Effect of spray volume and application frequency on insecticide efficacy against western flower thrips (*Frankliniella occidentalis*) and citrus mealybug (*Planococcus citri*) under greenhouse conditions

by

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Abstract

Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), and citrus mealybug, *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae), are major insect pests of greenhouse-grown horticultural crops. Both insect pests cause direct and indirect damage by feeding on plant leaves, stems, flowers, and fruits, which can lead to greenhouse producers experiencing significant economic losses. Insecticides are the primary management strategy used against these insect pests. Experiments were conducted under greenhouse conditions to determine the effect of spray volume and application frequency on insecticide efficacy against citrus mealybug on coleus (*Solenostemon scutellarioides*) and transvaal daisy (*Gerbera jamesonii*) plants and western flower thrips on transvaal daisy cut flowers. Three different spray volumes, two application frequencies (one or two applications), and three insecticides, each with a different mode of action, were evaluated for each insect pest.

In the citrus mealybug experiments, the insecticides acetamiprid, flonicamid, and cyfluthrin were used. The 75 mL spray volume consistently resulted in a higher mean citrus mealybug mortality than the 15 or 25 mL spray volumes. In two of the spray volume experiments, each spray volume resulted in mean citrus mealybug mortalities that were significantly different from each other. Mean percent citrus mealybug mortality was significantly higher after two insecticide applications (51.2% in the second experiment and 42.7% in the third experiment) than after one insecticide application (40.3% in the second experiment and 36.1% in the third experiment). Overall, acetamiprid was more effective against citrus mealybugs, based on percent citrus mealybug mortality, than flonicamid or cyfluthrin. Acetamiprid applied at 75 mL resulted in >70% mean citrus mealybug mortality in three of the spray volume experiments.

In contrast, flonicamid and cyfluthrin each resulted in <50% mean citrus mealybug mortality in all experiments.

In the western flower thrips experiments, the insecticides spinosad, chlorfenapyr, and flonicamid were used. In general, mean western flower thrips adult mortality increased as the spray volume of each insecticide increased. Application frequency affected western flower thrips adult mortality with spinosad resulting in 100% mortality and chlorfenapyr resulting in >98% mortality after two applications. Mean western flower thrips adult mortality for spinosad was >95% whereas chlorfenapyr was >67% across all experiments. In general, the mean percent western flower thrips adult mortality for flonicamid ranged between 50% and 80%. Therefore, based on percent mortality, flonicamid was less effective against western flower thrips adults than spinosad or chlorfenapyr.

Results from this study emphasize the importance of operational factors, especially spray volume and application frequency, in effectively managing insect pests. However, the effect of host plant architecture on spray volume and application frequency needs to be investigated. Proper use of insecticides against insect pests can reduce insecticide inputs and costs associated with labor. Citrus mealybugs and western flower thrips can be effectively managed using designated spray volumes and application frequencies, which will increase pest mortality and improve the suppression of pest populations.

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Chapter 1 - Introduction and Literature Review

Introduction

Citrus mealybug, *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae), and western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), are major insect pests of greenhouse-grown horticultural crops (Tommasini and Maini, 1995; Kirk, 2002; Franco et al., 2009; McKenzie, 1967). Both insect pests cause direct and indirect damage by feeding on plant leaves, stems, flowers, and fruits resulting in significant economic losses for greenhouse producers (Childers, 1997; Cloyd, 2009; Charles, 1982; Mani and Shivaraju, 2016; Reitz and Funderburk, 2012). The application of insecticides is the primary management strategy used against these insect pests in greenhouse production systems (Parrella, 1999).

Citrus mealybug feeds on plants in over 70 families (Ahmed and Abd-Rabou, 2010; Franco et al., 2009; McKenzie, 1967) by inserting their stylet-like mouthparts into plant tissue and removing fluids from the phloem, mesophyll, or both (Franco et al., 2009; Laflin and Parrella, 2004; Tanwar et al., 2007). Citrus mealybug feeding can result in direct and indirect damage. Direct damage is associated with removal of plant fluids, injection of toxins into plants during feeding, and secretion of honeydew, which is a clear, sticky liquid that serves as a substrate for black sooty mold (Mani and Shivaraju, 2016). Indirect damage caused by citrus mealybugs is affiliated with the transmission of plant viruses (Meyer et al., 2008; Cabaleiro and Segura, 1997; Tanwar et al., 2007).

Western flower thrips is another important insect pest encountered in greenhouse production systems that feeds on 60 families of horticultural crops (ornamentals and vegetables) worldwide (Kirk, 2002; Reitz, 2009; Robb and Parrella, 1995; Tommasini and Maini, 1995). Western flower thrips cause direct damage by feeding on leaves or flowers with their piercing-sucking mouthparts and by inserting eggs into plant tissues (Childers, 1997; Chisholm and Lewis, 1984; Harrewijn et al., 1996). Indirect plant damage is attributed to western flower thrips vectoring the tospoviruses, *Tomato spotted wilt* virus and *Impatiens necrotic spot* virus (Allen and Broadbent, 1986; Daughtrey et al., 1997; Pappu et al., 2009). Because western flower thrips reduce the aesthetic quality and marketability of greenhouse-grown horticultural crops, tolerance for western flower thrips is extremely low (Parrella and Jones, 1987). Consequently, greenhouse producers routinely apply insecticides to suppress western flower thrips populations (Loughner et al., 2005; Cloyd, 2009; Kontsedalov et al., 1998; Reitz and Funderburk, 2012). Greenhouse producers may rely exclusively on insecticides for the management of western flower thrips populations (Bielza et al., 2007). However, the effectiveness of insecticides against western flower thrips depends on implementing resistance management strategies, such as rotating insecticides with different modes of action (Cloyd, 2016; Bielza, 2008).

Greenhouse produces can implement a multitude of strategies to manage citrus mealybug or western flower thrips populations below damaging levels including: scouting, cultural control and sanitation, physical control, host plant resistance, and biological control (Charles, 1982; Mouden et al., 2017; Parrella and Jones, 1987). However, insecticides are the most common and effective means of managing these insect pests (Franco et al., 2009; Reitz and Funderburk, 2012; Venkatesan et al., 2016). Nonetheless, application methods, such as spray coverage and application frequency, can ensure the effectiveness of insecticides against these insect pests.

Literature Review

Biology of the citrus mealybug

The citrus mealybug, *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae), is a major insect pest of greenhouse-grown horticultural crops including ornamentals and vegetables (Franco et al., 2009; McKenzie, 1967; Laflin and Parrella, 2004). Citrus mealybugs are sexually dimorphic with females having five life stages: egg, three nymphal instars, and adult (Laflin and Parrella, 2004) whereas males have six life stages: egg, two nymphal instars, prepupa and pupa, and a winged adult (Gullan and Kosztarab, 1997; Franco et al., 2009; McKenzie, 1967). The citrus mealybug sex-pheromone [(1R)-(+)-cis-2,2-dimethyl-3-isopropenyl-cyclobutane methanol acetate] is produced by females and is used by winged adult males to locate females on host plants (Passaro and Webster, 2004; Moreno et al., 1984).

Development of the citrus mealybug depends on host plant and temperature. The highest oviposition rate and shortest incubation period for the citrus mealybug occurs at 30°C compared to 18 or 24°C when reared on the same host plant species (Ahmed and Abd-Rabou, 2010). The female citrus mealybug life cycle, from egg to adult, is completed in 21.4 days on citrus, 32.6 days on guava, and 38.8 days on grape at 30°C (Ahmed and Abd-Rabou, 2010). Female citrus mealybugs reared on rose, *Rosa hybrida* L., plants at 26.6°C (day) and 15.5°C (night) have an average development time of 6 days until egg hatch, 17 days until the second instar, 26 days until the third instar, and 32 days until the adult stage (Laflin and Parrella, 2004). Female citrus mealybugs develop faster than males. At temperatures averaging 20.3°C, the mean development time from egg to adult is 39 days for female citrus mealybugs and 45 days for males (Laflin and Parrella, 2004).

Citrus mealybugs are yellow to pink, and the body is covered with white, waxy filaments in the later instars and adult stage. Dermal pores on the citrus mealybug body produce curled filaments of hydrophobic wax that provide protection from predators, prevent water loss, and promote spacing of individuals within a colony (Cox and Pearce, 1983). Females produce an egg mass or ovisac that remains protected under the body cavity (Tanwar et al., 2007). One adult female citrus mealybug can lay over 400 eggs during her lifespan at 18°C (Copeland et al., 1985). Waxy filaments also constitute the ovisac and male cocoon that protect eggs and male pupae (Cox and Pearce, 1983).

Citrus mealybugs feed on plant fluids in the phloem sieve tubes with their piercing-sucking mouthparts (Franco et al., 2009). Large quantities of honeydew are excreted when feeding on host plants (Charles, 1982). First-instar nymphs (crawlers) eclose from eggs, have well-developed antennae and legs, and are more mobile than later instars and adults (Gullan and Kosztarab, 1997). Nymphs tend to establish on host plants close to where eggs have hatched, often promoting aggregate distributions (Gullan and Kosztarab, 1997). In general, citrus mealybugs feed on leaf undersides but will also feed on leaf uppersides and plant stems (Herrick and Cloyd, 2017). In greenhouse production systems, fertilization of host plants can affect citrus mealybug development. For instance, high nitrogen concentrations (200 and 400 ppm) can increase egg load, body size, and shorten development time compared to citrus mealybugs on coleus (*Solenostemon scutellarioides*) plants receiving low nitrogen concentrations (≤100 ppm) (Hogendorp et al., 2006).

Plant damage and management of the citrus mealybug

Citrus mealybugs feed on plants from 70 different families including: citrus (*Citrus* spp.), banana (*Musa acuminata*), custard apple (*Annona reticulata*), mint (*Mentha* spp.), tarragon (*Artemisia dracunculus*), persimmon (*Diospyros* spp.), many subtropical fruit trees, and ornamental plants in interior plantscapes (Franco et al. 2009). Inadvertent movement of plant material infested with nymphs is the primary means of mealybug dispersal in plant production systems (Tanwar et al., 2007). Citrus mealybugs use their piercing-sucking mouthparts to remove plant fluids from the phloem, mesophyll, or both (Franco et al. 2009). Extensive infestations can cause wilting, leaf chlorosis, leaf drop, premature fruit drop, stunted growth, and even death of host plants (Ahmed and Abd-Rabou, 2010; Mani and Shivaraju, 2016). During feeding, citrus mealybugs excrete large quantities of honeydew, a clear, sticky liquid that serves as a substrate for black sooty mold, which can inhibit photosynthesis and reduce overall aesthetic quality (Charles, 1982). Ants are attracted to the honeydew and will protect citrus mealybugs from natural enemies, such as the mealybug destroyer (*Cryptolaemus montrouzieri*), thus reducing the effectiveness of biological control programs (Copeland et al., 1985). Citrus mealybugs also cause indirect damage by transmitting plant viruses, such as *Banana streak OL (badna)* virus (BSOLV) (Meyer et al., 2008) and grapevine leafroll associated virus 3 (GLRaV-3) (Cabaleiro and Segura, 1997).

Insecticides are primarily used to manage citrus mealybug populations (Franco et al., 2009). However, granule or drench applications of systemic insecticides to the growing medium are not effective against citrus mealybugs, even at 8X the label rate (Herrick et al., 2019). Herrick et al. (2019) suggested that citrus mealybugs do not ingest lethal concentrations of the active ingredient because of their feeding behavior, resulting in inadequate suppression of citrus mealybug populations with systemic insecticides. Therefore, greenhouse producers must rely on applications of contact insecticides to manage citrus mealybug populations. However, contact insecticides have limited effectiveness against citrus mealybugs because of the hydrophobic waxy covering associated with later instars and adults, cryptic feeding behavior that results in a tendency to inhabit protected or hidden areas on plants, clumped spatial distribution among host plants, and difficulty in obtaining thorough coverage of all aboveground plant parts including leaves and branches (Copeland et al., 1985; Charles, 1982; Franco et al., 2009; Herrick and Cloyd, 2017; Venkatesan et al., 2016). Insecticides are most effective against early instar nymphs since they lack the hydrophobic waxy covering associated with adults and later instars (Ahmed and Abd-Rabou, 2010; Charles, 1982; Venkatesan et al., 2016). Mealybug eggs are difficult to kill with contact insecticides due to the protective, waxy ovisac (Venkatesan et al., 2016). Therefore, several insecticide applications may be needed to kill nymphs emerging from ovisacs. Insecticides are most effective against mealybugs that inhabit unconcealed locations on the host plant, which increases exposure to spray applications, and when most mealybugs are early-instar nymphs (Charles, 1982; Franco et al., 2009). Mealybugs can develop resistance to insecticides, especially if the same mode of action is used successively (Venkatesan et al., 2016). Consequently, greenhouse producers must detect infestations early, before outbreaks occur, and avoid spraying insecticides until a mealybug presence has been confirmed through visual plant inspections (Tanwar et al., 2007).

Biology of the western flower thrips

Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), have a life cycle consisting of an egg, two larval instars (1st and 2nd), two pupal instars (prepupae and pupae), and an adult (Cloyd, 2009; Tommasini and Maini, 1995; Moritz, 1997). Sex-determination in western flower thrips is controlled by a haplo-diploid breeding system in which haploid males develop from unfertilized eggs and fertilized eggs develop into diploid females (Moritz, 1997). Adults and larvae are approximately 2.0 mm in length and possess piercing-sucking mouthparts (Jensen, 2000; Moritz, 1997). In addition, western flower thrips tend to aggregate within flowers or terminal buds, which can negatively influence the effectiveness of scouting and insecticide applications (Cloyd, 2009; Reitz, 2009; Robb and Parrella, 1995).

Due to a high reproductive rate and short life cycle, overlapping generations can occur in a western flower thrips population during a single crop production cycle (Mouden et al., 2017; Reitz, 2009). The female western flower thrips adult lifespan is approximately 39 days under laboratory conditions (Li, 2019), and a single female can lay between 150 and 300 eggs throughout her lifespan (Trichilo and Leigh, 1988). Females insert eggs into plant tissues using a saw-like ovipositor (Childers, 1997). First instar larvae emerge from eggs 3 to 4 days after oviposition. The first-instar stage lasts 2 days, the second-instar stage lasts 4 days, and the pupal stages (prepupae and pupae) last 4 to 6 days before adult emergence (Kontsedalov et al., 1998; Gaum et al., 1994). Late second instar larvae reduce feeding and seek out a suitable location to pupate (Gaum et al., 1994) by dropping from plants and entering the growing medium or soil (Holmes et al., 2012). Only the adults and larvae feed on leaves, flowers, and fruits of host plants (Harrewijn et al., 1996; Loughner et al., 2005). Eggs inside plant tissues and pupae in the growing medium or soil are less susceptible to insecticide applications (Seaton et al., 1997; Cloyd, 2009; Robb and Parrella, 1995; Tommasini and Maini, 1995). Therefore, western flower thrips adults and larvae are more exposed to insecticides and natural enemies than the egg or pupal stages (Kontsedalov et al., 1998; Reitz and Funderburk, 2012).

