed on stock plants (Murphy et al. 2008). By comparison, female *E. eremicus* can disperse distances over 10 meters in the field and can find patches of whiteflies in a greenhouse, making them suitable for release when poinsettias are spaced out.

Biological control of *B. tabaci* on poinsettia Christmas crop by inoculative releases of *E. eremicus* can be an effective pest management tool (Hoddle and Van Driesche 1999a). However, the advantages of biological control using *E. eremicus* can be questioned by practitioners due to higher cost (Hoddle and Van Driesche 1999a, Van Driesche et al. 1999a, Stevens et al. 2000), when starting with infested material or experiencing unexpected influxes of whiteflies in open systems (Bellamy et al. 2004), when growers have to spray to control secondary pests or pathogens not controlled by *E. eremicus* alone, or if there is an unexpected alteration in the release of parasitoids due to a missed shipment or quality control issues when relying on a single natural enemy. In such cases, it may be beneficial to not have a biological control program based on the release of only *E. eremicus*, but rather the addition of a second natural enemy, *A. swirskii*.

1.8. Objectives

The purpose of this dissertation is to investigate whether the combination of *E. eremicus* and *A. swirskii* could be effective together in a seasonal inoculative biological control program to manage *B. tabaci* on poinsettias. More specifically, the main objectives are:

i. Determine *B. tabaci* densities on poinsettia cuttings received by growers and finished poinsettias at different retailers.

ii. Determine whether the combination of *E. eremicus* and *A. swirskii* can provide equal or superior suppression of *B. tabaci* compared to either natural enemy alone.

iii. Determine whether the combination of *E. eremicus* and *A. swirskii* can maintain *B. tabaci* suppression despite *B. tabaci* immigration and delays in natural enemy releases.

iv. Determine the effectiveness of a *B. tabaci* management program in commercial poinsettia production based on the release of *E. eremicus* and *A. swirskii* compared to conventional insecticidal control.

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2. WHITEFLY ABUNDANCE ON ROOTED POINSETTIA CUTTINGS AND FINISHED POINSETTIAS[1]

2.1. Overview

In this study, we surveyed the initial whitefly (Aleyrodidae) populations on rooted poinsettia [*Euphorbia pulcherrima* Willd. ex Klotz. (Malpighiales: Euphorbiaceae)] cuttings at two commercial greenhouse facilities in both 2016 and 2018 to determine the initial whitefly population at the beginning of poinsettia production and surveyed finished poinsettias at multiple retailers in Tyler, TX over 2 years to determine whitefly densities considered acceptable by the retailers. The initial whitefly population (mean ± SE) for all poinsettias was 0.02 ± 0.02 (2017) and 0.33 ± 0.13 (2018) nymphs per plant for producer facility A and 0.05 ± 0.05 (2017) and 0.02 ± 0.01 (2018) nymphs per plant for producer facility B. Out of the total 2417 rooted poinsettia cuttings inspected at both locations over 2 years, 29 cuttings had whitefly nymphs (1.2%), 18 had pupae (0.7%), and 23 had exuviae (1.0%). On finished poinsettias sampled at retailers, 4.38 to 40.38 immatures (nymphs + pupae) per plant were found within 60 s for any given retailer over the 2 years. We found poinsettias with as many as 220 immatures and 32 adults on a single plant at retailers. This study is the first to quantify densities of whiteflies at retail stores over multiple years.

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2.2. Introduction

Pest management decisions in an integrated pest management strategy rely on pest thresholds; however, thresholds have been poorly defined or investigated in greenhouse ornamental production, often resulting in prophylactic use of insecticides. For example, management of whiteflies on poinsettias has historically relied on regular applications of insecticides (Palumbo et al., 2001; Sharaf, 1986; Stevens et al., 2000), with some growers applying insecticides every 3 – 5 d (Hoddle and Van Driesche, 1996). However, relying on regular insecticide applications as a pest management strategy may be short-sighted due to risk of insecticide resistance (Palumbo et al., 2001; Schuster et al., 2010; van Lenteren, 2012), increasingly tighter federal and state pesticide regulations (U.S. Environmental Protection Agency, 2017), and increasing pressure from retailers against the use of specific insecticide classes by commercial growers (Friends of the Earth, 2017). Augmentative biological control, i.e., the regular release of natural enemies to reduce the target pest population to acceptable levels, is a promising strategy that has been increasingly adopted in many areas of the world, including parts of Europe, Asia and Latin America (Barratt et al., 2018). However, information related to starting pest densities at the grower and acceptable pest densities by the retailer needed for development of an augmentative biological control strategy in ornamental production in the United States is lacking.

Successful management of whiteflies (Hemiptera: Aleyrodidae) in an augmentative biological control program for poinsettias requires favorable conditions: lack of insecticide residue, few or limited pest species, and low starting whitefly densities (Van Driesche et al., 1999). Suppression of whiteflies has been considered unsuccessful using parasitic wasps, such as *Eretmocerus eremicus* Rose & Zolnerowich (Hymentopera: Aphelinidae),

when initial whitefly densities were greater than 1.0 whiteflies (all life stages) per poinsettia cutting (Van Driesche et al., 1999). Propagative plant materials have been suggested as a major source of whitefly populations in poinsettia production (Buitenhuis et al., 2016); however, there is limited published data to support this assertion. Dipping cuttings, or "immersion", in a pesticide mixture has been suggested as a method to start "clean" as a pre-requisite for a successful biological control program (Brownbridge et al., 2014; Buitenhuis et al., 2016; Krauter et al., 2017). More surveys of poinsettia cuttings from propagators will aid in determining if the cost of pre-emptive insecticide treatments of poinsettia propagative materials are justified.

Poinsettias at retailers are not likely completely free of pests, but the acceptable density of whiteflies at retailers has not be determine. The economic threshold on ornamentals has been generally defined as "low" (Stevens et al., 2000) or "essentially zero" (Bethke and Cloyd, 2009) because any pest injury is considered unacceptable. Documented final densities for whiteflies on poinsettias has been limited to retailers in Massachusetts (Hoddle and Van Driesche, 1996; Van Driesche et al., 1999). Implementation of augmentative biological control in poinsettia production requires a better understanding of current accepted whitefly densities at the retailers. In this study, we determine the starting infestation levels of whiteflies on rooted poinsettia cuttings at grower facilities and determine whitefly densities on finished poinsettias at the retailer.

2.3. Materials and methods

2.3.1. Whiteflies density on cuttings

To determine whether the initial population of whiteflies was low enough for biological control (1.0 or fewer live nymphs and pupae per cutting) (Van Driesche et al., 1999), we determined whiteflies densities at two grower locations in 2017 and 2018 within 50 miles of the Texas A&M AgriLife Research and Extension Center in Overton, TX. Two hundred newly rooted poinsettia cuttings (4 – 6 weeks post arrival from various propagators) were randomly selected for inspection during each visit at each grower for 3 consecutive weeks during the months of July and August. For each poinsettia cutting inspected, whitefly nymphs, pupae, and exuviae were counted using a 2.5x magnification head lens. In total, at least 2400 cuttings were inspected over the 2 years. Cuttings were sourced from propagators in Central and South America, which also supply cuttings to other parts of the United States and Canada. Cultivar names of each cutting were also recorded to determine any potential cultivar differences in initial whitefly densities. The two grower facilities are labelled “A” and “B” to maintain anonymity.

2.3.2. Whitefly density at retailers

Whitefly numbers on poinsettia plants sampled at retail stores were defined as commercially acceptable pest densities. We assumed that poinsettias on retailer shelves were considered acceptable by the retailer for sale, whereas unacceptable poinsettias would have been returned to the supplier or culled. Acceptable retailer whitefly densities were determined by scouting poinsettia crops at 10 different retailers in 2016 (8 Dec.) and seven

retailers in 2018 (13 – 14 Dec.) in Tyler, TX. Number of immatures (nymphs + pupae), exuviae, and whitefly adults were counted during 60 s per plant, between 10 – 30 plants per retailer, depending on availability. The source of the poinsettia (2018 only), pot size, price, bract color, and aesthetic rating was also recorded to tabulate any potential trends with whitefly density. Aesthetic rating was recorded by looking at the whole plant from a scale of 0 (whiteflies easily seen, occurrence of honeydew, development of sooty mold, plant stretched, canopy thinning, and yellowing leaves) to 10 (unable to detect whiteflies, no honeydew, no sooty mold present, compact plant, no thinning or yellowing leaves). We considered a rating of 7 or below to be a threshold where marketability is greatly reduced. To maintain anonymity of the sources of infested poinsettias and determine potential differences in acceptable whitefly densities based on clientele, retailers were categorized under one of four groups: big-box stores (physically large multinational establishments), independent garden centers, grocery store florists, and independent florists. Due to pricing and specialization, we anticipated the independent florist and garden centers to have lower whitefly populations than the big-box and grocery stores. Producers that supplied the poinsettias to retailers were anonymized with a single capital letter; however, it should be noted that producer A and B at the retailer are the same as facility A and B from our whitefly infestation on cuttings data.

2.3.3. Statistical analyses and interpretation of results

All statistical analyses were performed in [R] (v. 3.5.3, R Foundation for Statistical Computing, Vienna, Austria) using RStudio (v.1.2.5001, RStudio, Inc., Boston, MA). The

numbers of nymphs and pupae on rooted cuttings (all cultivars and sampling periods within year combined) were compared between years within each grower facility using Wilcoxon signed-rank test to determine if data from multiple years can be pooled together. The number of whitefly immatures and adults found on poinsettias at the retailers were compared between different producers using Wilcoxon rank sum test pairwise comparison with Benjamini-Hochberg correction (1995); we expected to find differences in final whitefly densities among producers. Graphical representations of results were generated using ggplot2 (Wickham, 2016).

2.3.4. Results and discussion

2.3.5. Whitefly density on cuttings

The number of nymphs counted on rooted poinsettia cuttings was significantly different between 2017 and 2018 at grower facility A ($P < 0.001$; Table 2.1); however, the number of nymphs (facility B), pupae (facility A), and exuviae (facility A and B) on rooted cuttings was not significantly different between 2017 and 2018 on infested poinsettia cuttings ($P = 0.320$, $P = 0.573$, $P = 0.055$, and $P = 0.084$, respectively). No pupae were found on any of the rooted cuttings at facility B for 2017 or 2018. Mean nymph numbers (± SE) per cutting for all poinsettias (whether showing or not showing signs of whitefly infestation) in 2017 was 0.02 ± 0.02 (facility A) and 0.05 ± 0.05 (facility B), and in 2018 was 0.33 ± 0.13 (facility A) and 0.02 ± 0.01 (facility B) nymphs per cutting.

Out of the total 2417 rooted poinsettia cuttings inspected at both locations over 2 years, 29 had at least one whitefly nymph (1.2%), 18 had at least one pupa (0.7%), and 23 had at least one exuvia (1.0%). Of the 19 rooted cutting cultivars observed, only nine had

any signs of infestations: Christmas Beauty Red (NPCW10158), Classic Pink, Classic Red, Enduring Marble (PER10603), Ice Crystal, Premium Lipstick Pink, Premium Picasso, Prestige Red, and Whitestar (Table 2.2). The highest number of nymphs counted on a rooted cutting overall was 54 nymphs on cultivar Whitestar from facility A in 2018. Caution should be used in drawing conclusions about cultivar susceptibility to whiteflies from the above data, as several factors, such as propagator conditions, sample size, or local sources of infestation could result in differences in whitefly densities. Differences in cultivars susceptibility should be tested through controlled studies.

Table 2.1. Total poinsettia cuttings inspected and infested, and mean (± standard error) nymphs, pupae, and whitefly exuviae on infested poinsettia cuttings from two facilities (A and B) over 2 years (2017 and 2018); at least 200 cuttings were inspected per visit using a 2.5x magnification head lens.

Year	Facility	Plants inspected (no./row)	Plants infested (no.)	Whiteflies on infested plants (mean ± SE[1])		
				Nymphs	Pupae	Exuviae
2017	A	610	9	1.22 ± 1*[2]	1 ± 0.41	0.44 ± 0.34
	B	600	1	28	0	0
2018	A	605	25	8.04 ± 2.65*	1.2 ± 0.44	1.52 ± 0.63
	B	602	6	1.67 ± 0.92	0 ± 0	0.5 ± 0.22

[1]Mean calculation only includes plants with at least one nymph or pupa.
[2]* significantly different between years within the same grower facility for specific whitefly life stage by pairwise comparisons using Wilcoxon rank sum test ($P < 0.05$).

Sufficient suppression of whiteflies on poinsettias can be achieved in an augmentative biological control program when starting populations are as high as 1.0 whiteflies (of all

stages) per poinsettia cutting (Van Driesche et al., 1999), which is 50-fold higher than the average number of whiteflies (all stages combined) we found on rooted cuttings for the location and year with the highest population (Facility A, 2018) of our sampled data. It should also be noted that the source of our observed whitefly densities were likely from a

Table 2.2. Total poinsettia cuttings inspected and infested, and mean (± standard error) nymphs, pupae, and whitefly exuviae on infested poinsettia cuttings by poinsettia cultivar.

			Whiteflies on infested plants (mean ± SE)[1]		
Cultivar	Plants inspected (no./row)	Plants infested (no.)	Nymphs	Pupae	Exuviae
Astro red	35	0	-	-	-
Christmas Beauty Cinnamon	14	0	-	-	-
Christmas Beauty North Pole	16	0	-	-	-
Christmas Beauty Princess	21	0	-	-	-
Christmas Beauty Red	412	3	11.33 ± 8.4	0 ± 0	0 ± 0
Christmas Cheer	39	0	-	-	-
Classic Pink	37	1	25	6	2
Classic Red	1047	23	4.78 ± 1.8	0.7 ± 0.3	0.22 ± 0.1
Classic White	111	0	-	-	-
Enduring Marble	43	1	0	0	1
Ice Crystal	101	2	0 ± 0	0 ± 0	1 ± 0
Maren	25	0	-	-	-
Premium Lipstick Pink	59	1	2	4	0
Premium Picasso	35	1	13	0	8
Premium Polar	19	0	-	-	-
Prestige Red	280	2	0	0.5 ± 0.5	0.5 ± 0.5
Snowflake Red	54	0	-	-	-
Whitestar	50	6	10.83 ± 8.8	1.17 ± 1.0	3.5 ± 2.1
Wintersun White	16	0	-	-	-

Data pooled from two facilities (A and B) over 2 years (2017 and 2018); at least 200 cuttings were inspected per visit using a 2.5x magnification head lens.
[1]Mean calculation only includes plants with at least one nymph or pupa.

combination of the propagators and from local natural populations, since we inspected cuttings that had been rooted at the local facility for 4 – 6 weeks prior to inspection. Despite the potential local source of whitefly populations and variation in initial whitefly numbers, our whitefly infestation count data from two different grower locations over 2 years suggests infestation levels on cuttings received from propagators are well within the acceptable range for initiating an augmentative biological control program.

While attempting to meet market demand, when given an opportunity, growers might choose cultivars that may start with lower infestation of whiteflies. Our study is not a controlled cultivar choice test by whiteflies and validation of cultivar differences requires additional data. In choice tests, whiteflies demonstrated preference and better performance (i.e., greater population growth) on light green leaf poinsettias compared to dark green leaf poinsettias (Medina-Ortega, 2011). Our survey found Whitestar cuttings tended to have the highest proportion of plants infested and amongst the highest mean nymphs, pupae, and exuviae compared to all other cultivars, followed by Classic Pink, Premium Picasso, Classic Red, and Premium Lipstick Pink. On the other hand, cultivars Classic White and Ice Crystal had practically no signs of whitefly infestations despite over 100 rooted poinsettias being inspected for each. It should be noted; however, that whitefly populations aggregate both within and between plants (Liu et al., 1993) and differences in initial infestation between cultivars may have been due to the relatively small sample size. Additionally, other factors such as propagator facility and location may have been explanatory variables for the observed initial infestation levels.

2.3.6. Whitefly density at retailers

We counted an average of 4.38 to 40.38 immature whiteflies per plant for all retailers in 2017 and 2018 combined (Table 2.3). We found up to 220 immatures on a single plant in 2018 at grocery store florist. We also found up to 32 adults on a single plant in 2018 at an independent garden center. The independent florist and garden center did not appear to have fewer whitefly immatures or adults compared to the grocery store florists and big-box stores over both years despite consistently higher marketability rating and price (Table 2.3); however, statistical inference was avoided due to the great differences in replicates (stores) and subsamples (plants within stores). All retailer types had a wide distribution of immature whitefly infestation levels (Figure 2.1), making it hard to identify an acceptable threshold for whitefly densities. Most poinsettias inspected were in 6 – 6.5-inch pots (big-box: 169, grocery store florist: 130, independent garden center: 51, independent florist: 15); however, some pots were 8 inches or larger (big-box: 17, independent florist: 5) and some were 4 inches (grocery store florist: 20). Even when poinsettias had up to 220 immatures (2018, grocery store florist), the store manager expressed that their poinsettias were considered relatively clean and whitefly-free compared to the previous year and were surprised to learn of whitefly populations on their poinsettias.

Significant differences among poinsettia producers for immature and adult whitefly populations were observed at retail stores (Table 2.4); however, only one (producer “H” from Canada) had no poinsettias infested with immatures or adult whiteflies. Excluding producer “H”, the percentage of poinsettias infested with immature whiteflies from different producers varied from 35% to 100%. We included the Store Unique Identifier (ID) in Table 4, as it is possible that poinsettias from one producer may become a whitefly

source for another producer's poinsettias at the retailer, rather than whiteflies coming from the producer. There was no clear pattern between level of infestation with immature whiteflies and poinsettia source location – poinsettias from Texas, California, Canada, and unknown source location all had some poinsettias with over 50 immatures per plant (Figure 2.2).

Table 2.3. Mean (± standard error) immature and adult whiteflies, proportion of finished color poinsettias infested with immatures and adult whiteflies, mean (± standard error) price, and median poinsettia aesthetic rating from different retailer types over 2 years.

Year	Type	Immatures (mean ± SE)	Adults mean ± SE)	Proportion immatures	Proportion adults	Mean price (mean ± SE)	Aesthetic rating 0 – 10 scale [median (min. – max.)]	Stores	Plants inspected (no./row)
2016	Big-box	4.38 ± 1.16	0.2 ± 0.07	0.43	0.13	5.71 ± 0.19	8 (5 - 9)	3	84
	Grocery florist	35.89 ± 3.63	0.83 ± 0.22	0.71	0.24	5.86 ± 0.13	7 (4 - 9)	6	103
	Garden center	16.2 ± 5.87	2.47 ± 0.7	0.63	0.50	7.08 ± 0.09	8 (7 - 9)	1	30
	Florist	8.6 ± 4.35	0.8 ± 0.59	0.40	0.30	57.5 ± 7.43	10 (8 - 10)	1	10
2018	Big-box	25.07 ± 3.21	1.21 ± 0.21	0.74	0.41	8.34 ± 0.3	10 (4 - 10)	4	102
	Grocery florist	33.63 ± 9.31	2.78 ± 1.1	0.81	0.56	6 ± 0	9 (7 - 10)	1	27
	Garden center	40.38 ± 10.77	9.1 ± 1.68	0.81	0.95	4.95 ± 0	9 (8 - 10)	1	21
	Florist	73 ± 22.08	6 ± 2.33	1.00	0.80	83.5 ± 12.27	10 (10 - 10)	1	10

Aesthetic rating was recorded by looking at the whole plant from a scale of 0 (whiteflies easily seen, occurrence of honeydew, development of sooty mold, plant stretched, canopy thinning, and yellowing leaves) to 10 (unable to detect whiteflies, no honeydew, no sooty mold present, compact plant, no thinning or yellowing leaves). Marketability of the plant was considered greatly reduced when aesthetic rating was 7 or below. The median, minimum (min.) and maximum (max.) aesthetic rating for all poinsettias within a retailer type is summarized. Finished poinsettias were inspected for whiteflies in 60 s per plant, with several plants (10 – 30) inspected per retailer over the 2 separate years (2016 and 2018).

Table 2.4. Mean (± standard error) immature and adult whiteflies and total proportion of finished color poinsettias infested with immature and adult whiteflies by producer.

Producer (ID)[1]	Store (ID)[2]	Source	Mean ± SE		Proportion		n
			Immatures	Adults	Immatures	Adults	
A	4	Texas	40.38 ± 10.8 ab[3]	9.1 ± 1.7 a[x]	0.81	0.95	21
B	2, 10, 11	Texas	23.94 ± 3.5 a	1.47 ± 0.4 b	0.69	0.39	62
C	7, 8	Texas	2.8 ± 0.8 c	0.11 ± 0.1 cd	0.35	0.11	46
D	1	Texas	45.15 ± 11.5 b	3.55 ± 1.4 ef	1.00	0.65	20
E	6	Texas	8.6 ± 4.4 ac	0.8 ± 0.6 bce	0.40	0.30	10
F	6	Texas	73 ± 22.1 b	6 ± 2.3 af	1.00	0.80	10
G	1, 2	Canada	20.84 ± 4.0 a	1.32 ± 0.3 be	0.84	0.48	31
H	3	Canada	0 ± 0 d	0 ± 0 d	0.00	0.00	20
I	12, 13	California	30.65 ± 6.0 ab	1.39 ± 0.4 be	0.76	0.39	46
J	9	NA	5.87 ± 3.0 c	0.53 ± 0.3 bc	0.47	0.27	15
P-Value			<0.001	<0.001			

Poinsettias were inspected for whiteflies for 60 s per plant at several retailers in Tyler TX over 2 years (2016 and 2018) and were split based on original poinsettia producer. The producer of poinsettia was anonymized using a producer identifier (producer ID). The geographic location (Source) and anonymized store identifier (ID) where each specific producer sold their poinsettias is also shown. The source of some poinsettias was not available (NA).

[1]A and B from Grower ID are the same A and B from Table 1.

[2]Rows with matching store ID numbers could be found within the same store.

[3]Any two means within a column not followed by the same letter are significantly different by pairwise comparison using Wilcoxon rank sum test ($P < 0.05$).

Thresholds for poinsettias have included virtually "zero" tolerance or undetectable populations, but neither have been defined based on acceptable whitefly populations at the retailers. Hoddle and Van Driesche (1996) found an average of 0.01 - 0.02 whitefly nymphs per leaf by inspecting six leaves from a total of 30 plants. In a similar study, Van Driesche et al. (1999) found an average between 0.55 – 0.98 nymphs per leaf on finished poinsettias. Both studies were conducted in Massachusetts, with information about the grower or original source lacking, and similar studies have not since been conducted. Our

survey of different retailers supports that “zero” or “undetectable” populations may not accurately describe marketplace whitefly thresholds on potted poinsettias, and there is a need to establish realistic and quantifiable thresholds for these and other ornamental plants. This study marks the first publication to our knowledge that provides a multi-year and multi-location survey of whiteflies on rooted poinsettias, and the first to consider poinsettia source, retailer types, and multiple years within a single study for whitefly thresholds on poinsettias.

