EFFICACY OF SYSTEMIC INSECTICIDES AGAINST THE CITRUS MEALYBUG, *PLANOCOCCUS CITRI*, AND PESTICIDE MIXTURES AGAINST THE WESTERN FLOWER THRIPS, *FRANKLINIELLA OCCIDENTALIS*, IN PROTECTED ENVIRONMENTS

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Abstract

Protected environments, such as greenhouses and interior plantscapes provide optimal conditions for arthropod (insect and/or mite) pests to survive, develop, and reproduce. Two commonly encountered insect pests in protected environments include the citrus mealybug (CMB), *Planococcus citri*, and the western flower thrips (WFT), *Frankliniella occidentalis*. It is difficult to mitigate CMB and WFT populations due to the behavioral characteristics of the insects and few pesticides that are registered for use in protected environments. This research involved two distinctly different studies. The objectives of the first study were to determine the efficacy and residual activity of systemic insecticides registered for use against CMB and to quantify CMB feeding locations. The objectives of the second study were to determine the compatibility and efficacy of commonly used binary pesticide mixtures against the WFT under both laboratory and greenhouse conditions.

To determine the efficacy of systemic insecticides against CMB, greenhouse experiments were conducted in which coleus, *Solenstemon scutellarioides*, plants were artificially infested with CMB. Drench applications of each designated treatment were applied to each plant. Results associated with drench applications of the systemic insecticides against CMB indicated minimal CMB mortality (<30%) for both preventative and curative drench applications of azadirachtin and spirotetramat. Thiamethoxam, a neonicotinoid-based insecticide, at the labeled and twice the labeled rate provided the highest CMB mortality; however, not until 21 days after treatment was this observed, and CMB mortality was <80%. In all cases, significantly more CMB were located on the stem of green coleus plants compared to the leaf top and bottom.

Pesticide mixture compatibility was determined using jar tests. In addition, phytotoxicity and efficacy of pesticide mixtures against WFT was determined through a series of laboratory and greenhouse experiments for each individual pesticide, and the mixtures to determine synergism, antagonism, or no effect. Results associated with the jar tests indicated that all the mixtures were compatible. Furthermore, the mixtures were not phytotoxic to the horticultural plant species evaluated. Laboratory results indicated that mixtures containing spinosad + bifenazate were antagonistic against WFT. Greenhouse experiments demonstrated significantly reduced efficacy associated with the abamectin + azadirachtin mixtures; however, each binary mixture provided approximately 80% mortality of WFT.

Table of Contents

List of Figures

List of Tables

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Dedication

I dedicate this work to my Dad, for introducing me to the world of horticulture.

Chapter 1 - Introduction and Literature Review

Introduction

Protected environments are those that maintain plants year round such as greenhouses and interior plantscapes (Manaker, 1997; Hammer, 2011). Plants commonly maintained in protected environments are either ornamental and/or horticultural, and are based on aesthetic appeal (Bethke and Cloyd, 2009). This allows for minimal to no visible damage, and any heavily-damaged plants are often disposed of, which may reduce profits (Bethke and Cloyd, 2009). A benefit of protected environments is that environmental parameters such as temperature, relative humidity, and light intensity can be controlled to some extent (Cloyd and Lindquist, 2001). Not only do protected environments provide ideal growing conditions for plants, they also provide optimal conditions for a wide array of arthropod (insect and/or mite) pests to survive, develop, and reproduce (Cloyd and Lindquist, 2001).

Arthropod pests may be introduced into protected environments when plants are transported from their native locations, where pests are cosmopolitan or by exchanging plant material (McKenzie, 1967; Kole and Hennekam, 1990). As a result, greenhouse producers and interior plantscape curators often encounter multiple arthropod pests throughout the year. Typically, greenhouse producers and interior plantscape curators apply pesticides (insecticides and/or miticides) to mitigate arthropod pest populations (Bethke and Cloyd, 2009; Franco et al., 2009). Pesticides provide rapid control/regulation of a number of arthropod pest species (Bethke and Cloyd, 2009). Currently, there are a limited number of products registered for use in protected environments, with most of the products registered for use in interior plantscapes classified as systemic insecticides (Bethke and Cloyd, 2009). These insecticides are translocated throughout the plant vascular tissue (Bennett, 1949), and are considered “safer” to use among

human traffic (Cloyd, 2010). However, there is little information available regarding the efficacy of the currently registered systemic insecticides.

Greenhouse producers also encounter difficulties mitigating arthropod pest populations because of the limited number of pesticides available. In general, there is an array of arthropod pest species present simultaneously in greenhouses capable of producing multiple generations per year (Cloyd, 2001; Cloyd and Lindquist, 2001). This is a concern as the current commercially available pesticides have a narrow-spectrum of target pest toxicity in order to comply with the Food Quality Protection Act (FQPA) (Sray, 1997). As such, multiple pesticides are necessary to mitigate the arthropod pest complex encountered. However, repeat applications are labor intensive and expensive (Jeppson, 1953); therefore, many greenhouse producers apply pesticide mixtures to manage the multitude of arthropod pests (Cloyd, 2009). In addition, due to the presence of multiple generations of arthropod pests each year, greenhouse producers may use pesticides with the same mode of action (MOA) within a cropping cycle. The indiscriminate use of pesticides with the same MOA, however, may increase the rate in which arthropod pest populations develop resistance (Georghiou and Taylor, 1977a; Brattsten et al., 1986). Consequently, it has been suggested to rotate or mix together pesticides with different MOA's to alleviate selection pressure and delay the onset of resistance (Georghiou and Taylor, 1977a).

Despite the pest management challenges encountered in both greenhouses and interior plantscapes, there is minimal information available on the efficacy of currently registered products. Therefore, the goal of this research is to provide quantitative data on the efficacy of currently available pesticides registered against two commonly encountered insect pests; the citrus mealybug, *Planococcus citri*; and the western flower thrips, *Frankliniella occidentalis*. This research will benefit the horticultural industry by prolonging the efficacy of insecticides

currently available and help greenhouse producers and interior plantscape curators to make better pest management decisions.

Literature Review

Interior plantscape environments

Interior plantscapes are protected environments that maintain tropical foliage plants for use in offices, homes, restaurants, shopping complexes, hotels, and medical buildings (Manaker, 1997). The practice of maintaining plants indoors has existed for centuries; however, in the 1970's, the importance of interior plantscapes became apparent (Manaker, 1997). Aside from the pleasing aesthetics plants provide indoors, plants also offer physiological and behavioral benefits to people including a reduction in stress levels and increased worker productivity (Relf, 1990; Lohr et al., 1996; Bringslimark et al., 2007). In addition, interior plantscapes in hospitals have led to multiple benefits for recovering patients including faster recovery times, reduced amounts of pain medications requested, lower anxiety and stress levels, and overall less pain when patients had a window view or plants were present in their hospital room (Park and Mattson, 2009). Also, indoor plants have been documented to improve air quality (Wood et al., 2002).

Due to the benefits of interior plantscapes, there is a demand for tropical foliage plants grown in North America especially in Florida, California, and Texas (McKenzie, 1967), with the wholesale value of indoor plants having increased from $37.6 million dollars anually in 1971 to approximately $287.4 million dollars in 2009 based on the annual income of the top 25 interior plantscape companies within the USA (Manaker, 1997; unpublished data, Interiorscape Magazine, 2009). However, arthropod pests may be introduced into interior plantscapes on plants shipped from these locations, where certain pests are cosmopolitan, and/or by exchanging plant material between indoor gardens (Kole and Hennekam, 1990). According to Cloyd and

Lindquist (2001), the primary arthropod pests of interior plantscapes include mealybugs, scales, aphids, thrips, whiteflies, fungus gnats, shore flies, leafminers, and spider mites. Both the citrus mealybug (CMB), *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae), and the longtailed mealybug (LTMB), *Pseudococcus longispinus* (Targioni-Tozzetti) (Hemiptera: Pseudococcidae), are two mealybug species commonly encountered in interior plantscape environments (McKenzie, 1967; Steiner, 1987; Cloyd and Lindquist, 2001).

Mealybug development

Female mealybugs have five life stages; egg, three instars, and adult (Franco et al., 2009). Both CMB and LTMB have similar life cycles except that LTMB are ovoviviparous (females give birth to live instars), whereas CMB are oviparous (females lay eggs) (Clausen, 1915; Franco et al., 2009). Citrus mealybug and LTMB males undergo six life stages and become winged adults (McKenzie, 1967; Cloyd and Lindquist, 2001). Previous research on citrus by Clausen (1915) indicated that LTMB females produce less than half of the offspring than other mealybug species such as the CMB. For instance, on average, a female CMB produces 483 eggs while average offspring produced by LTMB is 206 (Clausen, 1915). In addition, because LTMB females give birth to live offspring, there is a higher mortality rate during the first larval instar compared to other mealybug species (Clausen, 1915; Furness, 1976). However, the average time period for CMB and LTMB development for each instar is similar. The CMB takes approximatley 15-16 days to develop each life stage, whereas the LTMB takes appromately 13-17 days (Clausen, 1915).

Mealybug damage and management

Mealybugs cause both direct and indirect damage to plants. Direct damage occurs during feeding on plant vascular tissues (e.g., phloem), which results in an increased risk of infection

from plant pathogens, stunting, yellowing of leaves, leaf drop, and occasionally plant death (Kosztarab, 1996; Cloyd and Lindquist, 2001). Mealybugs are among the most destructive pests of interior plantscapes because of their feeding behavior (Kosztarab, 1996). For instance, mealybugs have been documented to have fewer intracellular punctures for longer durations compared to other phloem-feeding insects such as aphids and whiteflies (Calatayud et al., 1994). Furthermore, indirect damage is caused by the excretion of honeydew, a clear sticky liquid that is an ideal growing medium for black sooty mold fungi, which can reduce the ability of plants to photosynthesize (Kosztarab, 1996; Manaker, 1997). Also, honeydew is a supplimental food source for ants which may protect mealybugs from biological control agents or natural enemies such as parasitoids or predators (McKenzie, 1967; Kole and Hennekam, 1990). The potential economic losses associated with managing mealybug populations in indoor and outdoor environments are estimated to be $500 million dollars annually (Kosztarab, 1977).

Insecticides are typically applied to mitigate mealybug populations (Franco et al., 2009); however, there are a limited number of insecticides registered for use in interior plantscapes. This is a concern because arthropod pests may develop resistance to insecticides currently being used. Although there are no documented cases of insecticide resistance associated with either CMB or LTMB, resistance has been reported with other mealybugs in the genera, *Planococcus* and *Pseudococcus* (Whalon et al., 2012). Furthermore, most products registered for use in interior plantscapes are classified as systemic insecticides, however, minimal, if any information is available regarding efficacy of currently available systemic insecticides used in protected environments.

Systemic insecticides

Systemic insecticides are compounds absorbed by the plant and translocated throughout the vascular system (e.g., xylem and phloem). They may be applied as a foliar spray, drench, or granule to the soil/growing medium (Bennett, 1949; Cloyd et al., 2011). Systemic insecticides are primarily toxic to xylem- and phloem-feeding insect pests such as aphids, whiteflies, leafhoppers, soft scales, and mealybugs. There are a number of advantages associated with drench applications of systemic insecticides compared to foliar sprays including minimal exposure to workers and consumers, and less direct effects on natural enemies (e.g., parasitoids and predators) (Cloyd, 2010). Also, areas missed when applying a contact foliar insecticide as well as any new plant growth following an application are protected with systemic insecticides, thus eliminating the need for multiple applications (David and Gardiner, 1951; Jeppson, 1953; Reynolds, 1954; Brück et al., 2009; Byrne et al., 2010). Additionally, applying systemic insecticides as drenches decreases the amount of material lost due to evaporation, photolysis, and irrigation (Rudinsky, 1959; Cloyd et al., 2011).

However, despite these advantages, there are a number of factors that affect the efficacy of systemic insecticides including plant species, plant age, insect pest feeding behavior, water solubility, and growing medium type (Ripper et al., 1949; Glynne-Jones and Thomas, 1953; Jeppson, 1953; Bennett, 1957; Reynolds and Metcalf, 1962; Abdellatif et al., 1967; Pfluger and Schmuck, 1991; Wakita et al., 2005; Cloyd and Bethke, 2011; Cloyd et al., 2011). Also, environmental conditions and factors related to plants such as light intensity, temperature, relative humidity, plant size, root system establishment, and the physiological stage of development may impact the uptake of systemic insecticides (Jeppson, 1953; Wedding, 1953; Daane et al., 2006; Castle and Prabhaker, 2011; Cloyd et al., 2012). Regardless of these factors,

systemic insecticides are widely used in protected environments to mitigate hemipteran insect pests.

Azadirachtin and spirotetramat

Previous research by Ripper et al. (1949) and Hanna et al. (1952) documented that CMB were affected by systemic insecticides including bisdimethylamino-fluoro-phosphine and *bis* (*bis*dimethylaminophosphonous) anhydride. This research indicated that CMB will ingest lethal concentrations of systemic insecticides when feeding. The efficacy of azadirachtin as a systemic insecticide was discovered by Gill and Lewis (1971) and has been demonstrated against a variety of insect pests with piercing-sucking and chewing mouthparts including cabbage aphid (*Brevicoryne brassicae*), western flower thrips (WFT), (*Frankliniella occidentalis*), leafminer (*Liriomyza triflii*), and Japanese beetle (*Popillia japonica*) (Lindquist et al.,1986. Pavela et al., 2004; Thoeming et al., 2003; Vitullo and Sadof, 2007). Azadirachtin is known to act as an insect growth regulator (ecdysone antagonist), repellent, or antifeedant against insects (Mordue and Nisbet, 2000). It has been reported that plant height may be affected by certain systemic insecticides (Mellors et al., 1984). However, Vitullo and Sadof (2007) reported that azadirachtin had no significant effect on plant growth. Furthermore, factors such as growing medium type have been shown to affect the efficacy of azadirachtin, where the higher amount of organic matter present in the growing medium resulted in lower insect pest mortality (Thoeming et al., 2003).

Spirotetramat is in the tetramic acid group of systemic insecticides (Nauen et al., 2008; Yu, 2008; Brück et al., 2009) and inhibits lipid biosynthesis against both immature and adult stages of insects with piercing-sucking mouthparts (Yu, 2008; Brück et al., 2009). The efficacy of spirotetramat has been documented against populations of mealybugs, psyllids, sweet potato

whitefly (*Bemisia tabaci*) and a number of aphid species. Brück et al. (2009) found spirotetramat was efficacious against mealybugs on grapes (*Vitis* spp.). In addition, systemic activity of spirotetramat was effective against whiteflies on bell pepper (*Capsicum* spp.) plants. Also, eight aphid species were susceptible to foliar applications of spirotetramat when feeding on either lettuce (*Lactuca* spp.) plants or apple (*Malus* spp.) leaves (Brück et al., 2009). However, Kay and Herron (2010) reported that spirotetramat was not effective against WFT adults but was against nymphs. Despite the label information and use of azadirachtin and spirotetramat against mealybugs in protected environments, there is minimal quantitative data available associated with their efficacy against CMB under greenhouse conditions and whether these insecticides affect plant height.

Neonicotinoids

Neonicotinoid insecticides act as agonists on the nicotinic acetylcholine receptor leading to an overstimulation of the cholinergic synapses, resulting in hyper-excitation, convulsions, paralysis, and death of insects (Yu, 2008). Both imidacloprid and thiamethoxam have been evaluated under field conditions against the pink hibiscus mealybug, (*Maconellicoccus hirstus*) on mulberry (*Morus alba*) trees (Castle and Prabhaker, 2011). The researchers found that a single application of either imidacloprid or thiamethoxam eliminated mealybug infestations; however, efficacy varied on certain trees (Castle and Prabhaker, 2011). In addition, imidacloprid has been evaluated against the vine mealybug (*Planococcus ficus*) using drip- and furrow-irrigation application methods (Daane et al., 2006). It was concluded that the efficacy of the neonicotinoid insecticides varies against mealybugs depending on application method. However, Kay and Herron (2010) observed no efficacy with imidacloprid when applied as a drench against WFT. Although researchers have evaluated the efficacy of neonicotinoid insecticides under field

conditions, there is little quantitative data available on their efficacy in protected environments despite the number of insecticides commercially available.

Greenhouse environments

Greenhouses are classified as protected environments covered by glass or plastic, which provides a controlled environment for year round production of plants (Hammer, 2011). Commonly encountered arthropod pests of greenhouses include thrips, mealybugs, aphids, whiteflies, leafminers, fungus gnats, shore flies, and spider mites (Brødsgaard and Albajes, 1999). Western flower thrips (WFT), *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) is a major economically important insect pest of many horticultural crops grown in greenhouses (Robb and Parrella, 1988). The WFT was first described in the United States in 1895 in California (Pergande, 1895). However, it was not until 1983 that WFT was discovered in greenhouses in the Netherlands (Tommasini and Maini, 1995). Today, WFT is considered a worldwide insect pest of horticultural crops grown in greenhouse and outdoor production systems (Kirk, 2002).

Western flower thrips development

The WFT undergoes six life stages including an egg, two nymphal stages, prepupa, pupa, and adult (Lewis, 1997). Nymphs feed actively on all plant parts with their piercing-sucking mouthparts (Chisholm and Lewis, 1984; Hunter and Ullman, 1992). The prepupa and pupa life stages of WFT are typically located in the growing medium (Cloyd and Lindquist, 2001). The WFT populations are difficult to manage due to their life cycle. Furthermore, WFT can hibernate in plant crevices, which makes detection difficult for greenhouse producers. The WFT can cause both direct and indirect damage to plants while feeding (Ullman et al., 1993; Broadbent and Allen, 1995; Mound, 1996; Hull, 2009).

Western flower thrips damage and management

Direct damage is caused by WFT using their piercing-sucking mouthparts to withdraw plant fluids, resulting in deformation of leaves and flowers (Childers and Achor, 1995; Cloyd and Lindquist, 2001). The WFT cause indirect damage by transmitting two destructive tospoviruses; impatiens necrotic spot virus and tomato spotted wilt virus (Sether and DeAngelis, 1992; Broadbent and Allen, 1995; Daughtrey et al., 1997; Hull, 2009). Because WFT can vector viruses, it is important to maintain WFT populations at low levels (Bethke and Cloyd, 2009).

In order to manage/regulate WFT populations, greenhouse producers routinely apply insecticides (Bethke and Cloyd, 2009). However, there are a limited number of effective products available for use in greenhouse production systems. In addition, the relatively short life cycle (10 to 24 days depending on temperature) and extensive use of insecticides are factors that may lead to resistance development in WFT populations (Georghiou, 1977a; Georghiou, 1977b). The first instance of WFT resistance was reported in 1990; since then WFT has documented to be resistant to 23 active ingredients (Whalon et al., 2012). Out of the 23 active ingredients, 4 are registered for use in greenhouses which include abamectin, acephate, bifenthrin, and spinosad (Immaraju et al., 1992; Brødsgaard, 1994; Robb et al., 1995; Loughner et al., 2005; Bielza et al., 2008). In general, multiple arthropod pest species are commonly encountered simultaneously in greenhouses (Bethke and Cloyd, 2009). Since the Food Protection Quality Act (FPQA) was enacted in 1996, there have been increased restrictions on the registration and use of many broad-spectrum pesticides. As such, pesticides with narrow-spectrums of arthropod pest activity are becoming more widely available. However, greenhouse producers still need to manage the array of arthropod pests (Sray, 1997). Therefore, in order to deal with this situation, greenhouse producers apply mixtures of pesticides (Cloyd, 2009). A pesticide mixture is a combination of

two or more pesticides within a single solution and applied simultaneously (O'Connor-Marer, 2000; Cloyd, 2001). However, despite the trend in using pesticide mixtures, there is minimal data substantiating the compatibility, and synergistic/antagonistic effects of pesticide mixtures against WFT.

Pesticide mixtures

Pesticide mixtures are used by greenhouse producers to mitigate arthropod pest populations (Cloyd, 2009). Mixing pesticides is less expensive, in general, because a single application can be made as opposed to multiple applications of different pesticides (Warnock and Cloyd, 2005). In addition, pesticide mixtures may be synergistic, which increases toxicity against arthropod pests (Hewlett, 1968; O'Connor-Marer, 2000; Warnock and Cloyd, 2005; Cloyd, 2011). Pesticide mixtures have been recommended for use as a resistance management strategy based on the assumption that insects will not develop resistance to multiple modes of action simultaneously (Brattsten et al., 1986; Roush, 1989; Yu, 2008). However, there are concerns that pesticide mixtures may lead to developing multiple resistance mechanisms in arthropod pest populations (Tabashnick, 1989).

Efficacy of pesticide mixtures against western flower thrips

The efficacy of pesticide mixtures against agricultural pests is well documented (All et al., 1977; Koziol and Witkowski, 1982). However, less information is available on pesticide mixtures against greenhouse arthropod pests (Warnock and Cloyd, 2005). Cloyd et al. (2007) demonstrated that a number of pesticide mixtures provided control of the silverleaf whitefly (*Bemisia argentifolii*) and twospotted spider mite (*Tetranychus urticae*) nymphs under greenhouse conditions. In addition, synergistic and antagonistic effects have been quantified for agricultural insects using a combination index (Attique et al., 2006). Synergism is when the

toxicity of the pesticides used in the mixture is greater to the target pest when combined, compared to if the compounds were applied separately (Hewlett, 1968; O'Connor-Marer, 2000). Antagonism occurs when the level of efficacy is reduced after pesticides are combined into a mixture (O'Connor-Marer, 2000; Lindquist, 2002). Certain pesticide mixtures, such as those containing an organophosphate and a pyrethroid insecticide have demonstrated synergism when applied to resistant strains of the cotton bollworm, *Helicoverpa armigera* (Martin et al., 2003). The synergism between pyrethroids and organophosphates has been attributed to esterase inhibition, which prevents cleaving of the ester-linkage in pyrethroids; thus allowing the pyrethroid to kill insect pests (Gaughan et al., 1980; Zhao et al., 1996; Martin et al., 2003).

The efficacy of pesticide mixtures against WFT has also been evaluated. Synergism of pesticide mixtures against field populations of WFT has been documented (Bielza et al., 2007; Bielza et al., 2009). However, in these studies, the mixtures were not effective against susceptible populations of WFT. Warnock and Cloyd (2005) reported that mixtures containing spinosad, bifenazate, abamectin, imidacloprid, and azadirachtin were not antagonistic (based on percent mortality) when applied against WFT under greenhouse conditions. However, these studies were conducted without feedback from greenhouse producers on pesticide mixtures typically used to manage arthropod pests. Consequently, there is no quantitative information associated with synergism and/or antagonism or compatibility, of commonly used pesticide mixtures.

Objectives

This research will involve two distinct studies addressing insect pest management in protected environments. The first study will determine the efficacy of systemic insecticides applied as drenches to the growing medium against CMB. The second study will assess the

efficacy of various pesticide mixtures against WFT under both laboratory and greenhouse conditions.

Objective 1: Systemic activity of insecticides against the citrus mealybug

This study will involve a series of experiments designed to evaluate the efficacy of a number of systemic insecticides against CMB when applied as drenches to the growing medium of coleus (*Solenstemon scutellarioides*) plants under greenhouse conditions. The systemic insecticides used in the experiments are azadirachtin, spirotetramat, thiamethoxam, dinotefuran, and imidacloprid. Both azadirachtin and spirotetramat are labeled for use against CMB; however, there is minimal quantitative data available on the efficacy of these pesticides when applied as drenches. Two experiments will involve preventative (experiment 1) and curative (experiment 2) treatments of azadirachtin and spirotetramat, which will determine if application timing affects the efficacy of these systemic insecticides. Also, feeding behavior may influence the efficacy of systemic insecticides against CMB. Therefore, for green and red coleus plants treated curatively, feeding location (e.g., plant stem, leaf top, or leaf bottom) of CMB will be determined.

Two additional experiments will assess the residual activity, over an 8-week time period, of the neonicotinoid insecticides including thiamethoxam, dinotefuran, and imidacloprid at labeled and twice the labeled rate against CMB when applied as drenches to the growing medium. Similar to the previous experiments, feeding location of each CMB will be determined.

Objective 2: Efficacy of pesticide mixtures on the western flower thrips

This study will assess the compatibility and efficacy of commonly used pesticide mixtures against WFT under laboratory and greenhouse conditions. Each pesticide mixture will be evaluated for compatibility using jar tests, and applied to nine horticultural plant species to determine phytotoxicity (plant damage). In addition, a series of laboratory bean-dip bioassays

using the formulated pesticides individually and the formulated pesticides in mixtures to determine the LC_{50} values will be used to calculate a combination index (CI), which will quantitatively determine if binary pesticide mixtures are synergistic, antagonistic, or have no effect on adult WFT. Finally, greenhouse experiments will be conducted to determine the efficacy (based on percent mortality) of pesticide mixtures on WFT. This study will determine if the mixtures used by greenhouse producers are appropriate when applied to manage multiple arthropod pests and do not result in antagonistic effects against WFT.