Development time of western flower thrips, from egg to adult, takes 2 to 3 weeks at 20 to 25°C (Tommasini and Maini, 1995). When reared on green beans (*Phaseolus vulgaris*), all life stages of the western flower thrips develop faster as temperature increases, with the average time from egg hatch to adulthood consisting of 22.5 days at 15°C, 12.6 days at 20°C, and 8.4 days at 30°C (Lublinkhof and Foster, 1977). Gaum et al. (1994) reported higher population growth and net reproductive rates, shorter generation time, increased oviposition, and higher fecundity at 30°C on cucumber (*Cucumis sativus*). In addition to temperature, development time is affected by host plants, which may be associated with nutritional quality (Zhang et al., 2007). Western flower thrips populations had higher growth and development rates on impatiens (*Impatiens wallerana*) as foliar tissue phosphorus concentrations increased, indicating that nutrient content affects western flower thrips development and reproduction (Chen et al., 2004). Zhang et al. (2007) reported that at 27 ± 1°C and a 16:8 (L:D) hour photoperiod, developmental time from egg to adult was 9.2 days on cucumber leaves, 10.1 days on cabbage (*Brassica oleracea*) leaves, 10.4 days on kidney bean (*P. vulgaris*) leaves, 12.1 days on capsicum (*Capsicum annuum*) leaves, and 12.9 days on tomato (*Lycopersicon esculentum*) leaves.

Plant damage caused by western flower thrips

Western flower thrips feed on over 250 plant species within 60 different plant families (Tommasini and Maini, 1995). Adults and larvae use their piercing-sucking mouthparts to ingest fluids from the mesophyll and epidermal cells of plant tissues (Hunter and Ullman, 1989; Harrewijn et al., 1996) causing direct damage to plants that results in leaf, flower, and fruit scarring and distortion, discoloration of flowers, fruit deformation, sunken leaf tissues, and a characteristic “silvering” on leaves and flowers (Cloyd, 2009; Chisholm and Lewis, 1984; van

Dijken et al., 1994). Females insert eggs underneath the epidermal layer of leaves, flowers, or developing fruit using a saw-like ovipositor (Childers, 1997). Consequently, oviposition wounds resulting from extensive western flower thrips infestations can reduce the aesthetic quality and marketability of horticultural crops (Childers, 1997; Reitz, 2009). Western flower thrips also cause indirect damage by vectoring plant viruses, such as the tospoviruses: *Tomato spotted wilt* virus and *Impatiens necrotic spot* virus (Allen and Broadbent, 1986; Daughtrey et al., 1997; Mound, 1995; Pappu et al., 2009). First instar larvae acquire the virus, and adults transmit the virus throughout their lifespan (Tsuda et al., 1996; Van de Wetering et al., 1996).

The economic impact of western flower thrips is associated with their direct and indirect damage to host plants. Goldbach and Peters (1994) estimated that global annual losses related to the spread of *Tomato spotted wilt* virus alone exceeded $1 billion. In California, the costs of managing western flower thrips populations in cut flower production constitutes 7.5% of the total production costs (Murphy et al., 1998). The pest status of western flower thrips is attributed to damage to host plants caused by feeding and viral transmission and the ability to develop resistance to insecticides (Reitz, 2009).

Western flower thrips and insecticides

Greenhouse producers routinely apply insecticides to minimize direct and indirect damage caused by western flower thrips (Reitz, 2009; Murphy et al., 1998; Parrella and Jones, 1987). Continuous use of insecticides, however, creates intense selective pressure leading to the development of resistance in western flower thrips populations (Bielza, 2008; Jensen, 2000; Kontsedalov et al., 1998; Immaraju et al., 1992). In addition, producers may rely on only one insecticide for the management of western flower thrips. For instance, some greenhouse producers reported making more than 10 applications of insecticides with spinosad per cropping cycle (Bielza et al., 2007). Some greenhouse producers apply insecticides at 5-to-10-day intervals to suppress western flower thrips populations, but this leads to widespread development of resistance in greenhouse production systems (Murphy et al., 1998). The frequency of insecticide applications can influence the rate of resistance development, especially if insecticides with the same mode of action are used repeatedly (Bielza, 2008; Loughner et al., 2005). Consequently, western flower thrips populations worldwide have developed resistance to insecticides belonging to many chemical classes including: organophosphates, carbamates, pyrethroids, neonicotinoids, macrocyclic lactones, and spinosyns (Bielza et al., 2007; Cloyd, 2016; Immaraju et al., 1992; Jensen, 2000; Kontsedalov et al., 1998; Loughner et al., 2005; Robb and Parrella, 1995; Zhao et al., 1995).

Biological parameters of the western flower thrips can limit the effectiveness of insecticides. For example, the small body size (2.0 mm in length) and cryptic behavior (tendency to inhabit tight, enclosed areas on plants) protects western flower thrips from exposure to contact insecticides whereas the haplo-diploid breeding system promotes insecticide resistance (Cloyd, 2016; Jensen, 2000). Alleles in the haploid males are completely expressed and directly exposed to selection, which allows resistance alleles to become quickly fixed in a population (Denholm et al., 1998). Furthermore, a short generation time and high female fecundity contribute to the occurrence of multiple, overlapping generations with all life stages present simultaneously during a growing season (Moritz, 1997; Mouden et al., 2017), thus leading to the development of resistance in western flower thrips populations by exposing all life stages to an insecticide during each application.

Alternatives to insecticides for the management of western flower thrips are available, but they are often less efficacious and more costly than insecticides (Cloyd, 2015), which leads to an overreliance on insecticides by greenhouse producers (Murphy et al., 1998; Mouden et al., 2017). Insecticide resistance management is important for managing western flower thrips populations in greenhouse production systems. One of the primary strategies recommended to mitigate the development of insecticide resistance, in western flower thrips populations, is to rotate insecticides with different modes of action (Cloyd, 2016; Bielza, 2008; Gao et al., 2012; Jensen, 2000). However, greenhouse producers have a limited number of commercially available insecticides to choose from because of the high costs associated with developing and registering new insecticides (Sparks, 2013; Reitz and Funderburk, 2012). Therefore, preserving the effectiveness of currently available insecticides against western flower thrips is important for resistance management. In addition, operational factors under the direct control of greenhouse producers, such as spray coverage and application frequency, can influence the ability of insecticides to suppress insect populations.

Spray coverage and application frequency

There are several operational factors that can determine the effectiveness of insecticide applications against insect pests including spray coverage and application frequency (Dibble, 1962; McClure, 1977; Shelton et al., 2003; Shelton et al., 2006; Tipping et al., 2003). In fact, many failures to suppress insect pest populations can be attributed to incorrect insecticide dosage or improper coverage of plants due to applying an insufficient volume (Dibble, 1962). Spray coverage can influence the effectiveness of microbial agents and oils against insect pests. For instance, thorough coverage is necessary for control of insects using bacteria [*Bacillus* *thuringiensis* (Furlan et al., 2018)], fungal pathogens [*Beauveria bassiana* (Murphy et al., 1998)], and horticultural oils (Fondren and McCullough, 2005). Factors that influence the coverage of insecticide applications on plants include: spray volume, spray nozzle type, spray application method, and type of spray equipment (Dibble, 1962; Martini et al., 2012). One consequence of incomplete spray coverage is that target pests may avoid insecticide-treated areas, which will decrease the effectiveness of an insecticide against an insect pest (Martini et al., 2012). Spray coverage is determined by the amount of spray solution applied, total plant surface area including leaf undersides, and time spent spraying. Consequently, plants with greater surface area require a higher spray volume to obtain thorough coverage (D. Radosevich: personal observation).

In addition to spray coverage, frequency of insecticide applications can also impact effectiveness. The cultivar of cabbage (*Brassica oleracea* L.) and frequency of insecticide applications significantly affected the damage caused by the cabbage looper (*Trichoplusia ni*) (Story and Sundstrom, 1986). More frequent applications of permethrin, a pyrethroid insecticide, significantly reduced damage with the highest application frequency, three applications per growing cycle, resulting in the lowest percent leaf and head damage to cabbage in the spring and fall growing seasons (Story and Sundstrom, 1986). Cowpea (*Vigna unguiculata*) variety, number of insecticide applications, and timing of application significantly influenced crop yield in relation to losses caused by insect pests (Ajeigbe and Singh, 2006). The highest insecticide application frequency, three sprays throughout the cowpea growth cycle, provided the best protection against insect damage (Ajeigbe and Singh, 2006). Crop yields were higher when insecticides were applied more frequently because pest populations and their associated damage were reduced. As such, more frequent insecticide applications may provide better suppression of insect pests. Nonetheless, increasing application frequency can lead to the development of insecticide resistance (Bielza, 2008; Kontsedalov et al., 1998). To avoid insecticide resistance, greenhouse producers must rotate insecticides with different modes of action (Gao et al., 2012).

Few, if any, greenhouse studies have addressed the effect of spray volume and application frequency on insecticide efficacy against greenhouse insect pests. As a result, greenhouse producers may lack adequate knowledge concerning the volume and frequency of insecticide applications needed for sufficient suppression of target insect pests. Insecticides are often the primary strategy for managing insect pest populations in greenhouse production systems (Parrella, 1999). Therefore, it is imperative that greenhouse producers maximize the effectiveness of insecticide applications (based on mortality of the target insect pest) by properly implementing operational factors.

The failure to manage western flower thrips populations with insecticides is often blamed on resistance, yet failures may be due to incomplete spray coverage or poor application timing (Cloyd, 2016; Shelton et al., 2006). A better understanding of the influence of spray volume and application frequency on insecticide efficacy will help greenhouse producers suppress populations of citrus mealybugs and western flower thrips. In addition, proper stewardship of insecticides could diminish worker exposure to residues (Spear et al., 1975). Finally, increased mortality of citrus mealybug and western flower thrips populations may help lower the total number of insecticide applications needed and reduce labor and insecticide costs.

Objectives

Improved insecticide application methods will help greenhouse producers successfully manage insect pest populations and prevent plant damage. Operational factors, such as spray coverage and application frequency, are under the direct control of greenhouse producers and must be considered when using insecticides to manage insect pests. Therefore, the objectives and predictions associated with the following study are:

Objective 1: Determine how spray coverage (associated with volume) and application frequency influence insecticide efficacy against the citrus mealybug, *P. citri*.

Prediction: High spray volume (75 mL per plant) and more frequent insecticide applications (two per week) will result in greater percent mortality of citrus mealybugs on coleus (*Solenostemon scutellariodes*) and transvaal daisy (*Gerbera jamesonii*) plants. The greater percent mortality will be due to more citrus mealybugs coming into contact with insecticide residues when a higher spray volume is applied or when applications are more frequent. High spray volume and more frequent applications will allow more insecticide spray solution to contact individual citrus mealybugs on coleus and transvaal daisy plants, which will increase the level of mortality.

Objective 2: Determine how spray coverage (associated with volume) and application frequency influence insecticide efficacy against western flower thrips, *F. occidentalis*.

Prediction: Thorough coverage of transvaal daisy (*G. jamesonii*) cut flowers, based on a high spray volume (10 and 25 mL per flower), and more frequent insecticide applications (two applications) will result in greater percent mortality of western flower thrips adults. The greater percent mortality will be associated with more individual western flower thrips adults coming into contact with insecticide residues when higher spray volumes are applied or when applications are more frequent.

Chapter 2 - Effect of spray volume and application frequency on insecticide efficacy against the citrus mealybug (*Planococcus citri*) under greenhouse conditions

Introduction

The citrus mealybug, *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae), is an important insect pest of greenhouse-grown horticultural crops (Franco et al., 2009; McKenzie, 1967). Citrus mealybugs feed on plant fluids within the phloem sieve tubes using their piercing-sucking mouthparts, and extensive infestations can lead to wilting, leaf chlorosis, leaf drop, premature fruit drop, stunted growth, and even death of host plants (Mani and Shivaraju, 2016). Citrus mealybugs feed on leaf undersides but can also be found on leaf uppersides and plant stems (Herrick and Cloyd, 2017). During feeding, citrus mealybugs excrete honeydew, a clear, sticky liquid, that serves as a substrate for black sooty mold, which can inhibit photosynthesis and reduce overall aesthetic quality of plants (Charles, 1982). Indirect damage associated with citrus mealybugs involves vectoring plant viruses, such as *Banana streak OL (badna)* virus (BSOLV) (Meyer et al., 2008) and grapevine leafroll associated virus 3 (GLRaV-3) (Cabaleiro and Segura, 1997). Movement of infested plant material is the primary means of dispersal since nymphs can move among plants via contacting foliage (Tanwar et al., 2007). One adult female citrus mealybug can lay more than 400 eggs during her lifespan (Copeland et al., 1985), so it is important to manage citrus mealybug populations early before nymphs develop into adults.

Insecticides are the primary management strategy used against citrus mealybugs in greenhouse production systems (Parrella, 1999; Franco et al., 2009). However, systemic insecticides do not provide sufficient mortality of citrus mealybugs on greenhouse-grown horticultural crops, even at 8X the label rate, which may be associated with citrus mealybugs not ingesting lethal concentrations of the active ingredient due to their feeding behavior (Herrick et al., 2019). Therefore, greenhouse producers rely on foliar applications of contact insecticides. Nonetheless, contact insecticides have limited effectiveness against citrus mealybugs because (1) later instars and adults possess a hydrophobic waxy covering that provides protection from insecticide sprays (Copeland et al., 1985; Venkatesan et al., 2016), (2) it is difficult to obtain thorough coverage of all aboveground plant parts (leaves and stems), (3) citrus mealybugs have a cryptic feeding behavior and reside in protected or hidden areas on plants, and (4) citrus mealybugs have a clumped spatial distribution that reduces the exposure of individuals to insecticide applications (Charles, 1982; Franco et al., 2009; Herrick and Cloyd, 2017). Insecticides are most effective against early instar nymphs because they have not developed the hydrophobic waxy covering (Ahmed and Abd-Rabou, 2010; Charles, 1982; Venkatesan et al., 2016). In addition, insecticides are effective at managing citrus mealybug populations on host plants that provide fewer hiding areas, which increases exposure to spray applications (Franco et al., 2009). Factors that contribute to the efficacy of insecticide applications against target insect pests include: spray coverage (Dibble, 1962; McClure, 1977; Shelton et al., 2003; Shelton et al., 2006; Tipping et al., 2003; Martini et al., 2012) and frequency of application (Story and Sundstrom, 1986; Ajeigbe and Singh, 2006). High-volume insecticide applications that provide thorough coverage of host plants are important for suppressing mealybug populations (Venkatesan et al., 2016).