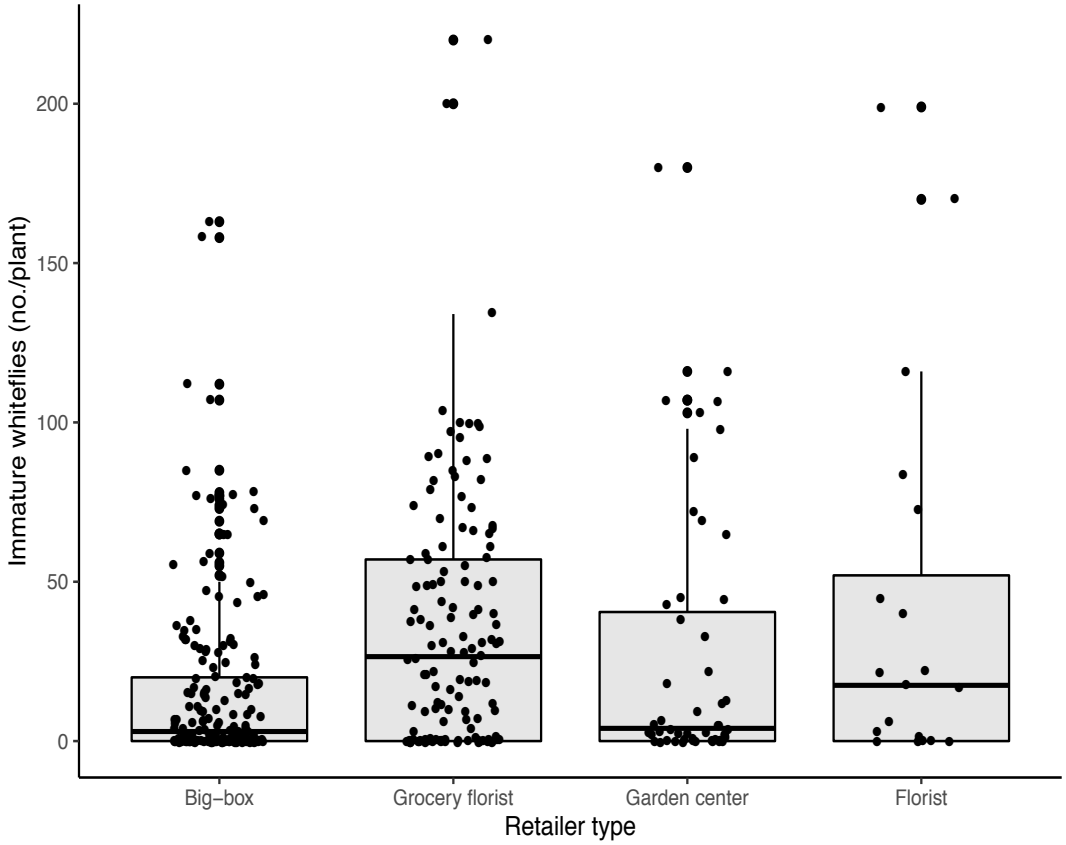

Figure 2.1. Immature whiteflies counted per finished color poinsettia by retailer group. Finished poinsettias were inspected for 60 s per plant, with several plants inspected per retailer over 2 separate years (2016 and 2018). Retailers were categorized into one of four types based on expected differences in retailer thresholds: big box (seven stores), grocery (seven stores), garden store (two stores), and florist (two stores).

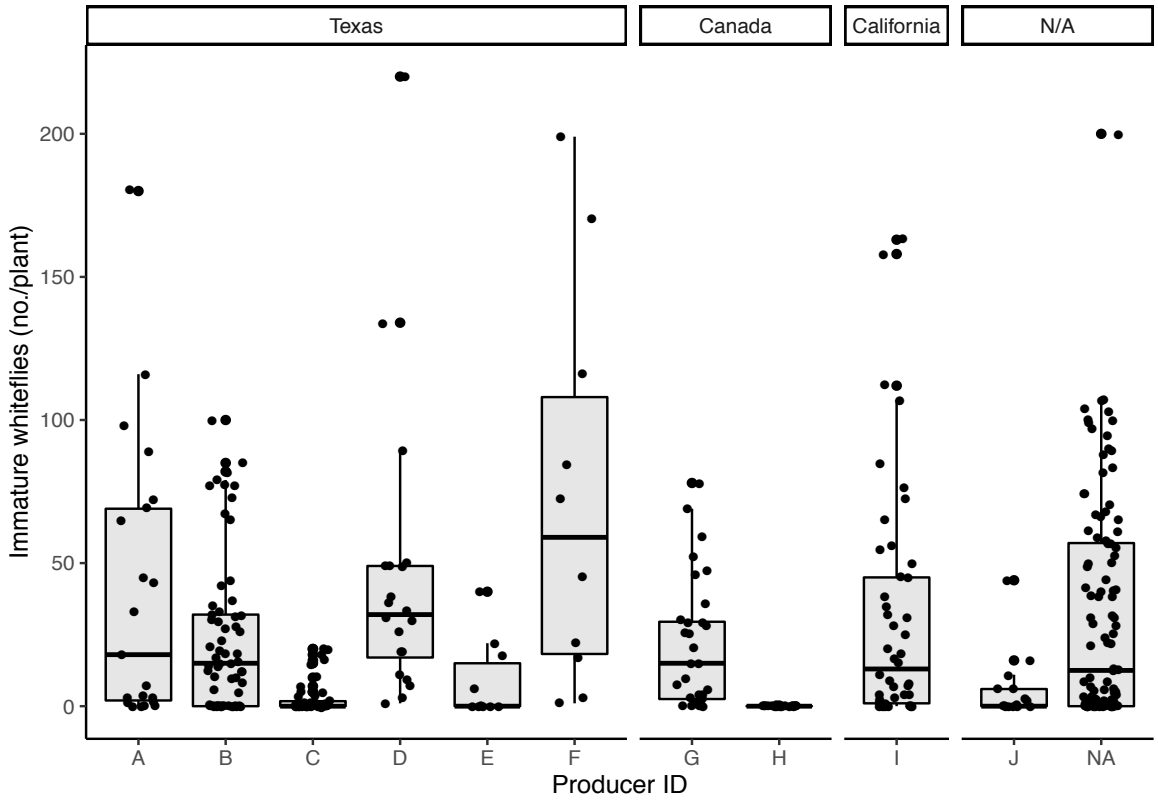

Figure 2.2. Number of immature whiteflies on finished color poinsettia by producer. Poinsettias were inspected for whiteflies for 60 s per plant. Several plants were inspected at different retailers in Tyler TX over 2 years (2016 and 2018) and were split based on original producer. Producer was anonymized using a producer identifier (producer ID). Poinsettia producer was not recorded in 2016 and all poinsettias were subsequently lumped under NA (for "not available"). NA is for plants inspected in 2016, in which the producer information was not collected (i.e., not available).

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3. A COMPARISON OF REPETITIVE RELEASES OF SINGLE OR MULTIPLE NATURAL ENEMY SPECIES ON THE SUPPRESSION OF *BEMISIA TABACI* INFESTING POINSETTIAS.[1]

3.1. Overview

The repetitive release of *Eretmocerus eremicus* Rose & Zolnerowich (Hymenoptera: Aphelinidae) to manage *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a promising strategy on poinsettias in protected culture. Management of *B. tabaci*, however, may be improved if releases include multiple natural enemy species that attack different *B. tabaci* life stages. In this study, we investigate whether suppression of *B. tabaci* on poinsettias is improved by the combination of *E. eremicus* and *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) compared to either natural enemy alone at release rates 1.2 – 2.7-fold the cost of conventional insecticide inputs. We found that all natural enemy treatments provided significant suppression when starting whitefly populations were below 13.7 ± 1.7 immatures per plant. The combination of *E. eremicus* and *A. swirskii* performed equally well compared to either natural enemy alone, in both a substitutive and additive design. These effects, however, were density dependent; neither natural enemy alone nor the combination of natural enemies suppressed whiteflies if initial whitefly density was above 40.8 ± 2.5 immature whiteflies per plant.

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3.2. Introduction

Seasonal inoculative biological control has been increasingly adopted for ornamentals in greenhouse production over the last couple decades (van Lenteren et al. 1996, Murphy et al. 2008, 2011, Barratt et al. 2018), with early adopters including the UK and the Netherlands (van Lenteren et al. 1988). In the USA, pesticides are still considered the most viable and economic solution for arthropod pest management (Bethke and Cloyd 2009). However, recent increases in pesticide restrictions from major retailers (Lowe's 2014), increases in pesticide-resistance (McKenzie et al. 2009, Dennehy et al. 2010, Xiao et al. 2012, Bass et al. 2015, Siegwart et al. 2015), unintended secondary pest outbreaks (Szczepaniec et al., 2013), and increased regulations required by the Environmental Protection Agency (United States Environmental Protection Agency 2017) call into question the long-term viability of relying on pesticides as the primary strategy for managing arthropod pests in protected culture in the USA. In order to facilitate a reduction in pesticide use and increased use of biological control in ornamentals, we need to elucidate the effect of natural enemy composition on pest suppression.

Poinsettias (*E. pulcherrima*) are popular holiday ornamental plants that are sold during November and December in the USA. Poinsettia sales were valued at $140M in 2015 alone, ranking second in value of potted flowering plants (United States Department of Agriculture 2016). Since poinsettias are sold as potted plants and desired mainly based on aesthetic value, the tolerance for pests or pest damage is considered essentially zero (Bethke and Cloyd, 2009). The main arthropod pests on poinsettias include thrips, mealybugs, spider mites, and whiteflies (Ecke et al. 2004), with whiteflies constituting the

main economic pest. A threshold of less than two whitefly nymphs per leaf is considered acceptable by commercial growers (Van Driesche et al. 1999a) and up to 73 ± 22 immatures at the retailer (Vafaie, Pemberton, Gu, Kerns, et al. 2020b) due to production of honeydew and sooty mold when whitefly densities are higher, drastically reducing marketability. Long-term management of whiteflies has historically been achieved through frequent and regular applications of insecticides (Sharaf 1986) and the cost to use a single natural enemy species in seasonal inoculative biological control to manage whiteflies on poinsettias in the USA were 30-fold higher than the cost of pesticide inputs (Hoddle and Van Driesche 1999a).

Recent work has demonstrated that increased natural enemy diversity can result in increased pest suppression when natural enemies occupy different niches (Snyder 2019). For example, aphid suppression was increased when natural enemies fed during different times of day (Gontijo et al. 2015), when natural enemies were more specialized on specific aphid host species rather than overlapping in species preference (Finke and Snyder 2008), or when natural enemies partitioned different parts of a plant canopy to find prey (Bográn et al. 2002, Straub and Snyder 2008). Likewise, the successful suppression of whiteflies using a parasitic wasp, *E. eremicus* (Hoddle and Van Driesche 1999a), may be improved by increasing natural enemy diversity.

Amblyseius swirskii controls several major ornamental and vegetable pests, including the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), whiteflies, and the broad mite, *Polyphagotarsonemus latus* (Banks) (Trombidiformes: Tarsonemidae) (Calvo et al. 2015). On cucumber leaf discs, *A. swirskii* fed primarily on eggs and 1st instar sweetpotato whiteflies, *B. tabaci* ((Nomikou et al. 2004), whereas *E.*

eremicus prefers to parasitize second and third instar whitefly nymphs (Bográn et al. 2002, Liu et al. 2015). Despite *A. swirskii*'s ability to manage *B. tabaci* populations (Gerling et al. 2001, Nomikou et al. 2002, Berndt et al. 2007, Calvo et al. 2011, 2012, Buitenhuis et al. 2015), the ability of this predator to disperse within a greenhouse is limited when plant canopies are not interconnected (Buitenhuis et al. 2010). By comparison, female *E. eremicus* can disperse distances over 10 meters in the field (Bellamy and Byrne 2001) and subsequently will be more likely to encounter patches of whiteflies in a greenhouse.

In this study, we investigate if the combination of *E. eremicus* and *A. swirskii* improve suppression of *B. tabaci* compared with either natural enemy alone at release rates 1.2 – 2.7-fold the cost of conventional insecticide inputs, using poinsettias as a model crop due to their long season, limited pest species, and relative monocrop.

3.3. Materials and methods

3.3.1. Cultures

Unrooted poinsettia cuttings (cultivars 'Prestige Red', 'Premium Red', and 'Prestige Early Red' for spring, summer, and fall trials, respectively) were sourced from Dümmen Orange (Dümmen NA Inc., Columbus, OH) and rooted under mist at the Texas A&M AgriLife Research and Extension Center IPM Greenhouse in Overton, TX using standard propagation protocols as published in Ecke et al. (2004). Greenhouse heaters were set to turn on when temperature decreased below 10 ºC and evaporated cooling pads were active above 26 ºC. Overhead fluorescent lights were programmed to come on for two hours from 10 pm – 12 am, to prevent bract formation for the spring and summer trials. Fall plants were allowed to flower naturally. After rooting (four to six weeks), cuttings were

transplanted into pots (15 cm diameter x 10 cm deep) with BM6 Custom Blend Potting Mix (Berger, Saint-Modeste, QC, Canada) and were continually fertigated through drip irrigation at 200 ppm nitrogen (Peters Professional 20-10-20 General Purpose Fertilizer, ICL Specialty Fertilizers, St. Louis, MO). *Bemisia tabaci* colonies, collected from infested poinsettias at retailers and local growers, were maintained on poinsettias in cages (2 – 3 poinsettias per cage; 60 cm x 60 cm x 60 cm, BugDorm-2120F, MegaView Science Co., Ltd., Talchung, Taiwan) in the IPM Greenhouse. *Bemisia tabaci*-free poinsettias were introduced into cages every two weeks to maintain a fresh supply of plant material to maintain the whitefly colony and old plant materials removed.

Eretmocerus eremicus and *A. swirskii* were sourced from BioBest (BioBest Group NV, Ilse Velden 18, Belgium). *Eretmocerus eremicus* were ordered in bulk with 5,000 pupae per bottle (Eretmocerus-System-5k). *Amblyseius swirskii* were ordered in 500 mL bulk bottles (Swirskii-System-25k), each containing 25,000 mites. Upon receipt, natural enemies were lab-stored until released the same day of arrival.

3.3.2. Experimental units

Each replicate contained a total of 12 individual poinsettia plants (sub-samples; three rows of four plants) grown in 15 cm x 10 cm pots, spaced at 30 cm centers within a single cage. Individual cages (150 cm x 120 cm x 90 cm) were fabricated with a PVC frame encased in thrips-proof netting (PAK 75 Anti-Insect Mesh Screen, PAKGlobal, LLC., Cornelia, GA) placed on benches inside the IPM Greenhouse. Benchtops were covered with row cover fabric (GR-RC05, Greenhouse Megastore, Danville, ILL) and the fabric was wrapped around the bottom of the PVC frame of the cages to prevent the movement of wasps

between cages. Previous work has demonstrated limited movement of *A. swirskii* beyond 30 cm (Buitenhuis et al. 2010). Each treatment was replicated four times (cages) per trial and the entire trial was repeated a total of three times: spring (April 16 – May 29), summer (July 17 – August 29), and fall (September 17 - November 13) of 2018.

3.3.3. Whitefly infestation

To establish baseline infestation of *B. tabaci* prior to natural enemy release, poinsettia transplants were placed under row cover fabric and infested using a protocol similar to Krauter et al. (2017). In brief, 18 adult whiteflies were aspirated into vials and placed in the middle of each row of nine poinsettias on two occasions, seven days apart. One week after the second introduction of whiteflies, the number of whitefly nymphs and pupae were counted on the entire plant and selectively allocated to a respective cage, to provide similar immature whitefly starting numbers in all cages. Trials started with relatively higher whitefly densities compared to starting densities in commercial poinsettia production (Vafaie, Pemberton, Gu, Kerns, et al. 2020a) to increase statistical power.

3.3.4. Natural enemy release

Natural enemies were released within 48 hrs after placing poinsettias in cages; one week after second introduction of whiteflies. The experimental treatments were designed to control for both intraspecific and interspecific interactions as described by Sih et al. (1998): (1) No natural enemies, (2) *E. eremicus* (1x rate), (3) *A. swirskii* (1x rate), (4) *E. eremicus* (1x rate) plus *A. swirskii* (1x rate), (5) *E. eremicus* (2x rate), and (6) *A. swirskii*

(2x rate). Treatments were replicated four times within each trial (spring, summer, and fall), resulting in a total of 24 cages across two adjacent greenhouse compartments. *Eretmocerus eremicus* were released weekly and *A. swirskii* released every four weeks. *Eretmocurus eremicus* was released in each cage by placing either 25 pupae (1x rate) or 50 pupae (2x rate) into a Petri dish (60 mm x 15 mm) and placing the Petri dish in the middle of the cage. *Amblyseius swirskii* were spread by measuring out either 2.5 mL (1x rate) or 5 mL (2x rate) of the bulk *A. swirskii* material and spreading evenly over the 12 plants within the cages. After the initial natural enemy release, spring and fall trials continued for 8 weeks. The summer trial was ended prematurely (week six) due to high whitefly numbers and rapid poinsettia quality decline in all treatments.

We determined emergence rates and proportion of females from *E. eremicus* pupae by placing 20 pupae (subsamples) into each of 18 small vented Petri dishes (60 mm x 15 mm; experimental unit) using a fine brush on 6 February, 2018. Petri dishes were evenly split between the IPM Greenhouse and a growth chamber (A1000, Conviron, Winnipeg, MB, Canada). Growth chamber temperature and humidity was programmed for 24 ºC, 8:16 hr (light:dark) cycle, and 70 % relative humidity. Petri dishes moved to the greenhouse were placed under the same cage fabric used in our trials, to determine emergence rate in experimental conditions. Petri dishes were removed after two weeks and placed in a -4 ºC freezer to kill any free-flying adults. Emerged *E. eremicus* adults were counted, sexed and summarized for each Petri dish. Although we evaluated several methods, we were unable to develop a reliable method for quantifying A. *swirskii* in our bulk carrier material, but rather relied on counting *A. swirskii* directly on poinsettias post-release.

3.3.5. Natural enemy release rate cost

Rates of *E. eremicus* and *A. swirskii* were determined based on economic comparison with local insecticide rotation input costs for poinsettias. Data from a local grower (Smith County, Texas, USA) and preliminary calculations based on another local grower insecticide rotations agree with a historical economic analysis of Eastern USA poinsettia greenhouses of a cost of $0.09 on average in inputs per 16.5 cm (diameter) pot (or $1.58/m^2) for a 17 to 18-week season (Stevens et al. 2000). Due to the relatively high mobility of *E. eremicus*, the cost per m^2 was used to determine release rates, whereas cost on a per pot basis was used for *A. swirskii*. Our cost comparison does not include the cost of shipping the natural enemies, as shipping costs can vary depending on quantity of natural enemies ordered and company used to source natural enemies, making estimating of shipping costs variable.

The price of *E. eremicus* provided by BioBest is $77.55 for 10,000 pupae (bulk), resulting in a cost of $1.89/m^2 to release 25 *E. eremicus* pupae (1x rate) per cage weekly for an 18-week crop; resulting in a 1.2-fold and 2.4-fold cost increase in our *E. eremicus* (1x rate) and *E. eremicus* (2x rate) releases compared to insecticide input costs, respectively. The price for *A. swirskii* from BioBest is $57.45 for a 500 mL bottle of 25,000 mites, resulting in a cost of $0.12/pot for 2.5 mL of *A. swirskii* carrier material (~123.2 mites) released every four weeks for an 18-week crop; resulting in a 1.3-fold and 2.7-fold cost increase in *A. swirskii* (1x rate) and *A. swirskii* (2x rate) releases compared to insecticide input costs, respectively. The release rates used in our combination treatment,

E. eremicus (1x rate) plus *A. swirskii* (1x rate), cost 2.5-fold higher than conventional insecticide inputs.

3.3.6. Data collection

A 3.5x magnification head lens was used to count all stages of whiteflies (nymphs, pupae, exuviae, and adults), *E. eremicus* adults, and *A. swirskii* mites on five upper and five lower leaves of every other poinsettia during each sampling period (weeks zero, two, four, six, and eight). When handled carefully, adult whiteflies and *E. eremicus* did not fly off of plants, making the counts of adults feasible. Due to larger canopy size and quantity of whiteflies after week four, counts were conducted within two days. Temperature and humidity data were collected using HOBO data loggers (U23 Pro V2, Onset Computer Corporation, Bourne, MA) placed in the middle bench of the greenhouse within a cage, and logged temperature and humidity every 30 minutes. At the end of the spring and fall trials, all poinsettias within each cage were photographed together for plant quality assessment. Based on visual inspection of the photographs, we created a rating with a top aesthetic score of 9 for each cage based on the following criteria: presence of whiteflies (-2), presence of sooty mold (-2), dead plants (-1), plant stunting or lack of uniformity (-1), upward curling of leaves (-1), yellowing of leaves (-1), and canopy thinning (-1).

3.3.7. Statistical analyses and interpretation of results

All statistical analyses were performed in R v.3.5.3 (R Core Team 2019) using RStudio (R Studio Team 2015). Additional packages used in addition to the R {base} were: lme4 (Bates et al. 2015), lmerTest (Kuznetsova et al. 2017), tidyverse (Wickham 2017), and emmeans (Lenth 2019). Normality of residuals was determined by visual inspection of Q-Q plots. The entire dataset and the R markdown script file are published and available for download [dataset] (Vafaie, Pemberton, Gu, Eubanks, et al. 2020).

Percentage emergence of *E. eremicus* pupae was calculated as total adults counted in the Petri dish after two weeks divided by 20 (total pupae put in each Petri dish). Percentage female was calculated as the number of (females / total adults)*100 counted in the Petri dish after the two weeks. Percent emergence and percent females were compared between growth chamber and greenhouse treatments using an ANOVA ($\alpha = 0.05$). Final aesthetic score of cages were compared using an ANOVA ($\alpha = 0.05$), with natural enemy treatment and temporal block (spring and fall) as interacting factors.

Mean temperature and relative humidity were calculated for each day. Mean, minimum and maximum of daily mean temperatures and relative humidity were summarized to two-week intervals corresponding to the two weeks prior to whitefly counting dates.

Due to an excessive number of zeros (zero-inflation) in whitefly counts inappropriate for standard parametric analyses (Tu and Liu 2014), we summed counts for each whitefly life stage to plant level and subsequently averaged to cage level (experimental unit) prior to statistical analyses. Whitefly count data was log-transformed ($\log(1+x)$), with x equal to mean whiteflies within a cage, and our model had a combination of fixed and nested random factors, making it suitable for analysis using generalized linear mixed models

(GLMM) with restricted maximum likelihood method (REML) (Bolker et al. 2009). We created the model using two interacting fixed factors (treatment and week number), and nested random factors (experimental unit ID nested within temporal block, spring and fall) to predict the log-transformed response variable (average number of whitefly immatures, pupae, or adults). Summer trial results were analyzed separately from spring and fall due to substantially higher immature whitefly starting populations. Each whitefly life stage was modeled separately. To determine the role of intraspecific interactions on whitefly populations, we performed planned contrasts ($\alpha = 0.05$) of the 1x rates vs 2x rates of conspecifics (i.e. *A. swirskii* 1x vs. *A. swirskii* 2x and *E. eremicus* 1x vs. *E. eremicus* 2x). To determine the role of interspecific interactions on whitefly populations, we performed planned contrasts of the 2x rates of conspecifics with the 2x combination treatment (i.e. *A. swirskii* 2x vs. *E. eremicus* + *A. swirskii* and *E. eremicus* 2x vs *E. eremicus* + *A. swirskii*).

To test for differences in vertical distribution of the whiteflies in the plant canopy by treatment, each plant was summarized as a proportion by dividing the total immatures on the upper canopy leaves (top five leaves) with the total immatures on the plant for each given sampling period. Proportion of immatures in the upper canopy was then averaged for each cage, log-transformed, and treatments compared using GLMM REML as described above for the spring and fall treatments.

Number of natural enemies was summed for all plants within each cage for each sampling period. Log-transformed mean *A. swirskii* were compared for *A. swirskii*-released treatments using GLMM REML as described above for the spring and fall trials. Mean *E. eremicus* was not compared between treatments due to small recovery in the cages.

3.4. Results

Eretmocerus eremicus pupae emergence was 60 ± 5% for pupae maintained in both the growth chamber and greenhouse ($F_{1,14} = 0.000$, P=1.000). Of the emerged wasps, the percent female was not significantly different when pupae were maintained in the growth chamber (54 ± 6%) or greenhouse (39 ± 5%) ($F_{1,14} = 2.923$, P=0.109).

When summarized to biweekly sampling period, mean daily temperature varied between 18 – 26 ºC (spring) and 16 – 26 ºC (fall) (Table 3.1). Maximum temperatures reached as high as 49ºC (spring) and 35 ºC (fall). Mean daily relative humidity varied between 69 – 78% (spring) and 84 – 90% (fall) (Table 3.1). Weeks zero and eight were not included in the table if the dataset was incomplete for the two weeks prior to the week number (i.e. HOBO data logger was not set up two weeks in advance or was removed just before the final assessment period).

Table 3.1 Mean temperature (min. – max.) and mean relative humidity (min. – max.) summarized for two weeks leading up to specific week of the trial for spring, summer, and fall. Weeks 0 and 8 were not included if the dataset was incomplete for the time period.

Block	Week	Mean temperature (min. – max.) (ºC)	Mean relative humidity (min. – max.) (%)
Spring	2	18 (13 - 25)	69 (60 - 81)
	4	18 (16 - 21)	74 (68 - 82)
	6	24 (22 - 26)	78 (72 - 88)
	8	26 (25 - 28)	78 (75 - 81)
Summer	2	30 (27 - 34)	68 (58 - 78)
	4	27 (25 - 29)	75 (65 - 88)
	6	29 (27 - 30)	75 (70 - 79)
	8	29 (26 - 31)	19 (1 - 54)
Fall	0	26 (25 - 27)	87 (82 - 92)
	2	24 (20 - 28)	90 (81 - 96)
	4	22 (13 - 26)	90 (82 - 95)
	6	16 (13 - 18)	84 (70 - 92)

Temperature and humidity data were logged every 30 minutes by a HOBO data logger (U23 Pro V2) that was placed in a cage in the middle of the greenhouse.