Chapter 2 - Systemic activity of insecticides against the citrus mealybug (*Planococcus citri*)

Introduction

Mealybugs are one of the most common insect pests of greenhouse and interior plantscape environments (McKenzie, 1967; Kole and Hennekam, 1990; Stauffer and Rose, 1997; Manaker, 1997). These protected environments provide optimal conditions including temperature and relative humidity for insect pests to survive, develop, and reproduce (Cloyd and Lindquist, 2001). Two commonly encountered mealybug species of greenhouses and interior plantscapes are the citrus mealybug (CMB), *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae), and the longtailed mealybug (LTMB), *Pseudococcus longispinus* (Targioni-Tozzetti) (Hemiptera: Pseudococcidae) (Steiner, 1987; Cloyd and Lindquist, 2001). Both CMB and LTMB cause damage to plants; however, the CMB is known to cause substantial economic damage (Cox, 1981). This may be associated with the ability of the females to produce twice as many offspring in a shorter time period than LTMB. Also, LTMB has a higher mortality rate during the first instar stage compared to CMB (Clausen, 1915).

Mealybugs are polyphagous insects that feed within the vascular tissue of plants using their piercing-sucking mouthparts, to withdraw plant fluids (Kosztarab, 1996; Cloyd and Lindquist, 2001). Direct damage to plants results in stunting, yellowing of leaves, leaf drop, increased risk of infection by plant pathogens, and occasionally plant death (Kosztarab, 1996; Manaker, 1997; Cloyd and Lindquist, 2001). Indirect damage to plants is caused by the excretion of honeydew, a clear sticky liquid that serves as a growing medium for black sooty mold fungi, which can reduce the ability of plant photosynthesis (Clausen, 1915; Kosztarab, 1996; Manaker, 1997; Cloyd and Lindquist, 2001). Honeydew is also a supplemental food source for ants, which

protect mealybugs from biological control agents or natural enemies such as parasitoids and predators (McKenzie, 1967; Kole and Hennekam, 1990).

Mealybug populations are difficult to mitigate due to both biological and behavioral characteristics (Franco et al., 2009). For instance, mature mealybugs possess a thickened waxy layer above the cuticle, which prevents penetration of most insecticides (Manaker, 1997; Cloyd and Lindquist, 2001). In addition, mealybugs tend to reside in protected areas on plants such as new leaves, petioles, and within the plant canopy, which are difficult to reach with foliar applications of insecticides (Clausen, 1915; Furness, 1976; Goolsby et al., 2000). Nonetheless, insecticides are primarily used to suppress CMB populations (Franco et al., 2009); however, there are only a limited number of insecticides registered for use in greenhouse and interior plantscape environments including contact and systemic insecticides (Bethke and Cloyd, 2009). Currently, a number of systemic insecticides are registered for use in protected environments that may have activity against CMB. Therefore, it is important to quantitatively determine the efficacy of these commercially-available systemic insecticides.

Systemic insecticides are compounds that can be absorbed by the plant and translocated through the vascular system thus becoming toxic to xylem- and phloem-feeding insect pests such as aphids, whiteflies, leafhoppers, soft scales, and mealybugs (Bennett, 1949). Systemic insecticides may be applied as a foliar spray, drench, or granule to the soil/growing medium (Cloyd et al., 2011). In protected environments, there are a number of advantages associated with using drench applications of systemic insecticides compared to foliar sprays. For instance, drench applications, which are applied directly to the soil or growing medium, alleviate exposure to public traffic affiliated with protected environments, thus reducing potential exposure to workers, consumers, and natural enemies such as parasitoids and predators (Cloyd, 2010). Also,

systemic insecticides are translocated through the plant vascular system, protecting growth that would have been missed when applying a contact foliar insecticide, as well as any new plant growth following an application (David and Gardiner, 1951). As such, this provides protection for extended periods of time (Jeppson, 1953; Reynolds, 1954; Byrne et al., 2010). Furthermore, drench applications of systemic insecticides may reduce the need for multiple insecticide treatments because the active ingredient is translocated through the plant protecting inaccessible areas and new growth (Jeppson, 1953; Brück et al., 2009). Also, by eliminating multiple spray applications, there is a reduction in labor and equipment costs (Jeppson, 1953). Additionally, applying systemic insecticides as drenches decreases the amount of material lost due to evaporation, photolysis, and irrigation (Rudinsky, 1959; Cloyd et al., 2011). The benefits associated with systemic insecticides, as noted above, make them appropriate compounds for use in greenhouse and interior plantscape environments; however, there are a number of factors that may influence the efficacy of systemic insecticides.

Factors, which may impact the efficacy of systemic insecticides include plant species, plant age, insect pest feeding behavior, water solubility, and growing medium type (Ripper et al., 1949; Jeppson, 1953; Bennett, 1957; Reynolds and Metcalf, 1962; Abdellatif et al., 1967). Water solubility allows the active ingredient to be translocated throughout the plant; therefore, the more water soluble an active ingredient, the quicker it is translocated (Glynne-Jones and Thomas, 1953; Cloyd and Bethke, 2011; Cloyd et al., 2011). Growing media with a high organic matter content can also affect the efficacy of systemic insecticides (Abdellatif et al., 1967; Gill and Lewis, 1971). For instance, Pfluger and Schmuck (1991) and Wakita et al. (2005) found that a growing medium containing >30% organic matter binds to imidacloprid and dinotefuran, thus reducing the efficacy of these systemic insecticides. In addition, factors related to the plant such

as size, establishment of the plant root system, and physiology may affect the uptake of a systemic insecticide (Daane et al., 2006; Castle and Prabhaker, 2011). Environmental conditions such as light intensity and relative humidity may affect the amount of active ingredient absorbed by the plant (Jeppson, 1953; Wedding, 1953). Despite these factors, systemic insecticides are widely used to mitigate hemipteran insect pests such as mealybugs.

The use of compounds with systemic activity against hemipteran insects was initially demonstrated by Ripper et al. (1949). The compound *bis*(*bis*dimethylaminophosphonous)-anhydride displayed systemic activity against aphids and other hemipteran insects with piercing-sucking mouthparts, including CMB, when applied to a number of plant species. However, it was noted that there may be variations in the lethal concentration ingested among different species of aphids. Similarly, Hanna et al. (1952) reported that the systemic compound, bisdimethylamino–fluoro–phosphine oxide, reduced populations of CMB and the cacao mealybug, *Pseudococcus njalensis* (Laing) from 42,971 to 35 on cacao (*Theobroma cacao*) trees over six weeks. These studies indicate that CMB populations may be reduced by compounds that exhibit systemic activity. However, there is minimal quantitative data associated with the effectiveness of current systemic insecticides labeled for use against mealybugs in greenhouse or interior plantscape environments.

Several products are promoted to have systemic activity and are labeled for use against CMB in greenhouses and interior plantscapes including the active ingredients azadirachtin (Azatrol®: PBI Gordon Corp.; Kansas City, MO) and spirotetramat (Kontos™: OHP Inc.; Mainland, PA), and the neonicotinoid insecticides, imidacloprid, (Marathon II®: OHP, Inc.; Mainland, PA), dinotefuran (Safari®: Valent; Walnut Creek, CA), and thiamethoxam (Flagship®: Syngenta; Greensboro, NC) (Maienfisch et al., 2001; Tomizawa and Casida, 2003; Jeschke et al.,

2011; Cloyd et al., 2011; Uneme, 2011). The systemic activity of azadirachtin was discovered by Gill and Lewis (1971). Azadirachtin is a compound derived from the seeds of the neem tree (*Azadirachta indica*), which affects certain insect pests by acting as an insect growth regulator (ecdysone antagonist), repellent, or antifeedant (Mordue and Nisbet, 2000). The oral LD_{50} of azadirachtin for rats is >5000 mg/kg indicating it is non-toxic to mammals (Yu, 2008); thus products containing azadirachtin are a safe option for use in greenhouses and interior plantscapes. Furthermore, azadirachtin is reported to have systemic activity when applied to the soil/growing medium, however, efficacy depends on the insect pest as well as the plant species (Larew, 1989).

The efficacy of azadirachtin as a systemic insecticide has been documented against a variety of insect pests with chewing and piercing-sucking mouthparts. Lindquist et al. (1986) demonstrated that a bare root treatment of a neem extract EC (emulsifiable concentrate) formulation (0.30% to 1.0%) to young plant cuttings significantly reduced leafminer (*Liriomyza triflii*) survival for up to 4 weeks. Similarly, Pavela et al. (2004) found that rape plants (*Brassica napis* subsp. *napus*) that had roots placed in solutions containing azadirachtin resulted in increased mortality of the nymphal stages of the cabbage aphid (*Brevicoryne brassicae*); however, there was little to no effect on adults. In addition, Vitullo and Sadof (2007) reported that azadirachtin suppressed defoliation by Japanese beetle (*Popillia japonica*) adults by <10% regardless of the application method (soil drench vs. foliar spray). Moreover, the study evaluated plant size and determined, when applied as a drench, there was no significant effect of azadirachtin on plant growth. Thoeming et al. (2003) evaluated the effect of azadirachtin on the western flower thrips (WFT), *Frankliniella occidentalis* and obtained >50% mortality of nymphs and adults with efficacy influenced by organic matter content in the growing medium. For

instance, when 200 mg of azadirachtin was added to a growing medium mixed in a 1:1 ratio with sand (low organic matter), there was 93% mortality compared to 76% mortality of WFT when using a high organic matter growing medium (Thoeming et al., 2003).

Spirotetramat is the active ingredient in the product Kontos™ (Nauen et al., 2008), which is in the tetramic acid group of systemic insecticides (Yu, 2008; Nauen et al., 2008; Brück et al., 2009). This insecticide has broad-spectrum activity against both immature and adult stages of certain insects with piercing-sucking mouthparts by inhibiting lipid biosynthesis (Yu, 2008; Brück et al., 2009). Spirotetramat is a unique systemic insecticide because it is translocated both acropetally (up) and basipetally (down) in plants (Schnorbach et al., 2008; Brück et al., 2009).

Brück et al. (2009) showed that populations of mealybugs, psyllids, sweet potato whitefly (*Bemisia tabaci*) and a number of aphid species were suppressed after applying spirotetramat. These insects possess piercing-sucking mouthparts and tend to feed within the phloem-sieve tubes (Cloyd et al., 2011). Brück et al. (2009) also found spirotetramat was efficacious (99% mortality) against mealybugs at a rate of 72-88 g ai/ha applied to grapes (*Vitis* spp.) when mealybugs were in the crawler stage. In addition, the systemic activity of spirotetramat lasted up to 4 weeks against whiteflies on bell pepper (*Capsicum* spp.) plants. Furthermore, eight aphid species were determined to be susceptible to foliar applications of spirotetramat when feeding on either lettuce (*Lactuca* spp.) plants or apple (*Malus* spp.) leaves (Brück et al., 2009).

In addition to azadirachtin and spirotetramat, neonicotinoid insecticides have systemic activity and are labeled for use in protected environments against CMB. Neonicotinoid insecticides were first commercially available in 1991 and are a class of insecticides, which includes multiple active ingredients labeled for use as drench applications to the growing medium including imidacloprid, dinotefuran, thiamethoxam, and clothianidin (Tomizawa and

Casida, 2003; Jeschke et al., 2011; Cloyd et al., 2011). Neonicotinoids have a different mode of action than other insecticide classes available, acting as agonists on the nicotinic acetylcholine receptor (Yu, 2008). This mode of action eventually leads to an overstimulation of cholinergic synapses, resulting in hyperexcitation, convulsions, paralysis, and death of insects (Yu, 2008). Insect pests typically targeted by neonicotinoid insecticides are xylem- and phloem-feeders with piercing-sucking mouthparts such as aphids, whiteflies, leafhoppers, soft scales, and mealybugs (Tomizawa and Casida, 2003; Nauen and Denholm, 2005; Cloyd et al., 2011).

While all neonicotinoid insecticides have a similar chemical structure and mode of action, effectiveness may vary when applied to the growing medium (Cloyd and Bethke, 2011). These variations may be influenced by factors associated with each insecticide and/or the plant, which affect the uptake of the insecticide by plant roots. For instance, water solubility, p*K*a, and log P_{oct} of each may impact movement through the plant (Reynolds and Metcalf, 1962; Cloyd and Bethke, 2011; Cloyd et al., 2011).

There are a number of benefits associated with neonicotinoid insecticides including low mammalian toxicity and extended residual activity or persistence, which results in control or regulation for long periods of time without the need for multiple applications (Tomizawa and Casida, 2003; Nauen and Denholm, 2005). As such, applying neonicotinoid insecticides as drenches in protected environments may be a safe and effective method of mitigating mealybug populations. Currently, little information is available on the efficacy of neonicotinoid-based insecticides against CMB when applied as drenches to the growing medium in protected environments; however, previous research has evaluated the efficacy of neonicotinoid insecticides against other mealybug species in field environments.

Both imidacloprid and thiamethoxam have been investigated under field conditions against the pink hibiscus mealybug (*Maconellicoccus hirstus*) on mature and young mulberry (*Morus alba*) trees (Castle and Prabhaker, 2011). The researchers found that a single application of either imidacloprid or thiamethoxam may eliminate a mealybug infestation; however, applications were not always effective on certain trees although all trees were the same species (Castle and Prabhaker, 2011). In addition, imidacloprid has been evaluated against the vine mealybug (*Planococcus ficus*) in California vineyards using drip- and furrow-irrigation application methods (Daane et al., 2006). A significant reduction (90 to 92%) in cluster damage was observed compared to the water control when using drip-irrigation. However, when using imidacloprid at the same rate, but applied using furrow-irrigation, there was only a 21 to 59% reduction in cluster damage (Daane et al., 2006).

Previous research assessed the systemic activity of azadirachtin, spirotetramat, and the neonicotinoid insecticides under field and laboratory conditions, although the efficacy of these insecticides under greenhouse conditions is currently not known. Therefore, the objectives of this study were to 1) determine the systemic activity of azadirachtin and spirotetramat against CMB when applied to the growing medium of both green and red coleus (*Solenstemon scutellarioides*) plants as either preventative or curative treatments under greenhouse conditions, 2) evaluate the residual activity of certain neonicotinoid insecticides against CMB, and 3) determine CMB feeding location on coleus plants.

Materials and Methods

This study was designed to assess the systemic activity of both azadirachtin and spirotetramat as preventative and curative treatments, and the residual activity of the neonicotinoid insecticides against CMB on coleus plants.

Citrus mealybug colony

Laboratory-reared colonies of CMB were maintained on butternut squash (*Cucurbita maxima*) under 24 ± 5°C, 50-60% relative humidity (RH), and 14:10 (L:D) hour photoperiod in the Department of Entomology at Kansas State University (Manhattan, KS). These colonies had not been exposed to pesticides for at least ten years.

Experiment one: Efficacy of systemic insecticides as a preventative treatment

Sixty green (cultivar; 'Lifelime') and 60 red (cultivar; 'Mariposa') coleus, *Solenstemon scutellarioides* plants were used. There were four treatments including two rates of azadirachtin (Azatrol®: PBI Gordon Corp.; Kansas City, MO) at 3.99 mL/946 mL (4%) and the labeled rate 8.13 mL/946 mL (8%), the labeled rate of spirotetramat (Kontos™: OHP Inc.; Mainland, PA) at 0.125 mL/946 mL, and a water control. Rooted cuttings of each cultivar were purchased from Euro American Propagators LLC (Bonsall, CA). Each rooted cutting was transplanted into a 15.2-cm container with Fafard® 2 Mix growing medium (Agawam, MA) containing Canadian sphagnum peat moss (65%), perlite, vermiculite, starter nutrients, wetting agent and dolomitic limestone. Plants were allowed to establish for approximately 3 weeks before insecticide treatments were prepared and applied as drenches to the growing medium of each plant on March 11, 2011. There were five replications per treatment with four treatments, for each evaluation week (three weeks in total). The insecticide treatments were prepared using tap water and each plant received 200 mL of the designated insecticide solution. One week following application, approximately fifteen 2^{nd} to 3^{rd} instar CMB, which were obtained from the laboratory-reared colony (described above), were randomly placed on each plant. Plants were irrigated just enough to avoid leaching, and fertilized weekly using Miracle-Gro® Water Soluble

All Purpose Plant Food (Scotts; Marysville, OH) at a ratio of 24:6:7 (N:P:K) at a rate of 3.9 mL/L.

Plants were assessed using a destructive sampling method 1, 2, and 3 weeks after artificial infestation and the number of live and dead CMB per plant as well as their locations on the plant (e.g., stem, leaf top, or leaf bottom) were recorded. Mealybugs that were prodded with a probe and did not move were determined to be dead. Male pupa and females that had successfully laid an egg mass were counted as live. Percent mortality was then calculated for each week (number of dead mealybugs per plant per treatment/total number mealybugs per plant per treatment). In addition, twenty plants (10 green and 10 red) were randomly selected to ascertain plant height the day of the treatment, and each plant was measured the day the plants were destructively sampled.

Experiment two: Efficacy of systemic insecticides applied as a curative treatment

This experiment was conducted similar to the previous experiment, with the only difference being CMB were placed onto coleus plants one week prior to application of the treatments. Again, sixty green (cultivar; 'Lifelime') and sixty red (cultivar; 'Mariposa') coleus rooted cuttings were purchased and transplanted into 15.2-cm containers with Fafard® 2 Mix growing medium. The treatments were the same as in the first experiment. Plants remained in the greenhouse for approximately 3 weeks to allow for establishment before being artificially infested with CMB (2^{nd} to 3^{rd} instars). After allowing the CMB to colonize or establish on the plants for one week, the insecticides were prepared and applied as drenches to the growing medium of each plant on May 20, 2011. Plants were assessed 1, 2, and 3 weeks following application of the treatments. In addition, feeding location on each coleus plant was determined on the stem, leaf top, or leaf bottom. Both live and dead CMB were recorded for each plant for

each assessment date. Plants were maintained similar to the first experiment. Percent mortality was assessed and plant height was measured as in the previous experiment.

Experiment three: Neonicotinoid systemic insecticides applied at labeled rates

This experiment consisted of 120 green (cultivar 'Lifelime') coleus cuttings purchased from Euro-American Propagators LLC, and transplanted into 15.2-cm containers with Fafard® 2 Mix growing medium. Plants had established for 3 weeks before the insecticide treatments were applied on July 21, 2011. There were three insecticide treatments using the labeled rates of each; imidacloprid at 1.7 fl. oz/100 gal. water (0.13 mL/946 mL), dinotefuran at 24 oz/100 gal. water (1.7 g/946 mL), and thiamethoxam at 8.5 oz/100 gal. water (0.6 g/946 mL). A water control was also included. There were 5 replications per treatment for each evaluation week (6 weeks total). Insecticide treatments were prepared using tap water and 200 mL of the designated treatment was applied to the growing medium of each plant. The first set of plants (20) was artificially infested with approximately twenty, 2nd to early 3rd CMB instars. Mealybugs remained on the plants for one week [14 days after treatment (DAT)] and then the first set of plants were destructively sampled to determine the number of live and dead mealybugs. Assessment dates were 14 (week 1), 21 (week 2), 28 (week 3), 35 (week 4), 42 (week 5), and 49 (week 6) DAT with plants artificially infested with CMB one week before destructive sampling. In addition, feeding location (plant stem, leaf top, or leaf bottom) on each coleus plant was recorded. Both live and dead CMB were recorded for each plant for each assessment date.

Experiment four: Neonicotinoid systemic insecticides applied at twice the labeled rate

This experiment was similar to experiment three (described above). However, the three insecticide treatments were applied at twice the labeled rate of each; imidacloprid at 0.26

mL/946 mL, dinotefuran at 3.4 g/946 mL, and thiamethoxam at 1.2 g/946 mL. A water control was also included. There were 5 replications per treatment for each evaluation week (6 weeks total). Treatments were applied, and assessments and feeding locations were determined similar to experiment three (described above).

Statistical analysis

Percent mortality and plant height data among the treatments were analyzed using SAS Statistical Software Program version 9.1 (SAS Institute, 2002) in an analysis of variance (ANOVA) with treatment as the main effect. Percent CMB mortality for each week was calculated by dividing the number of dead mealybugs per plant by the total number of mealybugs recovered on each plant. Percent mortality values were then normalized using arcsine square-root transformation and a one-way ANOVA was performed (SAS Institute, 2002). Significant treatment means associated with percent mortality and plant height were separated using Fisher's protected least significant difference (LSD) test at $P \leq 0.05$. All data presented are non-transformed.

Feeding location data were subjected to a mixed model ANOVA using a PROC MIXED procedure with week, treatment, replication, and location as the main effects and CMB as the random effect. Tests for significance were conducted for all main effects and for the treatment x location interaction. For variables having significant treatment x location interactions ($P \leq 0.05$), means were then sliced to show significance while holding each main effect constant (SAS Institute, 2002).

Results

Results associated with the application of azadirachtin and spirotetramat as preventative treatments on both green and red coleus are presented in Table 2.1. There were no significant differences in percent CMB mortality on green (F=3.16; df=3, 16; P=0.0536) coleus; however there were significant differences on red (F=3.31; df=3, 16; P=0.0472) coleus for week 2 associated with the first experiment. Higher CMB mortality was affiliated with plants treated with azadirachtin 8% (11%) and spirotetramat (15%) on green coleus and azadirachtin 4% and 8% (9%) and spirotetramat (19%) on red coleus (Table 2.1). In week 3, plants treated with spirotetramat had significantly higher CMB mortality than the other treatments on green (15%) (F=3.65; df=3, 16; P=0.0354) and red (19%) (F=3.68; df=3, 16; P=0.0346) coleus. Over the three week time period, the percent CMB mortality for plants treated with spirotetramat increased each week for green (4, 5, and 15%) and red (3, 7, and 19%) coleus. Additionally, there was a gradual increase in percent mortality (0, 1, and 4%) on green coleus over the three week time period for plants treated with 4% azadirachtin. However, results from plants treated with azadirachtin 8% on green coleus, and azadirachtin at both 4% and 8% on red coleus, showed no increase in percent mortality over time. There were no significant differences associated with plant height among the treatments, for both green and red coleus, across all three weeks (Table 2.2).

Results from experiment two, in which azadirachtin and spirotetramat were applied as curative treatments to both green and red coleus are presented in Table 2.3. Green coleus for week 1, treated with azadirachtin 4%, had 21% CMB mortality, which was significantly different (F=5.64; df=3, 16; P=0.0079) than the other treatments (Table 2.3). However, there were no significant differences among the treatments for green coleus in weeks 2 and 3. Red coleus for

week 2, treated with spirotetramat had 21% CMB mortality, which was significantly different (F=5.25; df=3, 16; P=0.0103) from the other treatments (Table 2.3). Also, week 3 spirotetramat had the highest percent CMB mortality (28%) on red coleus. There were no significant differences among the treatments associated with percent CMB mortality for week 1. Similar to the first experiment, percent mortality of CMB on plants treated with spirotetramat increased over the three week time period for both green (2, 14, and 22%), and red (7, 21, and 28%) coleus. However, there was no increase in CMB mortality over the three weeks associated with plants treated with azadirachtin with the exception of green plants treated with azadirachtin at 8%, which resulted in 12% mortality for weeks 1 and 2, and 21% mortality for week 3. There was a significant difference in plant height (F=3.39; df=3, 16; P=0.044) for green coleus in week 1, with plants treated with spirotetramat being significantly smaller than the other three treatments (Table 2.4).

Results associated with the feeding location (stem, leaf top, or leaf bottom) are presented in Figures 2.1-4. For CMB on green coleus plants, feeding location was significant (F=309.5; df=2, 162; P<0.0001). Furthermore, there was a significant treatment x location interaction (F=5.11; df=6, 162; P<0.0001). When the data were sliced, keeping treatment constant for all four treatments, significantly more CMB were found on the plant stem for each treatment: azadirachtin (4%) (F=75.67; df=2, 162; P<0.0001), azadirachtin (8%) (F=123.7; df=2, 162; P<0.0001) spirotetramat (F=46.0; df=2, 162; P<0.0001), and water (F=79.4; df=2, 162; P<0.0001) (Figure 2.1). In addition, when feeding location was held constant, the sliced data indicated that there was a significant difference in the number of CMB found on the leaf bottom (F=7.4; df=3, 162; P=0.0001) and the plant stem (F=4.3; df=3, 162; P=0.006) for the four treatments (Figure 2.2). Results for CMB feeding location on red coleus plants indicated the

main effects of treatment (F=4.0; df=3, 162; P=0.009) and location (F=93.7; df=2, 162; P<0.0001) were significant. In addition, the treatment x location interaction was significant (F=3.1; df=6, 162; P=0.007). Slicing the data, keeping treatment constant, revealed a significant affect for each treatment: spirotetramat (F=14.2; df=2, 162; P<0.0001), azadirachtin (4%) (F=24.3; df=2, 162; P<0.0001), azadirachtin (8%) (F=38.6; df=2, 162; P<0.0001), and water (F=26.1; df=2, 162; P<0.0001). Furthermore, the sliced data, when maintaining location constant, indicated a significant difference in the number of CMB feeding on the leaf bottom among the treatments (F=8.5; df=3, 162; P<0.0001). In addition, percent CMB found alive on the plant stem and leaf bottom were always higher compared to CMB found on the leaf top (Table 2.5).

Results affiliated with residual activity of the neonicotinoid insecticides (experiment three), based on percent CMB mortality, are presented in Table 2.6 and Figure 2.5. The first assessment made 14 DAT indicated significant differences among the treatments (F=6.92; df=3,16 ; P=0.0034). Both thiamethoxam (35%) and dinotefuran (25%) had significantly higher percent CMB mortalities than imidacloprid (17%) and the water control (3%). Percent CMB mortality for coleus treated with thiamethoxam and dinotefuran were not significantly different from each other although they were significantly higher than imidacloprid and the water control for assessment dates 14-35 DAT. Overall, there were no significant differences in percent CMB mortality between imidacloprid and the water control for all assessment dates (Figure 2.5).