Despite the importance of operational factors, no greenhouse studies have been conducted to assess the effect of spray coverage (associated with volume) and application frequency on insecticide efficacy against citrus mealybugs. Therefore, the objective of the following study was to determine how spray volume and application frequency impact the efficacy of insecticides against citrus mealybugs by conducting a series of greenhouse experiments. Insecticide efficacy was based on percent mortality of citrus mealybugs on host plants.

Materials and Methods

Citrus mealybug colony

Citrus mealybugs were obtained from a colony in the Department of Entomology at Kansas State University (Manhattan, KS). The citrus mealybug colony is maintained on butternut squash (*Cucurbita maxima*) purchased from local supermarkets and kept at 24 to 27°C, 50 to 60% relative humidity, and under constant light. The laboratory colony has been maintained for nearly 20 years and has never been exposed to insecticides.

Plant material and procedures

Coleus (*Solenostemon scutellariodes*) and transvaal daisy (*Gerbera jamesonii*) plants were used in a series of greenhouse experiments. The experiments tested the effect of spray volume and application frequency on insecticide efficacy against citrus mealybugs feeding on coleus and transvaal daisy plants. Coleus plugs (young plants: either seedlings or cuttings grown as single units in modular trays) were purchased from Ball Horticultural Company (West Chicago, IL, USA). Transvaal daisy plugs were obtained from Family Tree Nursery (Kansas City, MO). Upon arrival, plugs were transplanted into 15.2 cm diameter containers with a standard soilless growing medium. The coleus or transvaal daisy plants were arranged on a wire-mesh bench in a research greenhouse and irrigated with approximately 500 mL of water and fertilized as needed. In each experiment, the coleus or transvaal daisy plants were fertilized with Miracle-Gro® Water Soluble All Purpose Plant Food (Scotts Miracle-Gro Products, Inc.; Maryville, OH, USA) consisting of 24-3.5-13.2 (N-P-K) at a rate of 7.5 or 15 g/3.8 L of water. Each coleus or transvaal daisy plant received approximately 500 mL of the fertilizer solution. Plant size was similar so that differences in coverage could be determined based on the spray volumes tested.

After the coleus or transvaal daisy plants reached a certain size (based on plant height), they were positioned on the wire mesh bench in a research greenhouse so the leaves of adjacent plants were not touching to avoid cross-contamination among plants after they were infested with citrus mealybugs. Coleus plants were used as a host plant in experiments separate from the transvaal daisy plants, so host plant type was not a treatment factor. The spray volume experiment and application frequency experiment were conducted in different greenhouses. Each plant was artificially infested with 10 to 15 second to early third instar citrus mealybug nymphs, obtained from the laboratory colony, using a fine-point paintbrush. Citrus mealybug nymphs were placed among several different leaves on each plant. Nymphs were allowed to acclimate for two to three days before the designated treatments were applied. Greenhouse conditions were 22 to 24°C with a relative humidity between 60 and 70% under natural daylight.

All insecticide treatments were mixed in 946 mL of tap water, and spray applications were made to all designated coleus and transvaal daisy plants using a 946 mL plastic spray bottle. One experiment evaluated spray volume (25, 50, or 75 mL per plant; with equivalent application frequencies) over a four-week period with three applications of the designated treatment to each plant. For spray volume, 25 mL represented a low volume, 50 mL represented an intermediate volume, and 75 mL represented a high volume application. Another experiment tested frequency of application (one or two applications per week; with a spray volume of 50 mL per plant) over a two-week period. Using the appropriate spray volume, the aboveground plant parts (leaves and stems) of each plant were sprayed, especially the leaf undersides because citrus mealybugs tend to aggregate on the bottom of leaves (Herrick and Cloyd, 2017).

Across all experiments, there were four treatments including three insecticides registered for and commonly used against citrus mealybugs in greenhouses (R. Cloyd: personal communication) and a water control. All experiments were set up as a completely randomized design. One coleus or transvaal daisy plant was considered an experimental unit. In order to assess the growth of coleus plants over the course of each experiment, height (cm) and number of leaves were recorded twice for each coleus plant: once on the day the treatments were first applied and again on the day plants were destructively sampled (Table 2.1). The insecticides and application rates were: acetamiprid (TriStar® 30 SG: Cleary Chemical Corporation; Dayton, NJ) at 2.7 oz/100 gallons, flonicamid (Aria®: FMC Corporation; Philadelphia, PA) at 2.1 oz/100 gallons, and cyfluthrin (Decathlon® 20 WP: OHP, Inc.; Bluffton, SC) at 1.9 oz/100 gallons. All three insecticides have different modes of action (IRAC, 2019; Table 2.2).

For the spray volume experiment, treatments were applied to plants once every seven days for three weeks (three applications in total). On the fourth week, seven days after the final treatment application, whole coleus or transvaal daisy plants were destructively sampled in the greenhouse. The number of live, dead, and total number of citrus mealybugs associated with each plant were recorded. Percent mortality of citrus mealybugs was calculated by dividing the number of dead citrus mealybugs by the total number of citrus mealybugs recovered on each plant and multiplying by 100. First-instar nymphs, if present, were not counted. However, egg-laying females and male pupae were counted as alive since they survived the treatments long enough to develop beyond the second and third instar stage.

In the application frequency experiment, all plants were sprayed on the same day. Seven days after the first treatment application, the number of live, dead, and total number of citrus mealybugs associated with each plant designated to receive one application were recorded, and plants assigned to two applications per week were sprayed a second time. Seven days after the second application, the number of live, dead, and total number of citrus mealybugs associated with each plant were recorded for the remaining plants. Percent citrus mealybug mortality was calculated by dividing the number of dead citrus mealybugs by the total number of citrus mealybugs recovered on each plant and multiplying by 100.

Experiment 1: Effect of spray volume and application frequency on insecticide efficacy against citrus mealybug on coleus plants

Coleus plugs (cultivar: “Redhead”) were transplanted on August 16, 2018 into 15.2 cm diameter containers with a growing medium (Pro-Mix BX: Premier Tech Horticulture; Quakertown, PA, USA) composed of 75 to 85% coarse sphagnum peat moss, perlite, vermiculite, limestone, and a wetting agent. On September 19, 2018, all coleus plants were fertilized with Miracle-Gro® Water Soluble All Purpose Plant Food at a rate of 15 g/3.8 L of water. Once the coleus plants were tall enough (based on plant height) to determine differences in coverage using the designated spray volumes, each were assigned to a treatment. There were five replicates per treatment combination with 60 experimental units in the spray volume experiment and 40 experimental units in the application frequency experiment. All coleus plants were artificially infested with citrus mealybug nymphs on November 2, 2018.

In the application frequency experiment, all coleus plants were treated on November 5, 2018. Coleus plants receiving one application were destructively sampled in the greenhouse on November 12, 2018 while plants designated to receive two applications were sprayed a second time. On November 19, 2018, the number of live, dead, and total number of citrus mealybugs on each plant were recorded for the remaining coleus. In the spray volume experiment, all coleus plants were treated on November 7, 14, and 21, 2018 and then destructively sampled on November 28, 2018.

Experiment 2: Effect of spray volume and application frequency on insecticide efficacy against citrus mealybug on transvaal daisy plants

Transvaal daisy plugs (cultivar: “Mega Revolution Yellow Dark Eye”) were transplanted into 15.2 cm diameter containers on January 3, 2019 with a growing medium (Pro-Mix BX: Premier Tech Horticulture; Quakertown, PA, USA) composed of 75 to 85% coarse sphagnum peat moss, perlite, vermiculite, limestone, and a wetting agent. On March 7, 2019, the transvaal daisy plants were fertilized with Miracle-Gro® Water Soluble All Purpose Plant Food at a rate of 15 g/3.8 L of water. Transvaal daisy plants were fertilized a second time on April 11, 2019 at a rate of 7.5 g/3.8 L of water. During the growing period, flower buds were removed so that the transvaal daisy plants remained in the vegetative stage. Plants were randomly assigned to the treatments after they became large enough to observe differences in coverage between the three spray volumes (25, 50, and 75 mL). There were five replicates per treatment combination with 60 experimental units in the spray volume experiment and 40 experimental units in the application frequency experiment.

All transvaal daisy plants were infested with citrus mealybugs on April 22, 2019. In the application frequency experiment, plants were treated initially on April 24, 2019. Transvaal daisy plants assigned to one application per week were destructively sampled on May 1, 2019 while plants designated to receive two applications were treated a second time. On May 8, 2019, transvaal daisy plants that received two applications were destructively sampled. In the spray volume experiment, all transvaal daisy plants were treated initially on April 25, 2019 and treated once per week for two more weeks. All transvaal daisy plants in the spray volume experiment were destructively sampled on May 16, 2019.

Experiment 3: Effect of application frequency on insecticide efficacy against citrus mealybug on coleus plants

On January 21, 2019, coleus plugs (cultivar: “Redhead”) were transplanted into 15.2 cm diameter containers with a growing medium (Pro-Mix BX: Premier Tech Horticulture; Quakertown, PA, USA) composed of 75 to 85% coarse sphagnum peat moss, perlite, vermiculite, limestone, and a wetting agent. On March 25, 2019, all coleus plants were fertilized with Miracle-Gro® Water Soluble All Purpose Plant Food at a rate of 15 g/3.8 L of water. Once the coleus plants were the appropriate size, based on the coleus plants used in experiment 1, they were assigned to the treatments. There were five replicates per treatment combination for a total of 40 experimental units.

The experiment was conducted using coleus plants that were infested with citrus mealybugs on April 24, 2019. All plants received the first treatment application on April 26, 2019. Coleus plants designated to receive one application were destructively sampled on May 3, 2019 while plants designated to receive two applications were treated a second time. The remaining coleus plants were destructively sampled on May 10, 2019.

Experiment 4: Effect of spray volume on insecticide efficacy against citrus mealybug on coleus plants grown from cuttings

Since the coleus plants in experiment 4 originated from cuttings, the plant architecture differed from the coleus plants used in the other experiments. Plant architecture refers to the spatial arrangement of the aboveground parts of a plant including leaves, stems, and branches (Cloyd and Sadof, 2000). As the cuttings established, they developed two main branches and adopted a “V” shape. In addition, the coleus plants were shorter in height (cm) and had fewer leaves at the beginning of the experiment compared to the other coleus plants (Table 2.1). Different spray volumes were used (15, 50, and 75 mL) due to differences in plant architecture.

On June 19, 2019, cuttings were taken from coleus plants grown in experiment 3. Each cutting (12.7 to 15.2 cm in length) was taken from the terminal growth of a single coleus plant. Cuttings were placed into 15.2 cm diameter containers with a standard soilless growing medium. Eventually, the cuttings developed an established root system. The coleus plants were fertilized with Miracle-Gro® Water Soluble All Purpose Plant Food at a rate of 15 g/3.8 L of water on August 15, 2019. There were four replicates per treatment combination for a total of 48 experimental units.

For the experiment, 15 mL represented a low volume, 50 mL represented an intermediate volume, and 75 mL represented a high volume application. Each coleus plant was infested with citrus mealybug nymphs on August 16, 2019. The coleus plants were treated on August 19 and 26 and September 2, 2019. All coleus plants were destructively sampled on September 9, 2019.

Experiment 5: Effect of spray volume and application frequency on insecticide efficacy against citrus mealybug on coleus plants

On July 11, 2019, coleus plugs (cultivar: “Redhead”) were transplanted into 15.2 cm diameter containers with a growing medium (Berger BM1: Saint-Modeste; Quebec, Canada) composed of 75 to 85% coarse sphagnum peat moss, perlite, and vermiculite. The plants were fertilized with Miracle-Gro® Water Soluble All Purpose Plant Food at a rate of 15 g/3.8 L of water on August 15, 2019. All coleus plants were pruned for uniformity on October 2, 2019 by removing approximately 10.2 cm of terminal growth. There were four replicates per treatment combination with 48 experimental units in the spray volume experiment and 32 experimental units in the application frequency experiment. All coleus plants were infested with citrus mealybugs on October 11, 2019.

In the application frequency experiment, coleus plants received the first treatment application on October 13, 2019. Coleus plants that received one application were destructively sampled on October 20, 2019 while the remaining plants were treated a second time. The remaining coleus plants were destructively sampled on October 27, 2019. In the spray volume experiment, all coleus plants were treated on October 14, 21, and 28, 2019, and each coleus plant was destructively sampled on November 4, 2019.

Statistical analysis

There were two treatment factors affiliated with each experiment: treatment (three insecticides and a water control) (4 levels) and spray volume (3 levels) or application frequency (2 levels). Citrus mealybug percent mortality estimates were subject to a two-way analysis of variance (ANOVA) with treatment and spray volume or application frequency as the main effects. Statistical models were fitted using PROC GLIMMIX in a SAS software program (SAS

Institute, 2012). Significant treatment means were separated using Tukey's honestly significant difference (HSD) test at *P*=0.05. Pairwise comparisons were conducted using Tukey's HSD adjustment for multiple comparisons to avoid type I error rate inflation. Percent citrus mealybug mortality estimates were analyzed twice in each experiment: once with percent mortality estimates from all four treatments and again with the water control percent mortality estimates removed from the analysis. Data were analyzed without the water control percent citrus mealybug mortality estimates to more effectively compare the effects of spray volume and application frequency on insecticide efficacy.

Results

Experiment 1: Effect of spray volume and application frequency on insecticide efficacy against citrus mealybug on coleus plants

Percent citrus mealybug mortality associated with the treatments from the application frequency experiment are presented in Figures 2.1-2.2. In the application frequency experiment, the two-way interaction between treatment and application frequency was not significant (*F*=0.55; df=3, 32; *P*=0.65). The effect of application frequency on percent citrus mealybug mortality was not significant (*F*=1.31; df=1, 32; *P*=0.26), but there was a significant effect of treatment on percent citrus mealybug mortality (*F*=51.16; df=3, 32; *P*<0.0001). Within the insecticide treatments, there were no significant differences in percent citrus mealybug mortality between plants that received one application and those that received two applications (Figure 2.1). Citrus mealybug mortality on plants treated with flonicamid was not significantly different from the water control (Figure 2.1).

When the water control percent citrus mealybug mortality estimates were removed from the analysis, the two-way interaction was not significant (*F*=0.69; df=2, 24; *P*=0.51). In addition, the effect of application frequency was not significant (*F*=1.35; df=1, 24; *P*=0.26). The treatments had a significant effect on percent citrus mealybug mortality (*F*=39.99; df=2, 24; *P*<0.0001) with acetamiprid having a significantly higher percent citrus mealybug mortality than flonicamid or cyfluthrin (Figure 2.2). No first-instar nymphs were observed on coleus plants that were destructively sampled after one application, but at least 50 first-instar nymphs were present on each coleus plant that received two applications, regardless of treatment.