Due to shipping errors, natural enemy shipments were missed on a few occasions: *E. eremicus* on week seven (fall 2018) and *E. eremicus* on weeks two and six (spring 2018). When natural enemy shipments were missed, they were added to the following week, unless the subsequent week was the end of the trial (i.e. week seven).

Mean immatures per plant was between 12.2 ± 1.1 and 14.3 ± 1.9 at the beginning of the spring and fall trials. Log-transformed number of immatures, pupae, and adult whiteflies had a significant positive relationship with week overall for all life stages (Table 3.2), supporting that number of whiteflies increased for all treatments over time (Figure 3.1). Log-transformed number of immature whiteflies per plant in the untreated control could be described as exp (2.88 + 0.415 * week). Cages with natural enemies suppressed whitefly immatures, pupae, and adults (Table 3.3; Figure 3.1) over the duration of the trial compared to the untreated control. Doubling the rate of conspecifics (*E. eremicus* or *A. swirskii*) did not produce significantly different whitefly immatures, pupae, or adults (Table 3.3). *Eretmocerus eremicus* and *A. swirskii* were also not significantly different in their suppression of whitefly immatures, pupae, or adults (Table 3.3). Lastly, there was no support for interspecific interactions on whitefly suppression: *E. eremicus* (2x rate) vs. *E. eremicus* plus *A. swirskii* and *A. swirskii* (2x rate) vs. *E. eremicus* plus *A. swirskii* (Table 3.3).

Table 3.2 Generalized linear mixed model (GLMM) analysis with restricted maximum likelihood (REML) for effect of natural enemy treatment and week on log-transformed mean immature, pupae, and adult *B. tabaci* per plant for spring & fall and summer trials.

			Immatures		Pupae		Adults	
		DF	F	P-value	F	P-value	F	P-value
Spring & Fall	Treatment	5,41	5.86	<0.001	5.55	<0.001	5.37	<0.001
	Week	1,186	325.49	<0.001	473.98	<0.001	334.70	<0.001
	Treatment*Week	5,186	7.60	<0.001	3.69	0.003	13.37	<0.001
Summer	Treatment	5,18	0.66	0.658	0.22	0.950	0.67	0.650
	Week	1,66	521.41	<0.001	312.72	<0.001	1122.07	<0.001
	Treatment*Week	5,66	0.27	0.926	0.09	0.993	0.15	0.980

Trial (for spring & fall) was treated as a random blocking factor. Number of whiteflies for each life stage were summed to plant level and six poinsettias (subsamples) per cage (experimental unit) averaged prior to analysis.

Table 3.3 Summary of planned contrasts of treatments for log-transformed mean immature, pupa, and adult *B. tabaci* per plant for spring and fall trials.

Contrast (a - b)			Immatures		Pupae		Adults	
a	b	DF	Estimate	P-value	Estimate	P-value	Estimate	P-value
Control	All NE treatments	41	0.944	<0.001	0.720	<0.001	0.888	<0.001
E. eremicus	*E. eremicus* 2x	41	-0.192	0.421	-0.173	0.357	-0.017	0.948
A. swirskii	*A. swirskii* 2x	41	-0.030	0.898	0.034	0.856	-0.226	0.386
E. eremicus + *A. swirskii*	*E. eremicus* 2x	41	-0.319	0.184	-0.295	0.119	0.091	0.728
E. eremicus + *A. swirskii*	*A. swirskii* 2x	41	-0.295	0.219	-0.152	0.417	-0.459	0.083

Control refers to untreated control treatments and all NE treatments refers to all natural enemy treatments (*E. eremicus* and *A. swirskii*, in combination and alone at 1x and 2x rates).

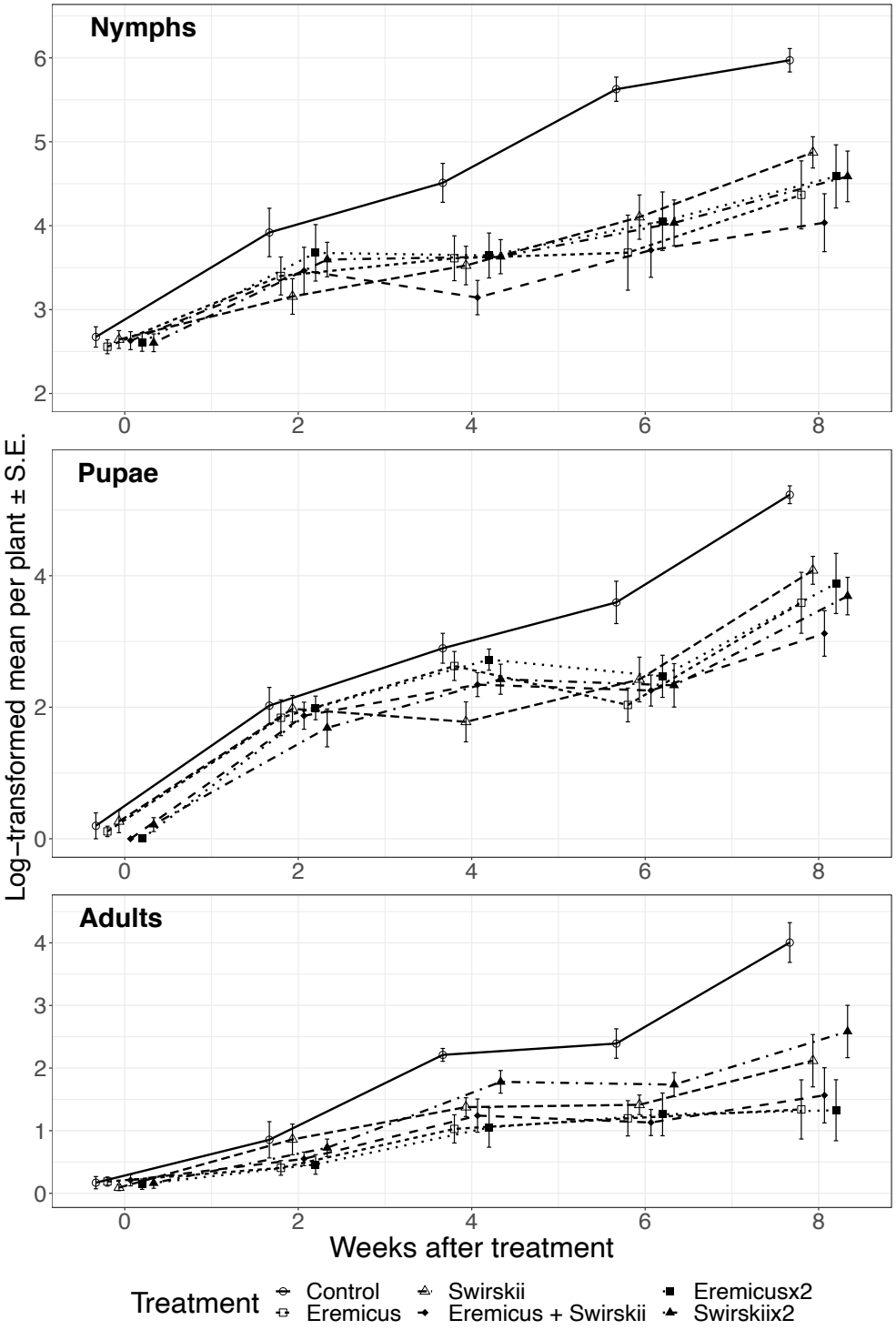

Figure 3.1 Log-transformed mean immature, pupae, and adult whiteflies per plant (± SE) for each treatment over 8 weeks for spring and fall trials. Number of whiteflies for each life stage were summed to plant level and six poinsettias (subsamples) per cage (experimental unit) averaged prior to plotting. Samples were collected within two days, but symbols are offset to better discern different treatments.

The mean immature whiteflies (± SE) at the end of the trial (week 8) per leaf was 52.1 ± 6.6 (N=8), 18.2 ± 5.1 (N=8), 18.0 ± 3.6 (N=8), and 8.4 ± 2.5 (N=8) for the untreated control, *E. eremicus* (2x rate), *A. swirskii* (2x rate) and *E. eremicus* + *A. swirskii* combination treatment, respectively. The mean final (week 8) adult whiteflies per leaf was 9.4 ± 2.7, 1.6 ± 1.4, 2.9 ± 1.0, and 1.2 ± 0.8 for the untreated control, *E. eremicus* (2x rate), *A. swirskii* (2x rate) and *E. eremicus* + *A. swirskii* combination treatments, respectively.

Mean proportion of immature whiteflies (± SE) on upper leaves was 0.108 ± 0.02, 0.124 ± 0.02, 0.343 ± 0.03, and 0.45 ± 0.02 for weeks 2, 4, 6, and 8, respectively, for all treatments combined. The number of immature whiteflies occupying the upper canopy significantly increased with week ($F_{1,138}$=203.14, P<0.001), but no significant interaction between week and natural enemy treatment ($F_{5,138}$=0.80, P=0.554).

Mean number of immatures per plant (± SE) for all treatments was between 40.8 ± 2.5 and 41.2 ± 2.8 at the start of the summer trial (Figure 3.2). Log-transformed number of immatures, pupae, and adults had a significant positive relationship with week overall for the summer trial, and natural enemy releases did not impact mean whitefly immatures, pupae, or adults (Table 3.2) for the duration of the 6-week trial. With treatment removed from the model, growth of the log-transformed change in immature whiteflies per plant could be described by exp (4.168 + 0.666 * week). After completing week four whitefly counts, a remedial application of Endeavor® Insecticide (pymetrozine) (Syngenta U.S., Greensboro, NC) at 37 g / 100 L was sprayed to reduce whitefly populations in all treatments. By week 6, mean number of immature whiteflies per plant (± SE) was between 1415.4 ± 220 and 2140.7 ± 462 and poinsettia plants in all treatments were in severe decline, resulting in a premature cessation of the summer trial.

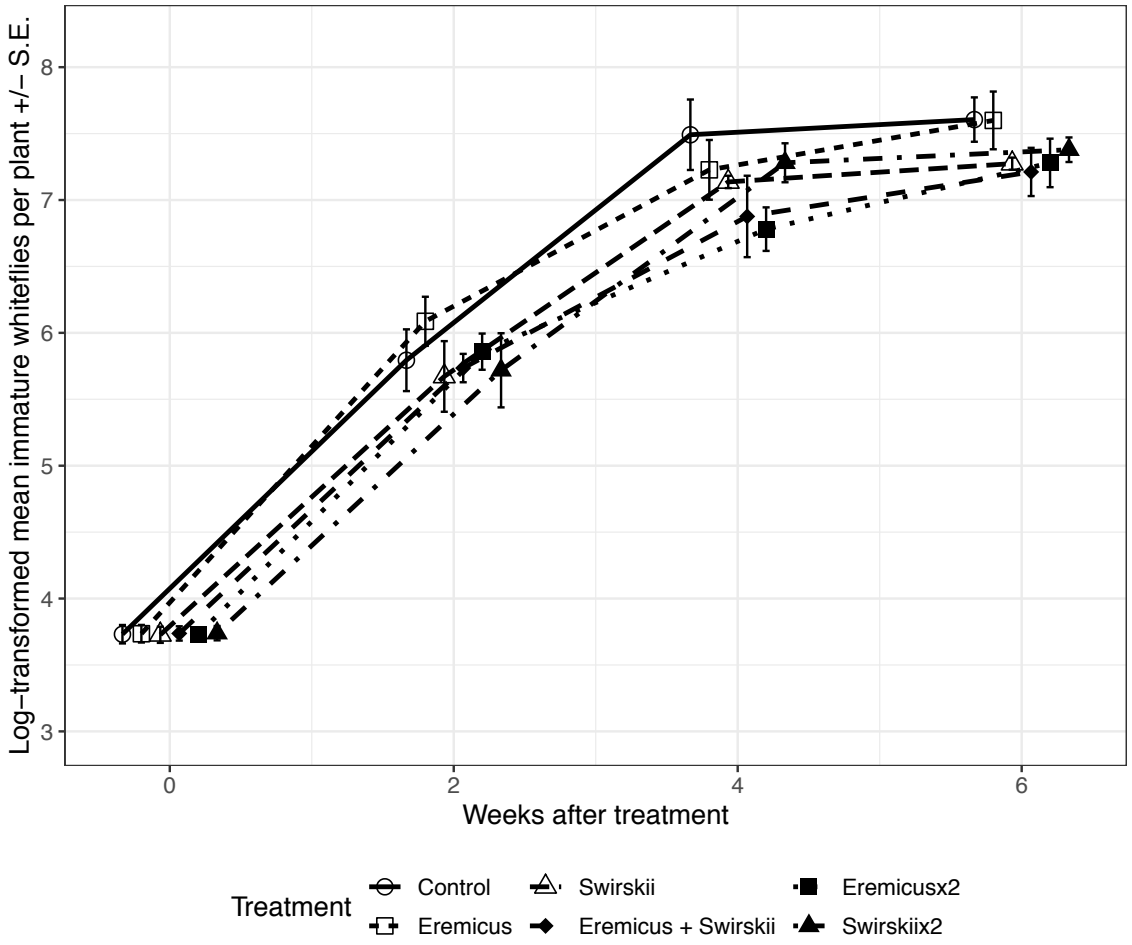

Figure 3.2 Log-transformed mean immature whiteflies per plant (±SE) for each treatment over 6-weeks for the summer trial. Number of immature whiteflies were summed to plant level and six poinsettias (subsamples) per cage (experimental unit) averaged prior to plotting. A remedial application of pymetrozine was made to all plants in all treatments at week 4. Samples were collected within two days, but symbols are offset to better discern different treatments.

Mean number of *A. swirskii* per cage increased over the duration of the trial for treatments where *A. swirskii* were introduced ($F_{1,113}$=25.51, P<0.001; Figure 3.3), and no week by treatment interaction ($F_{1,113}$=0.06, P=0.940). The number of *E. eremicus* adults observed within cages during the trial was very low; mean (± SE) between 2.1 ± 0.8 to 4.4

± 1.3 adults encountered per cage by week six for *E. eremicus* treatments (Figure 3.4). Occasional contamination (*E. eremicus* and *A. swirskii* in cages where they were not released) was observed, but contamination levels were considered negligible compared to quantities of natural enemies found in their own respective treatments.

There was no significant interaction between treatment and temporal block (spring and fall) on final plant aesthetic score ($F_{5,36}$=0.42, P=0.829; Table 3.4). Removing the interaction, there was no significant difference in final plant aesthetic score between the different treatments ($F_{5,36}$=1.63, P=0.174), however, there was a significance difference by trial block (spring and fall; $F_{1,36}$=18.67, P<0.001; Table 3.4).

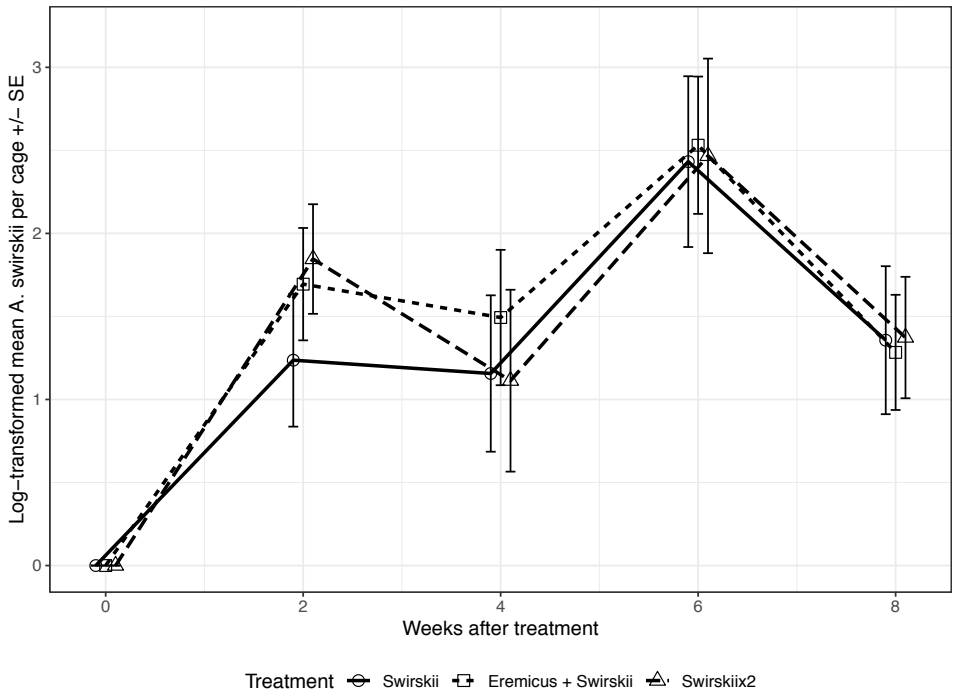

Figure 3.3 Log-transformed mean *A. swirskii* per cage (± SE) for each *A. swirskii* treatment over 8 weeks for the spring and fall trial. Number of *A. swirskii* was first summed to the level of experimental unit (cage) prior to plotting. Samples were collected within two days, but symbols are offset to better discern different treatments.

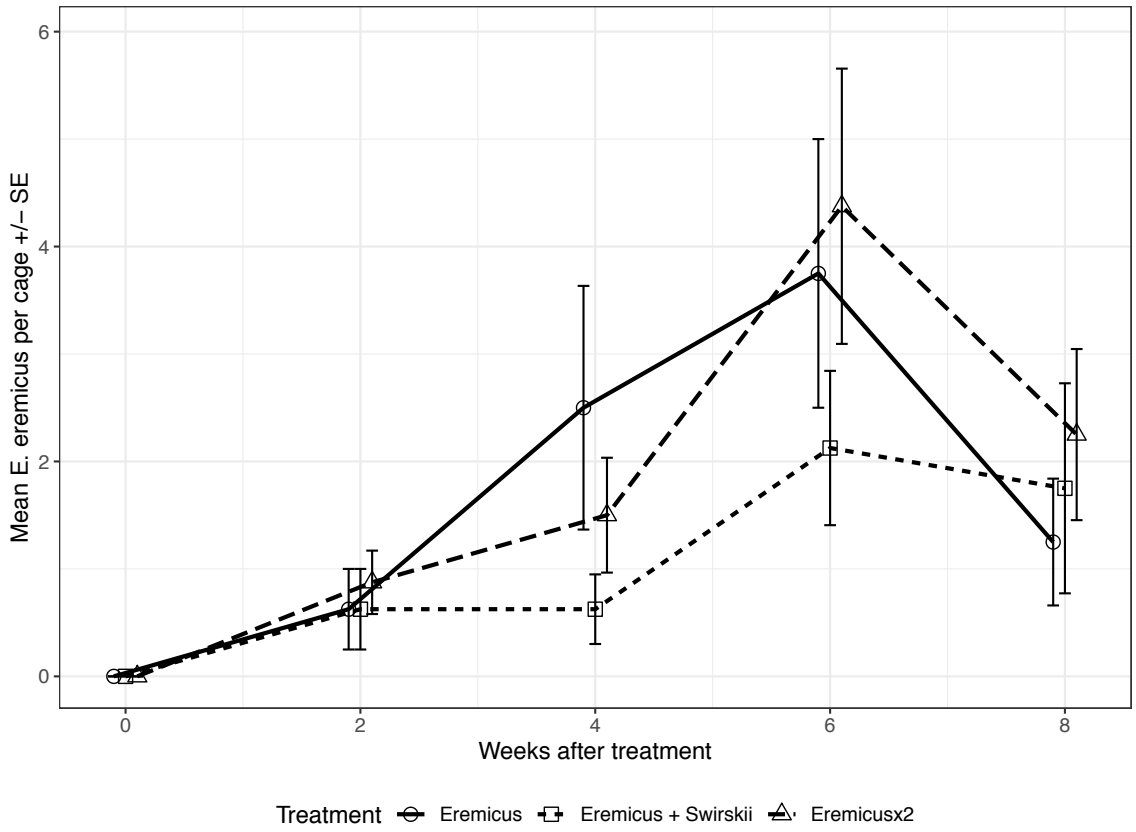

Figure 3.4 Mean *E. eremicus* per cage (± SE) for each *E. eremicus* treatment over 8 weeks for the spring and fall trial. Number of *E. eremicus* was first summed to the level of experimental unit (cage) prior to plotting. Samples were collected within two days, but symbols are offset to better discern different treatments.

Table 3.4 Total number of cages with visible signs of whiteflies, sooty mold, dead plants, plant stunting or lack of uniformity, leaf curling, leaf yellowing, and canopy thinning based on photos taken of poinsettias at the end of the spring and fall trials.

		Total cages with apparent signs of the following qualities								
Block	Treatment	Whiteflies	Sooty mold	Dead plants	Stunting	Leaf curling	Leaf yellowing	Canopy thinning	Mean final aesthetic score (0 - 9) (± SE)	N
Spring	Control	4	3	0	3	3	4	1	2.8 ± 0.9	4
	E. erem.[1]	4	1	0	4	2	4	0	4 ± 0.7	4
	A. swir.[2]	2	3	3	4	1	4	0	4 ± 1.3	4
	E. erem. + *A. swir.*	1	1	1	1	2	3	1	6 ± 2	4
	E. erem. 2x	1	1	2	2	2	4	1	5.2 ± 1.8	4
	A. swir. 2x	1	1	1	1	2	4	0	6 ± 1.4	4
Fall	Control	2	3	0	2	0	0	1	5.8 ± 1.1	4
	E. erem.	1	0	0	4	0	0	0	7.5 ± 0.5	4
	A. swir.	0	0	0	3	0	0	1	8 ± 0.4	4
	E. erem. + *A. swir.*	0	0	0	3	0	0	1	8 ± 0.4	4
	E. erem. 2x	1	0	0	4	0	0	0	7.5 ± 0.5	4

Aesthetic score (0 – 9) was estimated for each cage separately based on scores from the photographs and the mean (± SE) was calculated for each treatment within each trial block (spring and fall). Final aesthetic score was not statistically significant between treatments, but was significant between trial block (spring and fall; $F_{1,36}=16.60$, $P<0.001$).

[1]*E. eremicus*

[2]*A. swirskii*

3.5. Discussion

The lack of increased *B. tabaci* suppression despite the increased release rate of conspecifics (*E. eremicus* or *A. swirskii*) is not the first time this phenomenon has been observed for suppression of hemipterans (Crowder 2007). Density-dependent competition is frequently higher between conspecifics due to increased exploitative competition, whereas heterospecifics can utilize different feeding niches (Chesson 2000, Adler et al. 2018, Snyder 2019), commonly referred to as niche partitioning. Despite observing decreased egg-to-adult survivorship when number of female *E. eremicus* was tripled in greenhouse poinsettia trials, Hoddle et al. (1999) observed densities of immature whiteflies were more suppressed in the lower *E. eremicus* release rate greenhouse, attributed to high levels of parasitoid reproduction due to higher whitefly availability. The lack of increased suppression by increasing the rate of either *E. eremicus* or *A. swirskii* is valuable information to optimize economics for biological control practitioners; however, experimental evidence on life-history traits and interactions between biological control agents is currently lacking to make accurate predictions about optimal rates, timing, and species released (Plouvier and Wajnberg 2018).

The similarity in *B. tabaci* suppression by a single natural enemy species compared to the combination of the two natural enemies suggests a lack of interference competition between *E. eremicus* and *A. swirskii*, which may be explained by resource partitioning; *A. swirskii* attacks eggs and first instar nymphs (Nomikou et al. 2004), whereas *E. eremicus* prefers second and third instar nymphs (Bográn et al. 2002, Liu et al. 2015). Natural enemy composition can also alter resource allocation patterns to decrease exploitation competition (Bográn et al. 2002); however, the lack of differences between natural enemy treatments

on proportion of *B. tabaci* on the upper canopy did not support vertical niche stratification for *E. eremicus* and *A. swirskii*. Finding similar prey suppression by single species and multiple species of natural enemies means that the natural enemies are substitutable (Sih et al. 1998); equivalent management can be acquired by either our 2x rate natural enemy releases or the combination of the two natural enemies. The combination of *E. eremicus* and *A. swirskii* had a trend towards greater whitefly immature and pupae suppression compared to the 2x rates of a single natural enemy, supporting that the combination of the natural enemies was at least equivalent to single natural enemy species releases for whitefly suppression. The added benefit of adding *A. swirskii* to *E. eremicus* in a biological control program in poinsettias is the added target pests that *A. swirskii* can suppress, such as thrips (Seiedy et al. 2017), which are also considered an occasional pest on poinsettias. Without *A. swirskii*, outbreaks of thrips may be more common and require tandem pesticide applications in conjunction with natural enemy releases.