Furthermore, at 21 DAT there were significant differences among treatments (F=14.78; df=3,16 ; P=0.0001) and the highest percent CMB mortality for all three treatments; imidacloprid (33%), dinotefuran (64%), and thiamethoxam (77%). Significant differences among treatments were also observed 28 DAT (F=4.45; df=3,16; P=0.0186) and 35 DAT (F=4.08;

df=3,16 ; *P*=0.0249). There were no significant differences among the treatments 42 DAT (*F*=1.62; df=3,16 ; *P*=0.2234) and 49 DAT (*F*=0.78; df=3,16 ; *P*=0.5236) (Figure 2.5).

Results for CMB feeding location on coleus stems, leaf tops, or leaf bottoms are presented in Figures 2.6 and 2.7. There was a significant treatment x location interaction for mealybug feeding location (*F*=5.1; df=6, 339; *P*≤0.0001). Slicing tests for the significant interaction revealed a significant effect of each treatment on feeding location when held constant, thiamethoxam (*F*=17.7; df=2, 339; *P*≤0.0001), imidacloprid (*F*=67.4; df=2, 339; *P*≤0.0001), dinotefuran (*F*=37.9; df=2, 339; *P*≤0.0001), and the water control (*F*=78.4; df=2, 339; *P*≤0.0001) (Figure 2.6). Each treatment had significantly more CMB on the stem compared to the leaf top and leaf bottom (Figure 2.6). Furthermore, slicing the interaction, holding location constant, indicated a significant (*F*=10.4; df=3, 339; *P*≤0.0001) difference in the number of CMB located on the plant stem for each treatment when location was held constant. There were significantly fewer CMB located on the plant stem for the thiamethoxam and dinotefuran treatments compared to imidacloprid and water (Figure 2.7). In addition, percent CMB mortality was higher on the leaf top, compared to the plant stem and leaf bottom. Furthermore, thiamethoxam provided higher percent CMB mortality associated with the plant stem compared to dinotefuran and imidacloprid (Table 2.7).

Results associated with residual activity after doubling the labeled rates of the neonicotinoid insecticides (experiment four) are presented in Figure 2.8. The results indicated significant differences among the treatments 14 DAT (*F*=9.98; df=3,16 ; *P*=0.0006), 21 DAT (*F*=24.08; df=3,16 ; *P*<0.0001), 28 DAT (*F*=7.20; df=3,16 ; *P*=0.0028), 35 DAT (*F*=4.85; df=3,16 ; *P*=0.0138), and 42 DAT (*F*=11.34; df=3,16 ; *P*=0.0003). Thiamethoxam had the highest percent CMB mortality (38%) 21 DAT whereas dinotefuran had the highest percent

CMB mortality (28%) 28 DAT. Imidacloprid had a higher percent CMB mortality than the water control 14 and 21 DAT; however, there were no significant differences in percent CMB mortality between imidacloprid and the water control for all other assessment dates (Figure 2.8). There were no significant differences among any of the treatments 49 DAT (F=0.26; df=3,16 ; P=0.8554) (Figure 2.8).

Results for CMB feeding location on coleus (stems, leaf tops, or leaf bottoms) after doubling the labeled rates of the neonicotinoid insecticides are presented in Figures 2.9-2.11. There was a significant treatment x location interaction for feeding location (F=7.9; df=6, 339; $P\leq$0.0001). Slicing tests for the significant interaction revealed a significant effect of three of the treatments on feeding location when held constant, imidacloprid (F=19.0; df=2, 339; $P\leq$0.0001), dinotefuran (F=10.9; df=2, 339; $P\leq$0.0001), and the water control (F=36.8; df=2, 339; $P\leq$0.0001) (Figure 2.9). The thiamethoxam treatment did not have a significant effect on location (F=0.5; df=2, 339; P=0.5887). Each treatment had significantly more CMB on the stem and/or leaf bottom than the leaf top (Figure 2.9). Furthermore, slicing the interaction, and holding location constant, indicated a significant difference in the number of CMB located on the plant stem (F=8.5; df=3, 339; $P\leq$0.0001) and leaf top (F=6.6; df=3, 339; $P\leq$0.0002) for each treatment when location was held constant (Figure 2.10). There were significantly fewer CMB located on the plant stem for the thiamethoxam treatment whereas, significantly more CMB were located on the leaf top for the thiamethoxam treatment (Figure 2.7). Also, week had a significant impact on CMB location (F=14.8; df=10, 339; $P\leq$0.0001) (Figure 2.11). At the first assessment date, 14 DAT there were significantly more CMB located on the leaf bottom. At 21 and 28 DAT, there were significantly more CMB located on the plant stem and leaf bottom than the leaf top;

however, at 35, 42, and 49 DAT there were more CMB located on the plant stem compared to the leaf top and bottom (Figure 2.11).

Discussion

Both azadirachtin and spirotetramat were not effective against CMB based on low mortality values (<30%) across all three weeks regardless of the timing of application either preventative or curative. In addition, most CMB females were laying eggs at week 3 (Willmott, personal observation). The observed low mortality may be attributed to factors, which are known to influence the efficacy of systemic insecticides such as water solubility, growing medium type, insect feeding behavior, and plant species (Ripper et al., 1949; Jeppson, 1953; Bennett, 1957; Reynolds and Metcalf, 1962).

Although spirotetramat provided the highest percent CMB mortality on both green and red coleus after three weeks, mortality was <30%, which would be unacceptable under greenhouse and interior plantscape conditions. This is in contrast to Brück et al. (2009), in which spirotetramat, when applied to control mealybugs feeding on grapes, resulted in 92% and 99% mortality; however, information associated with the number days after treatment was not provided. Furthermore, in the current study, CMB mortality gradually increased from week 1 to week 3 for both green and red coleus plants treated with spirotetramat. This increased mortality over time may be related to the low water solubility of spirotetramat (0.029 g/L at 20°C, pH 7) (Babczinski and Hellpointner, 2008; Fischer and Weiß, 2008), which can affect the rate at which the active ingredient is translocated through the plant vascular system (Glynne-Jones and Thomas, 1953; Reynolds and Metcalf, 1962). For example, in a study by Reynolds and Metcalf (1962), disulfoton, which has a water solubility of 0.0066%, took twice as long to provide the same level of mortality as dimethoate, which has a water solubility of 2%. Therefore, due to the

low water solubility of spirotetramat, it may take longer than 3 weeks for the active ingredient to be translocated throughout the plant. Nevertheless, extending the experiments beyond 3 weeks was not practical because the generation time of CMB is approximately one month, and after the three week time period, there was a new generation of mealybugs.

Azadirachtin also has a low water solubility (0.00005 g/L) (Liu et al., 2005), which may have contributed to minimal mortality of CMB in both the preventative and curative treatments. However, unlike spirotetramat, the results associated with azadirachtin were not consistent, and varied over the three weeks. For example, in experiment one, azadirachtin when applied at the 4% rate on green coleus, demonstrated a slight increase in percent mortality over the three weeks. However, in experiment two, the percent CMB mortality for plants treated with azadirachtin (4%) was 21%, 6%, and 18% for weeks 1, 2, and 3. Therefore, additional factors other than water solubility may have affected the translocation of azadirachtin. For instance, growing medium type may have affected absorption of azadirachtin as reported by Thoeming et al. (2003). In addition, high organic matter content in the growing medium competes with the plants ability to absorb the active ingredient (Gill and Lewis, 1971). The growing medium used in both experiments was Fafard® 2 Mix growing medium containing Canadian sphagnum peat moss (65%), which is a source of organic matter. As such, further studies investigating the influence of organic matter type on systemic insecticide activity are warranted.

Plant height has been shown to be influenced by the systemic insecticide carbofuran resulting in taller soybean (*Glycine max*) plants (Mellors et al., 1984). However, results associated with plant height were similar to Vitullo and Sadof (2007) in that most of the treatments had no effect on plant height over the three week time period. The only significant difference in plant height was observed in experiment two for green coleus treated with

spirotetramat during week 1, however, by week 2 these differences were no longer observed. Therefore, it appears that azadirachtin and spirotetramat do not have any effect on plant height.

This study also evaluated the effectiveness of three neonicotinoid insecticides against CMB at the labeled and twice the labeled rates. The neonicotinoid insecticide, clothianidin was not evaluated; however, thiamethoxam is known to be converted into clothianidin in insects and plants (Nauen et al., 2003). At the labeled rate, both thiamethoxam and dinotefuran provided the highest level of CMB control 21-28 DAT. However, doubling the labeled rates of the neonicotinoid insecticides resulted in lower overall CMB mortality (<40%). The imidacloprid treatment was not significantly different than the water control for the first two assessment dates (14 and 21 DAT), although at the labeled rate, imidacloprid failed to provide adequate control of CMB, with mortality similar to the water control for all assessment dates. However, doubling the labeled rate of imidacloprid provided significantly less CMB mortality compared to thiamethoxam. Moreover, none of the treatments at the labeled and twice the labeled rate, provided >80% CMB mortality. The reason lower mortality was observed when doubling the labeled rate is unknown; however, it may be due to timing of the applications. For instance, using the neonicotinoid-based insecticides at the labeled rate was conducted from July to August; whereas the experiment using twice the labeled rate was conducted from February to March. Timing of application has been demonstrated by Jeppson et al. (1952, 1954) when systemic insecticides were ineffective when applied in January or February compared to applications made March through August.

The results associated with minimal mortality using imidacloprid contrast with what Castle and Prabhaker (2011) observed with pink hibiscus mealybug on mulberry trees, where there was a significant difference between the water control and both thiamethoxam and

imidacloprid. Moreover, Castle and Prabhaker (2011) reported no significant differences between thiamethoxam and imidacloprid. However, in the current study, imidacloprid provided minimal CMB mortality compared to thiamethoxam. These differences may be due to plant species, plant age, and treatment rates. For instance, rates for thiamethoxam and imidacloprid were determined based on the circumference of each mulberry tree, whereas in the current study, the same rate was applied to each plant regardless of size. In addition, Castle and Prabhaker (2011) observed 52% of the treated mulberry trees to be completely free of infestation after one application. However, this may be attributed to the fact that the trees were "severely pruned" in the autumn following treatment. Although it may seem reasonable to assume that trees would remain infested after pruning, without data confirming the presence of mealybugs, this may have contributed to the high mortality observed in the study.

Furthermore, results from the current study also contrast with Daane et al. (2006) with the vine mealybug, where imidacloprid was applied through both drip- and furrow-irrigated plots in April and May, which resulted in a significant reduction in cluster damage associated with the vine mealybug regardless of application method. However, Daane et al. (2006) also reported that furrow-irrigated plots, in April, only provided 21% reduction in cluster damage; however, this was still significantly different than the control. This reduction may be attributed to the widespread root zone of the furrow-irrigated fields resulting in less active ingredient being translocated throughout the plant (dilution effect). In the current study, root development was not likely responsible for the lack of CMB control on coleus plants because all test plants were the same age and size.

The highest percent CMB mortality for all three neonicotinoid insecticide treatments at the labeled rate, and thiamethoxam and imidacloprid at twice the labeled rate, occurred 21 DAT.

These results are different from Castle and Prabhaker (2011) where the highest mortality was observed 28 DAT. This difference may be because mulberry trees are a woody plant, and much larger than coleus plants, which are considered herbaceous (Liberty Hyde Bailey Hortorium, 1976). Despite this, in both studies, the treatments took 3-4 weeks before the highest mortality was observed, which suggest that factors such as water solubility and plant growth rate may affect translocation, thus influencing the efficacy of systemic insecticides. For instance, the water solubility of imidacloprid is 0.6 g/L at 20°C whereas the water solubility of dinotefuran and thiamethoxam is 39.8 g/L and 4.1 g/L at 20°C (Jeschke et al., 2011). Therefore, dinotefuran and thiamethoxam may have been absorbed and translocated throughout the plants more rapidly than imidacloprid.

In addition to percent CMB mortality, feeding location was also determined for three out of four experiments. More CMB were located on the plant stem of green coleus than the leaf top for experiments two and three, Also, in experiment four most CMB were located on the plant stem except for the first assessment date (14 DAT), where the majority of CMB were located on the leaf bottom. This difference may be due to plant size. For instance, experiment three was initiated when the mean plant height was 21.9 (± 0.5) cm whereas experiment four plant height mean was 8.6 (± 0.2) cm. Therefore, the stems of coleus plants in experiment four were much smaller compared to experiment three. Consequently, there was less area on the plant stems for CMB to migrate. Also, for red coleus in experiment two, there were more CMB located on the plant stem and leaf bottom. Therefore, low CMB mortality may be due to not ingesting a lethal concentration of active ingredient as suggested by Cloyd et al. (2012). If the active ingredient is not translocated uniformly to all plant parts, then CMB may not be ingesting lethal concentrations, which would result in minimal mortality. For example, CMB located on plant

stems tend to be less affected by systemic insecticides than those located on the leaf top (Willmott, personal observation).

Cloyd et al. (2012) recommended preventative drench applications of systemic insecticides to avoid insect outbreaks early in the crop production cycle. However, in the current study, preventative treatments had lower CMB mortality than the curative treatments. The reason for this lower mortality is unknown; however, it may be due to the timing of application. Previous research by Jeppson et al. (1952, 1954) suggests that timing of application may affect the efficacy of systemic insecticides. For instance, systemic insecticides were ineffective when applied in January or February compared to applications made March through August. In the current study, preventative treatments were applied in March whereas curative treatments were applied in May. Furthermore, Jeppson (1953) and Wedding (1953) suggested that factors such as light intensity and relative humidity may affect the amount of active ingredient absorbed by plants. Temperature was constant for both experiments (data not shown); however, no artificial lighting was used so light intensity was variable. Studies have reported that light intensity may impact movement and efficacy of systemic insecticides by influencing the transpiration rate, which is important in the translocation of the active ingredient within plants (Cloyd et al., 2012). Therefore, the curative treatments were applied during longer natural day lengths and as such plants may have absorbed a higher concentration of active ingredient.

Low CMB mortality may also be associated with feeding location as suggested by Cloyd et al. (2012). Thiamethoxam had the highest percent CMB mortality and when the data were sliced, a lower number of CMB were located on the plant stem than the other treatments, which supports the hypothesis of Cloyd et al. (2012). Therefore additional factors may be associated with higher numbers of CMB on the plant stem including plant chemistry and/or light intensity.

In conclusion, these studies have demonstrated quantitatively that both azadirachtin, spirotetramat, and the neonicotinoid insecticides (thiamethoxam, dinotefuran, and imidacloprid) at the labeled and twice the labeled rates do not provide acceptable control against CMB in greenhouses. This is the first study to quantitatively evaluate the systemic activity of azadirachtin and spirotetramat and the residual activity of the neonicotinoid insecticides against CMB under greenhouse conditions. Azadirachtin and spirotetramat demonstrated minimal, if any systemic activity against CMB over a three week time period. Both thiamethoxam and dinotefuran provided the highest CMB mortality; however, this will still <80%. It appears that it takes approximately 21-28 DAT for the active ingredient to be translocated within coleus plants, however, in all cases, CMB mortality was not sufficient. This may be associated with factors that could affect the efficacy of systemic insecticides (mentioned above), however, future research is needed to determine the primary factor(s) responsible. For instance, if water solubility or growing medium type are the primary factors, then these can be addressed by modifying production or management practices. However, if reduced efficacy is due to the feeding behavior of CMB, alternative pest management strategies such as sanitation and biological control may need to be implemented in addition to the use of systemic insecticides. Furthermore, pesticide manufacturers may have to consider modifying labeled rates to compensate for this phenomenon.

This study is also the first to determine CMB feeding location (stem, leaf top, and leaf bottom) on coleus plants. Most CMB were located on the plant stem and were alive, which indicates that CMB were not ingesting lethal concentrations at this location or an insufficient concentration of active ingredient is being translocated through the stem. Future research is warranted to quantify the concentration of active ingredients of each neonicotinoid insecticide using enzyme-linked immunosorbent assay (ELISA) tests in different plant parts, which would

determine if indeed feeding location is responsible for minimal mortality of CMB when using systemic insecticides.

Table 2.1. Mean percent mortality (± SEM) of citrus mealybug (*Planococcus citri*) for experiment one over three week assessment period (March 25 through April 8, 2011); one week after application of the treatments, and before both green and red coleus (*Solenstemon scutellarioides*) plants were artificially infested with citrus mealybugs (preventative); n = number of mealybugs per plant.

Treatment	Percent Mortality (± SEM)					
	n	Week 1	n	Week 2	n	Week 3
Green coleus						
Spirotetramat	50	4.0 (± 2.0) a[z]	66	5.0 (± 2.0) a	52	15.0 (± 7.0) a
Azadirachtin 4%	58	0.0 (± 0.0) a	67	1.0 (± 1.0) a	63	4.0 (± 3.0) ab
Azadirachtin 8%	51	6.0 (± 3.0) a	67	11.0 (± 5.0) a	61	0.0 (± 0.0) b
Water	58	0.0 (± 0.0) a	68	0.0 (± 0.0) a	56	0.0 (± 0.0) b
Red coleus						
Spirotetramat	90	3.0 (± 2.0) A[z]	85	7.0 (± 3.0) A	64	19.0 (± 4.0) A
Azadirachtin 4%	80	4.0 (± 3.0) A	67	9.0 (± 3.0) A	56	8.0 (± 3.0) B
Azadirachtin 8%	61	4.0 (± 3.0) A	78	3.0 (± 2.0) AB	68	9.0 (± 4.0) AB
Water	97	0.0 (± 0.0) A	74	0.0 (± 0.0) B	61	3.0 (± 2.0) B

[z]Means followed by the same lowercase or capital letter within a column are not significantly different from each other ($P \leq 0.05$) based on Fisher's protected least significant difference (LSD) mean separation test.

Table 2.2. Mean plant height (± SEM) of both green and red coleus (*Solenstemon scutellarioides*) plants in experiment one, one week after application of treatments. Assessment dates occurred from March 25 through April 8, 2011, with 5 replicate plants per treatment.

Treatment	Mean (± SEM) plant height (cm)		
	Week 1	Week 2	Week 3
Green			
Spirotetramat	16.1 (± 0.5) a[z]	18.6 (± 1.2) a	22.6 (± 0.7) a
Azadirachtin 4%	17.8 (± 0.5) a	20.8 (± 0.8) a	22.2 (± 1.4) a
Azadirachtin 8%	15.8 (± 0.7) a	18.0 (± 0.7) a	22.5 (± 1.5) a
Water	16.6 (± 0.9) a	19.4 (± 0.8) a	25.9 (± 0.7) a
Red			
Spirotetramat	28.6 (± 1.2) A[z]	34.2 (± 2.4) A	35.7 (± 1.9) A
Azadirachtin 4%	27.9 (± 2.9) A	33.4 (± 1.7) A	35.9 (± 2.2) A
Azadirachtin 8%	26.2 (± 2.4) A	35.1 (± 2.5) A	37.0 (± 1.3) A
Water	25.9 (± 1.8) A	34.7 (± 0.5) A	39.9 (± 1.8) A

[z]Means followed by the same lowercase or capital letter within a column are not significantly different from each other ($P \leq 0.05$) based on Fisher's protected least significant difference (LSD) mean separation test.

Table 2.3. Mean percent mortality (± SEM) of the citrus mealybug (*Planococcus citri*) over three week assessment period for experiment two (May 27 through June 10, 2011); one week after application of the treatments, and after both green and red coleus (*Solenstemon scutellarioides*) plants were artificially infested with citrus mealybugs (curative); n = number of mealybugs per plant.

Treatment	Percent Mortality (± SEM)					
	n	Week 1	n	Week 2	n	Week 3
Green coleus						
Spirotetramat	63	2.0 (± 2.0) b[z]	62	14.0 (± 3.0) a	50	22.0 (± 8.0) a
Azadirachtin 4%	47	21.0 (± 7.0) a	51	6.0 (± 4.0) a	48	18.0 (± 12.0) a
Azadirachtin 8%	59	12.0 (± 2.0) ab	50	12.0 (± 6.0) a	54	21.0 (± 9.0) a
Water	69	1.0 (± 1.0) b	59	5.0 (± 3.0) a	58	2.0 (± 2.0) a
Red coleus						
Spirotetramat	59	7.0 (± 2.0) A[z]	49	21.0 (± 3.0) A	25	28.0 (± 15.0) A
Azadirachtin 4%	61	9.0 (± 4.0) A	61	10.0 (± 3.0) B	63	7.0 (± 2.0) B
Azadirachtin 8%	61	4.0 (± 3.0) A	69	18.0 (± 5.0) AB	60	15.0 (± 3.0) AB
Water	51	2.0 (± 2.0) A	54	4.0 (± 2.0) C	60	7.0 (± 5.0) B

[z]Means followed by the same lowercase or capital letter within a column are not significantly different from each other ($P \leq 0.05$) based on Fisher's protected least significant difference (LSD) mean separation test.

Table 2.4. Mean plant height (± SEM) of both green and red coleus (*Solenstemon scutellarioides*) plants one week after application of treatments in experiment two. Assessment dates occurred from May 27 through June 10, 2011, with 5 replicate plants per treatment.

Treatment	Mean (± SEM) plant height (cm)		
	Week 1	Week 2	Week 3
Green			
Spirotetramat	13.7 (± 0.8) b[z]	16.3 (± 1.1) a	17.9 (± 1.4) a
Azadirachtin 4%	15.5 (± 0.7) ab	17.5 (± 1.3) a	18.7 (± 0.6) a
Azadirachtin 8%	15.8 (± 0.7) a	17.8 (± 1.2) a	20.9 (± 1.1) a
Water	16.6 (± 0.4) a	15.4 (± 1.3) a	18.8 (± 1.4) a
Red			
Spirotetramat	31.2 (± 0.4) A[z]	42.5 (± 3.3) A	52.8 (± 5.5) A
Azadirachtin 4%	37.3 (± 0.8) A	38.9 (± 1.2) A	44.0 (± 4.4) A
Azadirachtin 8%	32.3 (± 2.9) A	39.1 (± 1.0) A	45.2 (± 3.3) A
Water	32.1 (± 2.1) A	35.0 (± 2.4) A	49.0 (± 1.8) A

[z]Means followed by the same lowercase or capital letter within a column are not significantly different from each other ($P \leq 0.05$) based on Fisher's protected least significant difference (LSD) mean separation test.

Table 2.5. Percent live (L) and dead (D) citrus mealybugs, *Planococcus citri*, located on plant stem, leaf top, or leaf bottom on both green and red coleus (*Solenstemon scutellarioides*) plants, for each assessment week (1-3) for experiment two (curative treatments). There were five replications per treatment.

		Green						Red					
		1		2		3		1		2		3	
Location	Treatment	%L	%D	%L	%D	%L	%D	%L	%D	%L	%D	%L	%D
Plant stem	Spirotetramat	97.3	2.7	86.5	13.5	75.0	25.0	92.6	7.4	66.7	33.3	66.7	33.3
	Azadirachtin 4%	84.4	15.6	92.9	7.1	82.1	12.8	85.7	14.3	89.7	10.3	88.9	11.1
	Azadirachtin 8%	92.5	7.5	90.9	9.1	75.6	24.4	94.4	5.6	52.3	47.6	75.0	25.0
	Water	98.1	1.9	91.2	8.8	100.0	0.0	100.0	0.0	91.3	8.7	88.0	12.0
Leaf top	Spirotetramat	37.5	62.5	40.0	60.0	75.0	25.0	83.3	16.7	100.0	0.0	50.0	50.0
	Azadirachtin 4%	84.4	15.6	100.0	0.0	0.0	100.0	81.8	18.2	60.0	40.0	75.0	25.0
	Azadirachtin 8%	0.0	100.0	0.0	100.0	100.0	0.0	83.3	16.7	100.0	0.0	83.3	16.7
	Water	100.0	0.0	100.0	0.0	100.0	0.0	85.7	14.3	100.0	0.0	100.0	0.0
Leaf bottom	Spirotetramat	100.0	0.0	95.0	5.0	92.9	7.1	96.0	4.0	87.5	12.5	87.5	12.5
	Azadirachtin 4%	100.0	0.0	88.9	11.1	100.0	0.0	100.0	0.0	96.4	3.6	100.0	0.0
	Azadirachtin 8%	66.7	33.3	100.0	0.0	100.0	0.0	97.2	2.8	93.0	7.0	93.5	6.5
	Water	100.0	0.0	100.0	0.0	94.7	5.3	100.0	0.0	100.0	0.0	97.0	3.0

Table 2.6. Mean (± SEM) percent mortality of citrus mealybug, *Planococcus citri*, 14, 21, 28, 35, 42, and 49 days after treatment (DAT), using three neonicotinoid insecticides (thiamethoxam, imidacloprid, and dinotefuran). There were five replications per treatment per assessment time.