The results from the spray volume portion of experiment 1 are presented in Figures 2.3-2.4. In the spray volume experiment, the two-way interaction between treatment and spray volume was significant (*F*=6.61; df=6, 48; *P*<0.0001). Acetamiprid had a significantly higher percent citrus mealybug mortality at a spray volume of 75 mL (80.7%; n=50) than 50 mL (46.9%; n=58) or 25 mL (39.9%; n=60). Flonicamid applied at 75 mL had a significantly higher percent citrus mealybug mortality (46.3%; n=52) than 25 mL (27.8%; n=55) (Figure 2.3).

When the water control percent citrus mealybug mortality estimates were removed from the analysis, the two-way interaction between spray volume and treatment was significant (*F*=3.94; df=4, 36; *P*=0.0094). Acetamiprid applied at 75 mL had a significantly higher percent citrus mealybug mortality (80.7%; n=50) than the other insecticide treatments (Figure 2.4). On the coleus plants treated with acetamiprid at 75 mL, all citrus mealybugs counted as alive were male pupae, and no egg-laying females or first-instar nymphs were present. Nonetheless, first-instar nymphs and egg-laying females were observed on every other coleus plant (except for those treated with acetamiprid at 75 mL). More than 100 first-instar nymphs were present on each coleus plant treated with water.

Experiment 2: Effect of spray volume and application frequency on insecticide efficacy against citrus mealybug on transvaal daisy plants

In the application frequency experiment with transvaal daisy plants, the two-way interaction between treatment and application frequency was not significant (F=1.34; df=3, 32; P=0.28), but the main effects of application frequency (F=12.24; df=1, 32; P=0.0014) and treatment (F=105.76; df=3, 32; P<0.0001) on percent citrus mealybug mortality were significant. Two applications of cyfluthrin resulted in a significantly higher percent citrus mealybug mortality (37.9%; n=74) than one application (20.6%; n=74) (Figure 2.5). Percent citrus mealybug mortality for one application of cyfluthrin was not significantly different from mortality associated with the water control (Figure 2.5).

When the water control percent citrus mealybug mortality estimates were removed from the analysis, the two-way interaction was not significant (F=1.4; df=2, 24; P=0.26). However, the main effects of application frequency (F=13.94; df=1, 24; P=0.001) and treatment (F=89.68; df=2, 24; P<0.0001) on percent citrus mealybug mortality were significant. Acetamiprid had a significantly higher percent citrus mealybug mortality than either flonicamid or cyfluthrin (Figure 2.6). In addition, two insecticide applications resulted in a significantly higher percent citrus mealybug mortality than one application (Figure 2.7). On the transvaal daisy plants treated twice with acetamiprid, all citrus mealybugs counted as alive were male pupae. No first-instar nymphs were observed on transvaal daisy plants that were destructively sampled after one application. However, there were at least 50 first-instar nymphs on every plant that received two applications, regardless of treatment.

In the spray volume experiment with transvaal daisy plants, the two-way interaction between treatment and spray volume was not significant (F=2.01; df=6, 48; P=0.082), but the main effects of spray volume (*F*=12.23; df=2, 48; *P*<0.0001) and treatment (*F*=91.1; df=3, 48; *P*<0.0001) on percent citrus mealybug mortality were significant. Acetamiprid applied at 75 mL (73.9%; n=59) or 50 mL (70%; n=100) resulted in a significantly higher percent citrus mealybug mortality than acetamiprid applied at 25 mL (50.6%; n=78) (Figure 2.8). Percent citrus mealybug mortalities after 25 mL applications of flonicamid or cyfluthrin were not significantly different from the water control (Figure 2.8).

When the water control percent citrus mealybug mortality estimates were removed from the analysis, the two-way interaction was not significant (*F*=0.63; df=4, 36; *P*=0.65), but the main effects of spray volume (*F*=16.21; df=2, 36; *P*<0.0001) and treatment (*F*=44.95; df=2, 36; *P*<0.0001) on percent citrus mealybug mortality were significant. Acetamiprid had a significantly higher percent citrus mealybug mortality than either flonicamid or cyfluthrin (Figure 2.9), and percent citrus mealybug mortality was significantly higher when the insecticides were applied at 75 mL or 50 mL compared to 25 mL (Figure 2.10). On transvaal daisy plants treated with 75 mL of acetamiprid, male pupae and egg-laying females were present, but no live first-instar nymphs were observed on these plants. Egg-laying females and at least 50 first-instar nymphs were observed on each of the other transvaal daisy plants.

Experiment 3: Effect of application frequency on insecticide efficacy against citrus mealybug on coleus plants

The results from experiment 3 are presented in Figures 2.11-2.13. The two-way interaction between treatment and application frequency was not significant (*F*=0.44; df=3, 32; *P*=0.72), but the main effects of application frequency (*F*=5.31; df=1, 32; *P*=0.028) and treatment (*F*=143.57; df=3, 32; *P*<0.0001) on percent citrus mealybug mortality were significant.

Within the two-way insecticide treatment means, there were no significant differences in percent citrus mealybug mortality between coleus plants treated once and those treated twice (Figure 2.11). Applications of acetamiprid, regardless of the frequency, resulted in significantly higher percent citrus mealybug mortality compared to flonicamid and cyfluthrin (Figure 2.11). However, percent citrus mealybug mortality after one application of flonicamid or cyfluthrin was not significantly different from the mortality after two applications of water (Figure 2.11).

When the water control percent citrus mealybug mortality estimates were removed from the analysis, the two-way interaction was not significant (*F*=0.23; df=2, 24; *P*=0.79), but the main effects of application frequency (*F*=5.77; df=1, 24; *P*=0.024) and treatment (*F*=133.51; df=2, 24; *P*<0.0001) on percent citrus mealybug mortality were significant. Acetamiprid had a significantly higher percent citrus mealybug mortality than either flonicamid or cyfluthrin (Figure 2.12). In addition, two insecticide applications had a significantly higher percent citrus mealybug mortality than one application (Figure 2.13). None of the coleus plants destructively sampled after one application had any first-instar nymphs. However, all the coleus plants that received two applications were infested with first-instar nymphs by the end of the experiment. Coleus plants treated with acetamiprid had less than 50 first-instar nymphs per plant, and coleus plants treated with flonicamid, cyfluthrin, or water had more than 100 first-instar nymphs per plant.

Experiment 4: Effect of spray volume on insecticide efficacy against citrus mealybug on coleus plants grown from cuttings

Results associated with experiment 4 are presented in Figures 2.14-2.16. The two-way interaction between spray volume and treatment was significant (*F*=4.31; df=6, 36; *P*=0.0023).

Plants treated with 75 mL of acetamiprid had a percent citrus mealybug mortality (76.2%; n=28) that was significantly higher than 50 mL (44.7%; n=26) or 15 mL (30.8%; n=32) (Figure 2.14). For flonicamid and cyfluthrin, applications at 75 mL resulted in a significantly higher percent citrus mealybug mortality than 15 mL (Figure 2.14). Across all insecticide treatments, percent citrus mealybug mortality associated with 15 mL was not significantly different from the water control (Figure 2.14).

When the water control percent citrus mealybug mortality estimates were removed from the analysis, the two-way interaction between spray volume and treatment was not significant (F=1.22; df=4, 27; P=0.32). However, the main effects of spray volume (F=34.5; df=2, 27; P<0.0001) and treatment (F=15.09; df=2, 27; P<0.0001) on percent citrus mealybug mortality were significant. Among the insecticide treatments, acetamiprid had a percent citrus mealybug mortality that was significantly higher than flonicamid or cyfluthrin (Figure 2.15). All three spray volumes tested (15, 50, and 75 mL) resulted in citrus mealybug mortality levels that were significantly different from each other, and 15 mL applications had the lowest mortality whereas 75 mL applications had the highest mortality (Figure 2.16).

On two coleus plants treated with acetamiprid at 75 mL, one citrus mealybug male pupae was counted as alive on each plant. There were no live first-instar nymphs observed on coleus plants treated with acetamiprid at 50 or 75 mL of spray solution. All coleus plants treated with water had more than 100 first-instar nymphs at the end of the experiment. Coleus plants treated with 75 mL of flonicamid had less than 50 first-instar nymphs per plant. As spray volume increased, the number of first-instar nymphs on coleus plants treated with cyfluthrin decreased, especially on plants treated with 75 mL of spray solution.

Experiment 5: Effect of spray volume and application frequency on insecticide efficacy against citrus mealybug on coleus plants

In the application frequency experiment, the two-way interaction between treatment and application frequency was not significant (*F*=0.07; df=3, 24; *P*=0.98). The effect of application frequency was not significant (*F*=1.39; df=1, 24; *P*=0.25), but the effect of treatment on percent citrus mealybug mortality was significant (*F*=33.77; df=3, 24; *P*<0.0001). Acetamiprid, regardless of application frequency, had a percent citrus mealybug mortality similar to two applications of flonicamid (26.6%; n=61) or cyfluthrin (28.1%; n=78) (Figure 2.17). Percent citrus mealybug mortality for one flonicamid or cyfluthrin application was not significantly different from the mortality associated with the water control (Figure 2.17).

When the water control was omitted from the analysis, the two-way interaction between treatment and application frequency was not significant (*F*=0.09; df=2, 18; *P*=0.92). The effect of application frequency was not significant (*F*=0.88; df=1, 18; *P*=0.36), but the effect of treatment on percent citrus mealybug mortality was significant (*F*=12.2; df=2, 18; *P*=0.0004). Across the application frequencies, acetamiprid had a significantly higher percent citrus mealybug mortality than flonicamid or cyfluthrin (Figure 2.18). No first-instar nymphs were observed on coleus plants destructively sampled after one application, but egg-laying females and male pupae were present. Nonetheless, all coleus plants that received two applications of flonicamid, cyfluthrin, and water had more than 100 first-instar nymphs per plant. For coleus plants treated with two applications of acetamiprid, two plants had no first-instar nymphs while the other two plants had less than 50 first-instar nymphs per plant.

In the spray volume experiment, the two-way interaction between spray volume and treatment was significant (*F*=3.1; df=6, 36; *P*=0.015). For all three insecticides, percent citrus mealybug mortality associated with 75 mL was significantly higher than 25 mL (Figure 2.19). Furthermore, percent citrus mealybug mortality affiliated with flonicamid or cyfluthrin applied at 25 mL was not significantly different from mortality associated with the water control (Figure 2.19).

When the water control percent citrus mealybug mortality estimates were removed from the analysis, the two-way interaction between spray volume and treatment was not significant (F=0.65; df=4, 27; P=0.63); however, the main effects of spray volume (F=37.08; df=2, 27; P<0.0001) and treatment (F=24.46; df=2, 27; P<0.0001) on percent citrus mealybug mortality were significant. Among the insecticides, acetamiprid had a significantly higher percent citrus mealybug mortality while the mortalities for flonicamid and cyfluthrin were not significantly different (Figure 2.20). Percent citrus mealybug mortality across the spray volumes (25, 50, and 75 mL) were significantly different from each other, and 75 mL resulted in the highest mortality while 25 mL resulted in the lowest mortality (Figure 2.21).

Egg-laying female citrus mealybugs and male pupae were observed on coleus plants across every treatment when destructively sampled. Coleus plants treated with 50 or 75 mL of acetamiprid had first-instar nymphs that appeared to be morbid since they were immobile or upside down on the plant. However, less than 20 live first-instar nymphs were present on each coleus plant treated with 25 mL of acetamiprid. Coleus plants treated with 25 or 50 mL of flonicamid had more than 100 first-instar nymphs per plant, and plants treated with flonicamid at 75 mL had less than 50 first-instar nymphs per plant. All coleus plants treated with cyfluthrin or water had more than 100 first-instar nymphs per plant.

Discussion

This is the first study to evaluate the impact of spray volume and application frequency on insecticide efficacy against the citrus mealybug under greenhouse conditions. Results from this study emphasize the importance of operational factors associated with insecticide applications. In addition, greenhouse producers will be able to effectively manage citrus mealybug populations in greenhouse production systems. The study also compared the efficacy of three insecticides with different modes of action against the citrus mealybug. It is important to note that the laboratory colony of citrus mealybug used in the study has never been exposed to insecticides, so the possibility of insecticide resistance is highly unlikely.

Two insecticide applications resulted in significantly higher citrus mealybug mortality than one application in experiments 2 and 3. However, there was no effect of application frequency on citrus mealybug mortality in experiments 1 and 5. The differences among the experiments may be due to regular experimental error or variations in plant architecture. Coleus, *Solenostemon scutellariodes*, plants have a more complex architecture than transvaal daisy, *Gerbera jamesonii*, plants used in experiment 2, based on greater height and number of leaves (D. Radosevich: personal observation). In addition, transvaal daisy plants have a rosette-type of plant growth without branches (Dole and Wilkins, 2005), which may improve coverage of leaf undersides where citrus mealybugs are typically located (Herrick and Cloyd, 2017). Furthermore, citrus mealybugs may have fewer locations to hide on transvaal daisy plants, thus increasing exposure to spray applications. Higher citrus mealybug mortality after two insecticide applications for transvaal daisy plants may have been associated with the less complex plant architecture and smaller size, resulting in less spray volume required to cover all aboveground plant parts.

The current study found that additional insecticide applications do not always lead to better suppression of citrus mealybug populations. There are a number of factors that can influence the efficacy of insecticide applications, including: spray volume, spray nozzle type, spray application method, time spent spraying, and type of spray equipment (Dibble, 1962; Martini et al., 2012). The 50 mL spray volume used in the application frequency experiments may have not been enough to provide sufficient plant coverage, especially in experiment 5 where coleus plants were taller than those in the other experiments. Studies in agricultural systems have demonstrated that more frequent insecticide applications can substantially reduce insect damage to crops (Story and Sundstrom, 1986; Ajeigbe and Singh, 2006). In addition, more frequent insecticide applications can lead to higher citrus mealybug mortality (Herrick and Cloyd; unpublished data). However, increasing the frequency of insecticide applications may induce the development of resistance in citrus mealybug populations, especially if insecticides with the same mode of action are used in succession (Flaherty et al., 1982; Venkatesan et al., 2016). Consequently, greenhouse producers must develop rotation programs that avoid using insecticides with the same mode of action (Bielza, 2008; Loughner et al., 2005; Venkatesan et al., 2016).