Ability for any natural enemy treatment to suppress *B. tabaci* was density dependent. When the number of immature whiteflies was below a mean (± SE) of 13.7 ± 1.7 immatures per plant as in the spring and fall trials, all natural enemy treatments were able to significantly suppress whitefly population growth. Although whitefly populations still increased in all treatments, it should be noted that the initial whitefly density was substantially higher than the maximum 0.33 ± 0.13 nymphs per plant that has been historically observed on rooted cuttings in the region (Vafaie, Pemberton, Gu, Kerns, et al. 2020b) and whether the combination natural enemy treatment would provide acceptable suppression in commercial production of poinsettias has yet to be seen. We found our release rates provided negligible suppression of the whiteflies in the summer trial when the

starting densities were above a mean (± SE) of 40.8 ± 2.5 immatures per plant. Additional insecticides applied prior to or in tandem with the natural enemies may have decreased whitefly densities to sufficiently low levels for suppression by natural enemies (Gentz et al. 2010) and needs to be explored further.

Our *E. eremicus* emergence rate and female ratio were similar to past published literature (Van Driesche et al. 1999a), however, we were faced with two challenges for biological control: high temperatures and delays in natural enemy shipments. The warmer climate of sub-tropical regions is considered a challenge for augmentative biological control in protected culture (van Lenteren and Bueno 2003). With our temperatures reaching as high as 49 ºC in the spring trial and upper temperatures reaching 35ºC consistently, our results support previous work that *E. eremicus* and *A. swirskii* may be suitable natural enemies for suppression of *B. tabaci* in warmer climates (Greenberg et al. 2000, Qiu et al. 2004, Lee and Gillespie 2011).

In addition to exceptionally high temperatures, we experienced delayed shipments from the insectary on a few occasions, which could have been attributed to communication error with the vendor, internal communication errors at the vendor, or lack of natural enemies due to contamination, population crashes, or unanticipated high demand. Although the importance of sequence of introduction in multi-species interactions on predator-prey dynamics has been investigated (Sait et al. 2000), very few studies have determined the impact of delayed natural enemy releases in the middle of an augmentative biological control program on pest suppression. The impact of delayed releases of single compared to multiple natural enemies for *B. tabaci* suppression needs further investigation.

In conclusion, if starting whitefly populations are below 13.7 ± 1.7 immatures per plant, *E. eremicus* and *A. swirskii*, in combination or alone, can cause significant suppression of whiteflies when released at rates economically comparable to insecticide inputs. Although no significant improvement in suppressing whitefly populations was observed when adding *A. swirskii* to *E. eremicus*, the benefit may be more evident in the face of secondary pest outbreaks, such as thrips. Trials on a commercial scale with realistic starting whitefly densities and environmental conditions will be vital to determine whether the combination of the two natural enemies can compare to conventional insecticide inputs in both cost and ability to reduce whiteflies below marketable thresholds.

3.6. References

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4. INCREASING NATURAL ENEMY DIVERSITY TO RESPOND TO UNPLANNED CHALLENGES TO AUGMENTATIVE BIOLOGICAL CONTROL

4.1. Overview

Whether to increase natural enemy diversity or increase density of conspecifics for pest suppression in greenhouse augmentative biological control is currently unknown. In this study, we use sweetpotato whiteflies, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), on poinsettias, *Euphorbia pulcherrima* Willd. ex Klotzsch (Malpighiales: Euphorbiaceae), to determine whether the combination of *Eretmocerus eremicus* Rose & Zolnerowich (Hymentopera: Aphelinidae) and *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) is better for *B. tabaci* suppression compared to either natural enemy alone, and determine whether the combination treatment will maintain whitefly suppression when challenged with whitefly immigration or delayed natural enemy releases. The number of whiteflies on caged poinsettias treated with different natural enemy release rates (single or double rate), natural enemy diversity (one or two species), natural enemy delayed release (weeks 4 and 8), and whitefly immigration treatments (introduced at week 4 or week 8) were censused biweekly for 16 weeks. Increasing natural enemy diversity provided similar or better suppression of whiteflies compared to either natural enemy alone. Increasing natural enemy diversity also provided superior suppression of whiteflies when challenged with whitefly immigration or delays in natural enemy releases. Whitefly immigration or delays in *E. eremicus* releases did not increase whitefly populations, suggesting that suppression of whiteflies by *E. eremicus* alone is relatively robust. This study found no evidence for negative interactions between *E. eremicus* and *A. swirskii* for suppressing *B.*

tabaci and supports the use of multiple natural enemies in biological control instead of increasing density of natural enemy conspecifics for *B. tabaci* suppression.

4.2. Introduction

Seasonal inoculative biological control, the periodic release of natural enemies to manage pests below threshold in a short-term crop (van Lenteren 2000a), has become an increasingly attractive strategy for pest management in protected culture compared to conventional insecticide use due to pesticide resistance, absence of plant phytotoxic effects, lack of re-entry or pre-harvest intervals, and consumer demand (van Lenteren 2000b). However, immigration from neighboring crops is considered a major ecological factor limiting the success of augmentative biological control of pests in open plots (Bellamy et al. 2004, Collier and Van Steenwyk 2004, Liu et al. 2015), inspiring the need for insect screens to reduce movement of pests into greenhouses (Bethke and Paine 1991b, Bell and Baker 2009). Insect screens can also present challenges by requiring a sufficiently small screen hole size to prevent target pest immigration and added upfront costs, which can become obstacles to adoption by practitioners. Additionally, decreased natural enemy availability due to delayed shipments are not uncommon from different natural enemy suppliers due to challenges or errors anywhere along the communication and supply-chain: client-insectary miscommunication, insectary internal miscommunication, crash/collapse of insect cultures at the insectary, sudden unanticipated increased demand in natural enemies, delays or incorrect shipping. Even with direct communications with suppliers, shipments were delayed three times in a recent study (Vafaie, Pemberton, Gu, Kerns, et al. 2020c). Poorly timed natural enemy releases can result in ineffective pest management

(Collier and Van Steenwyk 2004, Tang et al. 2010). Previous studies predicted the impact of decreased natural enemy availability using model simulations; finding that successful management of leaf-miner populations before the end of the crop was unlikely when natural enemy releases were delayed more than 14 days (Heinz et al. 1993).

More recently, however, studies have investigated the use of generalist natural enemies and increasing natural enemy diversity to provide effective pest suppression that is more robust to pest immigration and variation in timing of releases of natural enemies (Messelink et al. 2012), where robustness is defined as "the capacity of a system to maintain a desired state despite fluctuations in the behavior of its component parts or its environment" (Mumby et al. 2014). In this study, we use sweetpotato whiteflies, *B. tabaci,* on poinsettias, *E. pulcherrima*, as the model system to determine whether adding the generalist predator, *A. swirskii*, to a specialist parasitic wasp, *E. eremicus*, will increase suppression of whiteflies despite being challenged with whitefly immigration or decreased natural enemy availability (i.e. delayed releases of natural enemies).

Poinsettia is an ornamental plant that is grown as potted annuals available from late-October to mid-December in North America. The main pest of poinsettias in the Southern United States of America is *B. tabaci*, with less common but also problematic pests including thrips (Thysanoptera: Thripidae), mealybug (Hemiptera: Pseudococcidae), and fungus gnat species (Diptera: Sciaridae). Published literature has focused on management of *B. tabaci* on poinsettias through releases of parasitic wasps, with *E. eremicus* showing the best economics and suppression in greenhouse settings (Hoddle and Van Driesche 1999b, 1999c, Van Driesche et al. 1999a, Van Driesche, Hoddle, Lyon, and Sanderson 2001); however, *A. swirskii* has demonstrated efficacy against *B. tabaci* in

greenhouse trials on other host plants more recently (Bolckmans et al. 2005, Calvo et al. 2012, 2015).

The characteristics of *A. swirskii*, such as longer lifespan, "standing army approach" strategy (Messelink et al. 2016), and feeding of different life stages than *E. eremicus*, may complement suppression of *B. tabaci* by *E. eremicus* when challenged with whitefly immigration and decreased natural enemy availability. Timing of releases of generalist predators have also been considered more forgiving compared to timing of specialist natural enemies due to longer lifespans, the ability to utilize alternative prey, and the ability of omnivorous generalist natural enemies to sustain themselves by consuming alternative foods such as pollen or nectar (Messelink et al. 2012). When given the choice to increase the quantity of natural enemies released or increase natural enemy diversity to provide more reliable pest suppression, practitioners have very few published studies to rely on.

In this study, we investigate the following objectives:

1. The effect of natural enemy composition (*E. eremicus* and *A. swirskii*, alone or in combination) on whitefly suppression.
2. Whitefly suppression by *A. swirskii* added to *E. eremicus* when challenged with early and late whitefly immigration.
3. Whitefly suppression by *A. swirskii* added to *E. eremicus* when challenged with decreased natural enemy availability.

4.3. Materials and Methods

4.3.1. Cultures

Bemisia tabaci colonies were collected and maintained at the Texas A&M AgriLife IPM Greenhouse in Overton, Texas as described in Vafaie et al. (2020). Prior to poinsettia infestation, four subsamples of 14 – 20 adult *B. tabaci* were collected in 95% ethanol and sent to Dr. Cindy McKenzie (United States Department of Agriculture – Agricultural Research Service, Fort Pierce, FL) for DNA barcoding using mitochondrial cytochrome c oxidase I (*mt*COI) as described in Shatter et al. (2009).

Unrooted 'Prestige Early Red' poinsettia cuttings were received on January 24th (2019) from Dümmen Orange (Coldenhovelaan 6, The Netherlands) and rooted as described in Vafaie et al. (2020). After rooting, cuttings were potted into pots (15 cm diameter x 10 cm deep) on February 24th using BM6 Custom Blend Potting Soil (Berger, Saint-Modeste, QC, Canada) and were continually fertigated through drip irrigation at 200 ppm nitrogen (Peters Professional 20-10-20 General Purpose Fertilizer, ICL Specialty Fertilizers, St. Louis, MO). On both March 11th and March 18th, poinsettias were infested with an average of 2 whitefly adults per pot by releasing two vials with 9 adult whiteflies each per row of 18 poinsettias (3 trays of 6 poinsettias) from our *B. tabaci* colony. Poinsettias were pinched on March 14th, leaving 5 – 7 internodes on each plant.

4.3.2. Experimental Design

On May 15th, we arranged 12 treatments (Table 4.1) in cubic cages (47.5 cm x 47.5 cm x 47.5 cm cages, BugDorm4454F, MegaView Science Co., Ltd., Taiwan) replicated 10 times each in a completely randomized complete block design on benches in two adjacent

greenhouse compartments to test three main hypotheses: i) an increase in natural enemy diversity will provide similar or superior whitefly suppression compared to either natural enemy alone, ii) an increase in natural enemy diversity will provide superior whitefly suppression when challenged with whitefly immigration (early or late immigration) compared to *E. eremicus* alone, and iii) an increase in natural enemy diversity will provide superior whitefly suppression when challenged with decreased natural enemy availability compared to *E. eremicus* alone. Treatments for objective 1 included 1) untreated control, 2) *E. eremicus* (1x rate), 3) *A. swirskii* (1x rate), 4) *E. eremicus* and *A. swirskii* combined, 5) *E. eremicus* (2x rate), and 6) *A. swirskii* (2x rate). The double rate of conspecifics treatments (Table 1; Obj. 1 #5 – 6) were included to control for intraspecific and interspecific interactions as described by Sih et al. (1998). Treatments for objective 2 were in a 2 x 2 factorial design for a total of four treatments, with two levels for natural enemy diversity (*E. eremicus* alone or *A. swirskii* added to *E. eremicus*) and two levels for whitefly immigration (8 adult whiteflies released per cage at week 4 or week 8 of the trial). Lastly, treatments for objective 3 were *E. eremicus* alone or *A. swirskii* added to *E. eremicus* with one-week delays in natural enemy releases at week 4 and 8 of the trial for both treatments. Each cage consisted of two poinsettias, spaced at approximately 25.5 cm centers. *Bemisia tabaci* nymphs and pupae were counted on all leaves (4 – 6) from one poinsettia per cage and treatments were assigned accordingly to start with similar mean whitefly populations between all treatments.

Table 4.1 Natural enemy release week, rate, and associated objective (obj.) for all treatments.

Objective	#	Treatment	Release weeks														
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	1	Untreated control															
1	2	*E. eremicus*															
	3	*A. swirskii*															
	4	*E. eremicus* + *A. swirskii*															
	5	*E. eremicus* 2x															
	6	*A. swirskii* 2x															
2	7	*E. eremicus* immigration week 4					*										
	8	*E. eremicus* + *A. swirskii* immigration week 4					*										
	9	*E. eremicus* immigration week 8									*						
	10	*E. eremicus* + *A. swirskii* immigration week 8									*						
3	11	*E. eremicus* delayed release															
	12	*E. eremicus* + *A. swirskii* delayed release															

Natural enemy release rates denoted by light grey (1X) and dark grey (2X) shading. The 1X *E. eremicus* rate was 47.3 ± 0.6 pupae and 2x rate was 91.1 ± 3.6 pupae released per cage. The 1X *A. swirskii* was 2.5 mL (~125 mites) and the 2X rate was 5 mL (~250 mites) mixed in bulk bran material spread over the two plants within the cage. For the whitefly immigration treatments, 8 adult whiteflies were added per cage to simulate early (immigration week 4) or late 8 (immigration week 8) immigration of whiteflies, denoted with asterisks. Delayed release treatments had natural enemy releases delayed by one week on weeks 4 and 8, and releases were subsequently added to the following week.
*Release 8 adult whiteflies in each cage.

4.3.3. Natural Enemy Release Rates

For the 1x rate treatments, *E. eremicus* pupa cards from Koppert Biological Systems (Howell, MI) were cut in half for approximately 30 pupae per cage, whereas intact cards (~60 pupae) were used for 2x rates. Actual number of pupae released per cage was quantified by counting the number of non-emerged pupae before placing them in cages and counting the number of non-emerged/dead pupae two weeks after release using a dissecting microscope. For *A. swirskii*, the carrier material was first thoroughly mixed in a Tupperware container before measuring out 2.5 mL (~125 mites) per cage for the 1x rate and 5 mL (~250 mites) for the 2x rate. Carrier material was spread evenly over both poinsettias within each cage. Treatment details are summarized in Table 4.1.

4.3.4. Data Collection

After the start of the trial, number of *B. tabaci* nymphs, pupae, and adults were counted every fortnight on four lower and four upper leaves from one poinsettia per cage. At week 14 after initial natural enemy release, poinsettias in all cages were photographed and aesthetic rating assessed based on the following criteria: noticeable canopy thinning, presence of foliar fungal pathogen(s), leaf curling/deformation, presence of sooty mold, and yellowing of leaves. For each photo, the aforementioned characteristics were marked as “Yes” (=1) or “No” (=0) and all scores subtracted from a perfect score of 5. The trial lasted for 16 weeks (July 15, 2019), although many of the poinsettias, especially the untreated control, were in great decline before the final assessment.

At the end of the trial, leaves with at least 10 exuviae from cages with *E. eremicus* (alone or in combination with *A. swirskii*) were removed and inspected under a dissecting

microscope to determine parasitization rate calculated as *E. eremicus* exuviae/(*E. eremicus* exuviae + *B. tabaci* exuviae). Parasitized exuviae can be distinguished due to their clean circular emergence hole compared to *B. tabaci* exuviae, which have a "T"-shaped emergence slit (McAuslane and Smith 2015). Temperature and relative humidity data were recorded at 30-minute intervals using a Hobo data logger (U23 Pro V2, Onset Computer Corporation, Bourne, MA) placed in the middle of both greenhouse compartments (Table 2).

4.3.5. Statistical Analyses

All statistical analyses were performed in R v.3.5.3 (RCore Team, 2019) using RStudio (RStudio Team, 2015). Additional packages used in addition to the R base were: lme4 (Bates et al. 2015), lmerTest (Kuznetsova et al. 2017), tidyverse (Wickham 2017), and emmeans (Lenth 2019). All whitefly count data were summed to the cage level and were normalized using log transformation (log(x+1)). By week 12, only 4 poinsettias from the untreated control remained alive, whereas all the other treatments had majority of poinsettias alive by week 14. Due to the high plant mortality in the poinsettias in the untreated control, two separate statistical models were used: one that only included data until week 12, specifically for determining the effect of the natural enemy treatments compared to the untreated control, and the other including data up till week 14 and did not include the untreated control, that was used for all natural enemy treatments pair-wise comparisons. Statistical model used for whitefly count data was a linear mixed-effect model using restricted maximum likelihood (REML) (Bolker et al. 2009), with treatment and week as the fixed effects, cage as the random effect, and whitefly counts as the

response variable. Separate statistical models were constructed for each whitefly life stage: nymphs, pupae, and adults. Plant aesthetic scores were compared using Kruskal-Wallis with Dunn's test for multiple comparisons with false discovery rate controlled by Benjamini-Hochberg adjustment (Benjamini and Hochberg 1995).

Mean, minimum and maximum daily temperature and relative humidity were summarized to two-week intervals that corresponded to our whitefly count dates. Values from assessment date and two weeks prior were used for each time interval in calculating mean, minimum and maximum.

To determine whether natural enemy treatments were effective at suppressing whitefly populations during the first 12 weeks, we performed a priori contrasts to compare the untreated control to natural enemy release treatments 2 – 6 (*E. eremicus* and *A. swirskii*, alone or in combination). To determine if increasing natural enemy diversity increases whitefly suppression, we performed a priori contrasts comparing the combination treatment (*E. eremicus* and *A. swirskii*) to the 2x rate of either natural enemy alone (substitutive model). Additionally, we conducted a priori contrasts to determine intraspecific interactions (1x vs 2x rate of the same natural enemy) on whitefly suppression.

To determine the effect of whitefly immigration on whitefly suppression by natural enemies, we performed a priori contrasts comparing immigration treatments to their non-immigration counterparts: a) *E. eremicus* early and late immigration vs *E. eremicus* treatment and b) *E. eremicus* and *A. swirskii* combination treatment early and late immigration vs *E. eremicus* and *A. swirskii* treatment. To determine if the addition of *A. swirskii* to *E. eremicus* provides better suppression of whiteflies when challenged with

early or late whitefly immigration, we performed three a prior contrasts: a) *E. eremicus* early whitefly immigration vs *E. eremicus* and *A. swirskii* combination treatment early immigration, b) *E. eremicus* late whitefly immigration vs *E. eremicus* and *A. swirskii* combination treatment late immigration, and c) *E. eremicus* whitefly immigration (early and late) vs *E. eremicus* and *A. swirskii* combination treatment immigration (early and late).

To determine the effect of delayed natural enemy release on whitefly suppression, we performed a priori contrasts comparing the delayed release treatments with their non-delayed counterparts: a) *E. eremicus* delayed release treatment vs *E. eremicus* treatment and b) *E. eremicus* and *A. swirskii* combination delayed release treatment vs. *E. eremicus* and *A. swirskii* combination treatment. To determine if the addition of *A. swirskii* to *E. eremicus* provides better suppression compared to *E. eremicus* alone when challenged with delayed releases of natural enemies, we also performed an a priori contrast comparing *E. eremicus* delayed release treatment with *E. eremicus* and *A. swirskii* combination delayed release treatment.

4.4. Results

All four *B. tabaci* samples were confirmed as 100% *B. tabaci* MEAM1 (B biotype). Temperature range was between 13ºC to 44ºC and relative humidity between 22% and 95% for the duration of the trial (Table 4.2). Temperature and relative humidity generally increased from the beginning until the end of the trial.

Table 4.2 Mean, minimum, and maximum daily temperature and humidity data summarized to two-week intervals related to sampling times.

Week	Mean temperature (C) (min - max)	Mean relative Humidity (%) (min - max)
2	21 (15 - 37)	59 (26 - 89)
4	22 (15 - 42)	60 (22 - 92)
6	23 (13 - 37)	78 (41 - 95)
8	23 (13 - 36)	79 (44 - 95)
10	27 (18 - 41)	76 (40 - 95)
12	26 (15 - 44)	74 (34 - 95)
14	28 (19 - 42)	78 (41 - 94)
16	29 (22 - 41)	78 (47 - 95)

Temperature and humidity data were logged using a Hobo data logger (U23 Pro V2, Onset Computer Corporation, Bourne, MA) in 30-minute intervals.

Mean ± SE number of *E. eremicus* pupae released in 1x and 2x rate cages throughout the trial was 47.4 ± 0.2 and 88.5 ± 0.2, respectively (Table 4.3). After subtracting the number of pupae from which no adults emerged two weeks later from the number of pupae initially released, mean ± SE number of *E. eremicus* adults that emerged within 1x and 2x rate cages was 36.7 ± 0.7 and 69.2 ± 4.2, respectively (Table 4.3).

Table 4.3 Number of *E. eremicus* pupae placed in 1X and 2X treatment cages, pupae recovered, and percent emergence.

Release rate	Mean pupae released (± SE)	Mean pupae recovered (± SE)	Mean percent emergence (± SE)	N
1	47.3 ± 0.6	10.5 ± 0.6	77.7 ± 1.3	13
2	91.1 ± 3.6	21.9 ± 1.8	74.3 ± 3.2	13

Number of *E. eremicus* pupae were counted before placing inside cages and were removed two weeks later to quantify number of pupae recovered. Mean pupae released, mean pupae recovered, and mean percent emergence were averaged for all 1X and 2X treatment cages for each week sampled (subsamples) prior to averaging over the duration of the experiment.

By week 16, the median percentage of exuviae from *E. eremicus* was between 0 and 80% (Table 4.4). The number of leaves with at least 10 exuviae (*B. tabaci* and *E. eremicus* combined) was limited in *E. eremicus*-released cages, resulting in a lack of samples and statistical power for further analysis.

4.4.1. Model Significance with and without Untreated Control

As described above, the first model for data analysis included all treatments through week 10 due to high plant mortality by week 12 in the untreated controls. Number of whitefly nymphs, pupae, and adults were significantly different between treatments ($F_{11,108} = 11.52$) and week ($F_{1,586} = 17.62$), and population growth over time was significantly different between treatments (treatment x week interaction; $F_{11,586} = 16.46$) ($p<0.001$ for all life stages, factors, and interaction). The population growth of whitefly nymphs, pupae, and adults were significantly lower for all natural enemy treatments compared to the untreated control for the duration of the trial (Figure 4.1; Table 4.5 – 4.7).

For the second model (untreated control excluded and up to week 14 included), number of whitefly nymphs, pupae, and adults were significantly different between treatments ($F_{10,99} = 9.89$) and week ($F_{1,745} = 58.46$), and population growth over time was significantly different among treatments (treatment x week interaction; $F_{10,745} = 26.52$) ($p<0.001$ for all life stages, factors, and interaction).

Table 4.4 Parasitization rate for *E. eremicus* in *E. eremicus* alone and *E. eremicus* and *A. swirskii* combination treatments at the end of the trial.

Treatment	Mean exuviae (+/- SE)	Median percentage *E. eremicus* exuviae (min - max)	N
E. eremicus	33.8 ± 10.2	80 (70 - 90)	6
E. eremicus + *A. swirskii*	37.6 ± 17.7	10 (0 - 30)	5
E. eremicus 2x	19	0	1
E. eremicus immigration week 4	32 ± 10.9	70 (20 - 80)	4
E. eremicus + *A. swirskii* immigration week 4	9.2 ± 4	80 (30 - 80)	5
E. eremicus immigration week 8	14 ± 3.7	70 (20 - 90)	4
E. eremicus + *A. swirskii* immigration week 8	11	30	1
E. eremicus delayed release	46.5 ± 21.5	60 (30 - 100)	2
E. eremicus + *A. swirskii* delayed release	27.3 ± 19.4	40 (30 - 90)	3

Only leaves with 10 or more exuviae (one leaf per cage, maximum) were inspected under a dissecting microscope for *E. eremicus* emergence hole. Rate of parasitization calculated as *E. eremicus* exuviae/total exuviae. Low replications due to lack of plants with more than 10 exuviae per leaf.