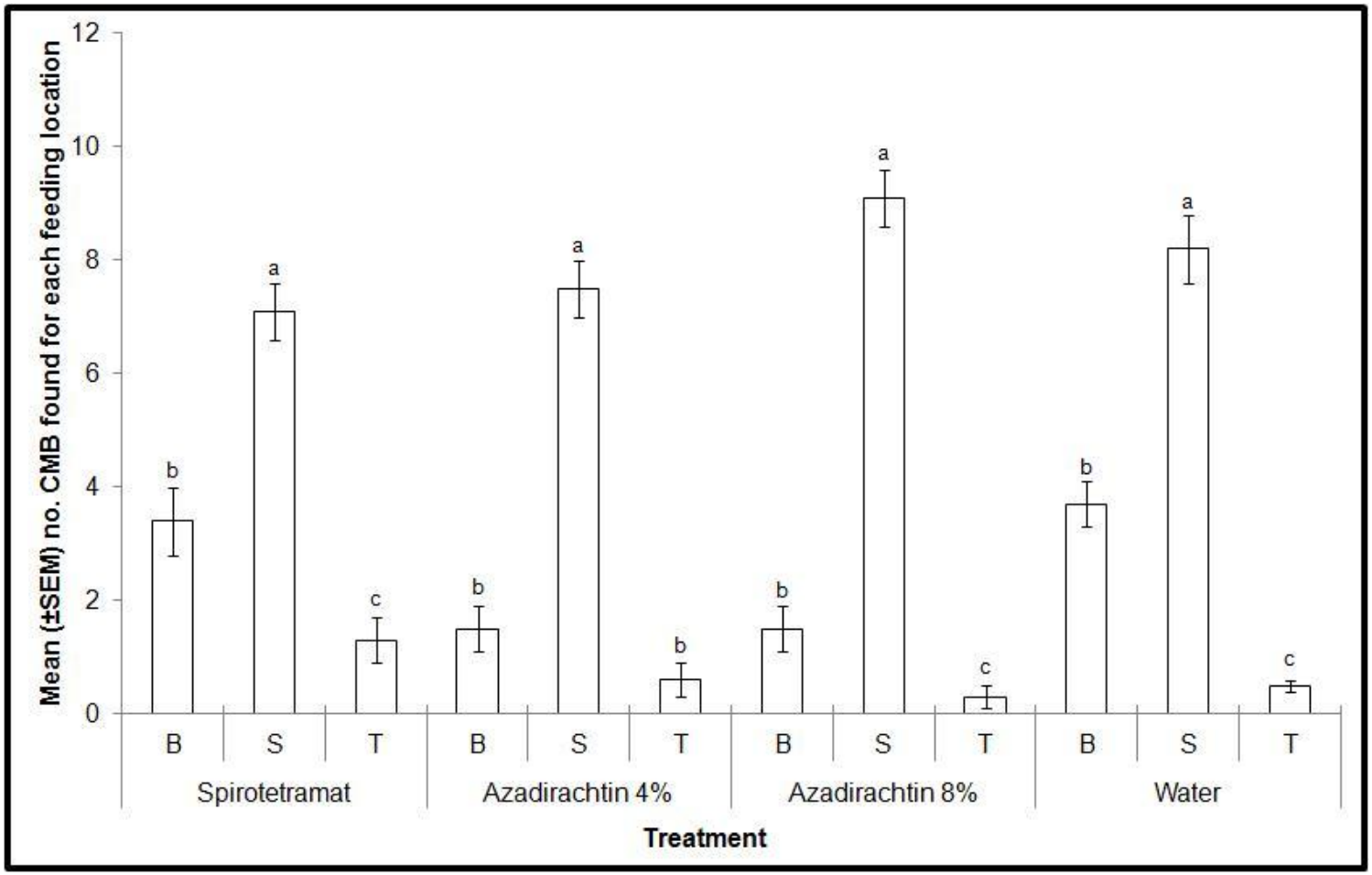
	Assessment time					
Treatment	**14 DAT**	**21 DAT**	**28 DAT**	**35 DAT**	**42 DAT**	**49 DAT**
Thiamethoxam	35.8 (± 4.0) a[z]	77.0 (± 8.0) a	65.0 (± 7.0) a	33.0 (± 7.0) a	12.0 (± 5.0) a	4.0 (± 2.0) a
Imidacloprid	17.0 (± 5.0) bc	33.0 (± 9.0) b	32.0 (± 7.0) ab	20.0 (± 4.0) ab	12.0 (± 6.0) a	4.0 (± 3.0) a
Dinotefuran	25.8 (± 8.0) ab	64.0 (± 2.0) a	53.0 (± 13.0) a	26.0 (± 8.0) a	13.0 (± 4.0) a	7.0 (± 3.0) a
Water	3.0 (± 2.0) c	7.0 (± 1.0) b	11.0 (± 3.0) b	4.0 (± 3.0) b	1.0 (± 1.0) a	1.0 (± 1.0) a

[z]Means followed by a common lower case letter within a column are not significantly different from each other ($P \leq 0.05$) based on Fisher's protected least significant difference (LSD) mean separation test.

Table 2.7. Percent live (L) and dead (D) citrus mealybugs, *Planococcus citri*, found on green coleus *Solenstemon scutellarioides* stem, leaf top, and leaf bottom; 14, 21, 28, 35, 42, and 49 days after treatment (DAT) associated with three neonicotinoid insecticides (thiamethoxam, imidacloprid, and dinotefuran) and a water control. There were five replications per assessment time.

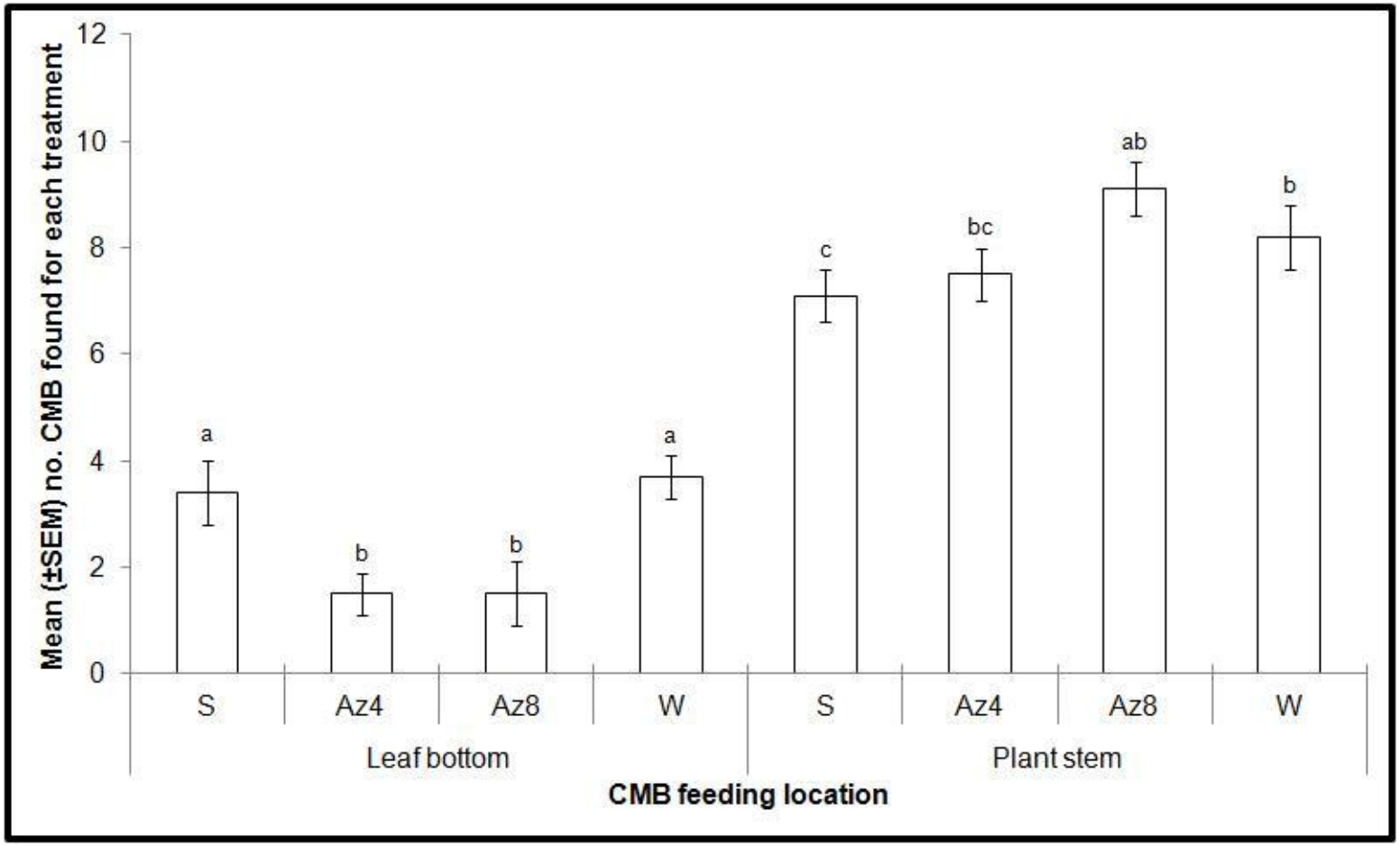
		Assessment time (DAT)											
		14		21		28		35		42		49	
Location	Treatment	%L	%D	%L	%D	%L	%D	%L	%D	%L	%D	%L	%D
Plant stem	Thiamethoxam	78.6	21.4	27.0	73.0	39.1	60.9	90.6	9.4	96.8	3.2	97.2	2.8
	Imidacloprid	89.7	10.3	73.1	26.9	90.0	10.0	87.5	12.5	91.9	8.1	97.9	2.1
	Dinotefuran	85.7	14.3	52.9	47.1	44.0	56.0	85.0	15.0	97.7	2.3	90.5	9.5
	Water	98.1	1.9	95.0	5.0	100.0	0.0	100.0	0.0	100.0	0.0	98.2	1.8
Leaf top	Thiamethoxam	26.7	73.3	14.3	85.7	10.3	89.7	21.1	78.9	64.3	35.7	80.0	20.0
	Imidacloprid	50.0	50.0	33.3	66.7	29.2	70.8	60.0	40.0	63.2	36.8	66.7	33.3
	Dinotefuran	37.5	62.5	9.1	90.9	20.0	80.0	56.3	43.8	46.7	53.3	90.9	9.1
	Water	83.3	16.7	77.8	22.2	56.3	43.8	81.3	18.8	87.5	12.5	100.0	0.0
Leaf bottom	Thiamethoxam	81.8	18.2	50.0	50.0	87.5	12.5	85.7	14.3	92.9	7.1	100.0	0.0
	Imidacloprid	90.9	9.1	30.0	70.0	85.7	14.3	93.3	6.7	100.0	0.0	100.0	0.0
	Dinotefuran	66.7	33.3	37.5	62.5	80.0	20.0	90.0	10.0	100.0	0.0	100.0	0.0
	Water	100.0	0.0	91.7	8.3	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0

Figure 2.1. Mean (± SEM) number of citrus mealybugs (CMB), *Planococcus citri*, found on leaf bottom (B), plant stem (S), and leaf top (T), of green coleus (*Solenstemon scutellarioides*) plants treated curatively with azadirachtin (4%), azadirachtin (8%), spirotetramat, and water over a three week time period.



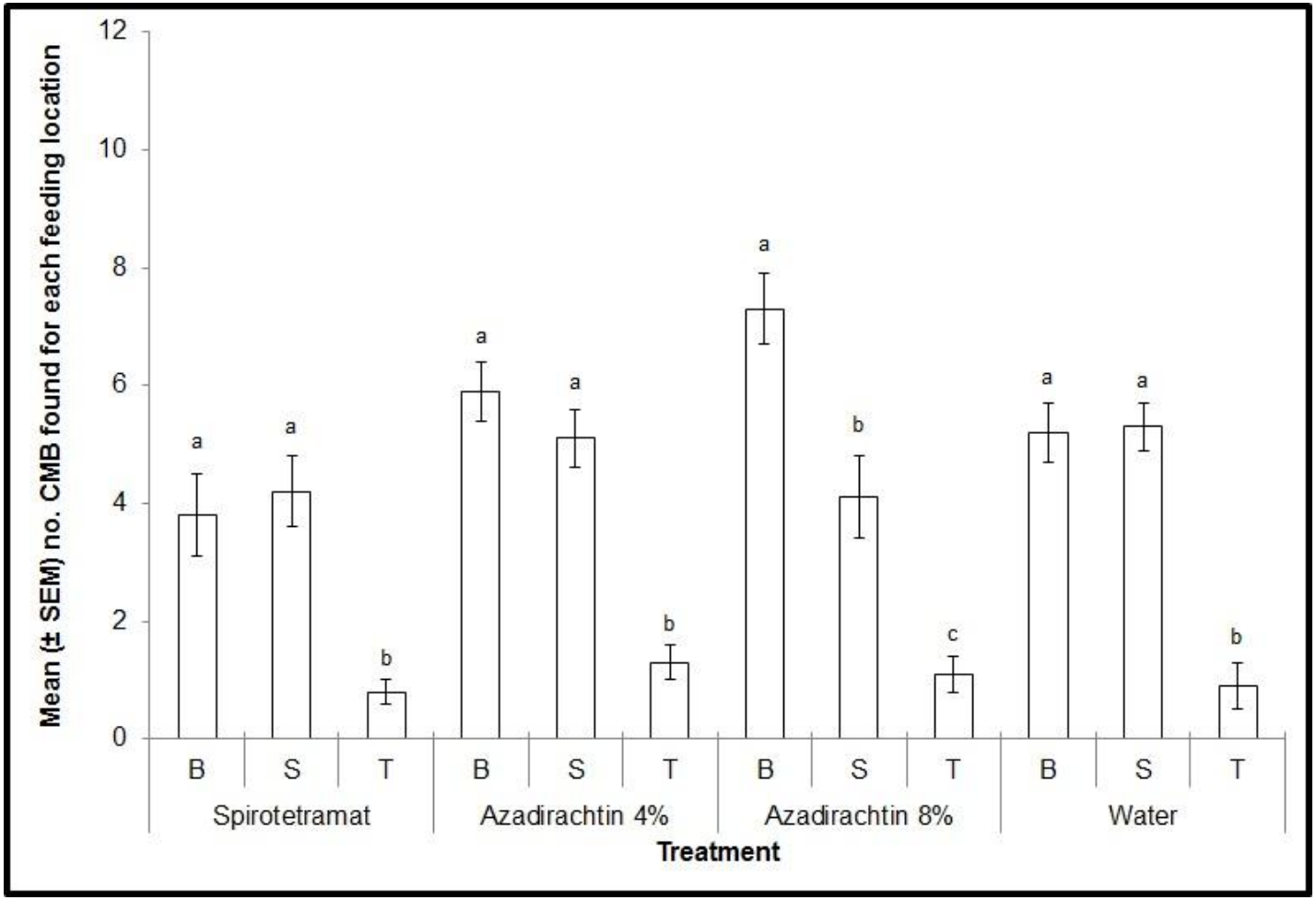
Bars with the same letter are not significantly different from each other ($P \leq 0.05$) based on a PROC MIXED procedure. Vertical lines indicate standard error of the mean (SEM).

Figure 2.2. Mean (± SEM) number of citrus mealybugs (CMB), *Planococcus citri*, found on leaf bottom and plant stem of green coleus (*Solenstemon scutellarioides*) plants treated curatively with spirotetramat (S), azadirachtin 4% (Az4), azadirachtin 8% (Az8), and water (W).



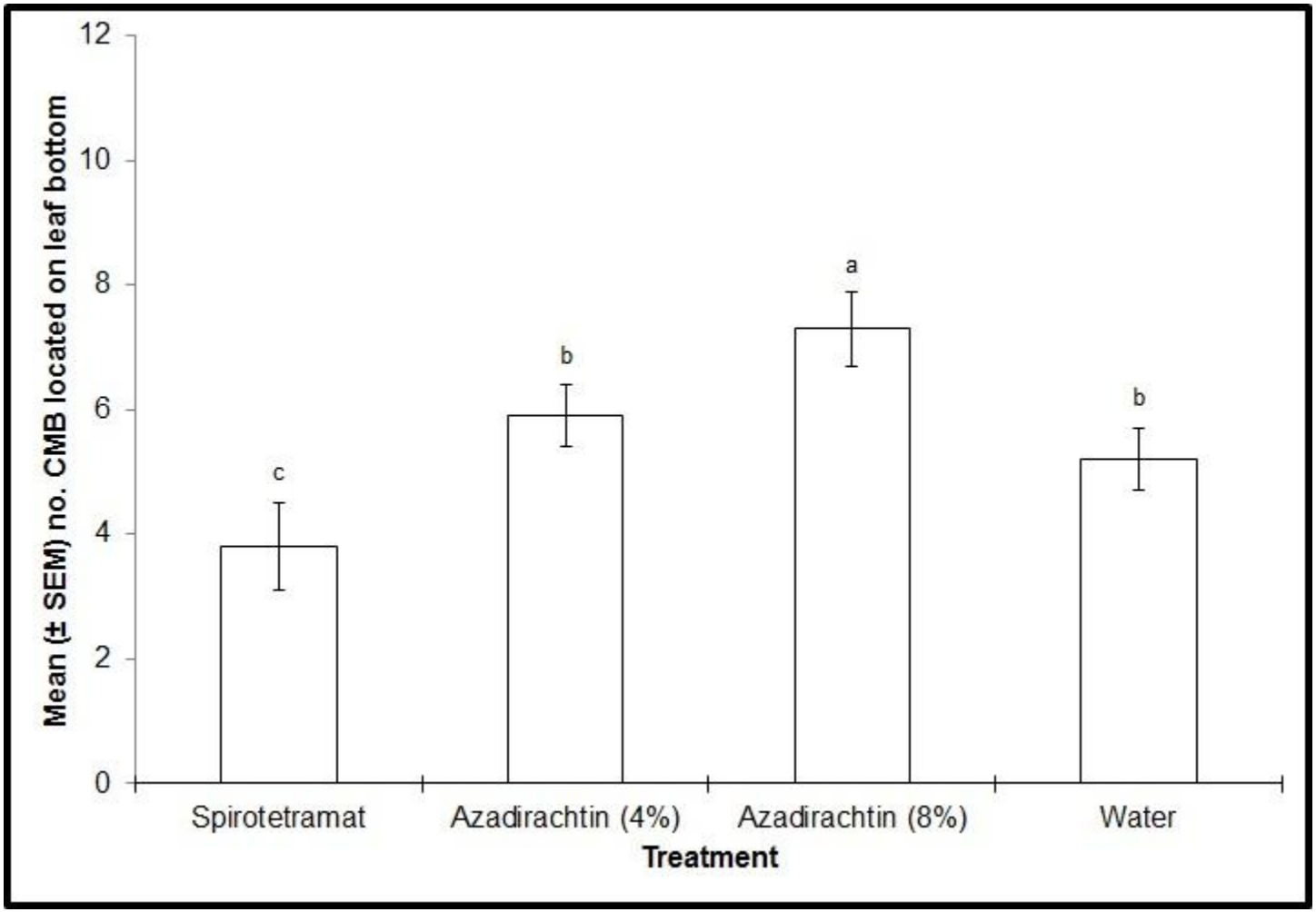
Bars with the same letter are not significantly different from each other ($P \leq 0.05$) based on a PROC MIXED procedure. Vertical lines indicate standard error of the mean (SEM).

Figure 2.3. Mean (± SEM) number of citrus mealybugs (CMB), *Planococcus citri*, found on leaf bottom (B), plant stem (S), and leaf top (T), of red coleus (*Solenstemon scutellarioides*) plants treated curatively with spirotetramat, azadirachtin (4%), azadirachtin (8%), and water over a three week time period.



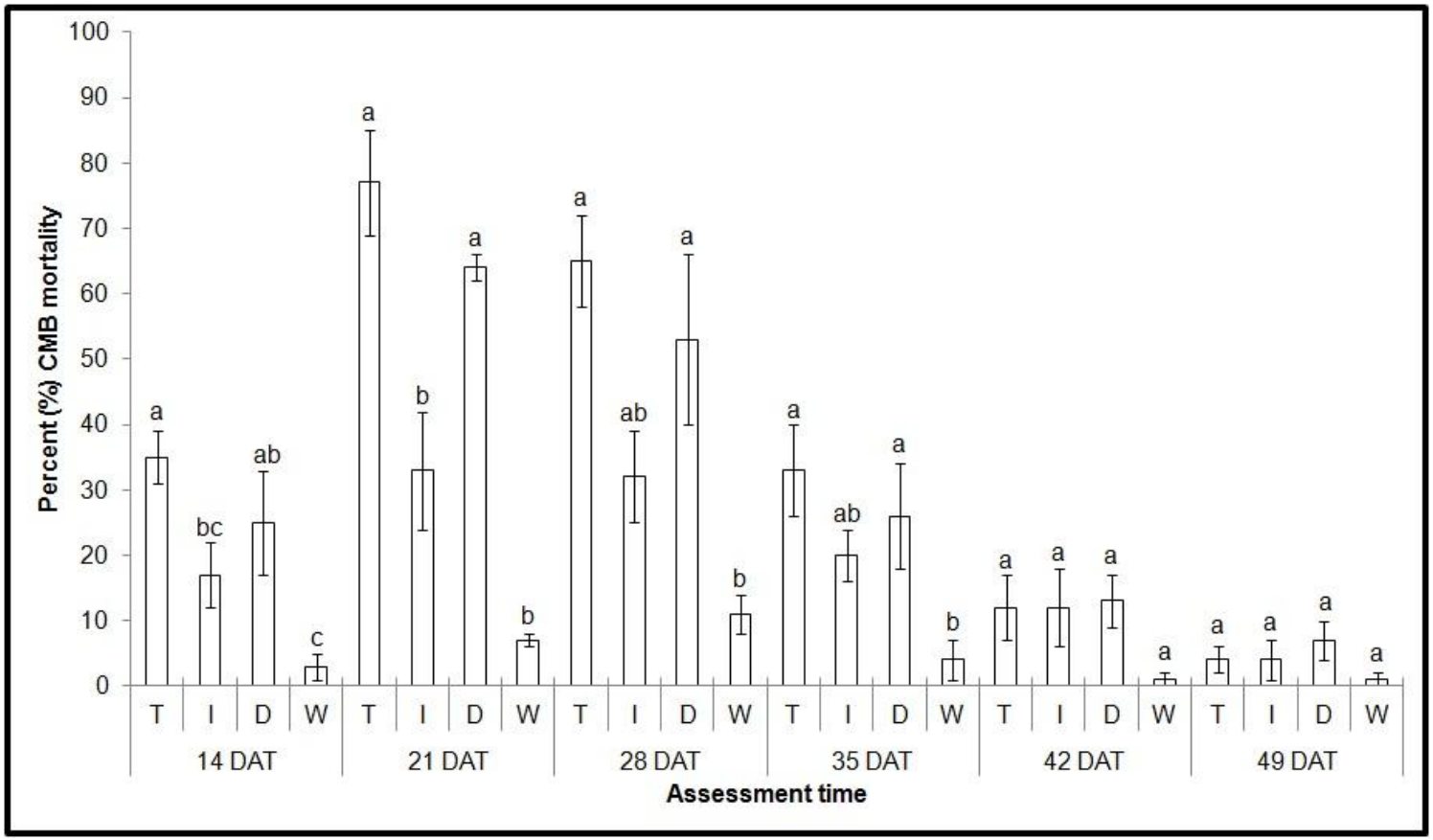
Bars with the same letter are not significantly different from each other ($P \leq 0.05$) based on a PROC MIXED procedure. Vertical lines indicate standard error of the mean (SEM).

Figure 2.4. Mean (± SEM) number of citrus mealybugs (CMB), *Planococcus citri*, found on leaf bottom of red coleus (*Solenstemon scutellarioides*) plants treated curatively with spirotetramat, azadirachtin 4%, azadirachtin 8%, and water.



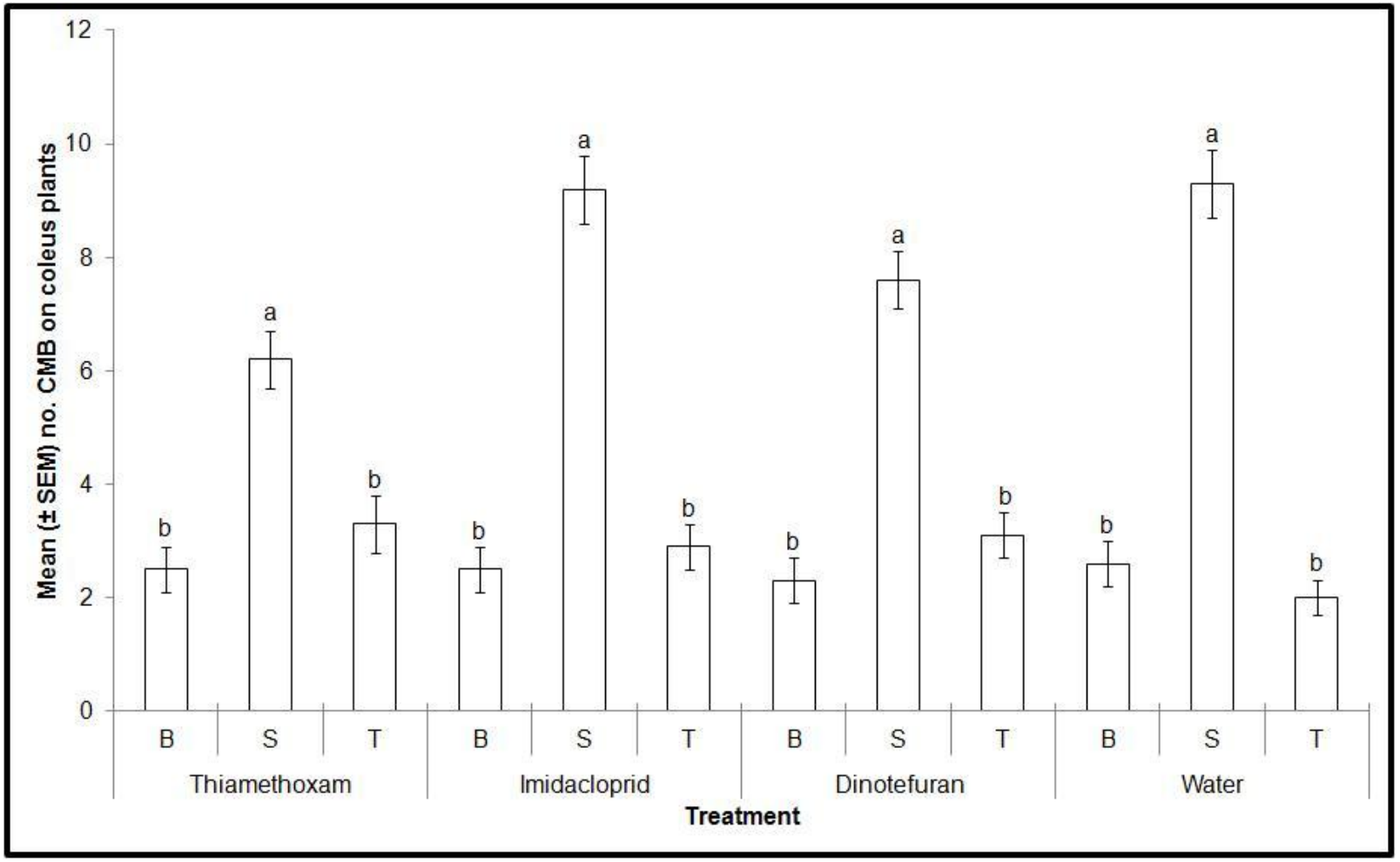
Bars with the same letter are not significantly different from each other ($P \leq 0.05$) based on a PROC MIXED procedure. Vertical lines indicate standard error of the mean (SEM).