Thorough spray coverage can increase pesticide efficacy (Dibble, 1962; McClure, 1977; Shelton et al., 2003; Shelton et al., 2006; Tipping et al., 2003; Martini et al., 2012). The current study demonstrated that a higher spray volume can lead to better coverage of plant parts, such as leaves and stems, based on percent citrus mealybug mortality. Across all experiments, the 75 mL spray volume consistently resulted in a higher percent citrus mealybug mortality than the 15 or 25 mL spray volumes, respectively. In experiments 4 and 5, citrus mealybug mortality associated with the 50 mL spray volume was significantly different from the other spray volumes (15, 25, or

75 mL). Plant size and architecture can influence the amount of spray volume required to obtain thorough coverage of all aboveground plant parts (D. Radosevich: personal observation). It is important that percent citrus mealybug mortality be at least 80% to minimize plant damage and reduce the number of individuals in subsequent generations (Herrick and Cloyd, 2017). Consequently, the insecticides that were less effective against citrus mealybugs, such as flonicamid and cyfluthrin, may need to be applied at higher volumes than acetamiprid to provide sufficient suppression of citrus mealybug populations. In addition, the 15 and 25 mL insecticide spray volumes resulted in citrus mealybug mortalities that were often similar to the water control. This was apparent in experiment 4 where cyfluthrin, a contact insecticide, applied at 15 mL had a lower mean percent citrus mealybug mortality than the water control. Thorough plant coverage associated with high spray volume is important in managing citrus mealybug populations with contact insecticides.

There was an effect of insecticide type on the abundance of first-instar citrus mealybug nymphs. Mealybug populations can be efficiently managed if insecticides are applied when early-instar nymphs are present because they lack the protective, waxy covering found on later-instar nymphs and adults (Charles, 1982). In general, plants treated with acetamiprid and flonicamid had fewer first-instar nymphs than plants treated with cyfluthrin, which may be affiliated with the translaminar activity of acetamiprid and flonicamid (Buchholz and Nauen, 2002; Morita et al., 2014). Translaminar activity refers to the ability of an active ingredient to penetrate leaf surfaces after a foliar application and form a reservoir within plant tissues, which results in insects being killed by ingesting the active ingredient when feeding on leaf undersides even if the insecticide was applied to the upper leaf surface (Buchholz and Nauen, 2002).

Acetamiprid has been shown to suppress cotton whitefly, *Bemisia tabaci*, adult and first-instar nymphal populations for up to ten days after a foliar application (Horowitz et al., 1998). If first-instar citrus mealybug nymphs were not killed by direct contact with the insecticide sprays, they may have ingested lethal concentrations of the active ingredient when feeding on leaves. Therefore, insecticides with translaminar activity may help alleviate problems with citrus mealybugs by killing nymphs, which reduces the number of potential egg-laying females. Acetamiprid consistently resulted in a higher citrus mealybug mortality than flonicamid and cyfluthrin. Applications of acetamiprid used against citrus mealybugs feeding on coleus plants can result in 84% mortality (Hogendorp and Cloyd, 2013). As previously mentioned, flonicamid and acetamiprid both possess translaminar activity, but the two active ingredients may have different rates of uptake in coleus and transvaal daisy leaves (Wang and Liu, 2007). The higher efficacy of acetamiprid may be related to the mode of action. For example, flonicamid acts as a selective feeding blocker that causes starvation by inhibiting the ingestion of phloem, as observed with the green peach aphid, *Myzus persicae* (Cho et al., 2011; Morita et al., 2014). However, the mode of action of flonicamid may delay the time required to kill insect pests. In contrast, acetamiprid acts on the nicotinic acetylcholine receptors in the central nervous system (Zhang et al., 2000), which may kill citrus mealybugs more quickly.

Operational factors can influence the success of pest management programs against insect pests. This study has demonstrated that spray volume and application frequency can influence insecticide efficacy against the citrus mealybug. Additional studies are needed to address the effect of plant architecture on spray volume and application frequency associated with citrus mealybug mortality. Future studies associated with spray volume and application frequency should compare the efficacy of other insecticides, such as horticultural oils and soaps, against citrus mealybugs. Because insecticides are the primary management strategy used against citrus mealybugs, greenhouse producers will benefit from improvements in operational factors, thus enhancing insecticide efficacy against citrus mealybugs and possibly other insect and mite pests. More effective insecticide applications that lead to higher mortality of citrus mealybugs will lower the inputs and labor costs associated with insecticides by effectively suppressing citrus mealybug populations. Proper stewardship of contact insecticides in greenhouse production systems can also alleviate issues associated with environmental contamination, worker safety, and insecticide resistance.

Table 2.1 Mean plant height (cm) and number of leaves of coleus, *Solenostemon scutellariodes*, plants used as a host for citrus mealybugs, *Planococcus citri*, in experiments (except experiment 2) before and after treatments were applied, and the mean change in plant height and number of leaves (after measurement – before measurement) throughout each experiment.

			Mean Plant Height (cm)			Mean Number of Leaves		
Experiment Number	**Experiment Type** [a]	**n**	**Before** [b]	**After** [c]	**Change**	**Before** [b]	**After** [c]	**Change**
1	AF	40	24.31	26.84	2.53	116.2	120.65	4.45
1	SV	60	26.52	32.87	6.35	120.47	125.33	4.86
3	AF	40	26.37	29.76	3.39	124.55	127.95	3.4
4	SV	48	23.91	42.36	18.45	58.2	122.38	64.18
5	AF	32	35.14	35.37	0.23	122.53	124.31	1.78
5	SV	48	32.31	32.91	0.6	124.44	128.46	4.02

[a] Experiment type: AF=Application Frequency and SV=Spray Volume.

[b] Before: plant height and number of leaves were measured on the day treatments were first applied.

[c] After: plant height and number of leaves were measured on the day coleus plants were destructively sampled.

Table 2.2 Insecticide treatments (active ingredient, trade name, rate used, and mode of action) used in the spray volume and application frequency experiments associated with citrus mealybugs, *Planococcus citri*.

Active Ingredient	**Trade Name**	**Rate Used**	**Mode of Action***
Acetamiprid	TriStar®	0.19 g/946 mL	Nicotinic acetylcholine receptor competitive modulator
Flonicamid	Aria®	0.15 g/946 mL	Chordotonal organ modulator
Cyfluthrin	Decathlon®	0.13 g/946 mL	Sodium channel modulator

*Modes of action are based on the classification scheme of the Insecticide Resistance Action Committee (IRAC, 2019).

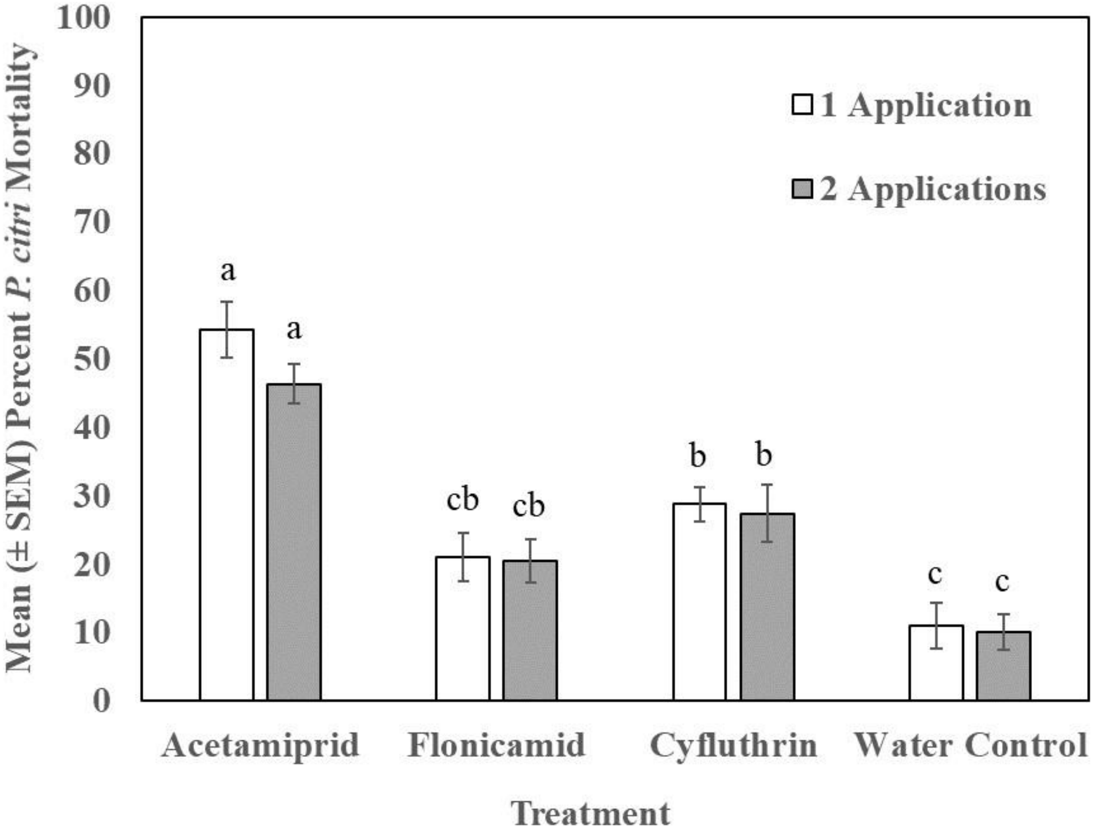

Figure 2.1 Mean (± SEM) percent citrus mealybug, *Planococcus citri*, mortality in experiment 1 after exposure to one or two applications of the following treatments at 50 mL of spray solution per plant: 1) acetamiprid (TriStar®) at 0.19 g/946 mL, 2) flonicamid (Aria®) at 0.15 g/946 mL, 3) cyfluthrin (Decathlon®) at 0.13 g/946 mL, and 4) water control. Means with the same letter are not significantly different (*P*>0.05) based on a Tukey's HSD test. Vertical bars represent the standard error of the mean (SEM).

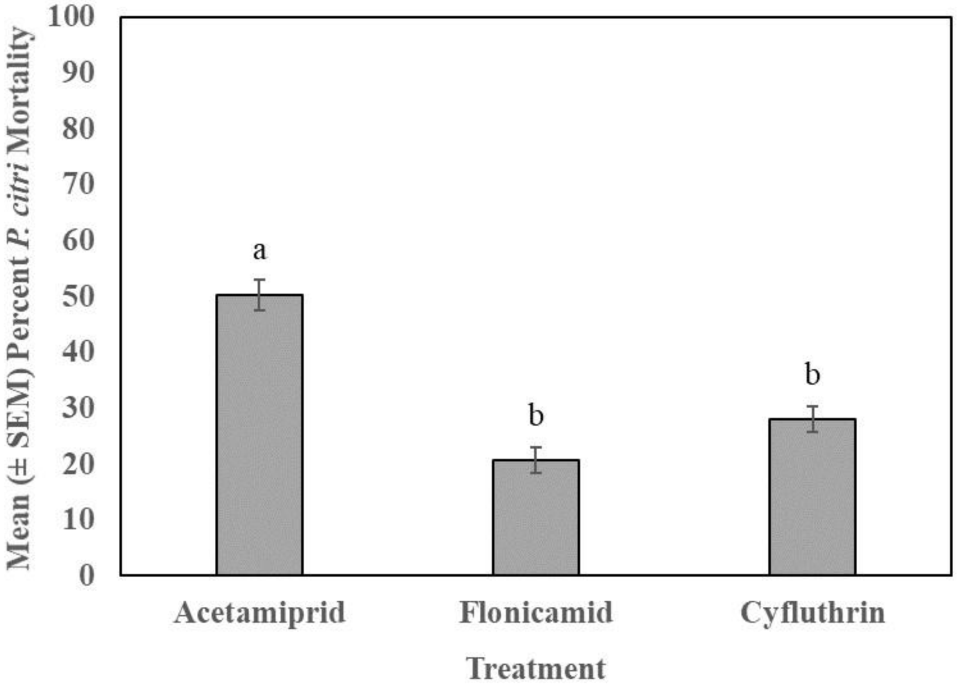

Figure 2.2 Mean (± SEM) percent citrus mealybug, *Planococcus citri*, mortality in experiment 1 after exposure to the following treatments at 50 mL of spray solution per plant: 1) acetamiprid (TriStar®) at 0.19 g/946 mL, 2) flonicamid (Aria®) at 0.15 g/946 mL, and 3) cyfluthrin (Decathlon®) at 0.13 g/946 mL. Percent citrus mealybug mortality was averaged across two application frequencies for each insecticide. Means with the same letter are not significantly different (*P*>0.05) based on a Tukey's HSD test. Vertical bars represent the standard error of the mean (SEM).

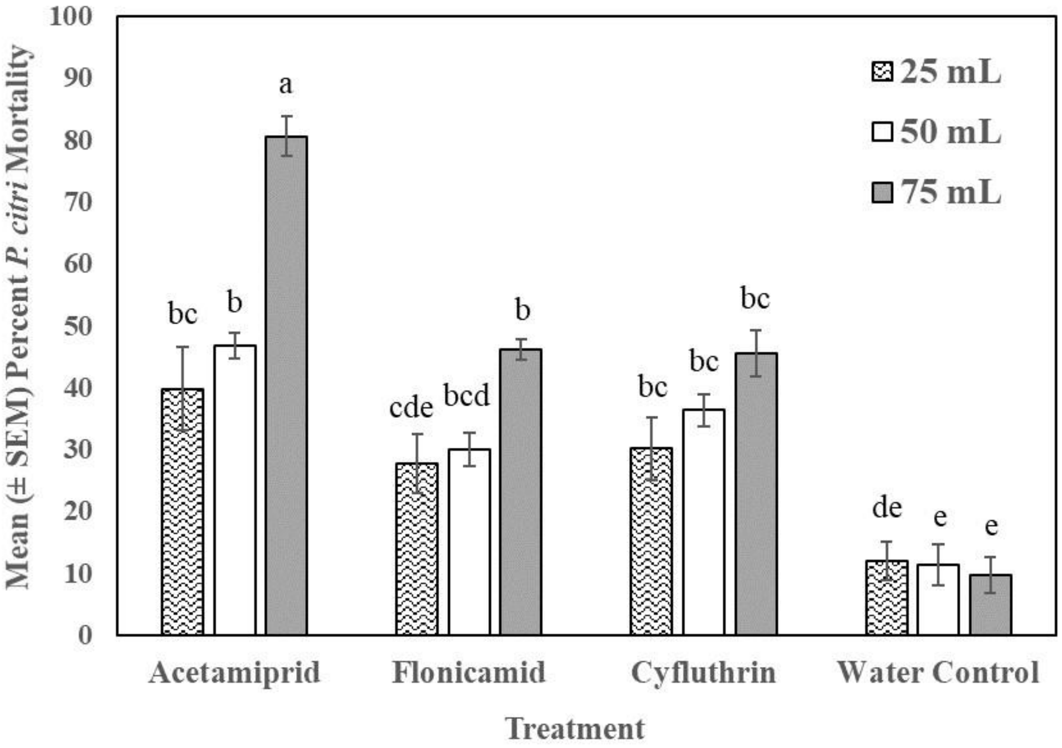

Figure 2.3 Mean (± SEM) percent citrus mealybug, *Planococcus citri*, mortality in experiment 1 after exposure to three weekly applications at spray volumes of 25, 50, or 75 mL associated with the following treatments: 1) acetamiprid (TriStar®) at 0.19 g/946 mL, 2) flonicamid (Aria®) at 0.15 g/946 mL, 3) cyfluthrin (Decathlon®) at 0.13 g/946 mL, and 4) water control. Means with the same letter are not significantly different (*P*>0.05) based on a Tukey's HSD test. Vertical bars represent the standard error of the mean (SEM).