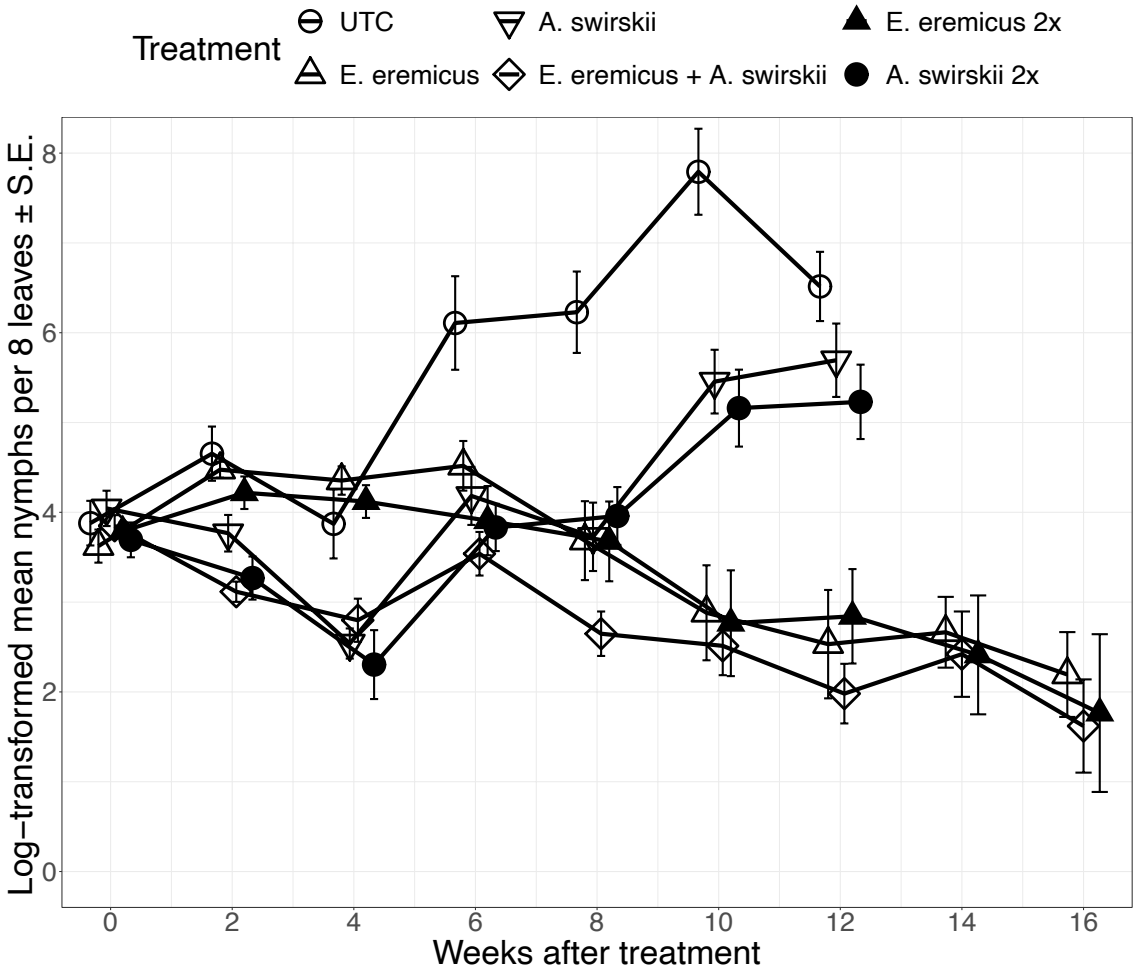

Figure 4.1 Mean log-transformed nymphs (± SE) counted on 8-leaves per cage for objective 1: untreated control (UTC), *E. eremicus* alone (1X and 2X rates), *A. swirskii* alone (1X and 2X rates), and *E. eremicus* and *A. swirskii* combination treatments (n=10) over 16 weeks. Untreated control and *A. swirskii* treatment counts ended early due to high plant mortality. All natural enemy treatments combined provided suppression of whiteflies compared to the untreated control for data including week 12 (P < 0.001). Increase rate of conspecifics did not result in increased whitefly suppression for *E. eremicus* or *A. swirskii* (P = 0.689 and P = 0.517, respectively). The combination treatment (*E. eremicus* and *A. swirskii*) provided increased whitefly suppression compared to both *E. eremicus* and *A. swirskii*, alone (P = 0.038 and P < 0.001, respectively).

4.4.2. Natural Enemy Composition | Objective 1

Overall, the combination of *E. eremicus* and *A. swirskii* consistently provided similar or superior suppression of whiteflies compared to either species alone (Figure 4.1; Tables 4.5 – 4.7). The single and double rate of *A. swirskii* provided similarly strong suppression of whiteflies early in the experiment (e.g., up to week 6; Figure 4.1), but provided relatively little suppression in weeks 10 and 12. Interestingly, *E. emericus* applied at either rate did not suppress whiteflies early in the experiment (i.e., up to week 4), but were as effective as the *E. erimicus* + *A. swirskii* treatment from week 10 until the end of the experiment (week 16) (Figure 4.1).

4.4.3. Whitefly Immigration | Objective 2

Overall, the combination of *E. eremicus* and *A. swirskii* provided superior suppression of whiteflies when challenged with whitefly immigration compared to *E. eremicus* alone (Figure 4.2; Tables 4.5 – 4.7), with the exception of suppression of whitefly adults with immigration at week 8 (Table 4.7). Immigration of whiteflies (week 4 and week 8) did not result in significant difference in whitefly nymph, pupa, or adult population growth compared to their non-immigration treatment counterparts (Figure 4.2; Tables 4.5 – 4.7).

Table 4.5 A priori contrasts for log-transformed whitefly nymphs for objectives 1 – 3.

Objective	Contrast (a – b)		Estimate	t-ratio	P-value
	a	b			
1	Untreated control	*E. eremicus* *A. swirskii* *E. eremicus* + *A. swirskii* *E. eremicus* 2x *A. swirskii* 2x	1.83	6.838	<0.001
	E. eremicus	*E. eremicus 2x*	0.15	0.401	0.689
	A. swirskii	*A. swirskii 2x*	0.23	0.651	0.517
	A. swirksii	*E. eremicus*	0.97	2.682	0.009
	E. eremicus + *A. swirskii*	*E. eremicus 2x*	-0.76	-2.109	0.038
	E. eremicus + *A. swirskii*	*A. swirskii 2x*	-1.64	-4.550	<0.001
2	*E. eremicus*	*E. eremicus* imm.W4 *E. eremicus* imm.W8	-0.19	-0.607	0.545
	E. eremicus + *A. swirskii*	*E. eremicus* + *A. swirskii* imm.W4 *E. eremicus* + *A. swirskii* imm.W8	0.37	1.179	0.241
	E. eremicus imm.W4	*E. eremicus* + *A. swirskii* imm.W4	1.52	4.234	<0.001
	E. eremicus imm.W8	*E. eremicus* + *A. swirskii* imm.W8	1.40	3.869	<0.001
	E. eremicus imm.W4 *E. eremicus* imm.W8	*E. eremicus* + *A. swirskii* imm.W4 *E. eremicus* + *A. swirskii* imm.W8	1.46	5.729	<0.001
3	*E. eremicus*	*E. eremicus* DR	-0.18	-0.510	0.611
	E. eremicus + *A. swirskii*	*E. eremicus* + *A. swirskii* DR	-0.06	-0.155	0.877
	E. eremicus DR	*E. eremicus* + *A. swirskii* DR	1.03	2.863	0.005

Untreated control vs. all natural enemy treatments contrast conducted using only up to week 12 linear mixed model with untreated control, whereas all other contrasts based on model including week 14 without untreated control. Different natural enemy combinations (Objective 1) compared to determine potential intraspecific and interspecific interactions on whitefly suppression.

Table 4.6 A priori contrasts for log-transformed whitefly pupae for objectives 1 – 3.

Objective	Contrast (a – b)		Estimate	t-ratio	P-value
	a	b			
1	Untreated control	*E. eremicus* *A. swirskii* *E. eremicus* + *A. swirskii* *E. eremicus* 2x *A. swirskii* 2x	1.63	6.776	<0.001
	E. eremicus	*E. eremicus 2x*	0.15	0.493	0.623
	A. swirskii	*A. swirskii 2x*	0.20	0.639	0.545
	A. swirksii	*E. eremicus*	0.51	1.644	0.103
	E. eremicus + *A. swirskii*	*E. eremicus 2x*	-0.51	-1.656	0.101
	E. eremicus + *A. swirskii*	*A. swirskii 2x*	-0.98	-3.166	0.002
2	*E. eremicus*	*E. eremicus* imm.W4 *E. eremicus* imm.W8	-0.24	-0.875	0.384
	E. eremicus + *A. swirskii*	*E. eremicus* + *A. swirskii* imm.W4 *E. eremicus* + *A. swirskii* imm.W8	0.39	1.473	0.144
	E. eremicus imm. W4	*E. eremicus* + *A. swirskii* imm.W4	1.43	4.612	<0.001
	E. eremicus imm.W8	*E. eremicus* + *A. swirskii* imm.W8	1.17	3.741	<0.001
	E. eremicus imm.W4 *E. eremicus* imm.W8	*E. eremicus* + *A. swirskii* imm.W4 *E. eremicus* + *A. swirskii* imm.W8	1.30	5.904	<0.001
3	*E. eremicus*	*E. eremicus* DR	-0.32	-1.038	0.302
	E. eremicus + *A. swirskii*	*E. eremicus* + *A. swirskii* DR	0.36	1.158	0.250
	E. eremicus DR	*E. eremicus* + *A. swirskii* DR	1.35	4.337	<0.001

Untreated control vs. all natural enemy treatments contrast conducted using only up to week 12 linear mixed model with untreated control, whereas all other contrasts based on model including week 14 without untreated control. Different natural enemy combinations (Objective 1) compared to determine potential intraspecific and interspecific interactions on whitefly suppression. Whitefly immigration (imm.; objective 2) contrasts whitefly suppression between *E. eremicus* alone and *E. eremicus* and *A. swirskii* combination treatments despite early (W4) and late (W8) immigration of whiteflies into cages. Delayed release (DR) treatments (objective 3) contrast whitefly suppression between *E. eremicus* alone and *E. eremicus* and *A. swirskii* combination treatment, despite delays in natural enemy releases.

Table 4.7 A priori contrasts for log-transformed whitefly adults for objectives 1 – 3.

Objective	Contrast (a – b)		Estimate	t-ratio	P-value
	a	b			
1	Untreated control	*E. eremicus* *A. swirskii* *E. eremicus* + *A. swirskii* *E. eremicus* 2x *A. swirskii* 2x	1.75	9.585	<0.001
	E. eremicus	*E. eremicus 2x*	-0.03	-0.126	0.900
	A. swirskii	*A. swirskii 2x*	0.17	0.681	0.497
	A. swirksii	*E. eremicus*	1.49	6.017	<0.001
	E. eremicus + *A. swirskii*	*E. eremicus 2x*	-0.25	-0.996	0.323
	E. eremicus + *A. swirskii*	*A. swirskii 2x*	-1.53	-6.212	<0.001
2	*E. eremicus*	*E. eremicus* imm.W4 *E. eremicus* imm.W8	-0.27	-1.245	0.216
	E. eremicus + *A. swirskii*	*E. eremicus* + *A. swirskii* imm.W4 *E. eremicus* + *A. swirskii* imm.W8	0.10	0.479	0.633
	E. eremicus imm.W4	*E. eremicus* + *A. swirskii* imm.W4	0.75	3.013	0.003
	E. eremicus imm.W8	*E. eremicus* + *A. swirskii* imm.W8	0.43	1.711	0.090
	E. eremicus imm.W4 *E. eremicus* imm.W8	*E. eremicus* + *A. swirskii* imm.W4 *E. eremicus* + *A. swirskii* imm.W8	0.59	3.338	0.001
3	*E. eremicus*	*E. eremicus* DR	-0.38	-1.522	0.131
	E. eremicus + *A. swirskii*	*E. eremicus* + *A. swirskii* DR	0.06	0.251	0.803
	E. eremicus DR	*E. eremicus* + *A. swirskii* DR	0.65	2.641	0.010

Untreated control vs. all natural enemy treatments contrast conducted using only up to week 12 linear mixed model with untreated control, whereas all other contrasts based on model including week 14 without untreated control. Different natural enemy combinations (Objective 1) compared to determine potential intraspecific and interspecific interactions on whitefly suppression. Whitefly immigration (imm.; objective 2) contrasts whitefly suppression between *E. eremicus* alone and *E. eremicus* and *A. swirskii* combination treatments despite early (W4) and late (W8) immigration of whiteflies into cages. Delayed release (DR) treatments (objective 3) contrast whitefly suppression between *E. eremicus* alone and *E. eremicus* and *A. swirskii* combination treatment, despite delays in natural enemy releases.

Table 4.8 Median (min. – max.) plant aesthetic score at week 14.

Treatment	Sum of plants with specified characteristic					Median Aesthetic Score (min. - max.)
	Canopy Thinning	Fungal pathogen	Curling leaves	Sooty mold	Yellowing Leaves	
Untreated control	9	10	8	10	9	0 (0 - 3)b
E. eremicus	5	6	3	3	3	3 (0 - 5)a
A. swirskii	5	4	3	10	5	2 (1 - 4)ab
E. eremicus + *A. swirskii*	0	6	1	0	2	4 (3 - 5)a
E. eremicus 2x	6	7	6	4	7	2 (0 - 5)ab
A. swirskii 2x	3	9	4	9	5	2 (0 - 5)ab
E. eremicus immigration week 4	6	6	2	3	5	2.5 (1 - 5)a
E. eremicus + *A. swirskii* immigration week 4	1	7	2	0	3	3.5 (2 - 5)a
E. eremicus immigration week 8	2	4	2	1	2	4 (0 - 5)a
E. eremicus + *A. swirskii* immigration week 8	2	3	3	0	3	4 (2 - 5)a
E. eremicus delayed release	5	7	5	5	4	2 (0 - 4)ab
E. eremicus + *A. swirskii* delayed release	2	9	3	1	4	3 (1 - 5)a

For each cage, plants lost aesthetic score points for signs of canopy thinning, signs of plant pathogens, curling leaves, sooty mold, and yellowing leaves. Each cage could receive a top score of 5 (presence of any characteristic results in -1 point) and median was calculated for all cages within a specific treatment. Aesthetic score followed by matching lower case letters are not significantly different according to Kruskal-Wallis with Dunn's test for multiple comparisons with false discovery rate controlled by Benjamini-Hochberg adjustment.

4.4.4. Delayed release | Objective 3

When challenged with delayed releases, the combination of *E. eremicus* and *A. swirskii* significantly reduced whitefly nymph, pupa, and adult populations compared to *E. eremicus* alone (Figure 4.3; Tables 4.5 – 4.7); supporting that an increase in natural enemy diversity provides superior whitefly suppression when challenged with decreased natural enemy availability compared to *E. eremicus* alone. Delayed releases of natural enemies for the *E. eremicus* alone or *E. eremicus* and *A. swirskii* combination treatment did not result in a significant difference in whitefly nymph, pupae, or adult population growth compared to their timely released counterparts (Tables 4.5 – 4.7).

4.5. Discussion

Increasing the quantity of natural enemies released did not increase suppression of *B. tabaci*, which supports our recent work (Vafaie, Pemberton, Gu, Kerns, et al. 2020c) and has been observed in several augmentative biological control programs that target Hemiptera (Hoddle et al. 1999, Crowder 2007). On the other hand, we observed that increasing natural enemy diversity, in a substitutive design, increased suppression of *B. tabaci* nymphs. This increase in suppression may be the first example supporting complementarity between *E. eremicus* and *A. swirskii* in suppressing *B. tabaci* in the published literature, suggesting that for these two natural enemies, increasing species diversity may be more beneficial than doubling the rate of conspecifics. Our recent study (Vafaie, Pemberton, Gu, Kerns, et al. 2020c) employed lower natural enemy densities and

lacked support for complementarity between *E. eremicus* and *A. swirskii*, suggesting that this phenomenon may be density dependent and needs further investigation.

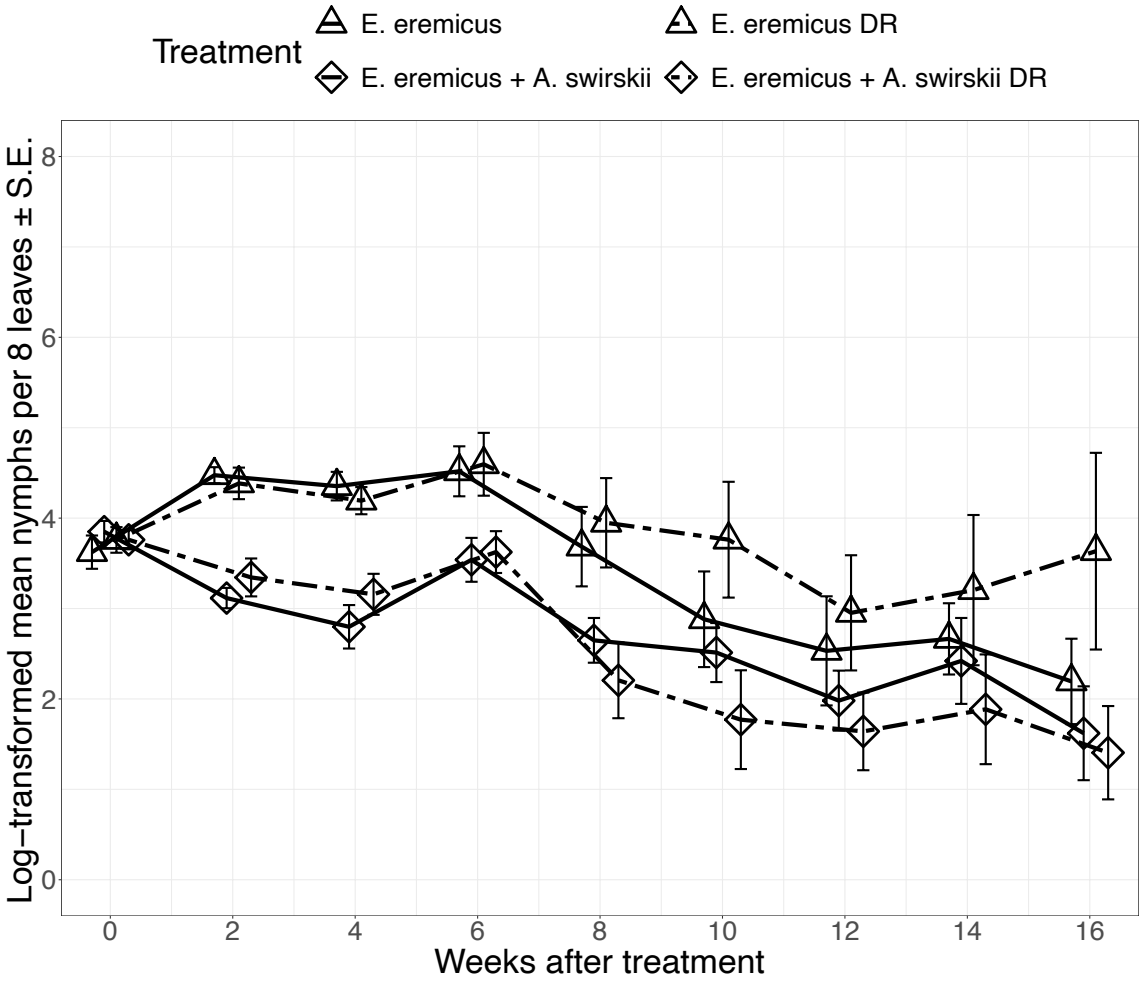

Figure 4.2 Mean log-transformed nymphs (± SE) counted on 8-leaves per cage for objective 2: *E. eremicus* alone or *E. eremicus* and *A. swirskii* combination treatments without and with whitefly immigration (imm. W4: week 4; imm. W8: week 8). Combination of *E. eremicus* and *A. swirskii* always resulted in greater whitefly suppression compared to *E. eremicus* alone (P < 0.001 for W4, W8, and both W4 and W8 immigration treatments combined).

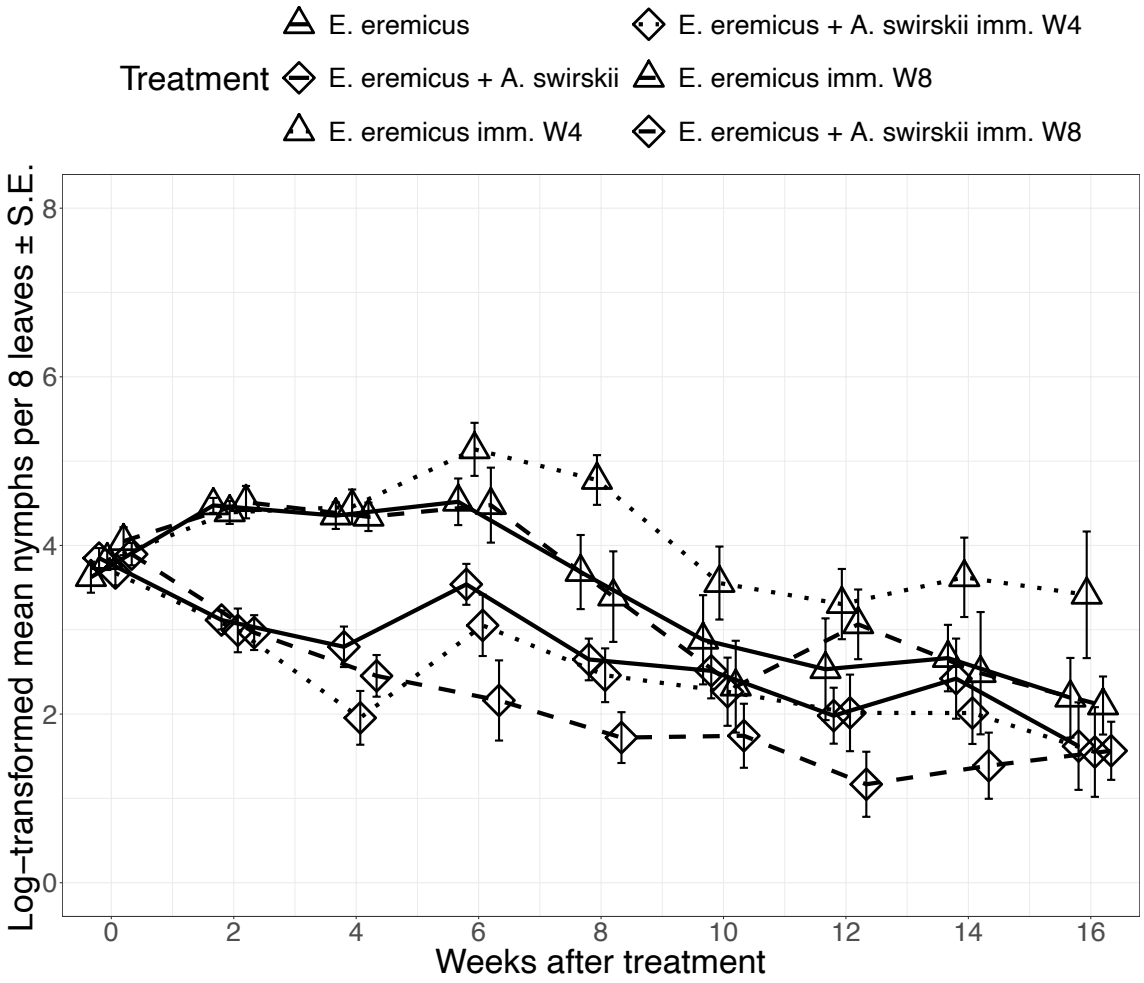

Figure 4.3 Mean log-transformed nymphs (± SE) counted on 8-leaves per cage for objective 3: *E. eremicus* alone or *E. eremicus* and *A. swirskii* combination treatments without and with delayed release (DR), simulating a one-week delay in natural enemy releases on weeks 4 and 8. Combination of *E. eremicus* and *A. swirskii* resulted in greater whitefly suppression compared to *E. eremicus* alone when challenged with delayed releases of natural enemies (P < 0.005).