Figure 2.5. Mean percent mortality (± SEM) of citrus mealybug (CMB), *Planococcus citri*, using three neonicotinoid insecticides [thiamethoxam (T), imidacloprid (I), and dinotefuran (D)] and water (W), on green coleus (*Solenstemon scutellarioides*) plants. There were five replications per treatment per assessment time [14, 21, 28, 35, 42, and 49 days after treatment (DAT)].



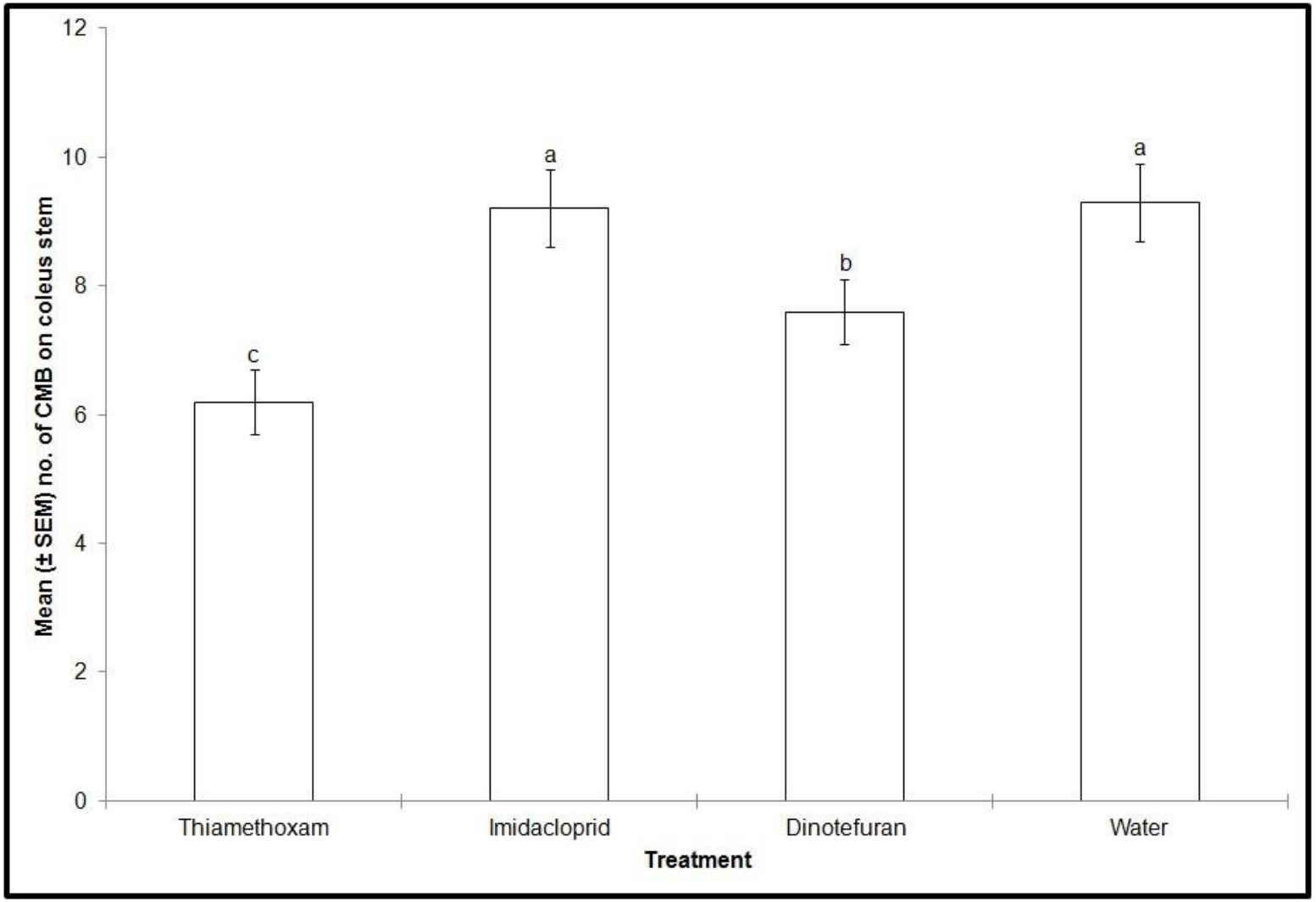
Bars with the same letter within an assessment time (DAT) are not significantly different from each other ($P \leq 0.05$) based on Fisher's protected least significant difference (LSD) mean separation test. Vertical lines indicate standard error of the mean (SEM).

Figure 2.6. Mean (± SEM) number of live and dead citrus mealybugs (CMB), *Planococcus citri*, located on leaf bottom (B), plant stem (S), and leaf top (T), of green coleus, (*Solenstemon scutellarioides*) plants for the thiamethoxam, imidacloprid, dinotefuran, and water treatments.



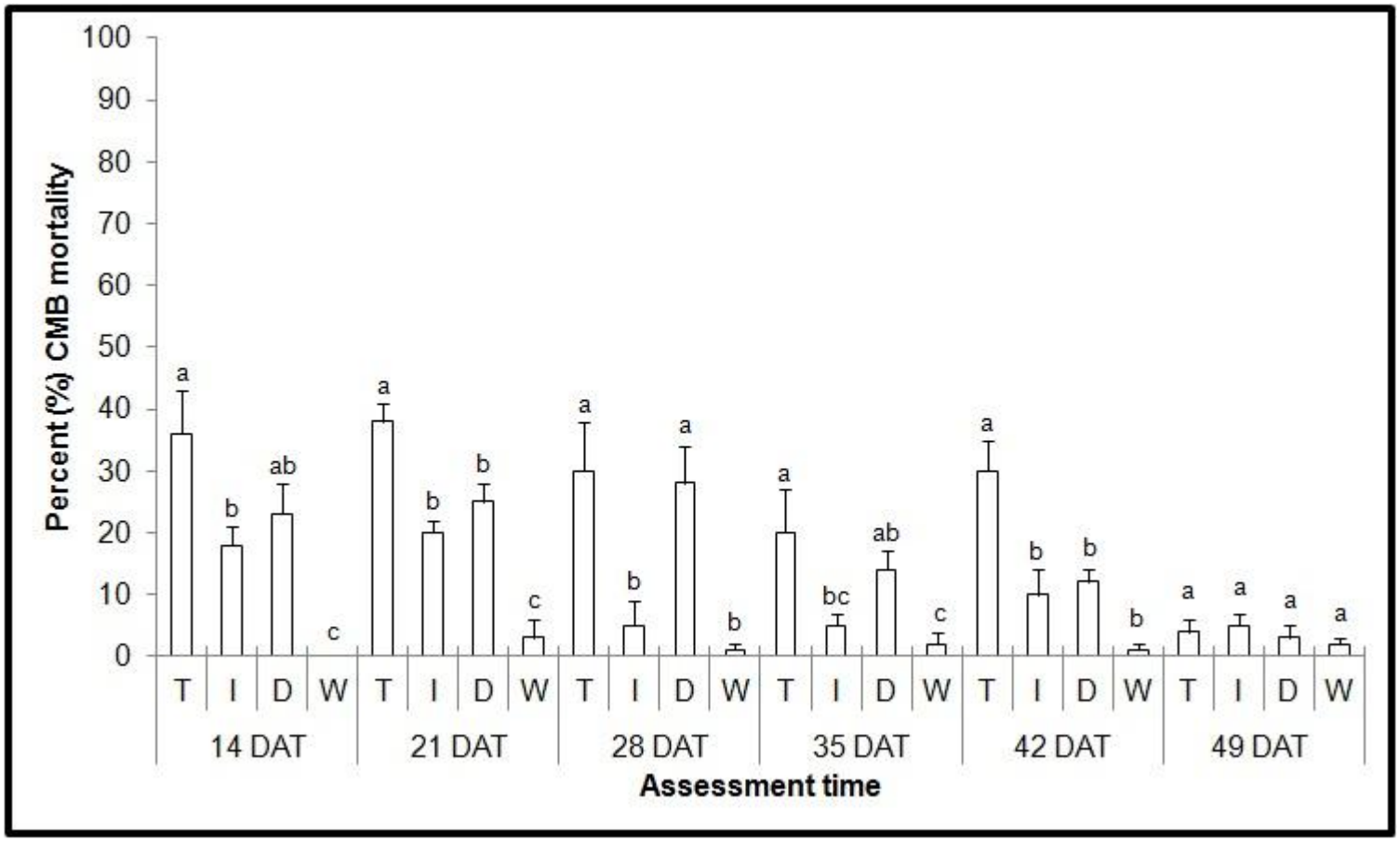
Bars with the same lower case letter are not significantly different from each other ($P \leq 0.05$) based on a PROC MIXED procedure. Vertical lines indicate standard error of the mean (SEM).

Figure 2.7. Mean (± SEM) number of live and dead citrus mealybugs (CMB), *Planococcus citri*, located on the plant stem of green coleus (*Solenstemon scutellarioides*) plants for each treatment.



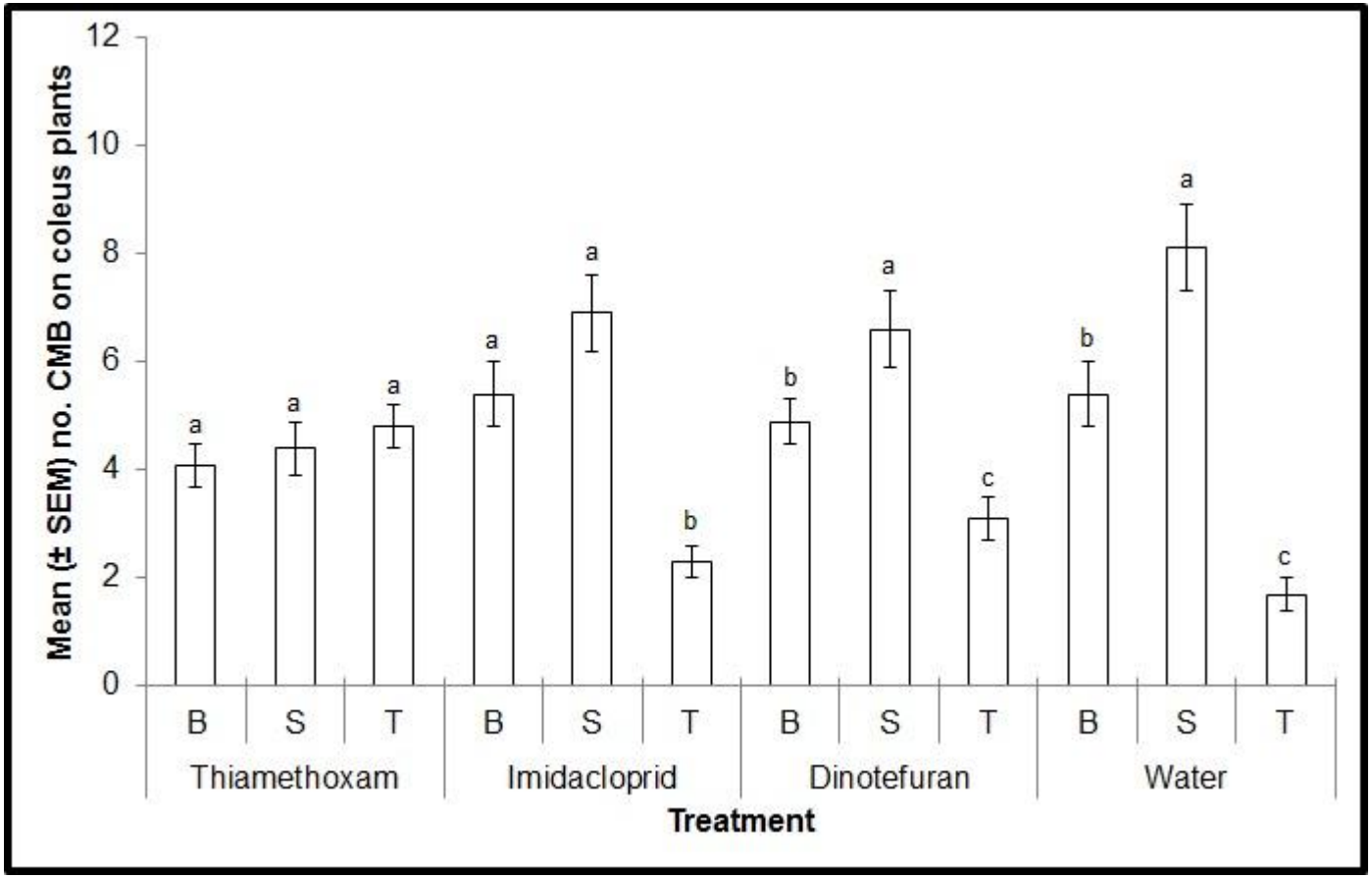
Bars with the same letter are not significantly different from each other ($P \leq 0.05$) based on a PROC MIXED procedure. Vertical lines indicate standard error of the mean (SEM).

Figure 2.8. Mean percent mortality (± SEM) of the citrus mealybug (CMB), *Planococcus citri*, after applying twice the labeled rates of three neonicotinoid insecticides [thiamethoxam (T), imidacloprid (I), dinotefuran (D)] on green coleus (*Solenstemon scutellarioides*) plants. There were five replications per treatment per assessment time [14, 21, 28, 35, 42, and 49 days after treatment (DAT)]. A water (W) control was also included.



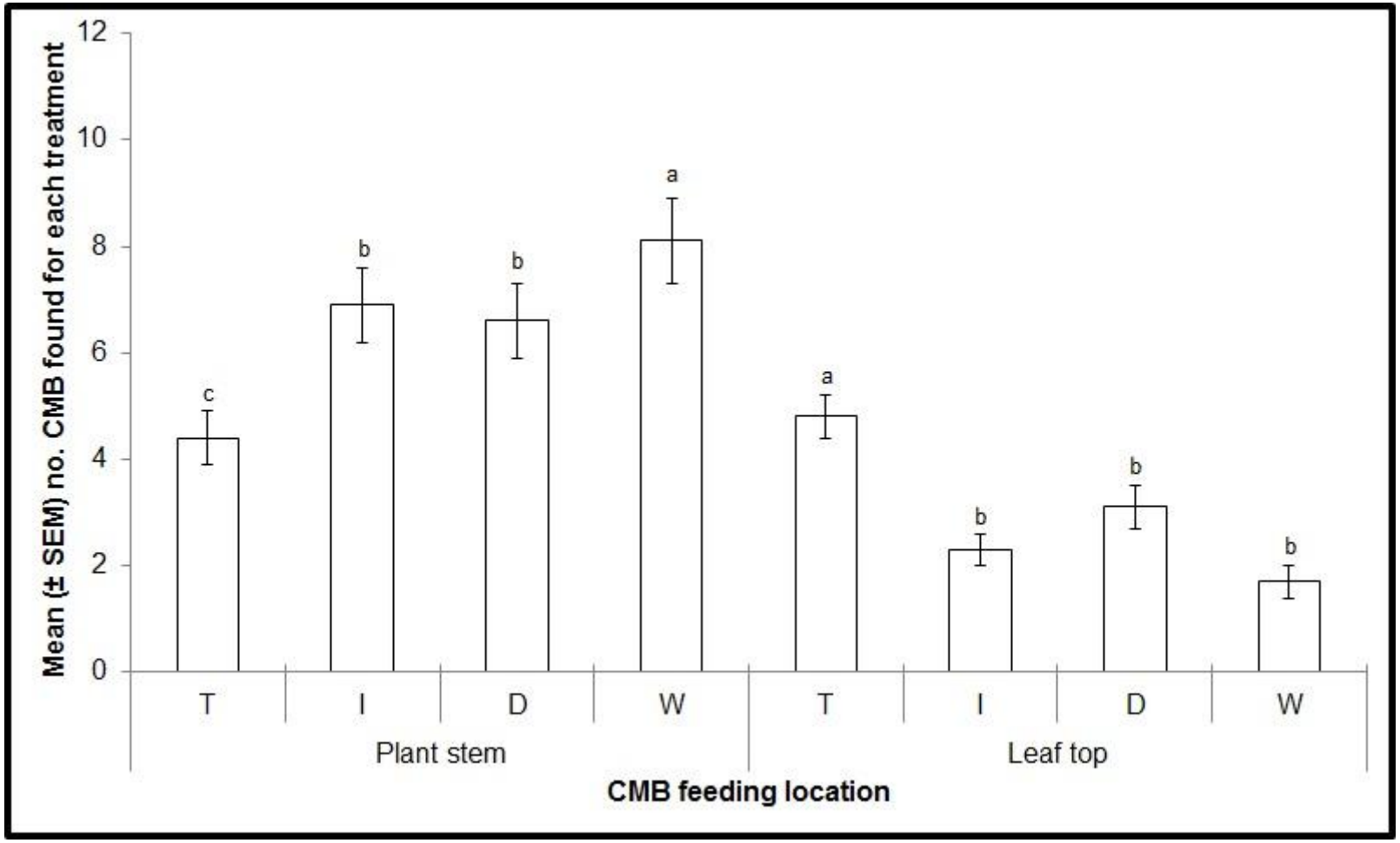
Bars with the same letter within an assessment time (DAT) are not significantly different from each other ($P \leq 0.05$) based on Fisher's protected least significant difference (LSD) mean separation test. Vertical lines indicate standard error of the mean (SEM).

Figure 2.9. Mean (± SEM) number of live and dead citrus mealybugs (CMB), *Planococcus citri*, located on leaf bottom (B), plant stem (S), or leaf top (T), of green coleus (*Solenstemon scutellarioides*) plants after applying twice the labeled rate of thiamethoxam, imidacloprid, dinotefuran, and a water control.



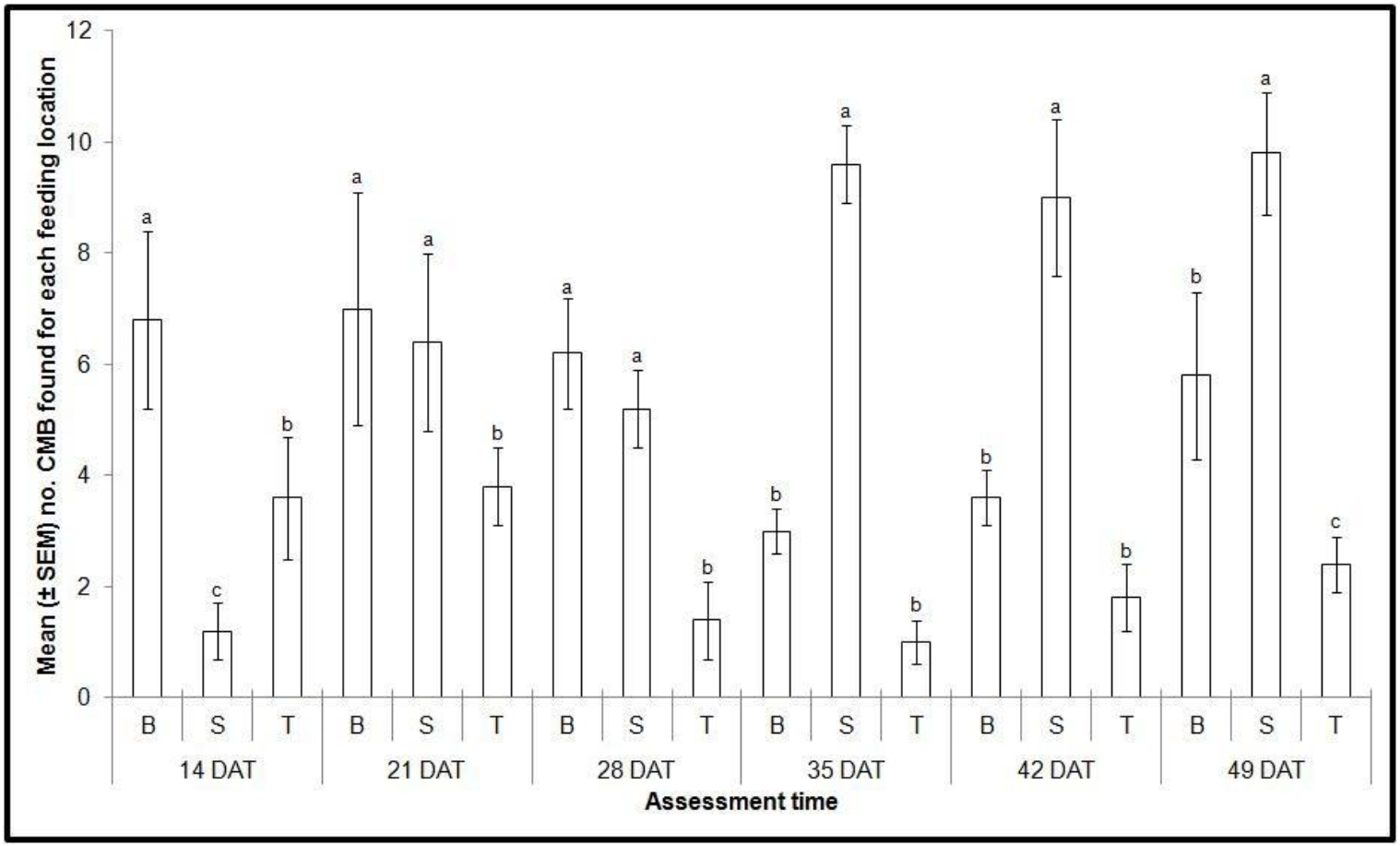
Bars with the same letter are not significantly different from each other ($P \leq 0.05$) based on a PROC MIXED procedure. Vertical lines indicate standard error of the mean (SEM).

Figure 2.10. Mean (± SEM) number of citrus mealybugs (CMB), *Planococcus citri*, found on plant stem and leaf top of green coleus (*Solenstemon scutellarioides*) plants after applying twice the labeled rate of thiamethoxam (T), imidacloprid (I), dinotefuran (D). A water (W) control was also included.



Bars with the same letter are not significantly different from each other ($P \leq 0.05$) based on a PROC MIXED procedure. Vertical lines indicate standard error of the mean (SEM).

Figure 2.11. Mean (± SEM) number of live and dead citrus mealybugs (CMB), *Planococcus citri*, located on leaf bottom (B), plant stem (S), or leaf top (T), after applying twice the labeled rates of three neonicotinoid insecticides [thiamethoxam (T), imidacloprid (I), and dinotefuran (D)] on green coleus (*Solenstemon scutellarioides*) plants. There were five replications per treatment per assessment time [14, 21, 28, 35, 42, and 49 days after treatment (DAT)]. A water (W) control was also included.



Bars with the same letter are not significantly different from each other ($P \leq 0.05$) based on a PROC MIXED procedure. Vertical lines indicate standard error of the mean (SEM).

Chapter 3 - Efficacy of pesticide mixtures on the western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae)

Introduction

Western flower thrips (WFT), *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), is an economically important insect pest of many horticultural crops grown in greenhouses worldwide (Robb and Parrella, 1988; Robb et al., 1995; Tommasini and Maini, 1995; Kirk, 2002). Western flower thrips are difficult to manage due to their life cycle, cryptic behavior, and reproductive capacity (Thoeming et al., 2003). There are six life stages including egg, two nymphal stages, prepupa, pupa, and adult (Moritz, 1997). Nymphs actively feed on leaves and flowers with their piercing-sucking mouthparts (Chisholm and Lewis, 1984; Hunter and Ullman, 1992), which are used to withdraw plant vascular fluids (Kirk, 1995). Western flower thrips continue to feed until the prepupa/pupa stage, which is typically located in the growing medium (Thoeming et al., 2003). As such, this life stage is difficult to control with insecticides. After pupation, adults emerge and feed primarily on flowers (Kirk, 1995). According to Robb and Parrella (1991), adults live up to 75 days at 20°C, and at 27°C fecundity is 228.6 eggs per female. Development from egg to adult is 9.3 days at 30°C (Robb and Parrella, 1991; Brødsgaard and Albajes, 1999). Protected environments, such as greenhouses, provide optimal conditions for WFT with a constant food supply and, in general, an absence of natural enemies (e.g., predators and parasitoids).