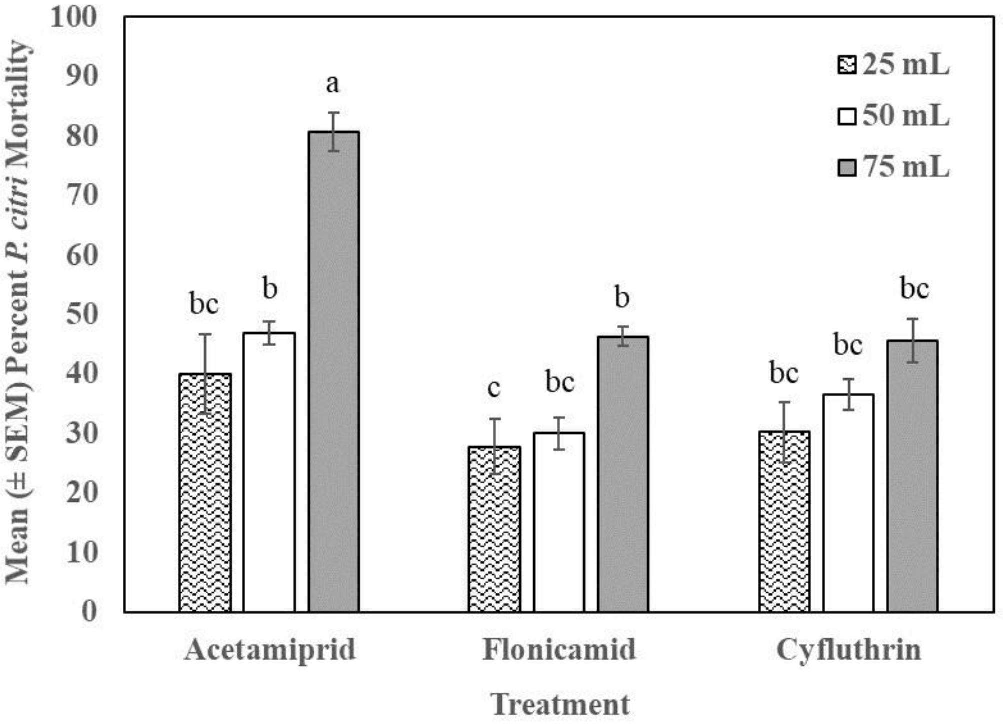

Figure 2.4 Mean (± SEM) percent citrus mealybug, *Planococcus citri*, mortality in experiment 1 after exposure to three weekly applications at spray volumes of 25, 50, or 75 mL associated with the following treatments: 1) acetamiprid (TriStar®) at 0.19 g/946 mL, 2) flonicamid (Aria®) at 0.15 g/946 mL, and 3) cyfluthrin (Decathlon®) at 0.13 g/946 mL. Means with the same letter are not significantly different (*P*>0.05) based on a Tukey's HSD test. Vertical bars represent the standard error of the mean (SEM).

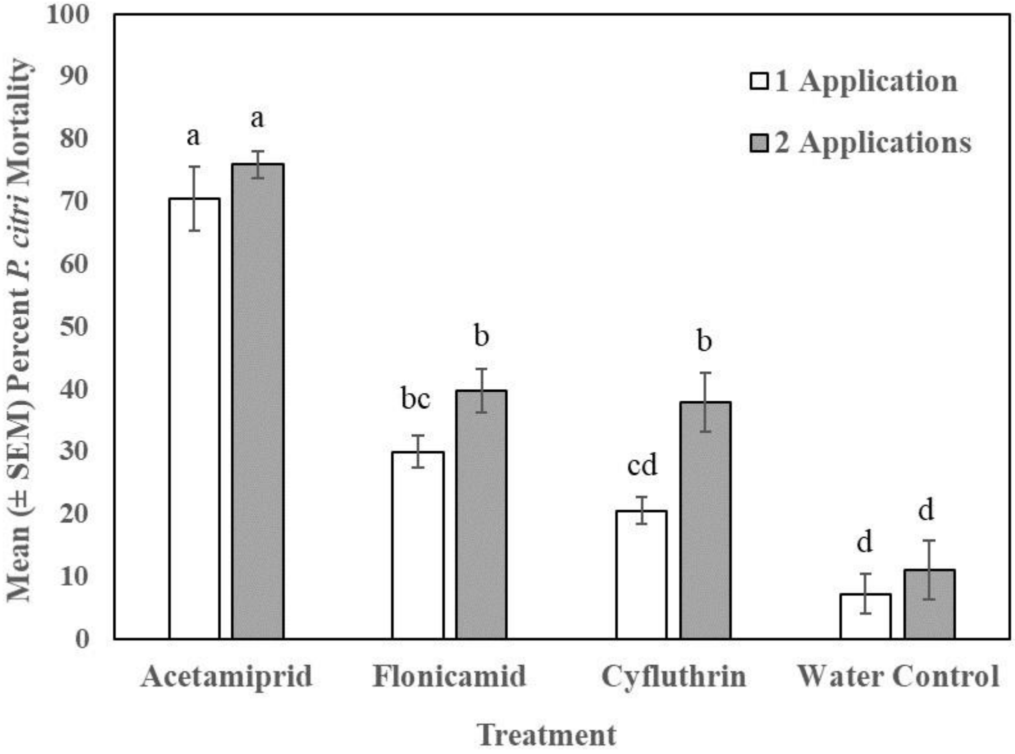

Figure 2.5 Mean (± SEM) percent citrus mealybug, *Planococcus citri*, mortality in experiment 2 after exposure to one or two applications at 50 mL of spray solution per plant associated with the following treatments: 1) acetamiprid (TriStar®) at 0.19 g/946 mL, 2) flonicamid (Aria®) at 0.15 g/946 mL, 3) cyfluthrin (Decathlon®) at 0.13 g/946 mL, and 4) water control. Means with the same letter are not significantly different (P>0.05) based on a Tukey's HSD test. Vertical bars represent the standard error of the mean (SEM).

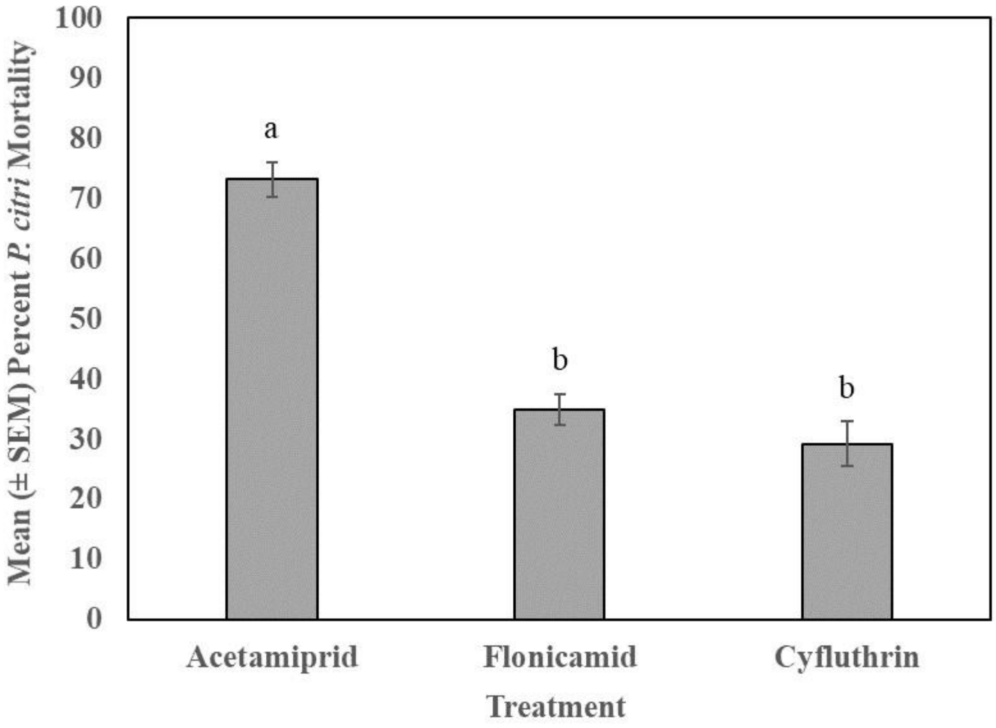

Figure 2.6 Mean (± SEM) percent citrus mealybug, *Planococcus citri*, mortality in experiment 2 after exposure to the following treatments at 50 mL of spray solution per plant: 1) acetamiprid (TriStar®) at 0.19 g/946 mL, 2) flonicamid (Aria®) at 0.15 g/946 mL, and 3) cyfluthrin (Decathlon®) at 0.13 g/946 mL. Percent citrus mealybug mortality was averaged across the application frequencies for each insecticide. Means with the same letter are not significantly different (*P*>0.05) based on a Tukey's HSD test. Vertical bars represent the standard error of the mean (SEM).

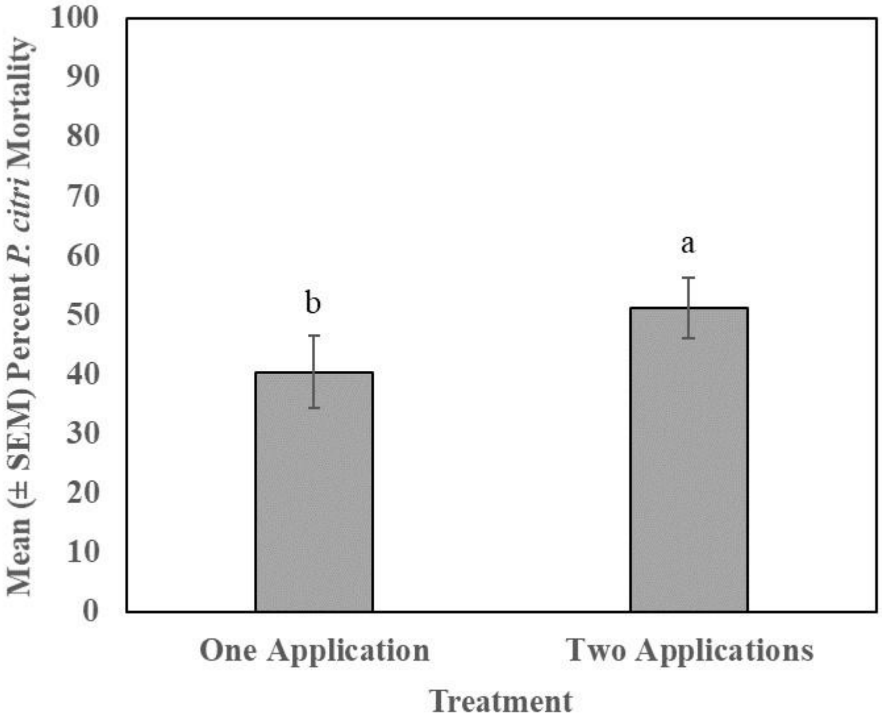

Figure 2.7 Mean (± SEM) percent citrus mealybug, *Planococcus citri*, mortality in experiment 2 after exposure to the following treatments at 50 mL of spray solution per plant: 1) acetamiprid (TriStar®) at 0.19 g/946 mL, 2) flonicamid (Aria®) at 0.15 g/946 mL, and 3) cyfluthrin (Decathlon®) at 0.13 g/946 mL. Percent citrus mealybug mortality was averaged across the three insecticides for each application frequency. Means with the same letter are not significantly different (P>0.05) based on a Tukey's HSD test. Vertical bars represent the standard error of the mean (SEM).

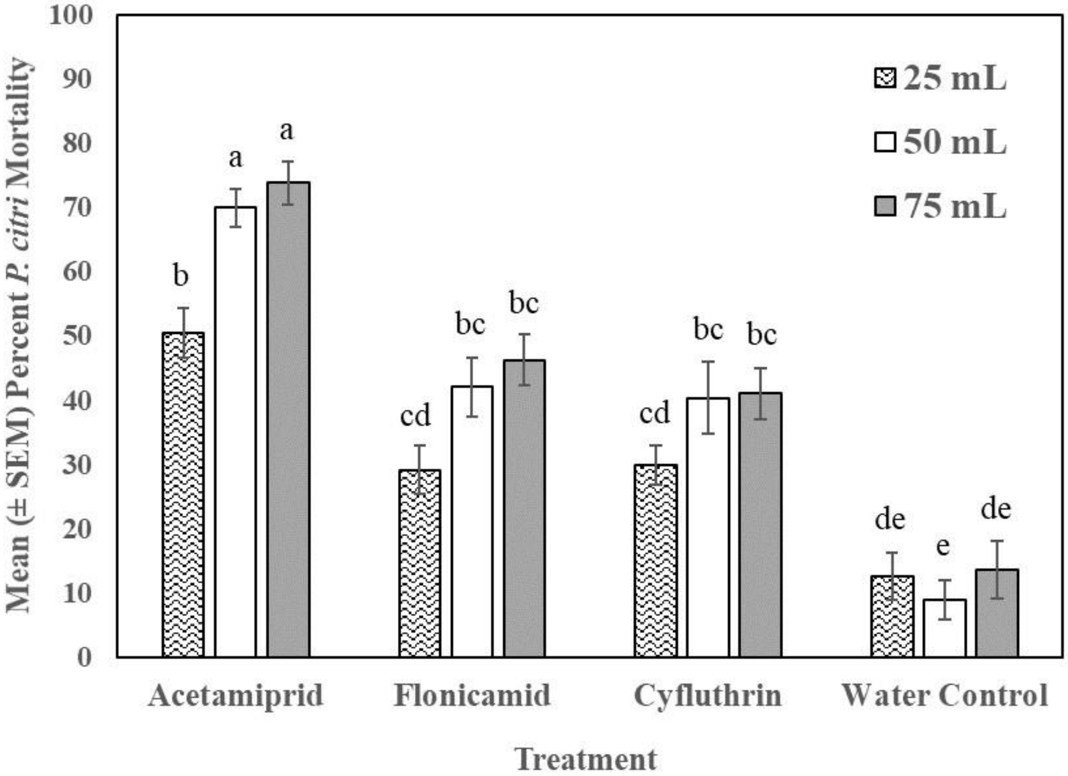

Figure 2.8 Mean (± SEM) percent citrus mealybug, *Planococcus citri*, mortality in experiment 2 after exposure to three weekly applications at spray volumes of 25, 50, or 75 mL associated with the following treatments: 1) acetamiprid (TriStar®) at 0.19 g/946 mL, 2) flonicamid (Aria®) at 0.15 g/946 mL, 3) cyfluthrin (Decathlon®) at 0.13 g/946 mL, and 4) water control. Means with the same letter are not significantly different (*P*>0.05) based on a Tukey's HSD test. Vertical bars represent the standard error of the mean (SEM).