Despite the practice of regular releases of *E. eremicus* for management of *B. tabaci*, very little published work has investigated the consequences of missed or delayed releases. Timing of natural enemy releases are designed to align with the target life stage that is most vulnerable (van Lenteren 2000a); however, *B. tabaci* can have several overlapping

generations within a greenhouse, which may explain why *E. eremicus* is often released on a weekly interval (Hoddle and Van Driesche 1996, 1999b, Hoddle et al. 1999). Additionally, early preventative control of *B. tabaci* is considered vital for successful augmentative biological control programs (Buitenhuis et al. 2016, Krauter et al. 2017). Predictive models estimated that a delay of over 14 days in the initiation of an augmentative biological control program using parasitic wasps, *Diglyphus begini* (Ashmead) (Hymenoptera: Eulophidae), for chrysanthemum leaf-miners, *Liriomyza triofolii* (Burgess) (Diptera: Agromyzidae), would result in a failure of the management strategy, regardless of release rates (Heinz et al. 1993). Our results suggest that delayed releases of both *E. eremicus* alone and in combination with *A. swirskii* are robust in suppressing *B. tabaci*. Practitioners would benefit from more studies determining the frequency or length of delay in natural enemy releases that would result in significantly decreased suppression of *B. tabaci*, so that curative measures can be taken as needed. Additionally, augmentative biological control will benefit from greater economic viability if the same number of natural enemies can be released on a less frequent basis (i.e. every two weeks) without any noticeable difference in whitefly suppression.

We chose to release four whitefly adults per plant within each cage to simulate whitefly immigration; however, the addition of whitefly adults may have been insufficient to cause a measurable increase in whitefly population compared to the already established population. Calvo et al. (2009) released 125 adult *B. tabaci* per m^2 every week to simulate pest immigration, equivalent to approximately 31 adult *B. tabaci* released weekly in our cages; however, a non-immigration treatment was not included in their study, making it

equally difficult to know whether their introductions resulted in a significant increase in whiteflies. In some instances, immigration within augmentative biological control systems can result in long-term stability and prevent predator extinction (Walde 1994), a phenomena supported by meta-population studies (Thiel and Drossel 2018). Although whitefly immigration into a greenhouse is widely considered as negatively impacting the success of augmentative biological control programs (van Lenteren 2000a, Yano 2004), some levels of immigration may be beneficial in sustaining natural enemy populations. Additional studies investigating different rates of whitefly immigration and their impact on the ability for natural enemies to provide sufficient suppression are needed.

An important consideration for implementation of biological control in a greenhouse is the environmental conditions (Shipp et al. 2011). Natural enemies still provided suppression of *B. tabaci* despite temperatures reaching highs of 44ºC by week 12. Optimal temperature for development time and reproduction for *E.* eremicus has been reported between 25 – 35ºC (McCutcheon and Simmons 2001) and 31.5ºC for *A. swirskii*, with an upper development threshold of 37.4 ± 1.12ºC (Lee and Gillespie 2011). The significant suppression of *B. tabaci* by the natural enemies and final aesthetic quality of our plants in the combination treatments provides some support that these two natural enemies may be suitable for use in warmer climates, such as Texas. It should also be noted that we recognize how the relatively small cages may create an artificial arena for our natural enemies; natural enemy interactions on prey suppression can vary greatly with spatial scale (Lin and Pennings 2018). A previous larger-cage study (12 plants in 1.2 m x 1.5 m cage) also supported a lack of increased suppression due to increased natural enemy

rates and similar suppression between combination treatments and single natural enemy treatments (Vafaie, Pemberton, Gu, Kerns, et al. 2020c).

In conclusion, we support that the combination of *E. eremicus* and *A. swirskii* is more effective for suppressing *B. tabaci* on poinsettias compared to either natural enemy alone; a pattern that was consistent with simulated whitefly immigration or delayed releases of natural enemies. Additional studies to determine the rate of whitefly immigration or frequency of delays in natural enemy releases that result in increased whitefly populations will help determine whether increased natural enemy diversity increases robustness of whitefly suppression compared to a single natural enemy species.

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5. USE OF MULTIPLE NATURAL ENEMIES TO MANAGE WHITEFLIES IN COMMERCIAL PRODUCTION OF COLOR POINSETTIAS

5.1. Overview

In this case study, we investigate the efficacy and economics of using two natural enemies in an integrated pest management (IPM) program to manage sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), in commercial poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) production. Two similar greenhouses at each of three different grower locations were designated as either IPM or conventional insecticide greenhouses in southeastern USA. In the IPM greenhouses, we released *Eretmocerus eremicus* Rose & Zolnerowich (Hymenoptera: Aphelinidae) weekly and *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) every four weeks, and selective insecticides were used to treat high whitefly densities as needed. In the conventional greenhouses, growers were autonomous in their insecticide application decisions. All whitefly stages were counted weekly on 50 randomly sampled poinsettias and 50 flagged (i.e. revisited) poinsettias in every greenhouse.

Whitefly densities were consistently similar or higher in the IPM managed greenhouses compared to their conventionally managed counterparts for the duration of the trial; however, whitefly densities were ultimately below those found at retailers in all greenhouses. The cost of inputs and labor for whitefly management in the IPM greenhouses was between 0.57 to 3.0-fold the cost of conventional management. Our study supports that releasing *E. eremicus* and *A. swirskii* can reduce insecticide applications by 25 – 78% and may be considered a feasible strategy to manage *B. tabaci* in commercial poinsettia production in place of conventional insecticidal control in southeastern USA.

5.2. Introduction

Poinsettias, *Euphorbia pulcherrima* Willd. ex Klotzsch, are an important seasonal ornamental crop sold for their aesthetics and colorful bracts between November and December in the USA with a wholesale value of $148,760,000 in the USA in 2018 (United States Department of Agriculture 2020). For the duration of the 12 to 18-week production cycle for poinsettias, growers must protect the poinsettias from a suite of common greenhouse pests, including whiteflies (Hemiptera: Aleyrodidae), thrips (Thysanoptera: Thripidae), mealybugs (Hemiptera: Pseudococcidae), and fungus gnats (Diptera: Sciaridae), with whiteflies frequently composing the predominant pest of poinsettias (Ecke et al. 2004).

5.2.1. Managing Whiteflies on Poinsettias

The two main species of whiteflies frequently found on poinsettias in the USA are the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae), and the sweetpotato whitefly, *Bemisia tabaci* (Gennadius); although the latter has been considered a "species complex" composed of morphologically indistinguishable species (Perring 2001, Shu-sheng et al. 2012). The taxonomy of *B. tabaci* is in need of revision, but currently the most pertinent *B. tabaci* species in greenhouse ornamentals in southern USA include the MEAM1 (formerly known as B biotype) and MED (formerly known as Q biotype) (Tay et al. 2012, McKenzie et al. 2014). *Bemisia tabaci* MEAM1 was problematic in greenhouse ornamentals in the USA since the early 1980s (Costa et al. 1993, Brown et al. 1995, Frewin et al. 2014, McKenzie et al. 2014), but populations were manageable with

available insecticides. *Bemisia tabaci* MED was first observed in the United States in 2004 and was characterized by insecticide resistance to several insecticides, such as pyriproxyfen, acetamiprid, buprofezin, fenpropathrin, acephate, imidacloprid, and thiamethoxam (Dennehy et al. 2005). For example, the lethal concentration of imidacloprid required to cause 50% mortality (LC_{50}) was found to be 83.8-fold higher for *B. tabaci* MED compared to MEAM1 (Luo et al. 2010). Specific rotations of insecticides can be used to manage both common cryptic species of *B. tabaci* (McKenzie et al. 2014); however, in circumstances where effective management with available insecticides was limited and *B. tabaci* MED was prevalent, growers had to resort to alternative whitefly management strategies, such as the use of biological control (Murphy et al. 2008).

Whiteflies on poinsettias are predominantly managed with frequent insecticide applications in southern USA. The lack of adoption of biological control in greenhouse ornamentals has frequently been attributed to concerns of cost or insufficient pest suppression (Stevens et al. 2000, Bethke and Cloyd 2009). Historically, retailer thresholds for pests on ornamentals were thought to be "zero" or undetectable (Stevens et al. 2000, Bethke and Cloyd 2009), which has recently been demonstrated as an incorrect assumption for whiteflies on poinsettias (Vafaie et al. 2020a). Average whitefly immatures reached as high as 73 nymphs per poinsettia at a florist, potentially raising the benchmark in the published literature for acceptable whitefly densities at retailers (Vafaie et al. 2020a). In some cases, more than 200 nymphs per plant and up to 100% infestation with whiteflies were detected at retailers (Vafaie et al. 2020a), despite current weekly insecticide applications, raising concerns about the sustainability of relying on insecticides for whitefly control on poinsettias. Furthermore, pressure from consumers, retailers (Friends

of the Earth 2017), and the Environmental Protection Agency (United States Environmental Protection Agency 2017) has increased demands to reduce insecticide use and increased pesticide application regulations, providing the impetus to study the potential for biological control to manage whiteflies on poinsettias in Texas, USA.

5.2.2. Commercial Scale Augmentation Biological Control

While quite a few studies have investigated augmentative biological control for management of *B. tabaci* on poinsettias (Heinz and Parrella 1994a, Hoddle and Van Driesche 1996, Hoddle et al. 1997, 1998, Van Driesche et al. 1999, Hoddle et al. 1999, Hoddle and Van Driesche 1999b, 1999a, Stevens et al. 2000, Van Driesche et al. 2001, 2002, Van Driesche and Lyon 2003), all have been conducted in cooler regions of the USA (Massachusetts, New York, and northern California) as compared to Texas, all investigated the release of single enemy species to manage *B. tabaci* (with the exception of Heinz and Parrella 1994a), and economic comparisons haven't been considered in over 17 years (Van Driesche and Lyon 2003). Additionally, our previous work supporting sufficient suppression of *B. tabaci* using natural enemy release rates economically comparable to insecticide inputs was conducted in small cages containing 2 or 12 poinsettias (Vafaie et al. 2020b and Vafaie et al. n.d., respectively). The spatial scale of augmentative biological control research can drastically alter the outcome due to factors such as Allee effects on prey and natural enemies, or physiological trade-offs that occur at different spatial scales (Courchamp et al. 1999, Kneitel and Chase 2004, Bajeux et al. 2017). Our goal of this study is to determine whether efficacious augmentative biological control could be maintained at the scale of commercial poinsettia production in Texas.

In the state of Texas, 81.1% of the greenhouse growing area is considered semi-open, hoop-houses with plastic film (single or multi-layer poly) (Vilsack and Reilly 2015). Due to the semi-open conditions, a seasonal inoculative biological control program was considered most suitable. A seasonal inoculative biological control program is defined as a regular release of natural enemies to suppress a target pest of a seasonal crop, with the expectation that the natural enemies will reproduce to provide residual control (van Lenteren and Bueno 2003). Our past work demonstrated that the combination of two natural enemies, *E. eremicus* and *A. swirskii*, may be effective at reducing whitefly populations (Vafaie et al. 2020b), despite whitefly immigration or delays in natural enemy releases (Vafaie et al. n.d.). Adding *A. swirskii* also provides the benefit of suppressing secondary pests, such as thrips (Ghasemzadeh et al. 2017) and twospotted spider mites (Acari: Tetranychidae) (Seiedy et al. 2017). In this case study, we investigate the efficacy and economics of using a combination of *E. eremicus* and *A. swirskii* in an integrated pest management program (IPM) to manage whiteflies on poinsettias compared to conventional whitefly management at three commercial grower facilities in Texas.

5.3. Materials and Approach

5.3.1. Cooperative Growers

Three different commercial growers were included in this trial, all within 80 kilometers of the Texas A&M AgriLife Research and Extension Center in Overton, Texas, USA (Table 5.1). Names and locations of growers were anonymized with capital letters "A", "B", and "C" to maintain confidentiality. At each grower, two greenhouses with similar conditions were chosen and designated as the IPM greenhouse and the

conventional insecticide greenhouse, respectively. Area of usable growing space (square meters), quantity of poinsettias at final spacing, type of greenhouse structure, and greenhouse cooling method are outlined in Table 5.1. Poinsettia cultivars were all 'Prestige Red' and 'Christmas Magic Red' for Growers B and C, respectively. Grower A had twenty cultivars in both greenhouses, which included: 'Premium Red', 'Jubilee Jingle', 'WinterSun White', 'Christmas Day', 'Frozen', 'Ice Punch', 'Protégé', 'Jingle Bell', 'Ice Crystal', 'Ferrara', 'Christmas Wish', 'Christmas Beauty Red', 'Autumn Leaves', 'Lipstick', 'Majestic Pink', 'Grand Italia', 'Red Glitter', 'Christmas Cheer', 'Premium Polar', and 'Premium Marble'.

Table 5.1. Greenhouse structure type, cooling method, growing space in square meters (sq. m.), and poinsettias at final spacing for conventionally and IPM managed greenhouses at the three different grower locations (A, B, and C).

	Conventional Control		IPM		Greenhouse type	Cooling
Grower	sq. m.	Poinsettias	sq. m.	Poinsettias		
A	1,151	2,451	1,142	3,722	Rigid Plastic	Evaporative cooling; active air flow
B	50	295	50	515	Rigid Plastic	Evaporative cooling; active air flow
C	186	2,256	186	559	Hoop-house with plastic film	Passive airflow

5.3.2. IPM and Conventional Whitefly Management

In the IPM greenhouses, *E. eremicus* pupae (Ercal, Koppert Biological Systems; Howell, MI) were released approximately weekly and *A. swirskii* (Swirski-Mite, Koppert

Biological Systems; Howell, MI) released approximately every four weeks. *Eretmocerus eremicus* were released as pupae placed in a saw-tooth pattern within a given greenhouse, hooked on metal stakes hanging just above the plant canopy (Figure 5.1A). *Eretmocerus eremicus* release density was 2.58 and 1.83 pupae per m^2 for growers A and B, respectively, per release, assuming 60 pupae per card. Release density of *E. eremicus* in greenhouse C was 1.94 pupae per m^2 for the first 5 releases, and subsequently reduced to 0.97 pupae per m^2 due to removal of half of the poinsettias from the greenhouse on October 30th, 2019. Our previous work demonstrated that the *E. eremicus* pupae cards utilized from Koppert Biological Systems actually contain an average of 91.1 ± 3.6 pupae per card with a mean emergence rate of 74.3 ± 3.2%, resulting in approximately 67.7 adult *E. eremicus* released per card (Vafaie et al. n.d.).

Amblyseius swirskii were released at a density of 44.1, 42.0, and 26.9 mites per m^2 in greenhouses A, B, and C, respectively, per release. *Amblyseius swirskii* was shipped in a carrier material, which was mixed in a large container to increase homogeneity of *A. swirskii* prior to loading aliquots into the Koppert Mini-Airbug hopper (Koppert Biological Systems; Howell, MI) for even spread on the poinsettia canopy (Figure 5.1B-C). To apply *A. swirskii* evenly, the applicator walked up and down each row of poinsettias at a consistent speed that was paced to provide limited extra carrier material after covering all poinsettias. Left-over carrier material was dispersed in whitefly hotspot areas as identified by monitoring (see "Weekly Assessments" below). In addition to releases of *E. eremicus* and *A. swirskii*, growers were permitted to apply select insecticides known to have relatively low negative impact on the natural enemies for suppression of whitefly hot spots.

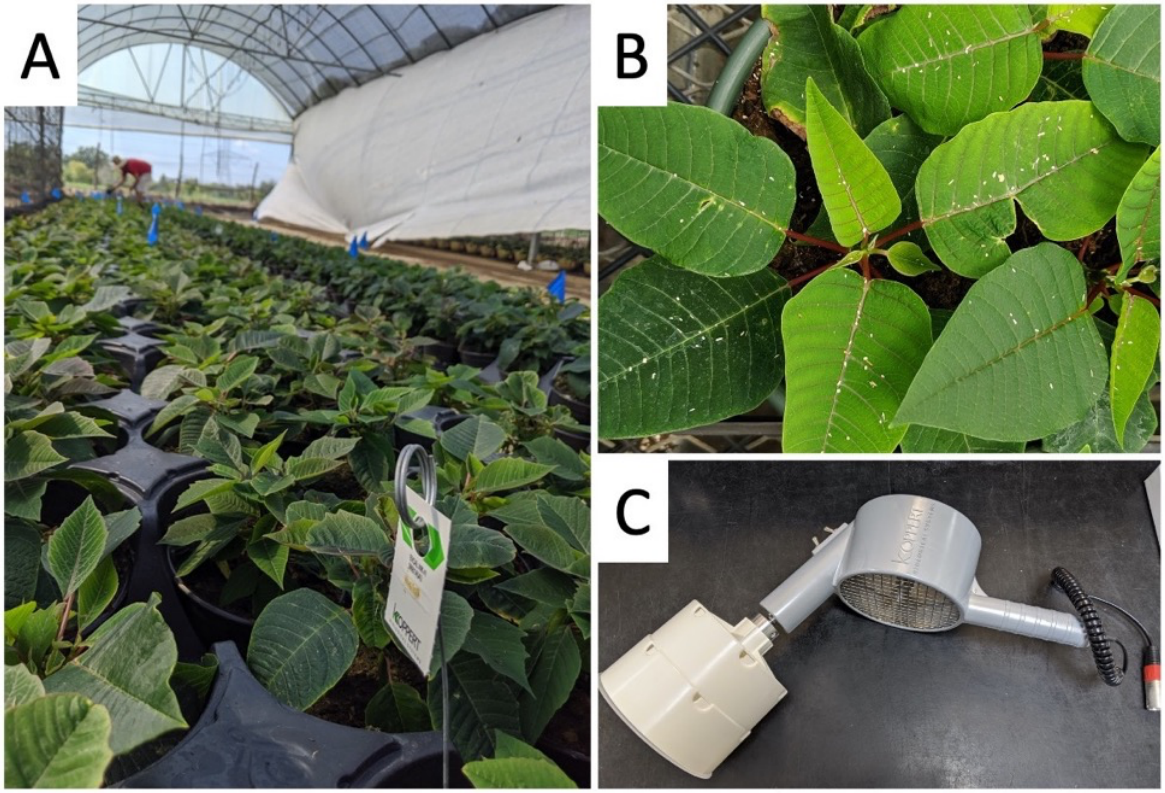

Figure 5.1. Photos of the *E. eremicus* pupae cards being hung just above the plant canopy and the flagged plants (blue flags) in the background (A), the *A. swirskii* carrier material on the poinsettias after broadcast application (B) made with the mini-airbug (Koppert Biological Systems)(C).

In the conventional greenhouses, growers were autonomous in insecticide use decision-making, with no restrictions on quantity or type of insecticides used. It should be noted that the growers had access to our monitoring data for both the IPM and conventional greenhouses, providing them with detailed information about whitefly densities that would otherwise not be available.

5.3.3. Weekly Assessments

Poinsettias were monitored starting from the time of transplanting until the time of shipping to retailers, with the exception of grower C, which was started 4 weeks after transplanting. The late start at grower C was due to a decision by the regional grower to move the trial to a different location due to unrelated crop protection and performance issues at the original location.

Within each greenhouse, a maximum of 20 leaves on 50 randomly sampled poinsettias were inspected using a 2.5x head lens. In addition to the randomly sampled poinsettias, 50 poinsettias were flagged and a single leaf tagged with string to track a single area of plant material over the length of the trial (Figure 5.1A). Number of whitefly immatures, pupae, exuviae, and adults, number of *E. eremicus* pupae and adults, and *A. swirskii* motiles were counted on all sampled plant material. Yellow sticky traps (between 1 per 25 sq. m. and 1 per 288 sq. m.) were also suspended just above the plant canopy and inspected weekly. Sticky traps were replaced when more than approximately 50% of the surface area was occupied by trapped insects. The number of whiteflies counted on a given sampling date was reduced by the maximum number of whiteflies found previously on the yellow traps to determine the quantity of new whitefly adults trapped within the given sampling period. The sampling period was recorded as week number, with week 2 starting on January 6, 2019.

5.3.4. Economic Evaluation

We used partial budget analysis to determine the cost of whitefly management, independent from other activities or inputs (Stevens et al. 2000). To determine an average

estimate of the cost of insecticide inputs, we averaged the price of insecticides from two different distributors and from a readily available online retailer (https://domyown.com) (Table 5.2). Prices for natural enemies were based on their source, and includes taxes and shipping. Shipping costs were estimated based quotes from the providers on an order quantity of 10 orders of either Ercal (USD $65) or Swirskii-mite (USD $80). We estimated the cost of insecticide inputs per square meter based on the insecticide label and usable grower space for each greenhouse, assuming 200 gallons of mixed insecticides per 0.40 hectares (1 acre).

We estimated labor cost using a reasonably average hourly wage of $17.50/hr for specialized labor (pesticide applicator), approximately 40 minutes for preparation and cleaning of insecticide application equipment and 100 minutes for an applicator to treat 856 m^2 of greenhouse space, as estimated by Stevens et al. (2000). There was no preparation time for releasing *E. eremicus* and minimal time for mixing and loading bulk *A. swirskii* material (6 minutes). We estimated the time it took to release natural enemies at each location and calculated time to treat each 1,000 m^2 on greenhouse as 15 and 50 minutes for *E. eremicus* and *A. swirskii*, respectively. Since an inventory of the total number of poinsettias was recorded during each visit, we were able to estimate the cost of management per poinsettia for any given application (Table 5.3).

Table 5.2. Mean cost (± standard error) of whitefly management products, the distributors, and active ingredients/species.

Product	Company	Active ingredient or species	Quantity per order	Mean cost per order ± S.E. ($, USD)
Ercal	Koppert	*E. eremicus*	3,000 pupae	49.50 (n=1)
Swirski-Mite	Koppert	*A. swirskii*	50,000 mites	103.25 (n=1)
Avid® 0.15 EC	Syngenta	Abamectin	3.8 L (128 fl. oz.)	590.33 ± 60.33 (n=3)
Capsil	Aquatrols®	Polyether	3.8 L (128 fl. oz.)	133.76 ± 44.16 (n=2)
Kontos®	OHP	Spirotetramat	0.2 L (8.45 fl. oz.)	181.15 ± 9.15 (n=3)
Marathon® II	OHP	Imidacloprid	0.2 L (8.45 fl. oz.)	141.05 ± 21.04 (n=2)
Merit® 2F	Bayer	Imidacloprid	3.8 L (128 fl. oz.)	59.52 ± 10.43 (n=2)
Rycar®	SePro	Pyrifluquinazon	0.2 L (8 fl. oz.)	179.3 ± 12.82 (n=3)
Safari® 20 SG	Valent	Dinotefuran	1.4 L (48 fl. oz.)	341.36 ± 8.06 (n=3)
Talus® 70DF	SePro	Buprofezin	1.4 L (48 fl. oz.)	328.05 ± 23.53 (n=3)
Azatin® O	OHP	Azadirachtin	0.9 L (32 fl. oz.)	218.74 ± 15.21 (n=3)
Mainspring®GNL	Syngenta	Cyantraniliprole	0.5 L (16 fl. oz.)	338.92 ± 13.92 (n=3)
Xxpire®	Corteva	Spinetoram and sulfoxaflor	0.5 L (16 fl. oz.)	224 ± 14 (n=2)
Conserve® SC	Corteva	Spinosad	1.0 L (32 fl. oz.)	141.68 ± 4.51 (n=3)
Endeavor®	Syngenta	Pymetrozine	0.4 kg (15 oz.)	169.31 ± 13.51 (n=3)

Prices for products acquired from up to two distributors and one online retailer (domyown.com).

Table 5.3. Total cost, cost per poinsettia, and cost per square meter (sq. m.) for the IPM and conventionally (conv.) managed greenhouses at growers A, B, and C.

					Input cost ($ USD)			Labor cost ($ USD)			Total ($ USD)	
		Product	Application frequency	Pot size (diam. in cm)	Per poinsettia	Per m^2	Total	Per poinsettia	Per m^2	Total	Per poinsettia	Per m^2
Grower A	IPM	Ercal	15	20	0.178	1.191	1,324.09	0.071	0.431	352.14	0.250	1.622
		Swirski-Mite	4									
		Rycar®	3									
		Talus® 70DF	1									
		Merit® 2F	1									
	Conv.	Rycar®	2	20 – 30	0.024	0.093	74.30	0.060	0.218	172.40	0.084	0.311
		Xxpire®	1									
		Merit® 2F	1									
Grower B	IPM	Ercal	12	15	0.134	0.870	43.18	0.153	0.948	47.05	0.287	1.819
		Swirski-Mite	2									
		Rycar®	2									
		Kontos®	1									
	Conv.	Safari® 20 SG	3	15	0.020	0.214	10.60	0.122	1.352	67.08	0.142	1.566
		Rycar®	1									
		Capsil	1									
		Marathon® II	1									
Grower C	IPM	Ercal	7	10	0.057	0.297	55.12	0.044	0.247	45.97	0.101	0.544
		Swirski-Mite	1									
		Kontos®	1									
		Avid® 0.15 EC	1									
	Conventional	Kontos®	1	10	0.029	0.314	58.26	0.148	0.875	162.50	0.177	1.189
		Avid® 0.15 EC	1									
		Mainspring®GML	2									
		Azatin® O	1									
		Conserve® SC	3									
		Endeavor®	1									

Cost of application per poinsettia was calculated based on the number of poinsettias in the greenhouse at the time of application. Size of poinsettia pots are given as a diameter of the top of the pot, rounded to the nearest centimeter.