Western flower thrips feed on all above ground plant parts causing both direct and indirect damage to plants (Ullman et al., 1993; Broadbent and Allen, 1995; Mound, 1996; Hull, 2009). Direct damage is caused when WFT feed on plant cells, resulting in deformation of leaves and flowers, which makes plants unmarketable (Childers and Achor, 1995; Cloyd and Lindquist,

2001). Furthermore, indirect damage is caused when adults transmit the tospoviruses: impatiens necrotic spot virus (INSV) and/or tomato spotted wilt virus (TSWV) (Sether and DeAngelis, 1992; Broadbent and Allen, 1995; Daughtrey et al., 1997; Hull, 2009). These two viruses infect more than 550 plant species (Sether and DeAngelis, 1992). Standards for greenhouse-grown crops are based primarily on aesthetics; thus, due to the potential of spreading viruses, the tolerance level for WFT is near zero (Bethke and Cloyd, 2009). Therefore, in order to mitigate populations of WFT, greenhouse producers continually rely on insecticide applications (Parrella, 1995; Brødsgaard and Albajes, 1999). Insecticides are useful in suppressing WFT populations, thus minimizing plant damage and reducing viral transmission (Bethke and Cloyd, 2009). In addition, a short developmental time allows for multiple generations per year and decreases the practicality of many management practices such as biological control (Lewis, 1997). Since only a limited number of insecticides are registered for use in greenhouses against WFT, it is difficult for greenhouse producers to develop rotation programs based on modes of action (Bielza et al., 2008). As such, the biological and operational factors (presented above) may increase the rate in which WFT populations develop resistance to insecticides (Georghiou and Taylor, 1977a; Georghiou and Taylor, 1977b).

Insecticide resistance has been associated with a number of active ingredients registered for use in greenhouses such as abamectin, acephate, bifenthrin, and spinosad (Immaraju et al., 1992; Brødsgaard, 1994; Loughner et al., 2005; Bielza et al., 2008). Another important factor affiliated with greenhouse arthropod (insect and/or mite) pest management is that producers typically encounter multiple pest species during a single cropping cycle. Therefore, the use of a variety of insecticides may be necessary to mitigate the multitude of arthropod pests that occur simultaneously in greenhouse environments. However, in order to comply with the Food Quality

Protection Act (FQPA) standards, most newly registered pesticides have narrow-spectrum arthropod pest activity (Sray, 1997). This can be costly to greenhouse producers due to the intensive labor involved in making multiple applications of pesticides (Cabello and Canero, 1994; Cloyd, 2009). Consequently, in order to delay the onset of resistance development and manage the multitude of arthropod pests including WFT, mealybugs, twospotted spider mite (*Tetranychus urticae*), aphids, whiteflies, and fungus gnats, greenhouse producers apply pesticides as mixtures (Cloyd, 2009).

A pesticide mixture is a combination of two or more pesticides in a single solution applied simultaneously (Brattsten et al., 1986; Roush, 1993; O'Connor-Marer, 2000; Cloyd, 2011). Greenhouse producers apply pesticides as mixtures to reduce labor costs because fewer applications are required (Cabello and Canero, 1994; O'Connor-Marer, 2000; Cloyd, 2009). In addition, pesticide mixtures may broaden the spectrum of pest activity; thus mitigating populations of the multiple arthropod pests encountered in greenhouses (Warnock and Cloyd, 2005). Furthermore, there is a possibility of synergism occurring between the pesticides used in the mixture (Ware and Whitacre, 2004; Warnock and Cloyd, 2005; Cloyd et al., 2007). Synergism occurs when the toxicity of the pesticides used in the mixture is greater to the target pest when combined, compared to if the compounds were applied separately (Hewlett, 1968; O'Connor-Marer, 2000). For synergism to occur, one of the compounds interferes with metabolic detoxification, which then increases toxicity of the other pesticide (Corbett, 1974). Pesticide mixtures have been recommended as a means of mitigating resistance (Brattsten et al., 1986; Roush, 1993). In fact, by mixing pesticides together, there is a potential to reduce the frequency of resistance developing in arthropod pest populations as long as there is no cross resistance (Brattsten et al., 1986; Roush, 1989). This is one of the reasons why greenhouse

producers continue to use pesticide mixtures regardless of the potential disadvantages/problems associated with their usage.

Problems that may occur when mixing pesticides include incompatibility, plant phytotoxicity, and antagonism (Cloyd, 2001). Pesticide incompatibility can cause problems for greenhouse producers by disrupting application equipment. Incompatibility is evident when flakes, crystals, or clumps develop thus indicating the pesticides will not mix together uniformly (O'Connor-Marer, 2000). In addition, when pesticides are incompatible there is uneven distribution throughout the solution resulting in non-uniform coverage when applied to plants (O'Connor-Marer, 2000). Phytotoxicity or plant injury is another potential problem associated with pesticide mixtures, which may reduce crop marketability. Furthermore, antagonism occurs when the level of efficacy is reduced when pesticides are combined into a mixture (O'Connor-Marer, 2000; Lindquist, 2002). However, despite these problems, greenhouse producers regularly apply pesticide mixtures in order to mitigate arthropod pest populations although data is somewhat limited on the advantages and/or disadvantages of pesticide mixtures when applied to suppress WFT populations (Cloyd, 2009).

A study conducted by Warnock and Cloyd (2005) evaluated two-, three-, and four-way pesticide mixtures against WFT under laboratory and greenhouse conditions. It was determined that mixtures containing spinosad, bifenazate, abamectin, imidacloprid, and azadirachtin had no antagonistic effects (based on percent mortality) when applied against 2-day old adult WFT. In addition, Cloyd et al. (2007) demonstrated that a number of pesticide mixtures provided >75% mortality of silverleaf whitefly (*Bemisia argentifolii*) nymphs 14 days after treatment, and ≥ 90% mortality of twospotted spider mite (*Tetranychus urticae*) nymphs, 7 days after treatment under greenhouse conditions. These results indicate that the pesticide mixtures were not antagonistic,

based on percent mortality, against either insect pest. Furthermore, in a laboratory experiment, synergistic effects between carbamates and pyrethroids were observed in two field populations of WFT with documented resistance to the pyrethroid, acrinathrin (Bielza et al., 2009). Based on these studies, pesticide mixtures applied under laboratory and greenhouse conditions may control WFT populations along with other commonly encountered arthropod pests in greenhouses. However, these studies were conducted without feedback from greenhouse producers on typically used pesticide mixtures. Furthermore, there is no quantitative information associated with synergism and/or antagonism of commonly used pesticide mixtures. Therefore, in order to obtain feedback from greenhouse producers on the most commonly used pesticide mixtures, surveys were conducted (Cloyd, 2009).

Greenhouse producers were surveyed three times, twice in 2007 and once in 2008, requesting what pesticide mixtures are being applied in greenhouses (Cloyd, 2009). Respondents indicated a wide-variety of pesticide mixtures including two-, three-, and four-way combinations (Cloyd, 2009). Three or more responses from greenhouse producers using binary pesticide mixtures indicated nine different combinations containing at least one pesticide registered for use against WFT (Table 3.1). The binary mixtures were categorized into three groupings; mixtures containing abamectin, mixtures containing spinosad, and mixtures containing neither active ingredient (Cloyd, 2009). In addition, many of the mixtures reportedly used contained at least one pesticide that was either not registered or not effective against adult WFT. Furthermore, there is no quantitative information demonstrating whether these mixtures are synergistic or antagonistic when applied to control WFT. Therefore, the objectives of this study were to 1) evaluate the most commonly used two- and three-way pesticide mixtures against WFT based on

the survey, and 2) determine the efficacy of currently used pesticide mixtures under both laboratory and greenhouse conditions against the WFT.

Materials and Methods

This study consisted of both laboratory and greenhouse experiments to assess nine of the most commonly used binary pesticide mixtures against WFT. Moreover, greenhouse experiments were conducted to evaluate the efficacy of three tertiary pesticide mixtures reportedly used in greenhouses to control WFT. Each of the binary and tertiary pesticide mixtures evaluated are presented in Table 3.1.

Western flower thrips colony

Laboratory-reared colonies of WFT were maintained on greenbean, *Phaseolus vulgaris*, under 24 ± 5°C, 50-60% relative humidity (RH), and 14:10 (L:D) hour photoperiod in the Department of Entomology at Kansas State University (Manhattan, KS). These colonies had not been exposed to pesticides for at least six years.

Pesticide compatibility

Jar tests were performed to determine visual compatibility of the binary pesticide mixtures used in the laboratory and greenhouse experiments. A procedure described by O'Connor-Marer (2000) was followed by mixing the pesticides in 500 mL Mason Ball (Broomfield, CO) jars. Each pesticide was prepared using the highest labeled rate or the recommended rate for WFT (Table 3.2). The pesticides were combined at a 1:1 volume ratio. Each mixture remained in a controlled laboratory environment for approximately 15 minutes. Compatibility was determined by visual observations.

Plant phytotoxicity

Binary pesticide mixtures were prepared at the highest recommended labeled rates for WFT, and for products not registered for WFT, the highest recommended labeled rate was used (Table 3.2). Mixtures were applied to *Tanacetum* spp., *Begonia* spp., *Petunia* spp., *Salvia* spp., *Tagetes* spp., *Impatiens* spp., *Vinca* spp., *Pansy* spp., and *Coleus* spp. plants to determine phytotoxicity of each binary mixture. All plants except *Tanacetum* spp. were planted into 10.2-cm containers using Fafard® 2 Mix growing medium (Agawam, MA) containing Canadian sphagnum peat moss (65%), perlite, vermiculite, starter nutrients, wetting agent and dolomitic limestone. The leaves and flowers of each plant were sprayed using a 946 mL plastic spray bottle (The Home Depot, Manhattan, KS) with approximately 15 mL of solution per plant. The *Tanacetum* spp. plants were potted into 15.2-cm containers using Fafard® 2 Mix growing medium and approximately 63 mL of spray solution was applied per plant for each treatment. There were five replications per treatment and a water control was included. The plants were maintained in a greenhouse at Kansas State University (Manhattan, KS), and assessed visually for phytotoxicity 7 days after treatment.

Technical grade residual bioassays

Glass residual bioassays were performed using each of the technical grade pesticides included in the mixtures to determine LC_{50} values. Technical grade active ingredients of abamectin, acephate, azadirachtin, bifenthrin, bifenazate, fenpropathrin, novaluron, pymetrozine, and spinosad were obtained from Chem Services Inc. (West Chester, PA). Each active ingredient was dissolved in acetone and 5 serial dilutions were made using acetone as a control (6 concentrations total) with four replications for each concentration. Then, 200 µl of each concentration was pipetted into a 7 mL glass vial and placed on a Roto-torque® (Cole-Parmer

Instrument Company; Vernon Hills, IL) rotator for 15 minutes or until dry in order to evenly coat the vials. After drying, a 1-mm piece of greenbean was added to the vial as a food source, and approximately 15 7-day old adult female WFT were added to each vial. The vials were covered with Parafilm® (Chicago, IL), which had approximately 50 holes in the top for ventilation, and then placed in a Percival Scientific (Perry, IA) growth chamber for 24 hours at 25°C and a 16:8 (L:D) hour photoperiod. After 24 hours, mortality was assessed by probing each individual with a needle and positioning on the dorsal side. Those WFT that did not move were considered dead.

Formulated pesticide bean-dip bioassays

Bean-dip bioassays were conducted to determine LC_{50} values for the formulated pesticides containing abamectin, acephate, azadirachtin, bifenazate, bifenthrin, fenpropathrin, novaluron, pymetrozine, and spinosad. Each pesticide was dissolved in deionized water and 5 serial dilutions were made using deionized water as a control (6 concentrations total) with four replications for each concentration. Greenbeans were cut into 2-mm pieces, inserted into the designated solution for 10 seconds, and allowed to dry on FisherBrand® (Pittsburgh, PA) qualitative-grade P8 9.0 cm circle filter paper. After drying, each greenbean slice was placed into a 7 mL glass vial. Then, approximately 15, 7-day old adult female WFT were counted and added to each vial. The vials were covered with Parafilm® (Chicago, IL) with 50 holes in the top for ventilation. Vials were placed in an environmental growth chamber for 24 hours at 25°C and a 16:8 (L:D) hour photoperiod. Mortality was determined similar to the previous experiment.

Formulated pesticide mixture bean-dip bioassays

Bean-dip bioassays (as described previously) were used to determine LC_{50} values for each formulated binary pesticide mixture (Table 3.1). The procedure used was similar to the

formulated pesticide experiment (described above); however, the pesticide mixtures were prepared using a 1:1 volume ratio. Each formulated pesticide was diluted with deionized water. Mortality was assessed similar to the previous laboratory experiments (described above).

Greenhouse efficacy experiments

Greenhouse experiments were conducted at Kansas State University (Manhattan, KS) to evaluate the efficacy of the formulated pesticides as well as the two-and three-way pesticide mixtures (Table 3.1). There were three experiments with each consisting of individual pesticides and binary mixtures. The first experiment included 10 treatments, the second experiment included 11 treatments, and the third experiment included 12 treatments (Table 3.2). There were five replications per treatment for each experiment.

Yellow cut transvaal daisy (*Gerbera jamesonii*) were obtained from Koehler & Dramm of Missouri (Kansas City, MO). Each flower was cut approximately 7.6 cm below the flower head and placed into a 22-mm glass vial containing tap water. Each vial was placed into a blue polypropylene container (250 mL) and surrounded with sand to ensure secure placement. Containers were placed on a wire-mesh greenhouse bench that had an open frame composed of polyvinyl chloride (PVC) pipe, which held a 50% black knit shade cloth (Hummert International; Earth City, MO) placed on top to protect the flowers from sunlight and preserve longevity.

After two days, approximately 20 WFT adults obtained from the laboratory-reared colony were aspirated into vials, added to each flower, and allowed to establish for two days prior to pesticide applications. Pesticide treatments were mixed, with tap water, at the recommended labeled rates for WFT, or for products not registered for WFT, the highest labeled rate (Table 3.3). The pesticide mixtures were prepared at a 1:1 volume ratio. Applications were made using a 946 mL plastic spray bottle (The Home Depot; Manhattan, KS). Each flower received

approximately 15 mL of the designated spray solution, after five days, WFT mortality was assessed using destructive sampling. Efficacy of each pesticide and pesticide mixture was based on percent mortality of WFT.

Statistical analysis

The LC_{50} values of technical grade, formulations, and mixtures of pesticides, which were determined in the laboratory, were calculated using a PROC PROBIT procedure (SAS Institute, 2002). A Pearsons Chi-Square (χ^2) value with $P \geq 0.05$ indicated no significant difference between the model and the observed regression lines. For the greenhouse efficacy experiments, percent mortality was determined by dividing the number of dead WFT per flower by the total number of WFT recovered from each flower. For statistical purposes, percent mortality values among the treatments were transformed using an arcsine square-root transformation procedure and then analyzed using an analysis of variance (ANOVA) with treatment as the main effect. Fisher's protected least significant difference (LSD) test at $P \leq 0.05$ was then used to identify significant differences among the treatments. In all cases, results are presented using non-transformed data.

Synergism or antagonism of the binary pesticide mixtures was evaluated based on combination index (CI) values as described by Chou and Talalay (1984). The following equation uses the LC_{50} values determined based on the mixture and individual pesticide:

$$CI = \frac{LC_{50}^{1m}}{LC_{50}^{1}} + \frac{LC_{50}^{2m}}{LC_{50}^{2}} + \left(\frac{LC_{50}^{1m}}{LC_{50}^{1}} \times \frac{LC_{50}^{2m}}{LC_{50}^{2}}\right)$$

The numerator is the LC_{50} value for the pesticides used in a mixture. In order to obtain this value, a ratio was calculated by dividing the LC_{50} of the first pesticide by the LC_{50} of the second pesticide. This ratio was factored into the LC_{50} value for the mixture to determine how

much of the mixture was associated with each pesticide (Attique et al., 2006). The denominator is the LC_{50} value for each of the formulated pesticides when used individually. Based on the calculation, a CI value > 1 indicates antagonism, < 1 synergism, and equal to 1 an additive effect.

Results

Pesticide compatibility and plant phytotoxicity

Each of the nine binary pesticide mixtures displayed no visible signs of incompatibility. Furthermore, none of the nine binary pesticide mixtures were visibly phytotoxic to any of the horticultural plants tested.

Technical grade residual bioassays

Results associated with the laboratory residual bioassays using the technical grade active ingredients for each pesticide are shown in Table 3.4. Three of the nine technical grade pesticides had a definitive LC_{50} value. Spinosad had the lowest LC_{50} value (1.57 µg/mL) indicating it was the most toxic against adult WFT, followed by bifenthrin (8.56 µg/mL) and acephate (282.11 µg/mL). We were not able to obtain definitive LC_{50} values for other pesticides because either there was no dose-response relationship or the mortalities at the concentration of the maximum solubility was still <50%. Therefore, the LC_{50} values for the pesticides were considered greater than the highest concentration tested. Pymetrozine was excluded because it did not dissolve in acetone, ethyl alcohol, or deionized water.

Formulated pesticide bean-dip bioassays

Results from the formulated pesticide bioassay using the bean-dip method are presented in Table 3.5. Ten pesticides were evaluated, with only four having a definitive LC_{50} value. Spinosad had the lowest LC_{50} value (0.44 µg/mL) indicating it was the most toxic to the baseline

population of WFT, followed by abamectin (148.80 µg/mL), acephate (720.86 µg/mL), and bifenthrin (1331.00 µg/mL). Similar to the technical grade bioassays, we were not able to obtain definitive LC_{50} values for other pesticides because either there was no dose-response relationship or mortalities at the concentration of the maximum solubility was still <50%. Therefore, the LC_{50} values for the pesticides were considered greater than the highest concentration tested. Low mortality (≤5%) was observed in the controls (vials treated with deionized water) indicating that the bean-dip method was appropriate for determining LC_{50} values.

Formulated pesticide mixture bean-dip bioassays

Results from the bean-dip pesticide mixture bioassays are presented in Table 3.6. Each mixture had a definitive LC_{50} value because they contained at least one pesticide that had an individual LC_{50} value. Those mixtures containing spinosad had the lowest LC_{50} values (≤1.79 µg/mL) indicating the highest toxicity to adult WFT. Mixtures containing abamectin had a range of LC_{50} values from 27.18 µg/mL (abamectin + azadirachtin [Azatin]) to 157.57 µg/mL (abamectin + bifenthrin). The mixture containing acephate + fenpropathrin had the highest LC_{50} value (382.40 µg/mL) demonstrating it was the least toxic to adult WFT. Calculations using the combination index equation (Chou and Talalay, 1984) indicated that eight pesticide mixtures were synergistic and one was antagonistic (Table 3.7). According to Chou (2006), three of the nine pesticide mixtures were classified as synergistic, three mixtures as moderately synergistic, and one mixture slightly synergistic (Table 3.8). There was one mixture classified as nearly additive, and one mixture was considered strongly antagonistic according to Chou (2006) (Table 3.8).

Greenhouse efficacy experiments

Results of the three greenhouse efficacy experiments are presented in Figures 3.1-3.3. For experiment one, there were significant differences among the treatments (F=34.4; df=11, 48; $P \leq 0.0001$). Abamectin and spinosad when applied individually resulted in almost 100% mortality of WFT. Although both azadirachtin (Azatin®) and bifenthrin when applied individually had significantly higher WFT mortality compared to the control, they were significantly less effective compared to abamectin and spinosad (Figure 3.1). Mixtures of abamectin + azadirachtin (Azatin®), abamectin + azadirachtin (Ornazin®), and abamectin + bifenthrin had reduced efficacy compared to the other mixtures but still provided ≥80% WFT mortality.

Results for experiment two are shown in Figure 3.2. There was a significant difference among the treatments (F=78.74; df=10, 44; $P \leq 0.0001$). The individual pesticides spinosad and acephate, and the mixtures of spinosad + pymetrozine, spinosad + bifenazate, spinosad + novaluron, and acephate + fenpropathrin, all resulted in nearly 100% WFT mortality. The remaining pesticides resulted in minimal WFT mortality and were not significantly different from the water control (Figure 3.2).

Experiment three results are presented in Figure 3.3. There was a significant difference among the treatments (F=56.45; df=11, 48; $P \leq 0.0001$). The individual pesticides abamectin, spinosad, acephate, and the three-way mixtures of spinosad + bifenazate + imidacloprid, and abamectin + acephate + fenpropathrin all provided ≥80% WFT mortality. Azadirachtin (Azatin®), bifenazate, tolfenpyrad, and fenpropathrin exhibited ≤15% WFT mortality. Imidacloprid was significantly higher in WFT mortality than the control although mortality was still <25%.

Discussion

This study evaluated pesticide compatibility, phytotoxicity, and efficacy of nine binary mixtures currently used by greenhouse producers (Cloyd, 2009). In addition, the efficacies of three, tertiary mixtures were evaluated under greenhouse conditions. As such, this study demonstrated that each of the binary mixtures were visibly compatible and showed no signs of phytotoxicity on the nine horticultural plant species evaluated. Information regarding those pesticide mixtures that are safe to apply to plants is extremely valuable to greenhouse producers because if mixtures cause phytotoxicity then this negates their usefulness.

Furthermore, this is the first study to evaluate synergistic and/or antagonistic effects of pesticide mixtures used by greenhouse producers under laboratory conditions by calculating the combination index. Attique et al. (2006) used a combination index to calculate synergistic and additive effects of pesticide mixtures containing bifenthrin against the diamondback moth (*Plutella xylostella*); however, this method has not been used to evaluate synergism and/or antagonism of pesticide mixtures used against the WFT.

Overall, our laboratory experiments using the active ingredient, individual formulated pesticide, and formulated pesticide mixtures, and our greenhouse experiments showed that spinosad, and those mixtures containing spinosad were the most toxic to adult WFT. These findings are similar to Warnock and Cloyd (2005) in which mixing spinosad with other pesticides did not affect the efficacy of spinosad against WFT. However, the pesticide mixture spinosad + bifenazate was considered strongly antagonistic under laboratory conditions. The reason there was no significant difference when this mixture was used in the greenhouse experiment, although antagonism was observed in the laboratory, was because the laboratory results evaluated LC_{50} values for WFT whereas the greenhouse experiments used the designated

pesticide labeled rates, which are substantially higher than the LC_{50} value. Therefore, 100% mortality would be expected when susceptible populations of WFT are exposed to mixtures containing spinosad. It has been documented that spinosad is effective against susceptible populations of WFT (Jones et al., 2005). However, spinosad resistance has been reported in field populations of WFT (Loughner et al., 2005). The WFT population evaluated in this study was laboratory-reared; therefore, further research is necessary to determine the efficacy of pesticide mixtures against field resistant populations of WFT.

It was not possible to calculate a LC_{50} value for abamectin when using the technical grade material, however, the formulated pesticide was effective against WFT. This suggests that the inert ingredients in the formulation such as butulated hydroxytoluene (BHT), n-methyl pyrrolidone, and mineral oil may be involved in enhancing mortality of WFT. For instance, Stansly and Liu (1994) observed a highly toxic effect of mineral oil against the silverleaf whitefly, *Bemisia argentifolii*. In both laboratory experiments, the mixtures of abamectin + azadirachtin (Azatin®) and abamectin + azadirachtin (Ornazin®) resulted in synergism, however, reduced efficacy was observed in the first greenhouse experiment when abamectin was combined with azadirachtin (Azatin®) or azadirachtin (Ornazin®) compared to abamectin alone. Reduced efficacy, when combining abamectin + azadirachtin has been observed against WFT (Warnock and Cloyd, 2005) and the beat armyworm (*Spodoptera exigua*) (Moar and Trumble, 1987). The mechanism responsible for these antagonistic effects is currently unknown; however, it may be due to azadirachtin interfering with the inert ingredients in the formulated pesticide containing abamectin. Furthermore, azadirachtin has antifeedant properties, which may influence the feeding behavior of WFT and thus affect mortality (Yu, 2008).

The binary mixtures containing fenpropathrin and acephate were synergistic under laboratory conditions and there was no reduction in efficacy in the greenhouse experiments. Synergism between pyrethroids and organophosphates has been observed when applied to a resistant strain of the cotton bollworm (*Helicoverpa armigera*) (Martin et al., 2003). This synergistic relationship occurs due to esterase inhibition, which p cleaving of the ester-linkage in pyrethroids, allowing the pyrethroid to remain lethal to insects (Gaughan et al., 1980; Zhao et al., 1996; Martin et al., 2003). Interestingly, when acephate + fenpropathrin were combined with azadirachtin (Azatin®) in a three-way mixture, there was significantly less WFT mortality in the greenhouse experiment. However, when acephate + fenpropathrin + abamectin were combined in a three-way mixture there was no reduced efficacy. As previously observed in the binary mixtures, pesticides combined with azadirachtin had reduced efficacy against WFT. Further research is warranted to understand if the antifeedant and repellant properties of azadirachtin are responsible for reduced efficacy when azadirachtin is included in tertiary pyrethroid/organophosphate mixtures.