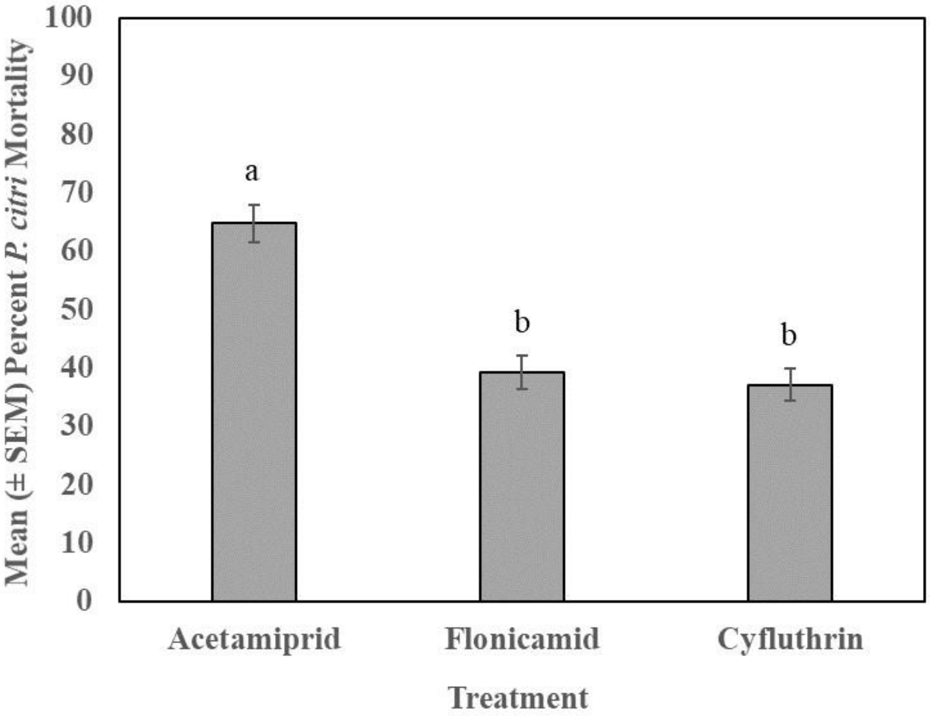

Figure 2.9 Mean (± SEM) percent citrus mealybug, *Planococcus citri*, mortality in experiment 2 after exposure to three weekly applications of the following treatments: 1) acetamiprid (TriStar®) at 0.19 g/946 mL, 2) flonicamid (Aria®) at 0.15 g/946 mL, and 3) cyfluthrin (Decathlon®) at 0.13 g/946 mL. Percent citrus mealybug mortality was averaged across the spray volumes (25, 50, and 75 mL) for each insecticide. Means with the same letter are not significantly different (*P*>0.05) based on a Tukey's HSD test. Vertical bars represent the standard error of the mean (SEM).

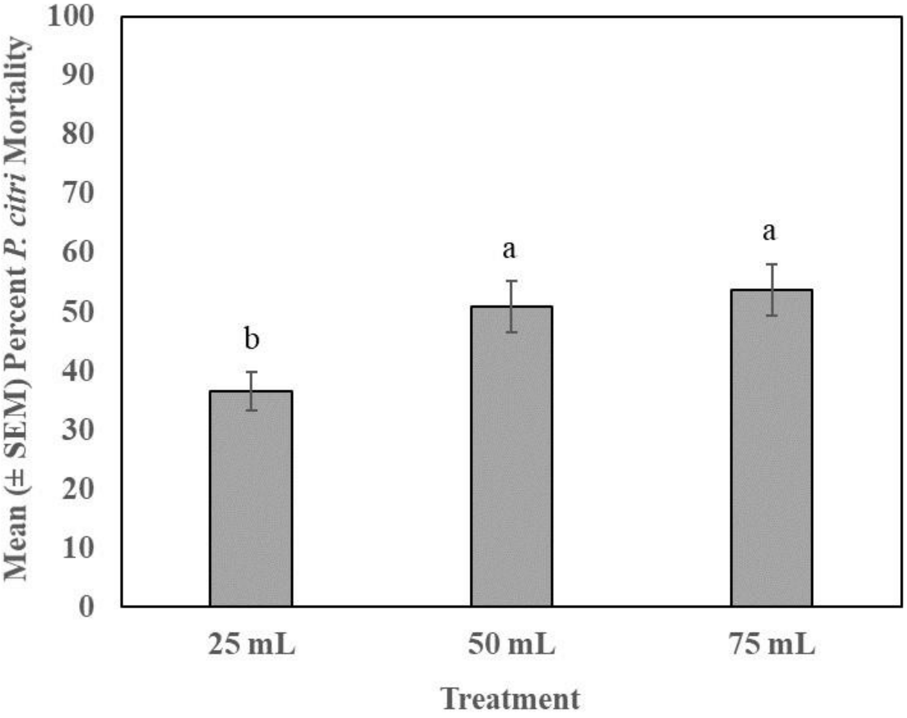

Figure 2.10 Mean (± SEM) percent citrus mealybug, *Planococcus citri*, mortality in experiment 2 after exposure to three weekly applications of the following treatments: 1) acetamiprid (TriStar®) at 0.19 g/946 mL, 2) flonicamid (Aria®) at 0.15 g/946 mL, and 3) cyfluthrin (Decathlon®) at 0.13 g/946 mL. Percent citrus mealybug mortality was averaged across the three insecticides for each spray volume. Means with the same letter are not significantly different (*P*>0.05) based on a Tukey's HSD test. Vertical bars represent the standard error of the mean (SEM).

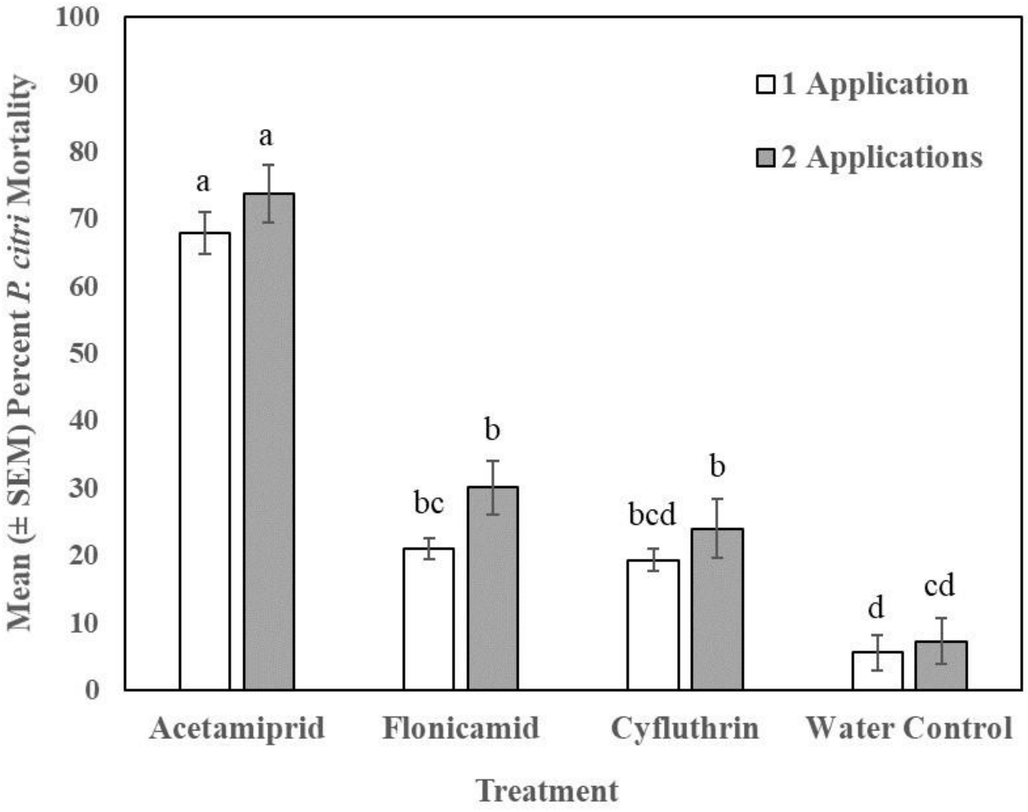

Figure 2.11 Mean (± SEM) percent citrus mealybug, *Planococcus citri*, mortality in experiment 3 after exposure to one or two applications of the following treatments at 50 mL of spray solution per plant: 1) acetamiprid (TriStar®) at 0.19 g/946 mL, 2) flonicamid (Aria®) at 0.15 g/946 mL, 3) cyfluthrin (Decathlon®) at 0.13 g/946 mL, and 4) water control. Means with the same letter are not significantly different ($P > 0.05$) based on a Tukey's HSD test. Vertical bars represent the standard error of the mean (SEM).

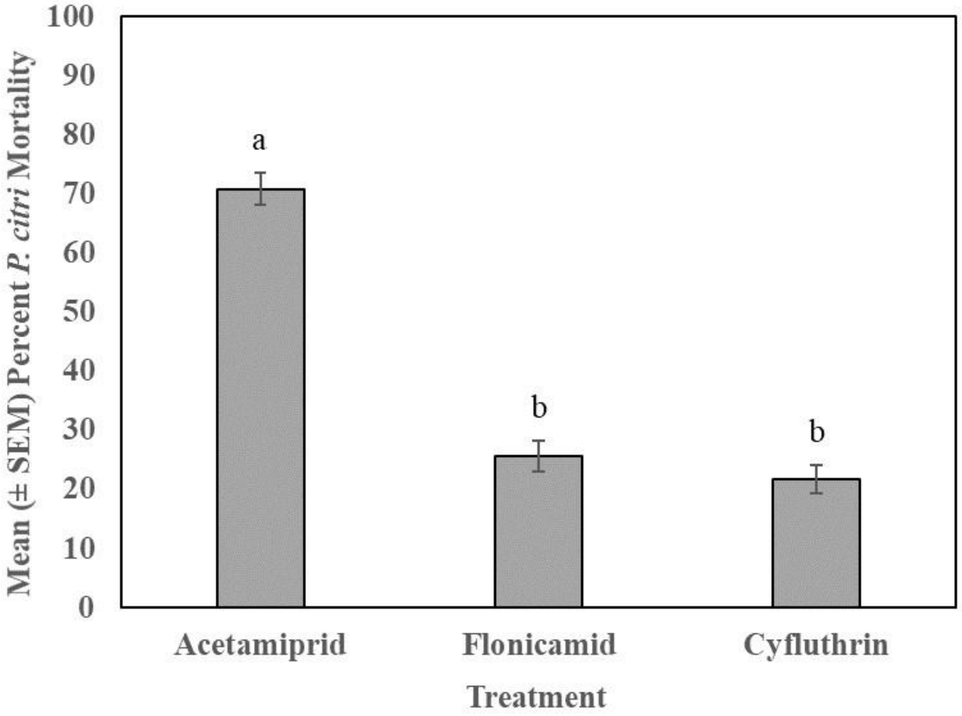

Figure 2.12 Mean (± SEM) percent citrus mealybug, *Planococcus citri*, mortality in experiment 3 after exposure to the following treatments at 50 mL of spray solution per plant: 1) acetamiprid (TriStar®) at 0.19 g/946 mL, 2) flonicamid (Aria®) at 0.15 g/946 mL, and 3) cyfluthrin (Decathlon®) at 0.13 g/946 mL. Percent citrus mealybug mortality was averaged across the application frequencies for each insecticide. Means with the same letter are not significantly different (*P*>0.05) based on a Tukey's HSD test. Vertical bars represent the standard error of the mean (SEM).

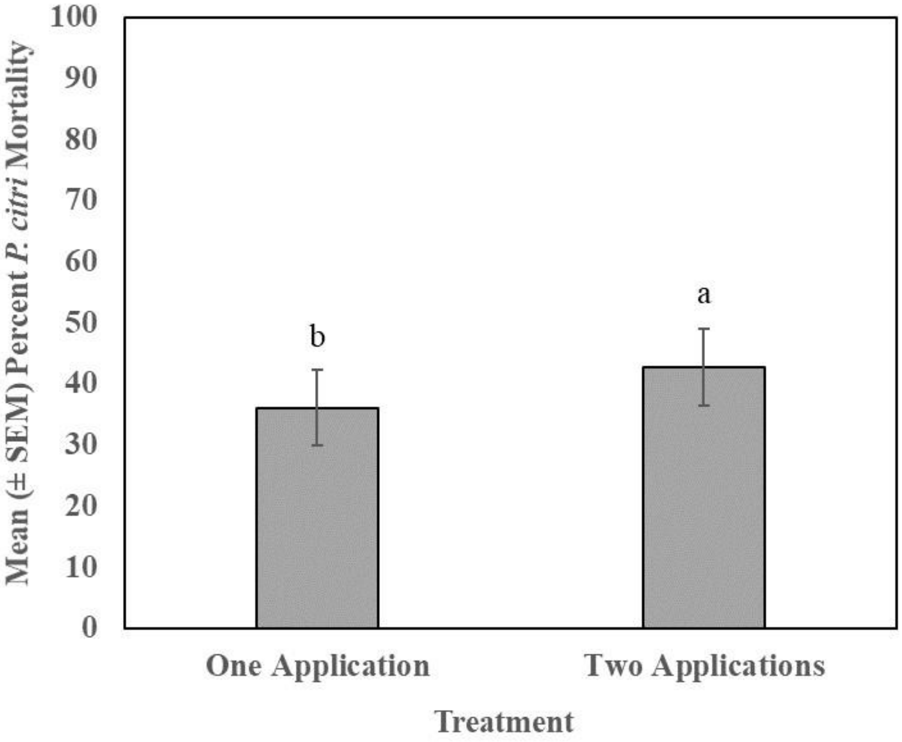

Figure 2.13 Mean (± SEM) percent citrus mealybug, *Planococcus citri*, mortality in experiment 3 after exposure to the following treatments at 50 mL of spray solution per plant: 1) acetamiprid (TriStar®) at 0.19 g/946 mL, 2) flonicamid (Aria®) at 0.15 g/946 mL, and 3) cyfluthrin (Decathlon®) at 0.13 g/946 mL. Percent citrus mealybug mortality was averaged across the three insecticides for each application frequency. Means with the same letter are not significantly different (*P*>0.05) based on a Tukey's HSD test. Vertical bars represent the standard error of the mean (SEM).