5.4. Major Findings

All greenhouses (IPM and conventional) at all grower locations had final whitefly densities below 73 immatures per plant and less than 69.1% of poinsettias infested, which is considered acceptable at retailers (Vafaie et al. 2020a). For all grower locations during the growing season, the IPM greenhouses had similar (grower B) or higher whitefly (growers A and C) immature numbers than the conventionally managed greenhouses for most weeks (Figure 5.2).

The proportion of poinsettias infested with immature whiteflies was consistently higher in IPM greenhouses than conventionally managed greenhouses over the duration of the study (0.94, 0.62, and 0.88 of the weeks sampled for growers A, B, and C, respectively), with the percentage of poinsettias with immature whiteflies reaching as high as 70% of randomly sampled plants for grower A on week 40 (Figure 5.3). The average difference in proportion of poinsettias infested with whiteflies between the IPM and conventionally managed greenhouses was 0.30, 0.06, and 0.09 for growers A, B, and C, respectively (Figure 5.3).

For grower A, the greater difference in proportion and number of immature whiteflies counted in the IPM greenhouse compared to the conventional greenhouse may be explained by lower overall whitefly pressure in the conventional greenhouse. Grower A used our monitoring data to inform whether to make insecticidal applications in the conventional greenhouse and thus, the lower whitefly densities are not due to prophylactic use of insecticides, but rather, a lack of whitefly pressure warranting insecticidal applications (Table 5.3). In a previous study, researchers introduced adult whiteflies in low-density greenhouses to produce similar starting initial whitefly pressure in

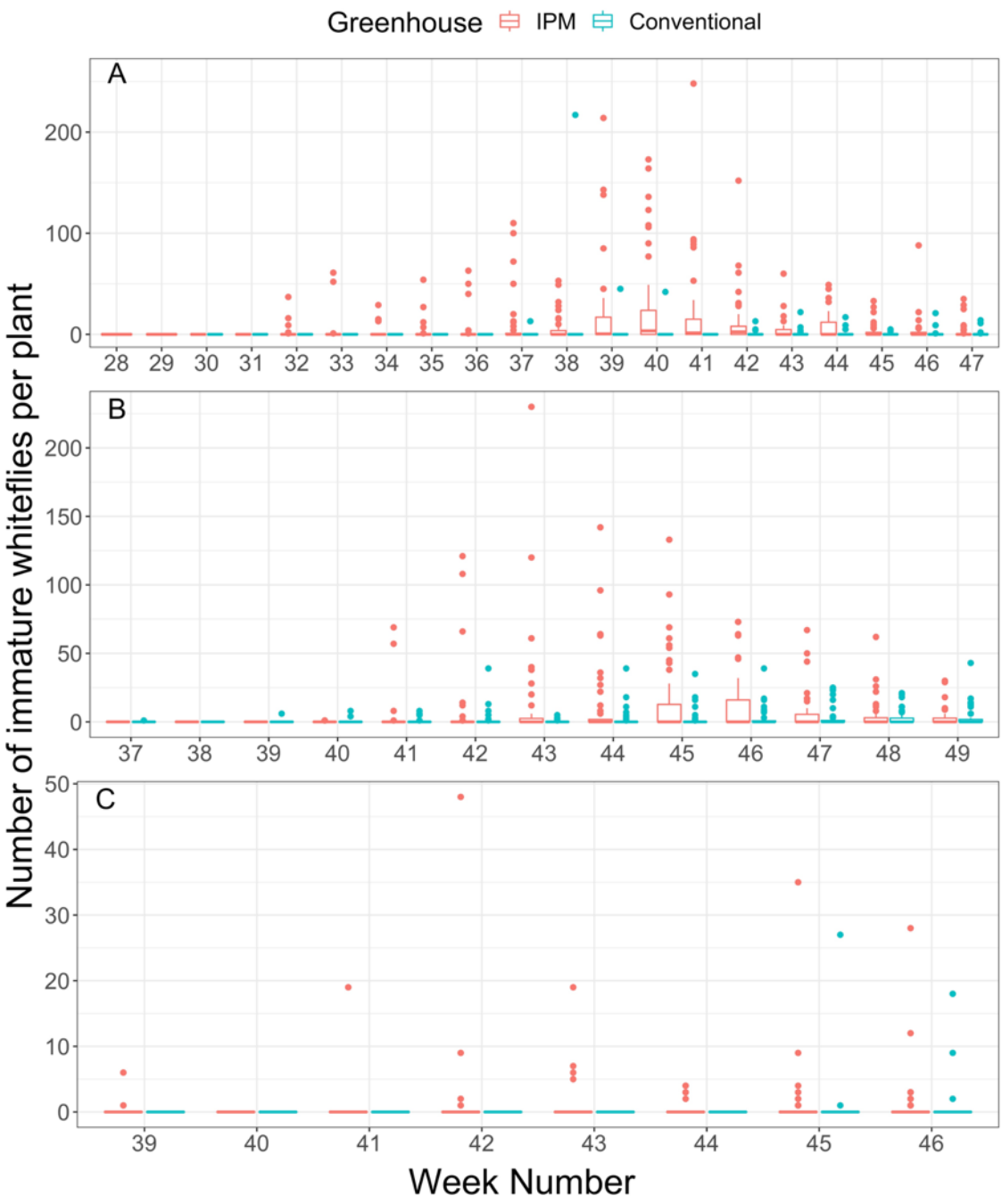

Figure 5.2. Number of immature whiteflies per plant either on randomly sampled poinsettias in the IPM or conventionally management greenhouse by calendar week number for growers A, B, and C. Number of immature whiteflies are represented by box plots, where dots represent extreme cases (i.e. outliers), vertical lines represent the top quartile of the counts, and the boxes (seen in grower A and B) represent the lower/middle and upper quartile of whitefly immatures per poinsettia.

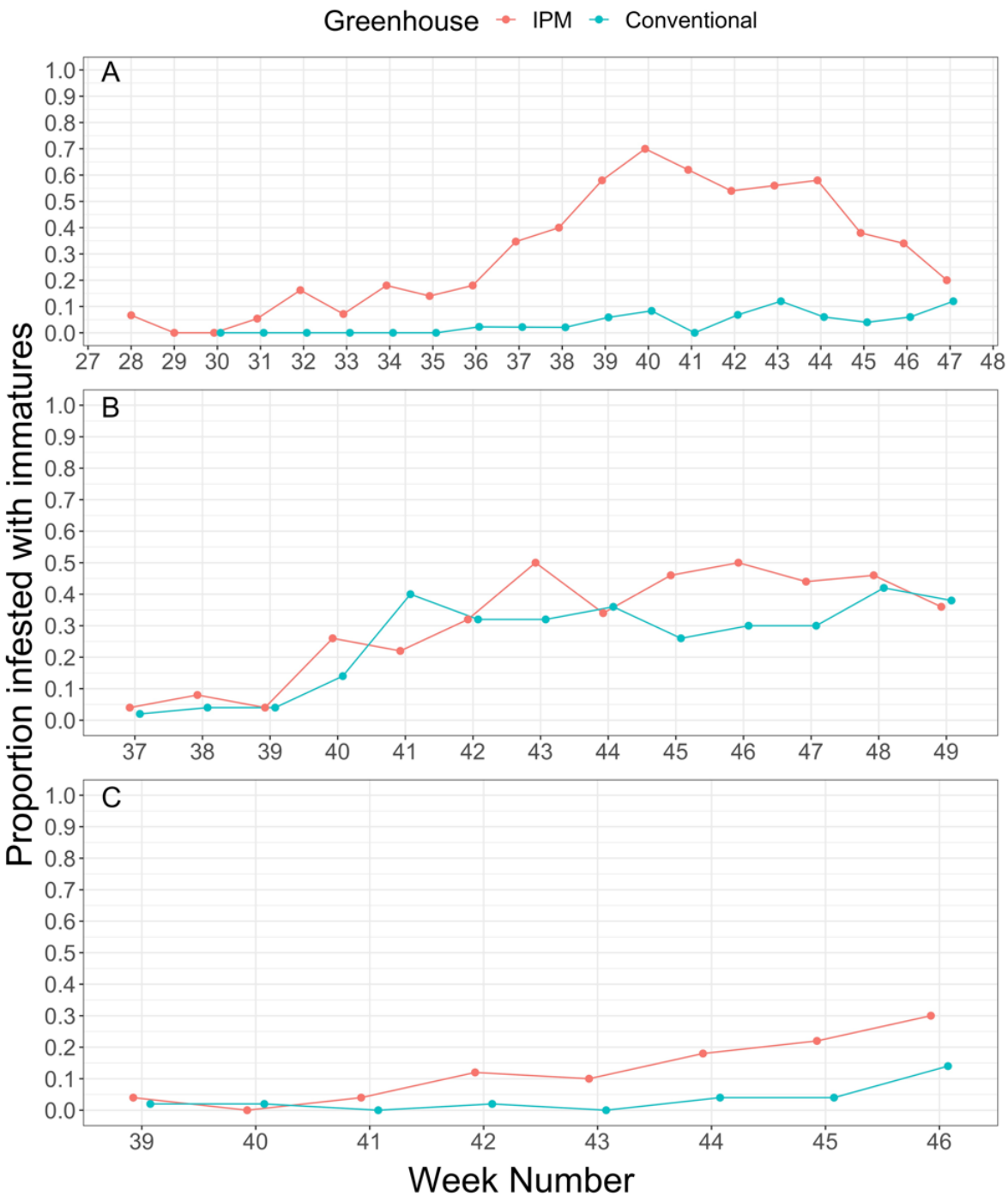

Figure 5.3. Proportion of poinsettias infested with whiteflies (any stage or density) on randomly sampled poinsettias in the IPM or conventionally management greenhouse by calendar week number for growers A, B, and C.

conventional and biological control managed greenhouses (Hoddle and Van Driesche 1999a, Van Driesche et al. 1999) to reduce the discrepancy in starting densities; however, introducing adult whiteflies into commercial poinsettia production was not considered an acceptable option in our case study.

In the IPM greenhouse (grower A), there was a specific cultivar that initially experienced an increase in whitefly density, which may have contributed to overall increased whitefly pressure in that particular greenhouse. Higher whitefly densities on specific cultivars could be attributed to whitefly populations from the propagators or due to differences in *B. tabaci* performance (Medina-Ortega 2011) or natural enemy performance on different poinsettia cultivars (Heinz and Parrella 1994b). Although that particular cultivar and initial whitefly infestation was relatively isolated to a few benches (out of 50 benches total), poinsettias were spaced throughout the entire IPM greenhouse at week 34, resulting in increased spread of whitefly infestations within the greenhouse (Figure 5.3A).

We also found fungus gnats on the yellow sticky traps and *Echinothrips americanus* Morgan (Thysanoptera: Thripidae) on poinsettias; however, densities were sufficiently low at all locations that no additional management was required by growers. At grower C, red imported fire ants, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) were found in numerous pots and had removed all *E. eremicus* pupae from the release cards within 2 hours of release on week 2 (Figure 5.4). The red imported fire ant is known for tending honeydew producing insects (Zhou et al. 2015) and engaging in intra-guild predation (Harvey and Eubanks 2005), which may be problematic in our trial by reducing the density of *E. eremicus* without providing any direct suppression of *B. tabaci*. Red imported fire ants were managed with granular incorporation of bifenthrin in all

greenhouses, as required by the Texas Department of Agriculture for potted plants being shipped out of fire ant quarantine areas (Miller 2018).

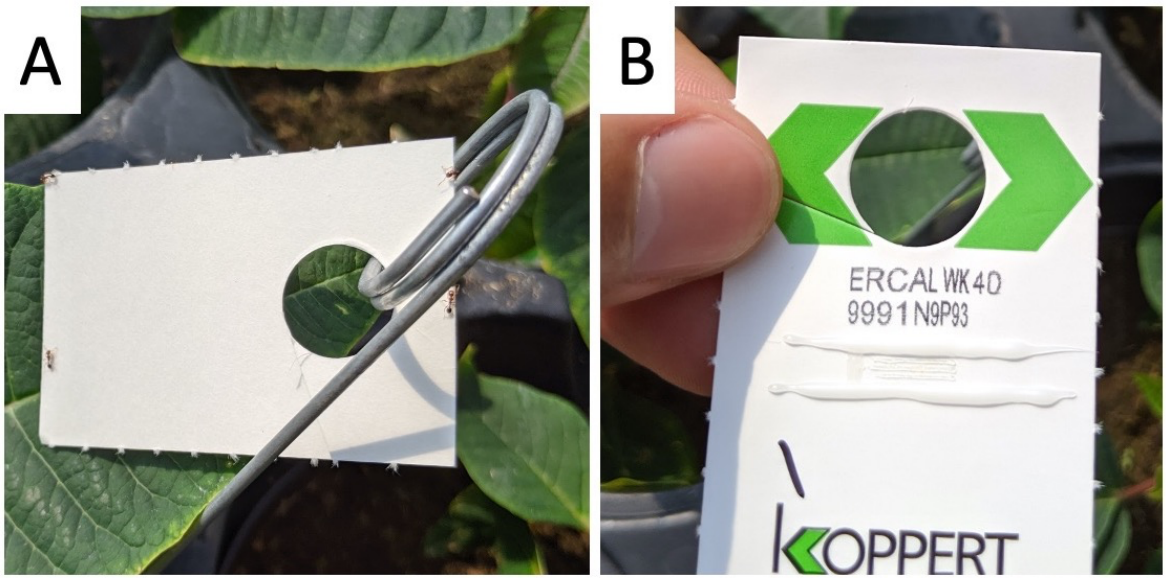

Figure 5.4. Photos of red imported fire ants (A) on the Ercal cards and after they had removed all the *E. eremicus* pupae (B). The glue that is designed to adhere the *E. eremicus* pupae to the card was all that remained.

When considering the cost of inputs and labor, the cost of the IPM strategy was between 0.57 and 3.0-fold the cost of conventional management (Table 5.3), demonstrating the economic competitiveness of using multiple natural enemies to manage *B. tabaci* in commercial poinsettia production. Previous studies calculated conventional management of *B. tabaci* in commercial poinsettia production to cost between $0.09 to $0.14 per 15-cm poinsettia when excluding labor costs (Hoddle and Van Driesche 1999b, Van Driesche et al. 2001, 2002, Van Driesche and Lyon 2003) and up to $0.27 per poinsettia when including labor costs (Stevens et al. 2000). Despite our insecticide input costs being substantially lower than the studies mentioned, the labor costs associated with releasing

natural enemies in this study compared to preparing and spraying insecticides greatly favored the IPM strategy (Table 5.3). For example, similar number of product applications at grower C (10 and 9 applications in the IPM and conventional strategy, respectively) resulted in 70.3% lower labor costs in the IPM strategy compared to the conventional strategy (Table 5.3).

Previous studies found augmentative biological control programs of *B. tabaci* on poinsettias to cost between 3- and 44-fold more than conventional insecticide management (Hoddle and van Driesche 1999, Hoddle and Van Driesche 1999a, Stevens et al. 2000, Collier and Van Steenwyk 2004), with the exception of Van Driesche and Lyon (2003). In their study, the cost of biological control was similar or even a bit lower than conventional insecticide management due to decreased release densities of *E. eremicus* and tandem use of insect growth regulators. We partially attribute the competitive economics of our IPM strategy to the lower overall release rates of natural enemies; with a range of 0.5 to 7.5 female *E. eremicus* per poinsettia (Van Driesche et al. 1999 and Van Driesche and Lyon 2003, respectively) in previous research compared to as low as a peak of 0.32 female *E. eremicus* per poinsettia in our study. The addition of *A. swirskii* to provide complementary suppression of *B. tabaci* (Vafaie et al. 2020b, n.d.) and tandem use of compatible insecticides (Van Driesche et al. 2001, Van Driesche and Lyon 2003) also contributed to the competitive economics of the IPM strategy. Acquiring equivalent or superior suppression of *B. tabaci* by decreasing the density of *E. eremicus* per release has been observed previously (Hoddle et al. 1999, Vafaie et al. 2020c, n.d.), which may be explained by intraspecific competition through host feeding or super-parasitism when whitefly densities are low (Hoddle et al. 1999, Javad et al. 2005). When compared to input

costs of other past insecticide rotations for whiteflies on poinsettias ($0.09 - $0.14/15 cm poinsettia) (Hoddle and Van Driesche 1999a, Van Driesche et al. 1999, 2001, Stevens et al. 2000, Van Driesche and Lyon 2003, Vafaie et al. 2020b) we see a 1.27-fold increase in cost at most by using the IPM management strategy. It should be noted that our economic analysis could be considered superficial by only looking at input and simplifying labor costs. More in-depth economic analyses may further favor strategies that rely on biological control due to decreased depreciation costs of pesticide application equipment, annual Worker Protection Standard and pesticide applicator training requirements, and non-market costs, such as long-term effects on worker health and water quality, which have been rarely quantified (Naranjo et al. 2019).

The vast majority of plants inspected across all grower locations and inspection dates had 5 or less whiteflies of any stage on them (Figure 5.5), with very few plants having more than 25 whiteflies of all life stages. The abundance of seemingly 'clean' plants can serve as a cautionary tale against limited monitoring of poinsettias. Distribution of *B. tabaci* on greenhouse-grown poinsettias is considered highly aggregated both between plants and within a plant (Liu et al. 1993a). We anticipated that flagging plants and revisiting the same plants weekly would provide more consistent and reliable monitoring data. Although the flagged plants consistently had lower standard error, flagged plants also failed to represent plants with high whitefly densities (Figure 5.6), perhaps supporting the use of randomly sampled plants in monitoring compared to revisiting flagged plants.

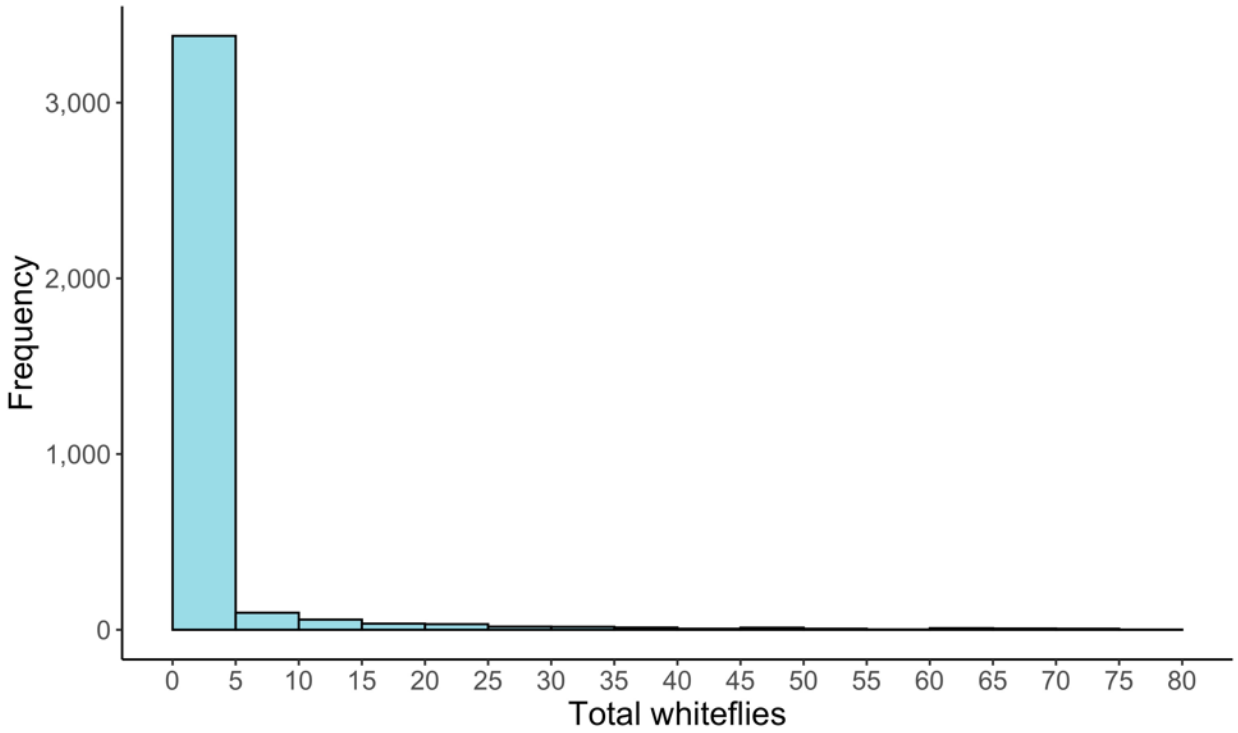

Figure 5.5. Frequency histogram for whitefly counts on randomly sampled poinsettias for all growers, locations, and weeks pooled together. The vast majority of poinsettias had less than 10 whiteflies. Each bin represents an interval of 10 whiteflies.

Yellow sticky traps consistently detected whitefly adults at the same time or earlier than detected through plant inspection for all growers and greenhouses (Figure 5.2 and Figure 5.7). However, yellow sticky traps were not great indicators of actual whiteflies densities; presence of whiteflies on sticky traps can be an indicator of a low whitefly population density or may be due to higher attraction to nearby plants (Berlinger 1980). Attempts to correlate yellow sticky trap catches with whitefly densities have been inconsistent, depending on factors such as whitefly species, crop, and density of yellow sticky traps (Pinto-Zevallos and Vänninen 2013a). To rely on yellow sticky cards as reliable indicators

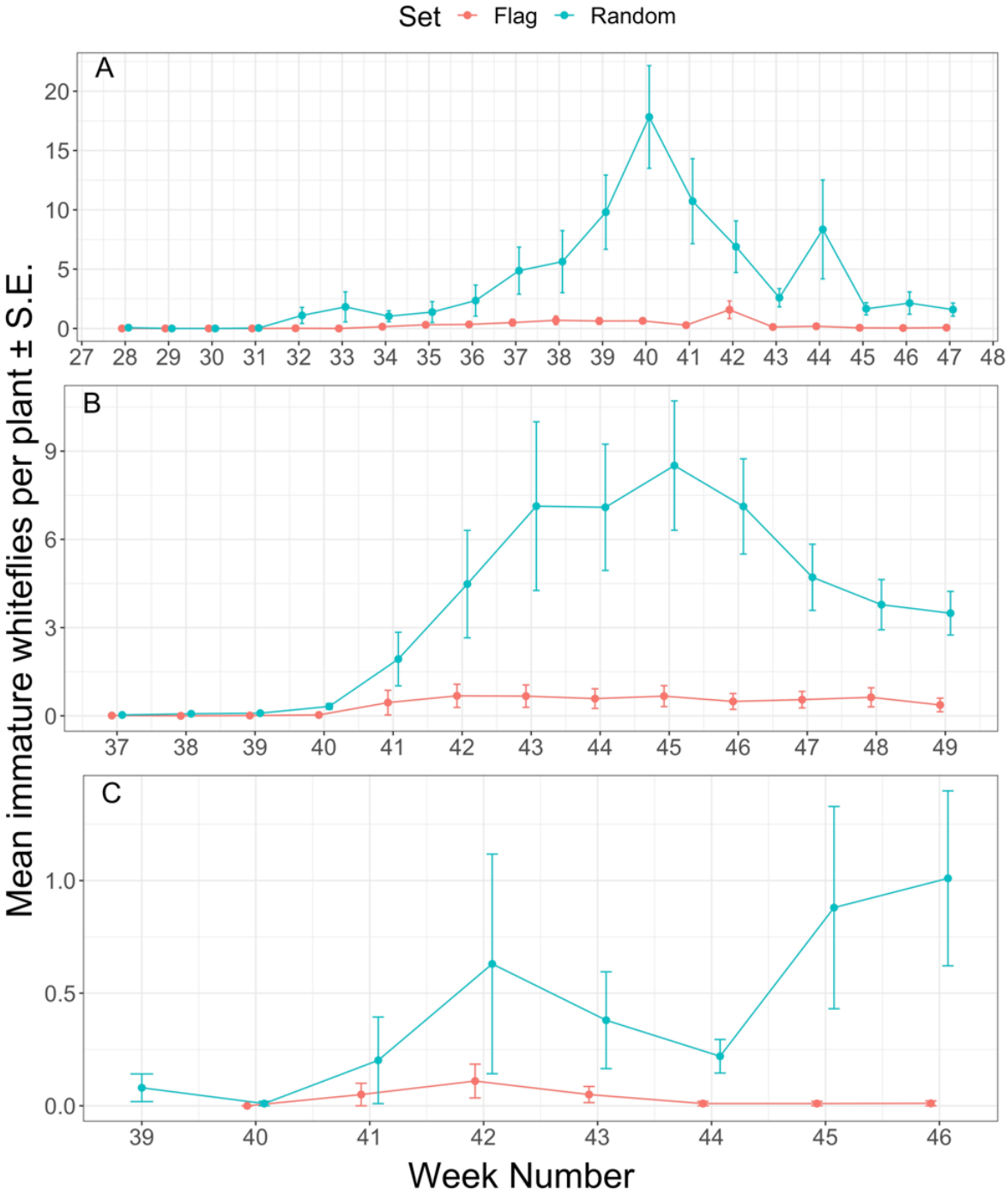

Figure 5.6. Mean immature whiteflies (± standard error) per poinsettia either on flagged plants or randomly sampled plants by calendar week number for growers A, B, and C. Fifty random and approximately 50 flagged plants were inspected each sampling period. Only select flagged leaves were inspected on the flagged poinsettias, whereas up 20 randomly selected leaves were inspected on randomly sampled poinsettias.