Active ingredients used in this study that were not effective against WFT in the laboratory and greenhouse experiments were azadirachtin, bifenazate, fenpropathrin, novaluron, and pymetrozine. This was expected as these pesticides are either insect growth regulators (novaluron and azadirachtin) (Yu, 2008) or selective-feeding blockers (pymetrozine) (Harrewijn and Kayser, 1997; Yu, 2008), which would have minimal effect on WFT adults. In addition, several pesticides (bifenazate and fenpropathrin) are not registered for WFT. However, when these pesticides were mixed with abamectin, spinosad, or acephate, which are registered for use against WFT, the mixtures were effective. For instance, in all the greenhouse experiments, >70% mortality was obtained.

In conclusion, this is the first study to demonstrate that the nine binary mixtures currently being used are visibly compatible and not phytotoxic when applied to a number of horticultural plants. Furthermore, this study quantitatively evaluated synergism and antagonism of binary pesticide mixtures using a combination index associated with WFT. Under laboratory conditions, seven of the nine most commonly used pesticide mixtures by greenhouse producers are synergistic. In addition, all nine of the evaluated binary pesticide mixtures provided >80% mortality of WFT under greenhouse conditions. As such, eight of the binary pesticide mixtures may be used by greenhouse producers who are attempting to mitigate multiple arthropod pest populations simultaneously with no antagonistic effects against WFT. The three-way pesticide mixtures varied in regards to WFT mortality under greenhouse conditions. Therefore, greenhouse producers should be cautious before applying three-way mixtures of pesticides. In addition, future research is warranted to determine the efficacy of these mixtures against field resistant populations of WFT. Overall, this study will assist greenhouse producers interested in applying pesticide mixtures against WFT populations.

Table 3.1. Commonly reported binary pesticide mixtures including trade names and common names used in greenhouses (Cloyd, 2009) that were evaluated in the pesticide compatibility, phytotoxicity, laboratory bioassays and greenhouse experiments. Three-way mixtures were only evaluated in the greenhouse experiments.

Trade names[z]	Common names
Avid + Menace	abamectin + bifenthrin
Avid + Conserve	abamectin + spinosad
Avid + Azatin	abamectin + azadirachtin
Avid + Ornazin	abamectin + azadirachtin
Orthene + Tame	acephate + fenpropathrin
Conserve + Endeavor	spinosad + pymetrozine
Conserve + Pedestal	spinosad + novaluron
Avid + Endeavor	abamectin + pymetrozine
Conserve + Floramite	spinosad + bifenazate
Abamectin + Orthene + Tame	abamectin + acephate + fenpropathrin
Conserve + Floramite + Marathon II	spinosad + bifenazate + imidacloprid
Orthene + Azatin + Tame	acephate + azadirachtin + fenpropathrin

[z]Company information: Avid® (Syngenta Crop Protection Inc.; Greensboro, NC); Azatin® (OHP Inc.; Mainland, PA); Conserve® (Dow AgroSciences, LLC; Indianapolis, IN); Endeavor™ (Syngenta Crop Protection, Inc.; Greensboro, NC); Floramite® (OHP Inc.; Mainland, PA); Marathon®II (OHP Inc.; Mainland, PA); Menace® (Nufarm; Burr Ridge, IL); Ornazin® (SePro Corp.; Carmel, IN); Orthene® (Valent U.S.A Corporation; Walnut Creek, CA); Pedestal™ (OHP Inc.; Mainland, PA); and Tame® (Valent U.S.A Corporation; Walnut Creek, CA).

Table 3.2. Common names of pesticides and pesticide mixtures used in the three greenhouse experiments. Experiments 1 and 2 evaluated binary mixtures, and experiment 3 evaluated three-way mixtures.

Experiment 1	Experiment 2	Experiment 3
abamectin	acephate	abamectin
bifenthrin	fenpropathrin	azadirachtin
spinosad	pymetrozine	spinosad
azadirachtin	bifenazate	bifenazate
azadirachtin	novaluron	imidacloprid
abamectin + bifenthrin	spinosad	acephate
abamectin + spinosad	spinosad + pymetrozine	fenpropathrin
abamectin + azadirachtin	spinosad + bifenazate	tolfenpyrad
abamectin + azadirachtin	spinosad + novaluron	abamectin + acephate + fenpropathrin
water control	acephate + fenpropathrin	spinosad + bifenazate + imidacloprid
	water control	acephate + fenpropathrin + azadirachtin
		water control

Table 3.3. Common name, trade name, chemical class, labeled rates, rates used per 16 oz, percent active ingredient, and labeled rate (μg/mL) for each of the pesticides used in mixtures against the western flower thrips, *Frankliniella occidentalis.*

Common name	Trade name[z]	Chemical class	Labeled rate per 100 gal	Rate per 16oz[y]	Percent (%) active ingredient	Labeled rate (μg/mL)
abamectin	Avid	macrocyclic lactone	8.0 fl oz	0.30 mL	2.0	11.2
acephate	Orthene	organophosphate	10-2/3 oz	0.38 g	75.0	599.3
azadirachtin	Azatin	botanical	16.0 fl oz	0.59 mL	3.0	39.7
azadirachtin	Ornazin	botanical	8.0 fl oz	0.30 mL	3.0	20.2
bifenazate	Floramite	carbazate	8.0 fl oz	0.30 mL	22.6	149.8
bifenthrin	Menace	pyrethroid	21.7 fl oz	0.80 mL	7.9	135.4
fenpropathrin	Tame	pyrethroid	16.0 fl oz	0.59 mL	30.9	359.5
imidacloprid	Marathon II	neonicotinoid	1.7 fl oz	0.06 mL	21.4	31.8
novaluron	Pedestal	benzoyl urea	8.0 fl oz	0.30 mL	10.0	62.2
pymetrozine	Endeavor	pyridine azomethine	5.0 oz	0.78 g	50.0	187.2
spinosad	Conserve	spinosyn	6.0 fl oz	0.22 mL	11.6	56.2
tolfenpyrad	Hachi-Hachi	carboxamide	22.0 fl oz	0.81 mL	15.0	257.4

[z]Company information: Avid® (Syngenta Crop Protection Inc.; Greensboro, NC); Azatin® (OHP Inc.; Mainland, PA); Conserve® (Dow AgroSciences, LLC; Indianapolis, IN); Endeavor™ (Syngenta Crop Protection, Inc.; Greensboro, NC); Floramite® (OHP Inc.; Mainland, PA); Hachi-Hachi® (SePro Corp.; Carmel, IN); Marathon®II (OHP Inc.; Mainland, PA); Menace® (Nufarm; Burr Ridge, IL); Ornazin® (SePro Corp.; Carmel, IN); Orthene® (Valent U.S.A Corporation; Walnut Creek, CA); Pedestal™ (OHP Inc.; Mainland, PA); and Tame® (Valent U.S.A Corporation; Walnut Creek, CA).

[y]Rate used in pesticide incompatibility, phytotoxicity, and greenhouse experiments.

Table 3.4. LC_{50} values, slope (± SEM), and *P* values for nine technical grade pesticides used in laboratory glass residual bioassays on western flower thrips (WFT), *Frankliniella occidentalis*; n = total number of WFT evaluated per treatment. There were four replications per treatment.

Pesticide (common name)	n	Slope (± SEM)	$P > \chi^2$	LC_{50} (95% CI) µg/mL
abamectin	294	0.13 (± 0.08)	0.49[z]	>40000.00
acephate	294	2.11 (± 0.30)	0.06	282.11 (207.91, 362.20)
azadirachtin	293	-7.35 (± 25229.56)	0.43	>4000.00
bifenazate	295	0.05 (± 13245.76)	0.64	>4000.00
bifenthrin	293	1.61 (± 0.22)	0.09	8.56 (5.39, 12.93)
fenpropathrin	293	0.69 (± 0.25)	0.29	>8000.00
novaluron	295	0.05 (± 0.18)	0.33	>4000.00
pymetrozine[y]	-	-	-	-
spinosad	295	3.53 (± 0.48)	0.10	1.57 (1.29, 1.87)

[z] $P > \chi^2 \geq 0.05$ indicates no significant difference between the observed regression line and the expected model.

[y]Technical grade pymetrozine did not dissolve in acetone, ethyl alcohol, and/or deionized water and was thus excluded.

Table 3.5. LC_{50} values, slope (± SEM), and *P* values for ten formulated pesticides on western flower thrips (WFT), *Frankliniella occidentalis*, in bean-dip laboratory bioassays; n = total number of WFT evaluated per treatment. There were four replications per treatment.

Common name	n	Slope (± SEM)	$P > \chi^2$	LC_{50} (95% CI) µg/mL
abamectin	300	0.72 (± 0.19)	0.32[z]	148.80 (83.79)
acephate	299	4.02 (± 0.54)	0.07	720.86 (603.80, 852.59)
azadirachtin (Azatin)	303	0.13 (± 0.42)	0.65	> 634.01
azadirachtin (Ornazin)	300	0.34 (± 1.01)	0.83	> 319.65
bifenazate	303	0.08 (± 0.44)	0.27	> 2396.52
bifenthrin	312	2.14 (± 0.26)	0.62	1331.00 (1100.00, 1639.00)
fenpropathrin	296	0.74 (± 0.41)	0.80	> 5751.66
novaluron	296	0.27 (± 0.32)	0.84	> 994.56
pymetrozine	304	0.11 (± 0.29)	0.36	> 3000.00
spinosad	294	3.96 (± 0.80)	0.06	0.44 (0.35, 0.54)

[z] $P > \chi^2 \geq 0.05$ indicates no significant difference between the observed regression line and the expected model.

Table 3.6. LC_{50} values, slope (± SEM), and *P* values, for nine binary pesticide mixtures on the western flower thrips (WFT), *Frankliniella occidentalis*, in bean-dip bioassays under laboratory conditions; n = total number of WFT evaluated per treatment. There were four replications per treatment.

Common name	n	Slope ± (SEM)	$P > \chi^2$	LC_{50} (95% CI) µg/mL
abamectin + azadirachtin (Azatin)	297	0.57 (± 0.18)	0.19[z]	127.04 (69.16, 649.45)
abamectin + azadirachtin (Ornazin)	305	0.83 (± 0.18)	0.39	27.18 (12.71, 41.71)
abamectin + bifenthrin	304	0.78 (± 0.22)	0.09	157.57 (61.64, 282.35)
abamectin + pymetrozine	298	0.28 (± 0.19)	0.89	68.90 (53.43, 92.85)
spinosad + abamectin	295	2.88 (± 0.38)	0.07	0.37 (0.30, 0.46)
spinosad + bifenazate	292	2.73 (± 0.36)	0.20	1.79 (1.41, 2.14)
spinosad + novaluron	301	3.24 (± 0.39)	0.86	0.34 (0.30, 0.40)
spinosad + pymetrozine	294	1.81 (± 0.31)	0.35	0.38 (0.21, 0.53)
acephate + fenpropathrin	295	2.71 (± 0.42)	0.06	382.40 (301.34, 485.68)

[z] $P > \chi^2 \geq 0.05$ indicates no significant difference between the observed regression line and the expected model.

Table 3.7. Synergism and antagonism calculations for nine binary pesticide mixtures against laboratory-reared colonies of the western flower thrips, *Frankliniella occidentalis* based on a combination index (CI).

Mixture (common names)	$LC_{50}1$ (µg/mL)[z]	$LC_{50}2$ (µg/mL)[y]	Ratio[x]	CI[w]
abamectin + azadirachtin (Azatin)	148.80	634.01	1 : 0.23	0.75
abamectin + azadirachtin (Ornazin)	148.80	319.65	1 : 0.47	0.34
abamectin + bifenthrin	148.80	1331.00	1 : 0.11	0.97
abamectin + pymetrozine	148.80	3000.00	1 : 0.05	0.44
spinosad + abamectin	0.44	148.80	1 : 0.003	0.84
spinosad + bifenazate	0.44	2396.52	1 : 0.0002	4.07
spinosad + novaluron	0.44	994.56	1 : 0.0004	0.77
spinosad + pymetrozine	0.44	3000.00	1 : 0.0001	0.86
acephate + fenpropathrin	720.86	5751.66	1 : 0.13	0.48

[z] $LC_{50}1$ = lethal concentration that kills 50% of WFT population using the first pesticide alone.

[y] $LC_{50}2$ = lethal concentration that kills 50% of WFT population using the second pesticide alone.

[x] Ratio: $LC_{50}1/LC_{50}2$

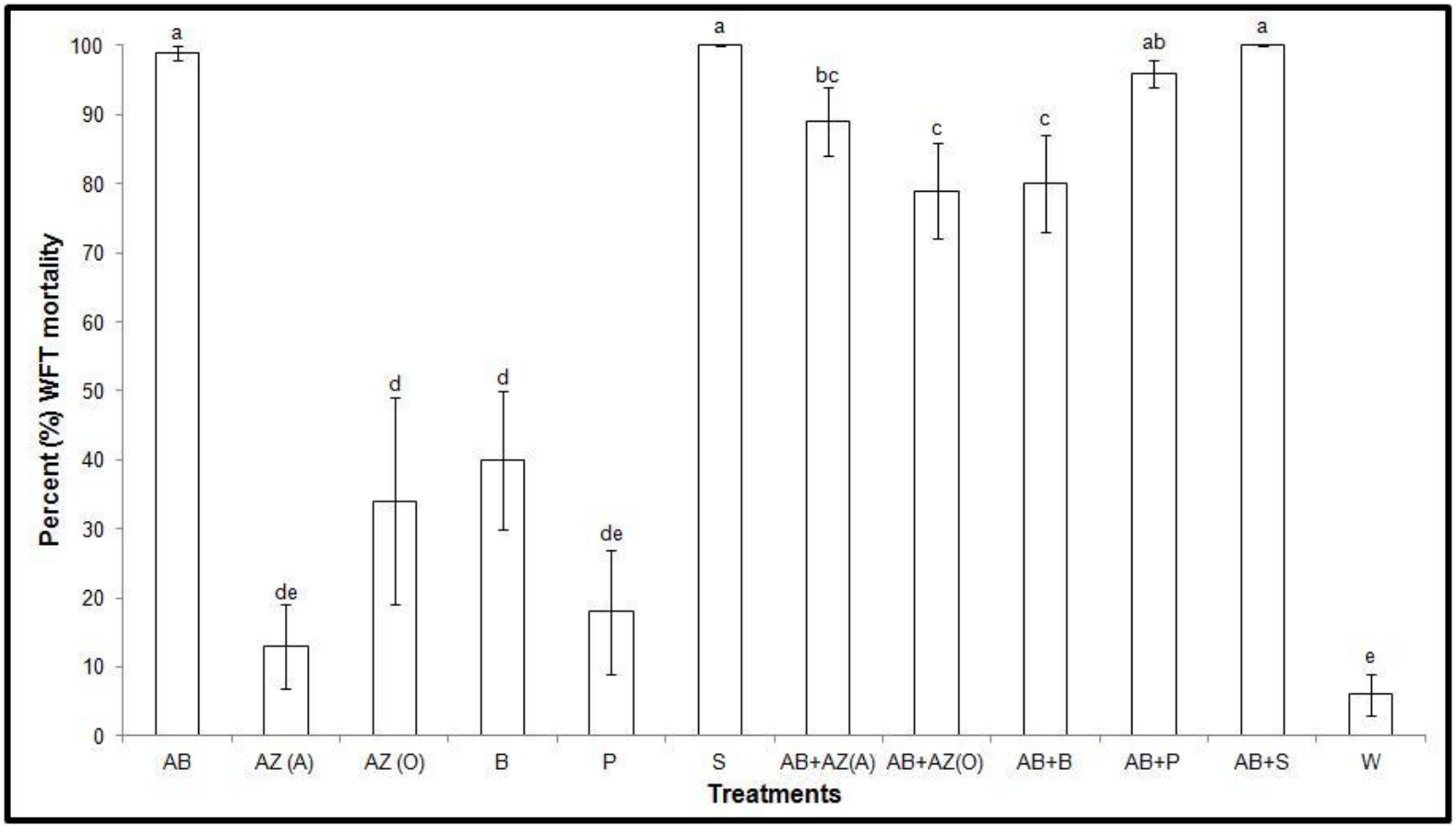
[w] CI = combination index at LC_{50}.

Table 3.8. Classification of combination index (CI) values based on Chou (2006) calculations for nine binary pesticide mixtures on laboratory-reared colonies of western flower thrips, *Frankliniella occidentalis.*

Mixture (common names)	CI[z]	Classification
abamectin + azadirachtin (Azatin)	0.75	moderate synergism
abamectin + azadirachtin (Ornazin)	0.34	synergism
abamectin + bifenthrin	0.97	nearly additive
abamectin + pymetrozine	0.44	synergism
spinosad + abamectin	0.84	moderate synergism
spinosad + bifenazate	4.07	strong antagonism
spinosad + novaluron	0.77	moderate synergism
spinosad + pymetrozine	0.86	slight synergism
acephate + fenpropathrin	0.48	synergism

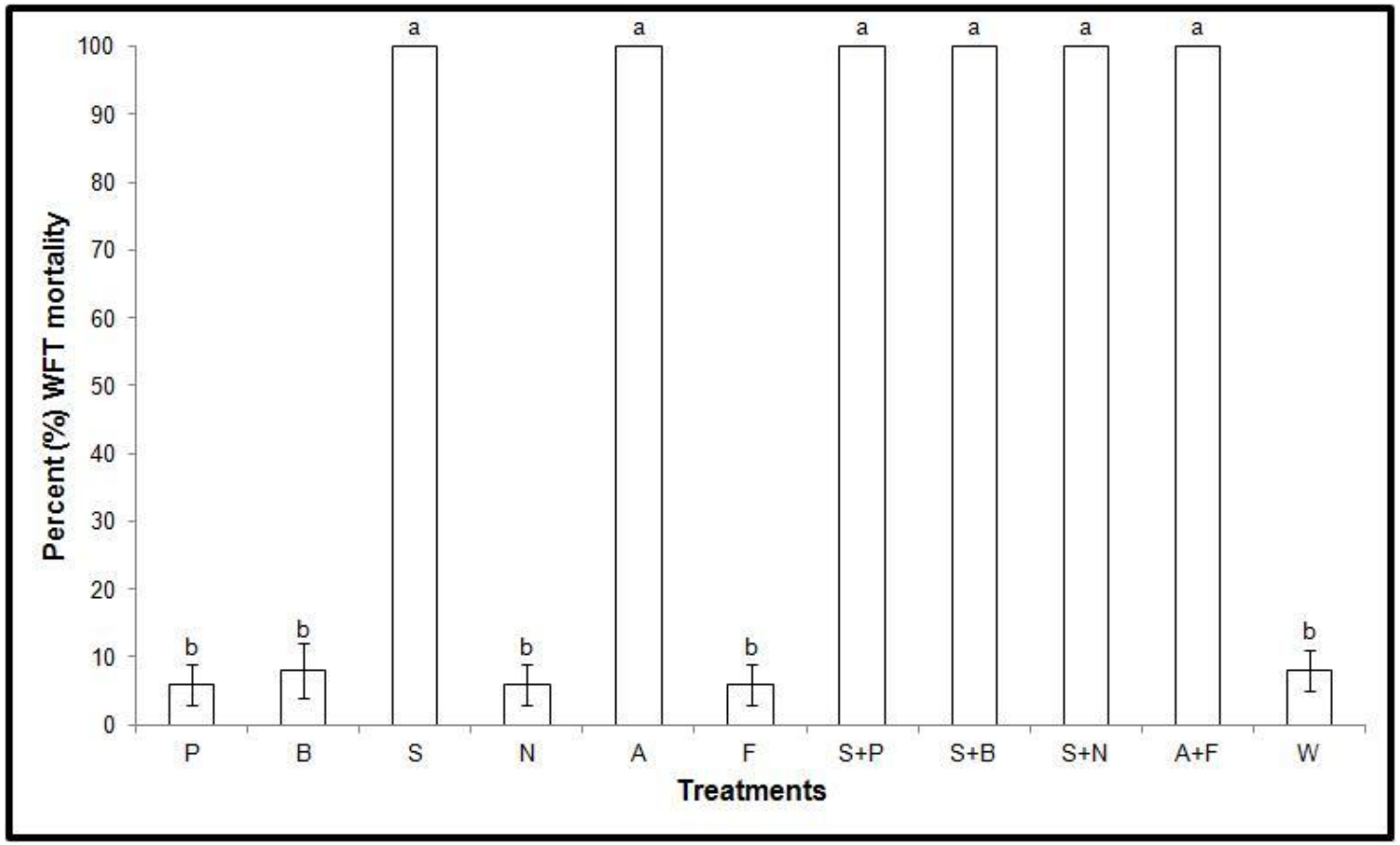
[z]CI = combination index at LC_{50}.

Figure 3.1. Percent western flower thrips (WFT), *Frankliniella occidentalis* mortality associated with six formulated pesticides and four pesticide mixtures applied as foliar sprays under greenhouse conditions. Treatments included abamectin (AB), azadirachtin (Azatin) [AZ (A)], azadirachtin (Ornazin) [AZ (O)], bifenthrin (B), pymetrozine (P), spinosad (s), and water (W). Assessments were made five days after application with five replications per treatment.



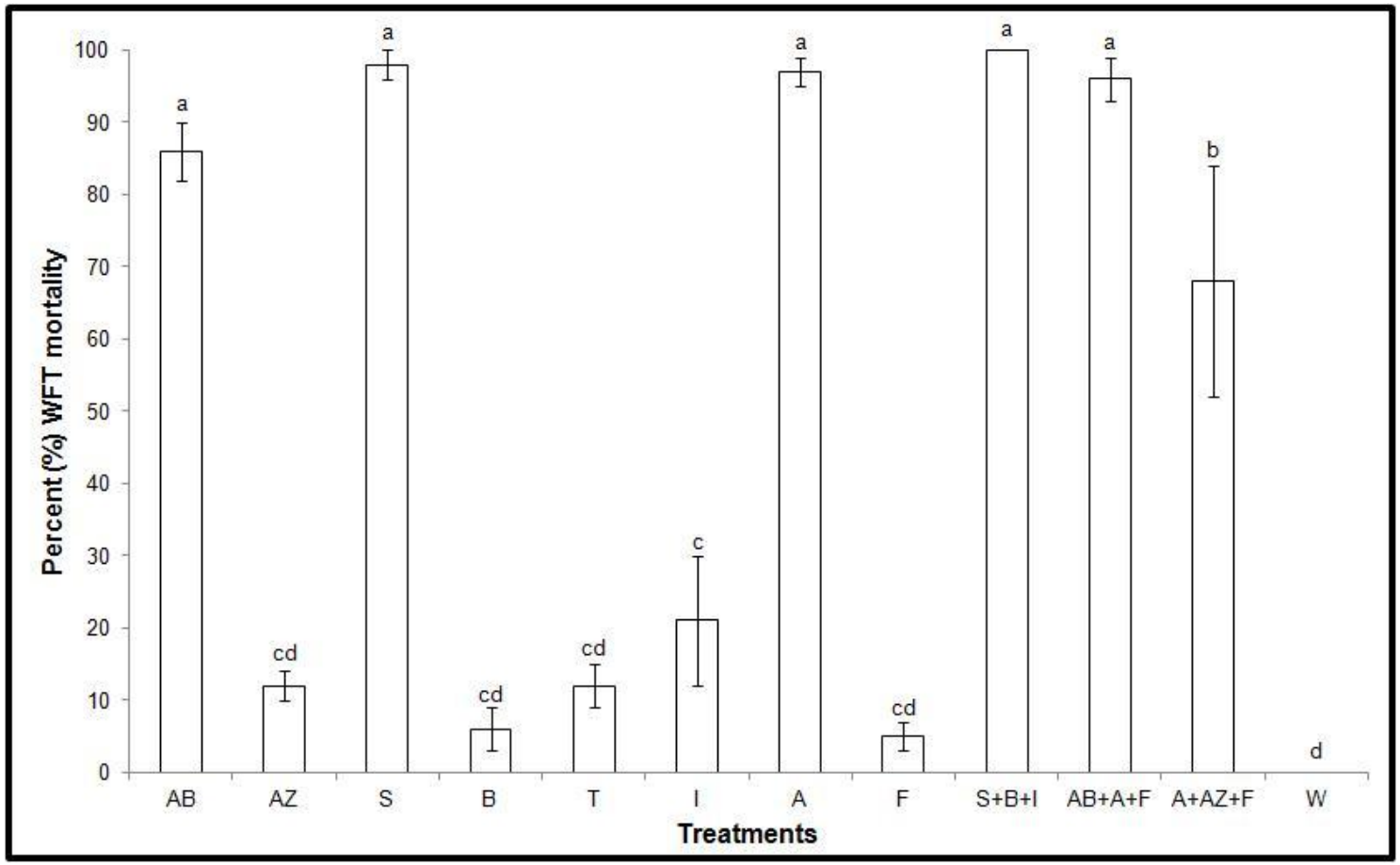
Bars with the same letter are not significantly different from each other ($P \leq 0.05$) based on Fisher's protected least significant difference (LSD) mean separation test. Vertical lines indicate standard error of the mean (SEM).

Figure 3.2. Percent western flower thrips (WFT), *Frankliniella occidentalis* mortality associated with six formulated pesticides and five pesticide mixtures applied as foliar sprays under greenhouse conditions. Treatments included pymetrozine (P), bifenazate (B), spinosad (S), novaluron (N), acephate (A), fenpropathrin (F), and water (W). Assessments were made five days after application with five replications per treatment.



Bars with the same letter are not significantly different from each other ($P \leq 0.05$) based on Fisher's protected least significant difference (LSD) mean separation test. Vertical lines indicate standard error of the mean (SEM).

Figure 3.3. Percent western flower thrips (WFT), *Frankliniella occidentalis* mortality associated with eight formulated pesticides and three tertiary pesticide mixtures applied as foliar sprays under greenhouse conditions. Treatments included abamectin (AB), azadirachtin [Azatin (AZ)], spinosad (S), bifenazate (B), tolfenpyrad (T), imidacloprid (I), acephate (A), fenpropathrin (F), and water (W). Assessments were made five days after application with five replications per treatment.



Bars with the same letter are not significantly different from each other ($P \leq 0.05$) based on Fisher's protected least significant difference (LSD) mean separation test. Vertical lines indicate standard error of the mean (SEM).

Chapter 4 - Summary and Conclusions

This research involved two distinctly different studies designed to address insect pest management against two commonly encountered insect pests in protected environments. The first study consisted of four experiments designed to evaluate the efficacy and residual activity of systemic insecticides registered for use against the citrus mealybug (CMB), *Planococcus citri*. Systemic insecticides are used against a wide variety of insect pests; however, minimal information exists on the efficacy of currently available pesticides against CMB in protected environments. This study clearly demonstrated that systemic insecticides including azadirachtin, spirotetramat, thiamethoxam, dinotefuran, and imidacloprid applied as drenches to the growing medium provided <80% mortality against CMB feeding on green and red coleus (*Solenstemon scutellarioides*) plants. Both azadirachtin and spirotetramat, only provided <20% CMB mortality when applied preventatively. Slightly higher mortality was observed when applied curatively treatments; however, this was still <30%. For the neonicotinoid insecticides, the highest percent CMB mortality was observed 21 to 28 days after treatment (DAT) for thiamethoxam; however this was <80%. Also, CMB tended to feed more on the plant stems compared to the leaf top on green coleus, and the plant stem and leaf bottom of red coleus compared to the leaf top.

This study provides valuable information on systemic insecticides that will allow greenhouse producers and interior plantscape curators to more efficiently deal with CMB. This research differs from previous studies performed outdoors that demonstrated the efficacy of systemic insecticides against arthropod pests with piercing-sucking mouthparts (Thoeming et al., 2003; Pavela et al., 2004), in that minimal mortality was observed in the current study. However, Calatayud et al., (1994) demonstrated that although mealybugs have piercing-sucking mouthparts, their feeding behavior differs from other insects with piercing-sucking mouthparts

such as aphids and whiteflies, which may influence CMB susceptibility to systemic insecticides. Furthermore, this study is the first to quantify CMB feeding location. It was found that most CMB feed on plant stems of green coleus. Therefore, feeding behavior, in addition to feeding location, may affect the efficacy of systemic insecticides against CMB. Future research should assess if CMB feeding behavior influences the ingestion of active ingredient of pesticides in different plant parts or tissues. To determine this, enzyme-linked immunoabsorbant (ELISA) tests can be used to determine the concentration of active ingredient in plant stems and leaves. Because most CMB fed on the plant stem, determining the concentration of active ingredient translocated to the plant stem and leaves will reveal if CMB are ingesting lethal concentrations of systemic insecticides. At this point, the reason why most CMB were located on plant stems is unknown; however, it may be related to stem nutrient content, or there is less light emitted on the plant stem compared to the leaves indicating that CMB may be avoiding direct light exposure.

Future research should also concentrate on how growing medium type influences the efficacy of systemic insecticides. Previous research has suggested that growing medium type may interfere with the absorption of insecticides (Abdellatif et al., 1967; Gill and Lewis, 1971; Thoeming et al., 2003). The current study evaluated systemic insecticides applied to a growing medium containing Canadian sphagnum peat moss (65%); consequently, this may reduce the efficacy of systemic insecticides. In conclusion, greenhouse producers and interior plantscape curators should use supplementary insect pest management strategies to mitigate CMB populations in protected environments in addition to drench applications of systemic insecticides.

The second study was designed to evaluate binary pesticide mixtures currently used in greenhouse environments against western flower thrips (WFT), *Frankliniella occidentalis*. A series of laboratory and greenhouse experiments were conducted to evaluate pesticide mixtures.

Jar tests indicated all of the mixtures were visibly compatible and not phytotoxic to nine different horticultural plant species including *Tanacetum* spp., *Begonia* spp., *Petunia* spp., *Salvia* spp., *Tagetes* spp., *Impatiens* spp., *Vinca* spp., *Pansy* spp., and *Coleus* spp. In addition, three out of the nine technical grade active ingredients and four out of nine formulated pesticides, produced definitive LC_{50} values. Each of the pesticide mixtures had a definitive LC_{50} value. For both the technical grade bioassays and the formulated bioassays spinosad was the most toxic to WFT. Furthermore, most of the pesticide mixtures were synergistic; however, one mixture (spinosad + bifenazate) was antagonistic against WFT. All pesticide mixture greenhouse experiments resulted in >70% WFT mortality.

Pesticide mixtures have been used against WFT without a good understanding if they are synergistic or antagonistic. This study provides important information to greenhouse producers because it is the first to assess the commonly used pesticide mixtures against WFT. Similar to Warnock and Cloyd (2005), none of the mixtures were antagonistic, based on percent WFT mortality, in the greenhouse experiments. However, greenhouse producers should avoid using mixtures of spinosad + bifenazate as this mixture was antagonistic under laboratory conditions. In addition, the efficacy of pesticide mixtures may vary when applied to field populations of WFT as observed by Bielza et al., 2008. Therefore, future research should involve using field resistant populations of WFT because WFT have developed resistance to a number of insecticides registered for use in greenhouses including abamectin, acephate, bifenthrin, and spinosad (Immaraju et al., 1992; Brødsgaard, 1994; Bielza et al., 2009). This information can be used to assist greenhouse producers on what pesticide mixtures are appropriate and which ones should be avoided.

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Appendix A - Interior plantscape arthropod pest survey

Materials and Methods

Interior plantscape survey

A survey was posted online on SurveyMonkey.com by the National Foliage Foundation (NFF), Orlando, FL. The NFF composed a list of interior plantscape companies within the United States and Canada and distributed the survey via email. The survey was available online to interior plantscape curators from October 8, 2009 through January 4, 2010. The survey was used to obtain information from interior plantscape companies regarding arthropod (insect and/or mite) pests found on plants and the pesticides (insecticides and/or miticides) currently used to regulate them. Based on the responses, we determined which interior plantscape arthropod pests are the most problematic and difficult to regulate with pesticides, along with which products are currently used to deal with the commonly encountered arthropod pests. The survey also requested information on how the insecticides are applied (foliar spray application and/or drench to the growing medium), if the spray solution pH was monitored, and if interior plantscape curators thought pesticides are less effective against arthropod pests than they were 5 years ago.

Results and Discussion

The survey was posted online for approximately 3 months from October 8, 2009 through January 4, 2010, and in that period 64 interior plantscape curators responded (we do not know the number that actually received the survey). Based on the 64 responses obtained, the three most common interior plantscape insect pests encountered were the longtailed mealybug (LTMB), *Pseudococcus longispinus* (Targioni-Tozzetti) (71.9%), citrus mealybug (CMB), *Planococcus citri* (Risso) (29.7%), and brown soft scale (BSS), *Coccus hesperidium* L. (28.1%) (Table A.1).

In addition, many of the interior plantscape companies that responded to the survey indicated using pesticides to mitigate these insect pests. The most widely-cited chemical class used by the respondents was the neonicotinoid insecticides (Table A.2). The neonicotinoid insecticide most commonly used for control of any of the reported insect pests was imidacloprid (Marathon®II; OHP Inc.; Mainland, PA; and Merit®; Bayer Environmental Science; Research Triangle, NC), which was reported 33 times. The second most commonly used insecticide was dinotefuran (Safari®, Valent Professional Products U.S.A.; Walnut Creek, CA) reported 14 times. There were 40 different materials reported from the 64 respondents (Table A.2). Additionally, imidacloprid was the most typically used neonicotinoid insecticide for control of LTMB, CMB, and BSS compared to dinotefuran and acetamiprid (Table A.3). Imidacloprid is widely used because it was the first active ingredient available and may be more familiar to interior plantscape curators.

In addition to information regarding arthropod pests and pesticide usage, information was obtained regarding the most common host plants for LTMB, CMB, and BSS. Results indicated that the five most cited host plants were *Epipremnum* spp., *Aglaonema* spp., *Dracaena* spp., Palms, and *Ficus* spp. (Table A.4). Also, when asked how pesticides were applied to plants, most of the respondents indicated applying pesticides as both sprays to the plant foliage and drenches to the growing medium (35.9%). Furthermore, approximately 9.4% of the respondents only applied pesticides as drenches to the growing medium (Table A.5), which may be associated with certain benefits including human safety; however, there is minimal quantitative information regarding the efficacy of systemic insecticides when applied as drenches. Another item the survey requested feedback on was monitoring the spray solution pH. Monitoring pH is important because alkaline hydrolysis can occur if the pH is above 7.0 which leads to degradation of the

pesticide and a reduction in efficacy. Very low percentages (6.3%) of respondents actually check the pH of the spray solution before applying pesticides (Table A.6).

In addition to inquiring about arthropod pests and application techniques, the respondents were asked if they thought that insecticides/miticides currently available are less effective against arthropod pests than they were five years ago; 31.3% of respondents indicated “yes” and 34.4% indicated “no”. The remaining respondents denoted that they do not use pesticides or did not respond to the survey question (Table A.7). The reason that pesticides may be less effective against arthropod pests compared to 5 years ago is unknown, although, it may be due to circumstantial factors that affect the pesticide efficacy, which include application method, timing of application, coverage of plant parts, and monitoring spray solution pH.

In conclusion, little information was previously known regarding the current arthropod pest complex, products most commonly used to mitigate arthropod pests, and application techniques used in interior plantscapes. The information obtained from this survey will benefit interior plantscape curators by increasing their knowledge of the current arthropod pests most commonly encountered and the pesticides used to mitigate these pest populations. This survey identified that both LTMB and CMB are widely encountered insect pests, and that neonicotinoid insecticides are primarily used against them.

Table A.1. Survey responses from 64 interior plantscape companies (=respondents) associated with what are considered to be the major arthropod pests commonly encountered in interior plantscapes; n = number of responses.

Question	Arthropod pests	n	Percent (%)
What do you consider the major arthropod pest(s) in your interior plantscape?	Longtailed mealybug, *Pseudococcus longispinus*	46	71.9
	Citrus mealybug, *Planococcus citri*	19	29.7
	Brown soft scale, *Coccus hesperidium*	18	28.1
	Fungus gnats	9	14.1
	Mites	7	10.9
	Thrips	3	9.4
	Aphids	2	3.1

Table A.2. Survey results from 64 interior plantscape companies (=respondents) within the USA and Canada associated with the materials used to control arthropod (insect and/or mite) pests in interior plantscapes; n = number of responses.

Active ingredient	Trade name	n	Percent (%)
imidacloprid	Marathon/Merit	33	51.6
dinotefuran	Safari	14	21.9
potassium salts of fatty acids	Insecticidal soap	11	17.2
bifenthrin	Talstar	7	10.9
isopropyl alcohol	Rubbing alcohol	7	10.9
acetamiprid	TriStar	5	7.8
clarified hydrophobic extract of neem oil	Neem Oil	5	7.8
cyfluthrin	Decathlon	4	6.3
capsaicin	Hot Pepper Wax	4	6.3
paraffinic oil	Ultra Fine Spray Oil	3	4.7
kinoprene	Enstar II	3	4.7
pyrethrin	1600 X-clude	3	4.7
paraffinic oil	Horticultural Oil	3	4.7
limonene	Citrus Cleaner	2	3.1
olive oil	Castile Soap	2	3.1
potassium salts of fatty acids	End-All II	2	3.1
potassium salts of fatty acids	Trounce	2	3.1
canola oil	Pyola	1	1.6
pine oil	PineSol	1	1.6
sodium tallowate	Ivory Dish Detergent	1	1.6
chlorpyrifos	DuraGuard	1	1.6
peppermint oil	Peppermint Oil Soap	1	1.6
diflubenzuron	Adept	1	1.6
bifenthrin	Cross Check	1	1.6
buprofezin	Talus	1	1.6
spinosad	Conserve	1	1.6
azadirachtin	Azatin	1	1.6
bifenthrin	Bifen I/T	1	1.6
refined petroleum oil	Sunspray	1	1.6
mineral oil	Exoexempt	1	1.6
pyrethrin	Pyrenone	1	1.6
bendiocarb	Closure	1	1.6
mineral oil	Mineral Oil	1	1.6
sodium dodecylbenzene sulphonate	Sunlight Soap	1	1.6
sodium hydroxide	Murphy Oil Soap	1	1.6
ethyl alcohol	Sparkle	1	1.6

Miscellaneous products included oils (n=4); Brand X (n=3); and soaps (n=1).

Table A.3. Survey responses from 64 interior plantscape companies (=respondents) within the USA and Canada that reported encountering brown soft scale (*Coccus hesperidium*), citrus mealybug (*Planococcus citri*), and longtailed mealybug (*Pseudococcus longispinus*) and using neonicotinoid insecticides for control.

Insect pest	Neonicotinoid insecticide active ingredient		
	imidacloprid	dinotefuran	acetamiprid
Brown soft scale	7	2	2
Citrus mealybug	7	4	1
Longtailed mealybug	26	12	3

Table A.4. Survey responses from 64 interior plantscape companies (=respondents) regarding preferred host plants of brown soft scale (*Coccus hesperidium*), citrus mealybug (*Planococcus citri*), and longtailed mealybug (*Pseudococcus longispinus*).

	Host plant				
Insect pest	***Aglaonema* spp.**	***Epipremnum* spp.**	***Dracaena* spp.**	***Ficus* spp.**	**Palms**
Brown soft scale	7	5	6	7	6
Citrus mealybug	6	5	4	5	2
Longtailed mealybug	22	19	14	9	9

Table A.5. Summary of survey responses from 64 interior plantscape companies (=respondents) within the USA and Canada regarding the method of pesticide application (foliar spray, drench to the growing medium, or both); n = number of responses.

Question	Response	n	Percent (%)
Does your company apply insecticides as sprays to the foliage, drenches to the growing medium, or both?	spray and drench	23	35.9
	spray	17	26.6
	drench	6	9.4
	no response	18	28.1

Table A.6. Summary of survey responses from 64 interior plantscape companies (=respondents) within the USA and Canada associated with monitoring the pH of the spray solution prior to application of an insecticide; n = number of responses.

Question	Response	n	Percent (%)
Does your company monitor the pH of the spray solution?	yes	4	6.3
	no	44	68.8
	no response	16	39.1

Table A.7. Summary of survey responses from 64 interior plantscape companies (=respondents) within the USA and Canada regarding the proposed current efficacy of pesticides (insecticides/miticides) used against arthropod (insect and/or mite) pests compared to 5 years ago; n = number of responses.

Question	Response	n	Percent (%)
Are the pesticides currently being used less effective against arthropod pests than they were 5 years ago?	yes	20	31.3
	no	22	34.4
	no response	22	34.4

Appendix B - Life cycle of the longtailed mealybug (*Pseudococcus longispinus*) on four host plants

Materials and Methods

Host plant evaluations for longtailed mealybug

Preliminary data from the online survey was used to determine the most commonly encountered arthropod pests of interior plantscapes and susceptible host plants. The survey was posted online from October 8, 2009 through January 4, 2010 by the National Foliage Foundation on SurveyMonkey.com, as described previously (refer to Appendix A). Data from the 64 respondents indicated that longtailed mealybug (LTMB), *Pseudococcus longispinus*, is the most prevalent insect pest of interior plantscapes. Furthermore, the three most susceptible host plants of LTMB based on the survey results were, *Aglaonema* spp., *Epipremnum* spp., and *Dracaena* spp.

Longtailed mealybug colony

Colonies of LTMB were obtained from Southern Tropic of Plants Ltd. (Winnipeg, Manitoba, Canada) with no previous record of being exposed to pesticides. Colonies were maintained on *Epipremnum* spp. in a laboratory under 24 ± 5°C, 50-60% relative humidity (RH) and 14:10 hour (L:D) photoperiod in the Department of Entomology at Kansas State University (Manhattan, KS).

Experiment one: Four host plants

The three most susceptible host plants indicated in the survey (*Aglaonema* spp., *Epipremnum* spp., and *Dracaena* spp., and a fourth, *Croton spp*., was used based on information from the scientific literature (Cloyd and Lindquist, 2001). Five of each plant species were

purchased from a retail florist (HyVee Floral; Manhattan, KS) for a total of 20 plants. Plants were placed into a laboratory at Kansas State University, Department of Entomology (Manhattan, KS) on May 6, 2011. After thoroughly examining each plant to ensure no mealybugs were present, approximately 2-3 adult female longtailed mealybugs (LTMB), *Pseudococcus longispinus* were placed on to each plant. Plants were randomly arranged on a bench counter top. Mealybug development was monitored weekly. After approximately three months (August 8, 2011), plants were destructively sampled to determine the number and approximate life stages of the LTMB on each plant. Females that died after offspring were evident were removed from the plant. In addition, HOBOware® Pro Data Loggers (Bourne, MA) were used to record temperature, light intensity, and relative humidity.

Experiment two: Longtailed mealybug development on Aglaonema spp. as a host plant

Based on results from experiment one, *Aglaonema* spp. was used to evaluate susceptibility as a host plant (described above) for LTMB. This experiment was conducted using 10 *Aglaonema* spp. plants (5 of cultivar "Gemini" and 5 of "Juliette") in a laboratory at Kansas State University, Department of Entomology (Manhattan, KS). Plants were purchased from a retail florist (HyVee Floral; Manhattan, KS), and had not been sprayed with any pesticides for at least two weeks. Twenty 2nd to 3rd LTMB instars were placed on each plant. Plants were monitored regularly and watered as needed. Temperature, relative humidity and light intensity were recorded using HOBOware® Pro Data Loggers, as in experiment one.

Results and Discussion

Results from experiment one are shown in Figure B.1. Overall, LTMB placed onto *Aglaonema* spp. produced the highest number of females (n=52) during the three month time

period. The total number (males and instars) present on *Aglaonema* spp. plants was 67, which was the highest number compared to the other 3 host plants. The plant with the second highest total number of LTMB was *Dracaena* spp. (n=51); however, 44 were male cocoons. Both *Croton* spp. and *Epipremnum* spp. had very few LTMB (1 and 13, respectively).

Results confirming *Aglaonema* spp. as a susceptible host plant were similar to those reported in the survey. Both *Aglaonema* spp. and *Epipremnum* spp. were indicated to be the most commonly infested host plants by LTMB (n=19). The mean temperature, RH, and light intensity in the laboratory were 24.0°C, 51%, and 7.2 lumens/ft^2 respectively. Temperature fluctuations in the laboratory ranged from 20.8°C to 35.1°C (± 14.3°C).

Along with having the highest number of adult female LTMB after 3 months, it is easier to detect LTMB on *Aglaonema* spp. compared to the other plants evaluated. For instance, the plant has dark-green foliage, creating a contrast that makes it easy to locate all instars of LTMB. In addition, size is simpler to maintain than both *Dracaena* spp. and *Epipremnum* spp. For example, *Dracaena* spp. plants have many nodes and protected places for LTMB to hide, which increases the amount of time required to locate LTMB. In addition, *Epipremnum* spp. has many long runners (vines), which increase the structural complexity making it difficult and time consuming to locate LTMB.

For experiment two, the *Aglaonema* cultivar "Gemini" had 77 and "Juliette" had 83 LTMB (male and instar) after a three month time period (Figure B.2). Both cultivars contained more LTMB instars than males and adult females. There were fewer LTMB in experiment two compared to experiment one, but this may be attributed to using 2^{nd}-3^{rd} instars in the second experiment whereas adults were used in experiment one. It appeared that three months was sufficient time for LTMB to develop; however, it may not have been long enough for a second

generation to develop. In addition, the mean temperature, relative humidity, and light intensity in the laboratory for experiment two were 23.9°C, 33%, and 4.4 lumens/ft^2, respectively. Unlike experiment one, temperature fluctuations were less ranging from 19.7°C to 27.2°C (± 7.5°C).

In conclusion, *Aglaonema* spp. and *Dracaena* spp. are susceptible host plants and conducive for LTMB development. However, due to the excessive number of nodes and difficulty in detecting LTMB on *Dracaena* spp., *Aglaonema* spp. was selected for further evaluations. The *Aglaonema* cultivars "Gemini" and "Juliette" were suitable for LTMB development. Future research should determine other host plants to assess the development of LTMB under different environmental conditions. This information may assist interior plantscapes in more effectively dealing with LTMB by timing pesticide applications accordingly.

Figure B.1. Total number (male and instar) of longtailed mealybug (LTMB), *Pseudococcus longispinus*, on four host plants (*Aglaonema* spp., *Croton* spp., *Dracaena* spp., and *Epipremnum* spp.) maintained indoors (24.0°C, 51% relative humidity, and 7.2 lumens/ft^2) from May 6, 2011 to August 8, 2011. Vertical bars represent the standard error of the mean (SEM).

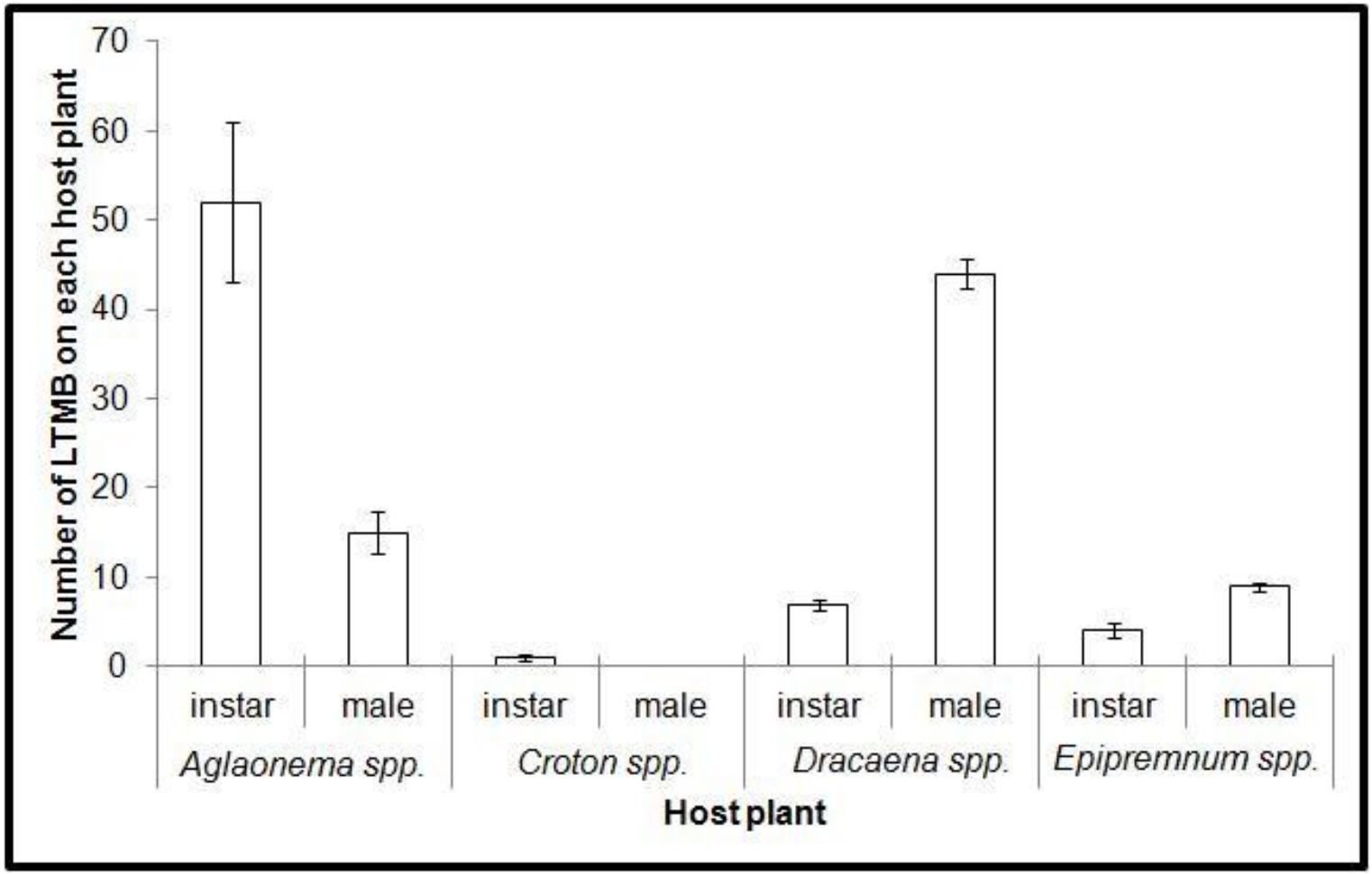

Figure B.2. Total number (instar, female, and male) of longtailed mealybug (LTMB), *Pseudococcus longispinus* on *Aglaonema* spp., cultivars "Gemini" and "Juliette"; maintained indoors (23.9°C, 33% relative humidity, and 4.4 lumens/ft^2) from September 19 to October 19, 2011. Vertical bars represent the standard error of the mean (SEM)