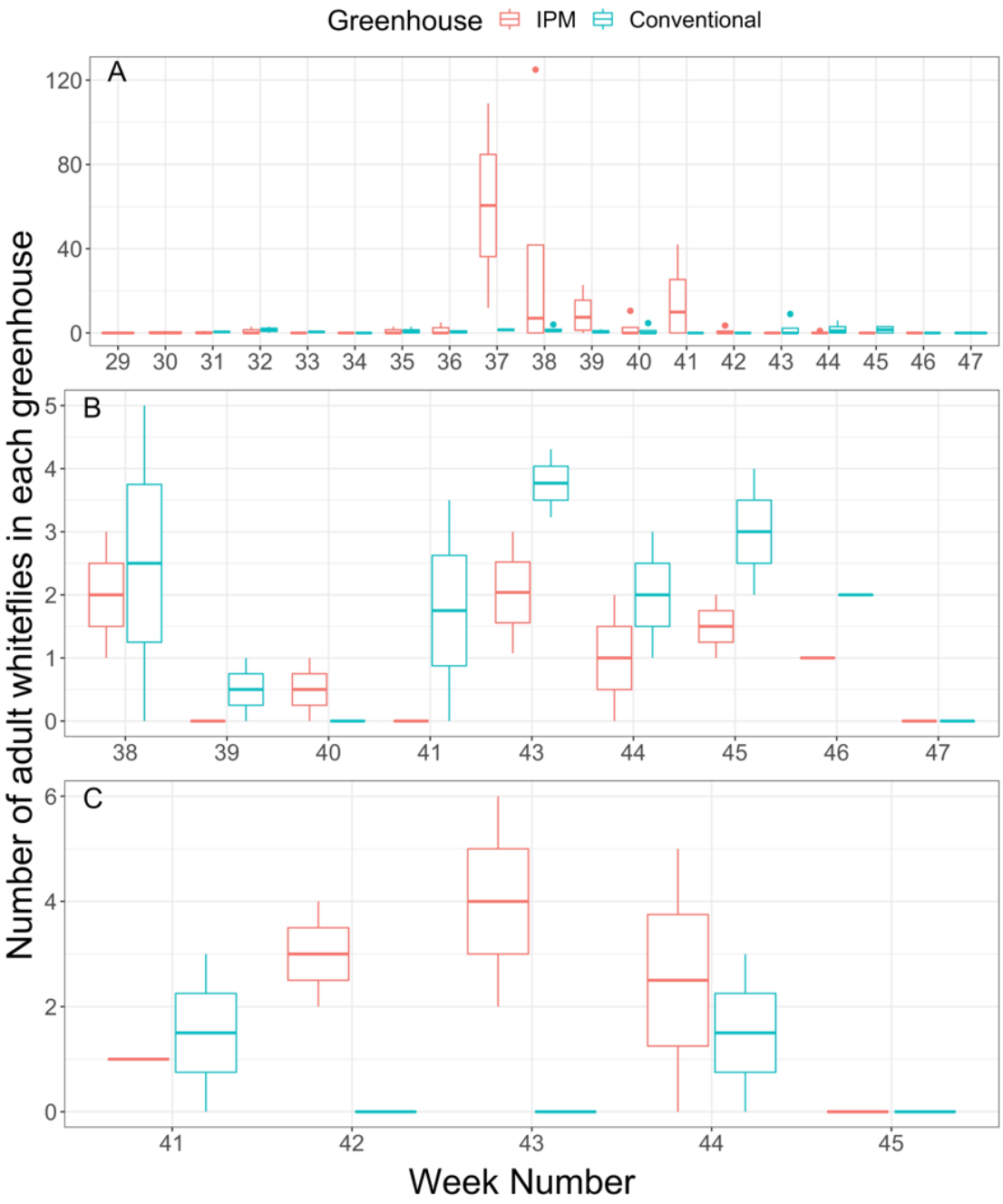

Figure 5.7. Number of adult whiteflies on yellow sticky traps from IPM or conventionally management greenhouse by calendar week number for growers A, B, and C. Number of adult whiteflies on sticky traps are represented by box plots, where dots represent extreme cases (i.e. outliers), vertical lines represent the top or bottom quartile, and the boxes represent the lower, middle (i.e. median), and upper quartile for adult whiteflies counted. The number of whiteflies counted on a given sampling date was reduced by the maximum number of whiteflies found previously on the yellow traps to determine the quantity of new whitefly adults trapped within the given sampling period.

of whitefly densities, one trap needs to be used per 18 – 20 plants in greenhouse tomato production (Gillespie and Quiring 1987), which would be considered impractical in poinsettia production. Our density of yellow sticky traps ranged from 1 per 25 to 287 m^2, which is within the sticky trap densities suggested by suppliers, researchers, and extension officers worldwide (1 trap per 93 to 500 m^2) (McDonough et al. 1999, Pinto-Zevallos and Vänninen 2013b). Due to the highly aggregated distribution of *B. tabaci* on poinsettias (Liu et al. 1993a), we would not suggest using less than 1 sticky trap per 300 m^2 to detect the presence of *B. tabaci*, and additional traps would certainly be needed to better estimate densities and spatial distribution of *B. tabaci* populations within the greenhouse.

To reduce labor costs associated with scouting, growers may consider "presence/absence" sampling, also known as 'binomial sampling', rather than counting all whitefly individuals on a given plant. Binomial sampling has been investigated for *B. tabaci* on cantaloupe (Tonhasca et al. 1994), watermelon (Lima et al. 2017), greenhouse ornamentals (Liu et al. 1993a, 1993b, Burns et al. 1999), cotton (Diehl et al. 1994, Naranjo et al. 1996), and greenhouse vegetable crops (Spinner et al. 2011), frequently correlating log variance of whitefly counts between plants with mean densities of *B. tabaci* within plants using Taylor's power law method (Taylor 1961). Across all growers, greenhouses, and weeks, we found a strong correlation between proportion of poinsettias infested and log-transformed mean whitefly immatures ($p<0.001$, adjusted $r^2=0.898$; Figure 5.8). The number of immature whiteflies found on poinsettias could be described by the equation: $Y = e^{(1.886x)}$, where y is the average number of whitefly immatures per plant, e is the exponential function, and x is the proportion of plants that have immature whiteflies. We also found a similar correlation when comparing proportion of plants

infested with log-transformed maximum number of mean whitefly immatures ($p<0.001$, adjusted $r^2=0.777$); $y_{max}=\exp^{(10.4098x)}$), where y_{max} is the maximum number of whitefly immatures found on a plant within a sampling period.

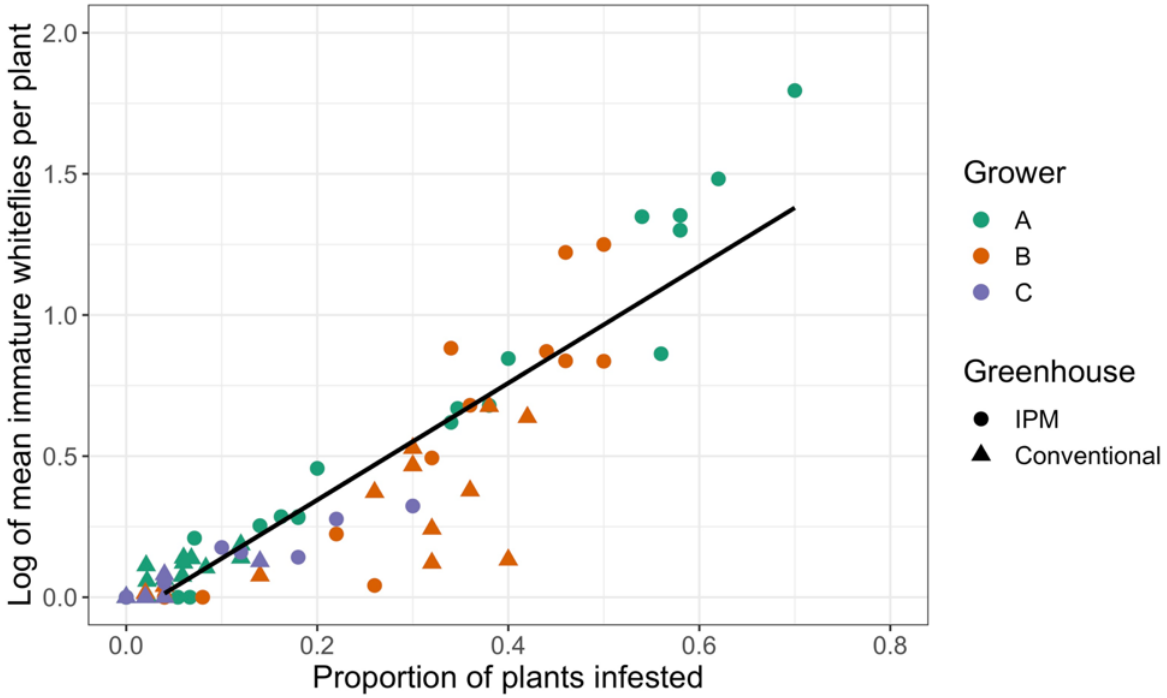

Figure 5.8. Relationship between average immature whiteflies observed per plant (log-scale) and proportion of plants infested with whiteflies. Initial model composed of proportion of plants infested with immature whiteflies as a fixed factor, greenhouse nested within grower, and week number as random factors, and log-transformed mean immature whiteflies per plant as the response variable in a generalized linear mixed model (GLMM) using the lmer (Kuznetsova et al. 2017) function in R Studio (R Studio Team 2015). All random factors were considered non-significant and were removed from the final model.

Growers may consider curative insecticide applications when the proportion of plants infested exceeds 0.4 (or 40%), as the highest number of immatures on a single plant predicted starts to exceed densities found at the retailer (~70 nymphs

per plant) (Vafaie et al. 2020a). However, caution should also be exercised when using presence/absence sampling, as the predictive power of this method decreases with decreasing sampling size and time spent inspecting a given plant (Burns et al. 1999). Growers can benefit from future research investigating minimum sample size and validating the robustness of using binomial sampling to predict whitefly density.

5.5. Recommendations

Growers cannot rely on yellow sticky traps alone to determine whitefly densities, but can use yellow sticky traps (1 trap per 300 m^2 at minimum) as good indicators of when to start scouting poinsettias for whiteflies within greenhouse-grown poinsettias. Growers can reduce labor costs associated with scouting for whiteflies on poinsettias by using "presence/absence" sampling rather than counting all individuals on a plant as a predictor of average whitefly density or maximum immature whiteflies per plant; however, additional validation is needed to determine how many poinsettias need to be sampled and for how long to reliably predict whitefly densities. Whiteflies are unevenly distributed within a greenhouse, so care should be taken to sample throughout the greenhouse to detect any areas that may have high whitefly densities.

Whitefly management was most effective throughout the growing season using conventional management strategies; however, both IPM and conventional management strategies provided sufficiently low whitefly densities and proportion of plants infested for retail and were economically comparable. Both strategies relied on effective monitoring data to make spray application decisions rather than prophylactic use of insecticides. Based on susceptibility of local *B. tabaci* populations to currently available insecticides, the

current cost of natural enemies, and recommended natural enemy release rates, growers in Texas, USA may consider releasing *E. eremicus* weekly (between 0.97 to 2.58 pupae per m^2) and *A. swirskii* every four weeks (between 26.9 to 42 mites per m^2) with focused insecticidal treatments when *B. tabaci* populations continue to increase over several consecutive sampling periods. Added benefits of reduced insecticide use include lack of re-entry interval, less concerns with phytotoxicity or insecticide residues, and decreased concern for pesticide applicator health, especially during high temperatures.

5.5.1. References

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6. CONCLUSIONS

6.1. Main Findings

The main objective of this dissertation was to investigate whether the combination of *Eretmocerus eremicus* Rose & Zolnerowich (Hymenoptera: Aphelinidae) and *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) could effectively suppress *Bemisia tabaci* (Gennadius) (Hemipera: Aleyrodidae) on poinsettias, *Euphorbia pulcherrima* Willd. ex Klotz. (Malpighiales: Euphorbiaceae), in a seasonal inoculative biological control program on poinsettias. Poinsettia cuttings coming from propagators have been considered a major source of whitefly infestation (Buitenhuis et al. 2016); however, few studies have conducted surveys to check this assumption. Additionally, pest thresholds on ornamental plants shipped to retailers have commonly been considered "essentially zero" (Bethke and Cloyd 2009). In my initial objective, I tested these two assumptions about *B. tabaci* on poinsettia cuttings received by growers and whitefly densities on finished color poinsettias at retailers. A survey of poinsettia cuttings received by two growers over two years revealed very low initial *B. tabaci* densities, with 41 out of 2,417 (1.7%) cuttings having any signs of infestation and an average of 0.10 nymphs per cutting. Despite low starting populations and regular applications of insecticides during poinsettia production, retailer whitefly densities were as high as 73 nymphs per plant counted within 60-seconds and at least 40% of poinsettias observed were infested at any given retailer. The vast majority of producers of poinsettias (11 of 12 growers) and from all geographic locations (Texas, Canada, and California) were infested with immature *B. tabaci* at the retailer. The starting *B. tabaci* densities and acceptable densities at the retailers set our standard for 'successful' management of *B. tabaci* in poinsettia production.

In my second objective, I investigate whether increasing natural enemy diversity (two species) can provide superior suppression of *B. tabaci* compared to either natural enemy alone. Natural enemies were selected based on characteristics that support complementarity due to differences in feeding preferences (niche partitioning) (Bográn et al. 2002, Nomikou et al. 2004, Liu et al. 2015, Snyder 2019). The combination of *E. eremicus* and *A. swirskii* provided equivalent suppression of *B. tabaci* compared to either natural enemy alone; however, the combination treatment had the lowest final mean immature *B. tabaci* density (8.4 ± 2.5 per poinsettia). Doubling the density of released natural enemy conspecifics did not significantly increase *B. tabaci* suppression, supporting that increasing diversity of natural enemies (*E. eremicus* with *A. swirskii*) may be more favorable for *B. tabaci* suppression. The ability for the combination natural enemy treatment to maintain *B. tabaci* populations below densities found at retailers was density-dependent, with successful suppression of *B. tabaci* when starting populations were below 14.3 ± 1.9 immature *B. tabaci* per poinsettia and failing when densities exceeded a starting population of 40.8 ± 2.5 immature *B. tabaci* per poinsettia.

In my third objective, I investigate whether the combination of *E. eremicus* and *A. swirskii* can maintain *B. tabaci* suppression when challenged with delays in natural enemy releases and whitefly immigration. Both poor timing of natural enemy releases (Heinz et al. 1993, Collier and Van Steenwyk 2004, Tang et al. 2010) and whitefly immigration (Bethke and Paine 1991a, Bellamy et al. 2004, Collier and Van Steenwyk 2004, Bell and Baker 2009, Liu et al. 2015) can disrupt biological control; however, whether the negative impact of these two challenges can be decreased by increasing natural enemy diversity had not been investigated. In greenhouse caged experiments, the combination of *E. eremicus*

and *A. swirskii* maintained superior suppression of *B. tabaci* compared to *E. eremicus* alone despite being challenged with whitefly immigration (at week 4 or 8 of the trial) and delays in natural enemy releases (at weeks 4 and 8 of the trial). Additionally, negative intraspecific interactions were further exacerbated at higher natural enemy densities (compared to the second objective), resulting in significantly superior suppression of *B. tabaci* when using the combination of the natural enemies compared to increasing the density of either natural enemy alone.

Lastly, in my fourth objective I conducted a case study to compare the efficacy of a *B. tabaci* management program based on the release of *E. eremicus* and *A. swirskii* compared to conventional insecticide use in commercial poinsettia production. Despite whitefly densities being similar or consistently higher in the natural enemy-released greenhouses compared to conventionally managed greenhouses, final *B. tabaci* densities and proportion of poinsettias infested were well below retailer thresholds found in the first objective (73 nymphs/poinsettia and 0.69 proportion infested). The cost of inputs in the biological control greenhouses were lower ($0.057) or higher ($0.178) than frequently reported input costs for 15.2-cm potted poinsettias ($0.09). Economics of biological control were favored when labor costs associated with pesticide applications were included in the partial budget analysis and when some tandem insecticides were used to decrease high whitefly densities. No other common pests of poinsettia were detected during the commercial trials, which could have been due to lack of pest pressure or suppression of secondary pests by *A. swirskii*.

Prior to this dissertation, research on seasonal inoculative biological control of *B. tabaci* on poinsettias focused on single species natural enemy releases, namely *Encarsia*

formosa (Gahan) (Hymenoptera: Aphelinidae) and *E. eremicus*, in cooler climate regions (i.e. Massachusetts, New York, and New England) and our understanding of how whitefly immigration or delays in natural enemy releases would impact final *B. tabaci* densities managed by natural enemies was limited. This research demonstrates that increasing natural enemy diversity, specifically *E. eremicus* and *A. swirskii*, is more beneficial than doubling the rate of conspecific natural enemies to manage *B. tabaci* on poinsettias, even when challenged with whitefly immigration or delays in natural enemy releases, and that releases of these natural enemies to manage *B. tabaci* in warm climates of Texas can be economically viable and produce poinsettias acceptable for retail.

6.2. Future Research

6.2.1. Scouting Tools

Sampling techniques for *B. tabaci* remain relatively unchanged over the last 30 years (Ohnesorge and Rapp 1986, Moerkens et al. 2019). Yellow sticky traps are still considered unreliable indicators of *B. tabaci* populations in poinsettias at low *B. tabaci* densities (Pinto-Zevallos and Vänninen 2013a) and manual inspection of poinsettia leaves is still common practice for estimating whitefly densities; a method that is labor-intensive and subsequently considered costly. New methodologies, such as the use of light emitting diodes (LEDs) (Chen et al. 2004, Chu et al. 2004) or adding specific patterns to yellow sticky traps (Kim and Lim 2011) appear to increase the reliability of the trap as an early indicator of *B. tabaci* presence. Further methods are being developed to automate detecting and counting whiteflies on yellow sticky traps from images (Moerkens et al. 2019), which could further decrease the time spent monitoring. However, special attention needs to be

given to increasing trap catch efficiency for *B. tabaci* without adversely affecting biological control efforts by trapping more natural enemies (Hoelmer et al. 1998, Karut and Kazak 2007). Traps constructed of plastic cups coated with Tanglefoot® (The Scotts Company LLC, Ohio, USA) and a 530 nm lime-green LED inside increased *B. tabaci* trapped by 100% and decreased *E. eremicus* and *En. formosa* trapped compared to yellow sticky traps in greenhouse tomatoes (*Lycopersicon esculentum*) and bell pepper (*Capsicum annuum*) (Chu et al. 2003, Nombela et al. 2003), but has not yet been tested in greenhouse ornamentals.

Research should also focus on increasing reliability of presence/absence (i.e. binomial) sampling in estimating whitefly densities in poinsettias. Effective and reliable binomial sampling methods have been developed for *B. tabaci* on field-grown watermelons (*Citrullus lanatus*) (Lima et al. 2017) and cantaloupes (*Cucumis melo*) (Tonhasca et al. 1994), providing economic and time-effective methods to estimate *B. tabaci* populations and make management decisions. Preliminary results from my fourth objective suggest that binomial sampling may be a great indicator of mean and maximum *B. tabaci* densities in poinsettias; however, the minimum amount of time needed to determine presence/absence on a poinsettia, minimum sample size (i.e. number of poinsettias), and model validation in different locations across years is needed to produce a reliable method to estimate *B. tabaci* densities using presence/absence sampling in poinsettias.

6.2.2. Natural Enemy and Habitat Diversity

Increasing natural enemy diversity through conservation biological control in landscapes can increase herbivore suppression if the natural enemies occupy distinct feeding niches (Snyder 2019). However, the realized niche and interactions between natural enemies can be context-dependent, with favorable outcomes for herbivore suppression in augmentative biological control programs in large complex landscapes compared to small simple ones (Perez-Alvarez et al. 2019). Interactions between natural enemies in biological control have commonly been investigated in Petri dish and small scale experiments (Messing et al. 2006), which can be beneficial for isolating specific interactions or behavioral characteristics. However, the realized niche and interactions between organisms in an ecosystem is scale- and context-dependent (Perez-Alvarez et al. 2019), making predictions based on small-scale experiments unreliable. On the other hand, commercial-scale trials are less feasible and cost-prohibitive. To determine the density and diversity of natural enemies to release for optimal herbivore suppression, future research needs to characterize how interactions between natural enemies change when manipulating the scale of simplistic ecosystems.

In some instances, habitat diversity can be increased to conserve natural enemies in greenhouse production, such as in banker plant systems. Banker plants are long-lasting systems designed to sustain predators or parasitoids by providing consistent sources of nutritional resources, such as prey or pollen (Huang et al. 2011). One of the intents of banker plant systems are to decrease the cost and increase predictability by combining factors of augmentative and conservation biological control (Frank 2010). Banker plant systems have shown promise in managing *B. tabaci* on greenhouse vegetables using

papaya or ornamental pepper (*C. annuum* varieties 'Masquerade, 'Red Missile', and 'Explosive Ember') to maintain *Encarsia sophia* (Xiao et al. 2011) or *A. swirskii* (Xiao et al. 2012), respectively. The lack of studies investigating banker plant systems to manage *B. tabaci* on greenhouse ornamentals (Frank 2010) provides opportunities for further research.

6.2.3. Tandem Insecticide Use

The use of selective and low-residual insecticides, in combination with natural enemies, can reduce the risk of crop loss in instances of high pest pressure (Gentz et al. 2010). The cost of biological control using *E. eremicus* or *En. formosa* was up to 44-fold the cost of conventional insecticide rotations in studies prior to the year 2000 (Table 1.1). A combination of reducing wasp release densities and tandem use of compatible insecticide drastically decreased the cost of biological control compared to conventional insecticide rotations, eventually bringing the price on par or even lower than conventional insecticide rotations (Van Driesche and Lyon 2003). For example, two mid-season applications (one week apart) of an insect growth regulator, buprofezin, allowed for a three-fold decrease in quantity of *E. eremicus* required to manage *B. tabaci* in commercial poinsettia crops, resulting in a ~3-fold decrease in cost compared to a program relying solely on *E. eremicus* (Van Driesche, Hoddle, Lyon, and Sanderson 2001).

Similarly, high populations of *B. tabaci* later in the season can be problematic when relying solely on natural enemies for suppression (Hoddle et al. 2001a, Hoddle et al. 2001b), resulting in management that greatly benefits from a toolbox of natural enemy-compatible insecticides. This toolbox is increasing with the work of the IOBC-Pesticides

and Beneficial Organisms Working Group, which has developed standardized guidelines to test side-effects of pesticides on natural enemies. Further investigation in thresholds and timing of natural enemy-compatible insecticides would be valuable to increasing economics and mitigating risk associated with sudden increases in *B. tabaci* populations in a biological control program.

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