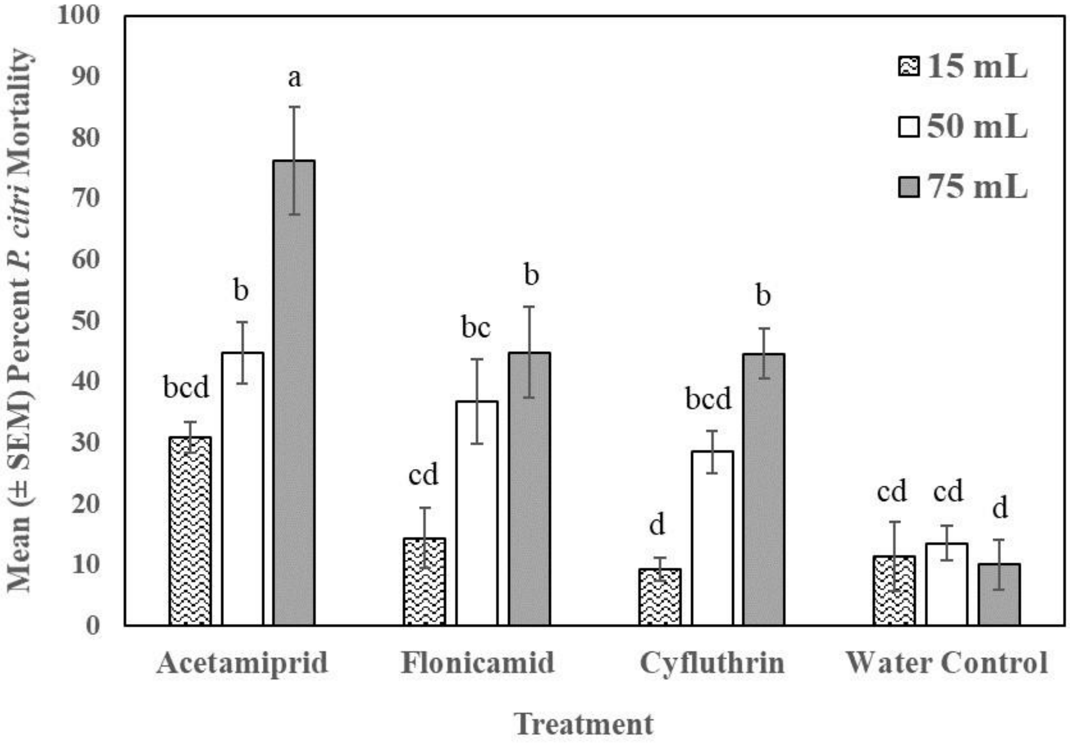

Figure 2.14 Mean (± SEM) percent citrus mealybug, *Planococcus citri*, mortality in experiment 4 after exposure to three weekly applications at spray volumes of 15, 50, or 75 mL associated with the following treatments: 1) acetamiprid (TriStar®) at 0.19 g/946 mL, 2) flonicamid (Aria®) at 0.15 g/946 mL, 3) cyfluthrin (Decathlon®) at 0.13 g/946 mL, and 4) water control. Means with the same letter are not significantly different (*P*>0.05) based on a Tukey's HSD test. Vertical bars represent the standard error of the mean (SEM).

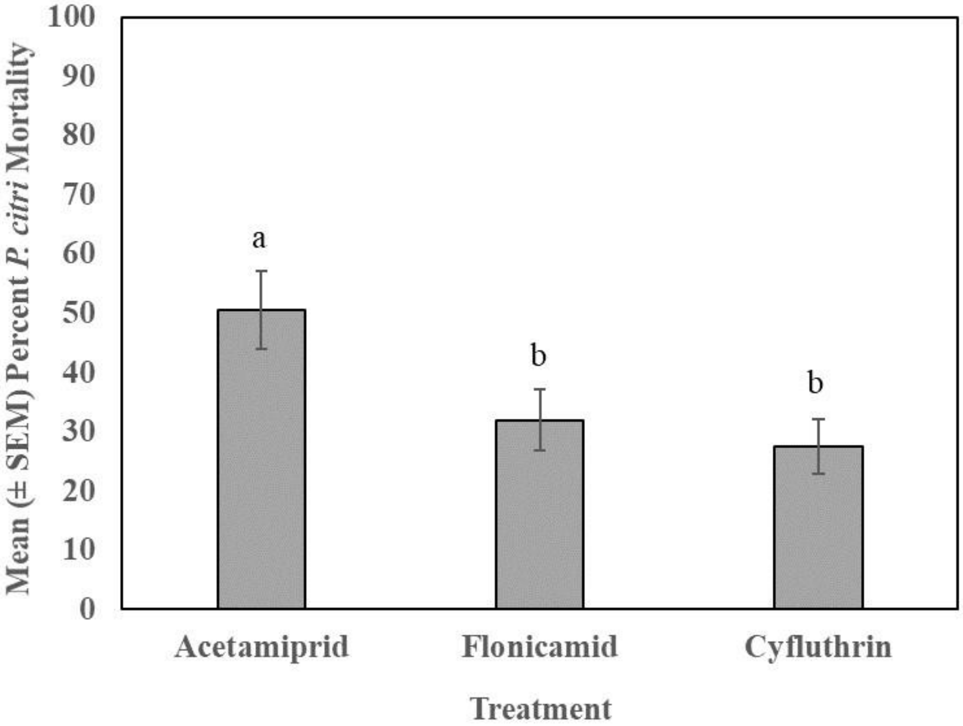

Figure 2.15 Mean (± SEM) percent citrus mealybug, *Planococcus citri*, mortality in experiment 4 after exposure to three weekly applications of the following treatments: 1) acetamiprid (TriStar®) at 0.19 g/946 mL, 2) flonicamid (Aria®) at 0.15 g/946 mL, and 3) cyfluthrin (Decathlon®) at 0.13 g/946 mL. Percent citrus mealybug mortality was averaged across the spray volumes (15, 50, and 75 mL) for each insecticide. Means with the same letter are not significantly different (*P*>0.05) based on a Tukey's HSD test. Vertical bars represent the standard error of the mean (SEM).

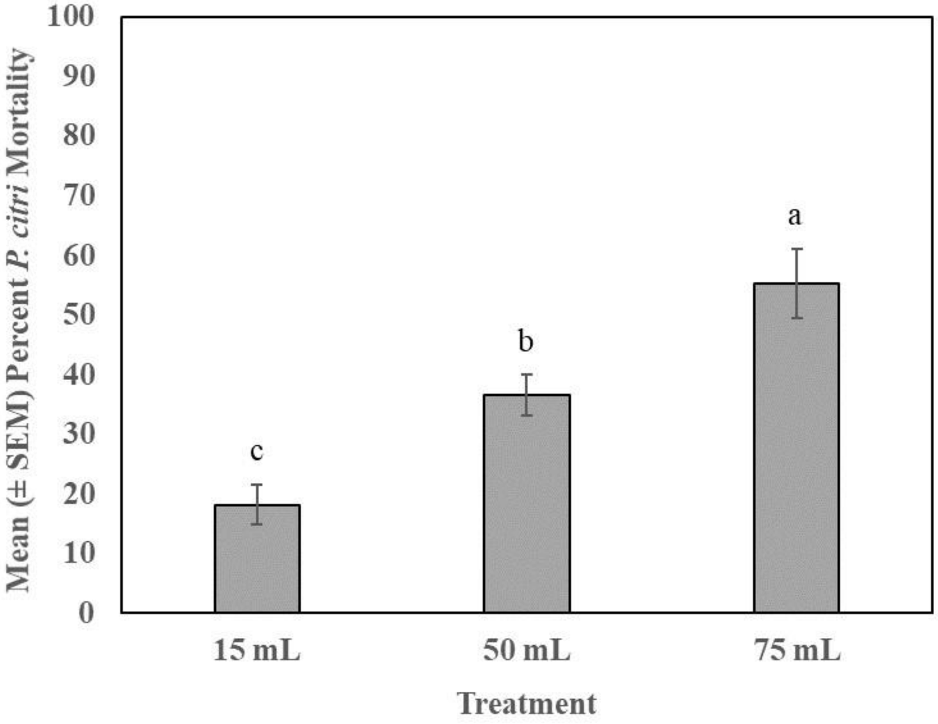

Figure 2.16 Mean (± SEM) percent citrus mealybug, *Planococcus citri*, mortality in experiment 4 after exposure to three weekly applications of the following treatments: 1) acetamiprid (TriStar®) at 0.19 g/946 mL, 2) flonicamid (Aria®) at 0.15 g/946 mL, and 3) cyfluthrin (Decathlon®) at 0.13 g/946 mL. Percent citrus mealybug mortality was averaged across the three insecticide treatments for each spray volume. Means with the same letter are not significantly different (*P*>0.05) based on a Tukey's HSD test. Vertical bars represent the standard error of the mean (SEM).

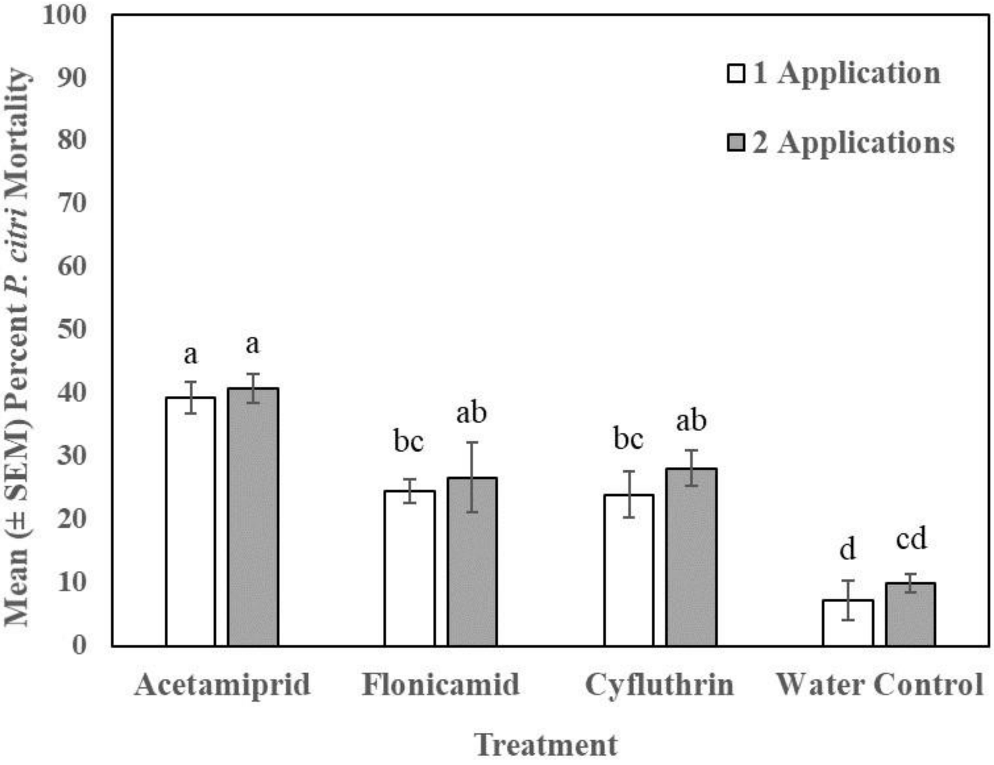

Figure 2.17 Mean (± SEM) percent citrus mealybug, *Planococcus citri*, mortality in experiment 5 after exposure to one or two applications of the following treatments at 50 mL of spray solution per plant: 1) acetamiprid (TriStar®) at 0.19 g/946 mL, 2) flonicamid (Aria®) at 0.15 g/946 mL, 3) cyfluthrin (Decathlon®) at 0.13 g/946 mL, and 4) water control. Means with the same letter are not significantly different (*P*>0.05) based on a Tukey's HSD test. Vertical bars represent the standard error of the mean (SEM).

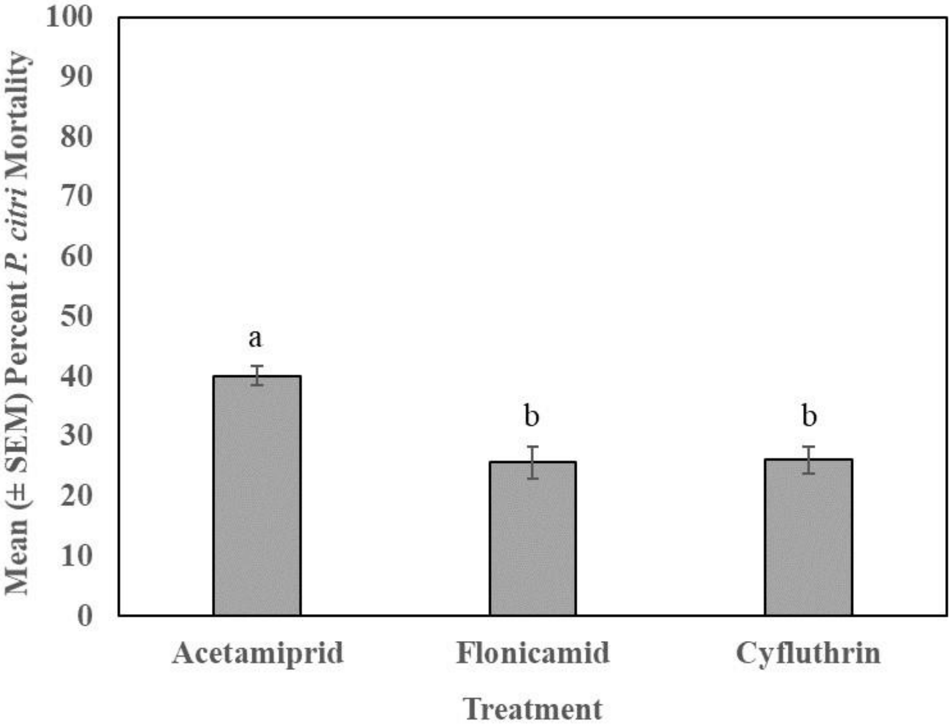

Figure 2.18 Mean (± SEM) percent citrus mealybug, *Planococcus citri*, mortality in experiment 5 after exposure to the following treatments at 50 mL of spray solution per plant: 1) acetamiprid (TriStar®) at 0.19 g/946 mL, 2) flonicamid (Aria®) at 0.15 g/946 mL, and 3) cyfluthrin (Decathlon®) at 0.13 g/946 mL. Percent citrus mealybug mortality was averaged across the two application frequencies for each insecticide. Means with the same letter are not significantly different (*P*>0.05) based on a Tukey's HSD test. Vertical bars represent the standard error of the mean (SEM).

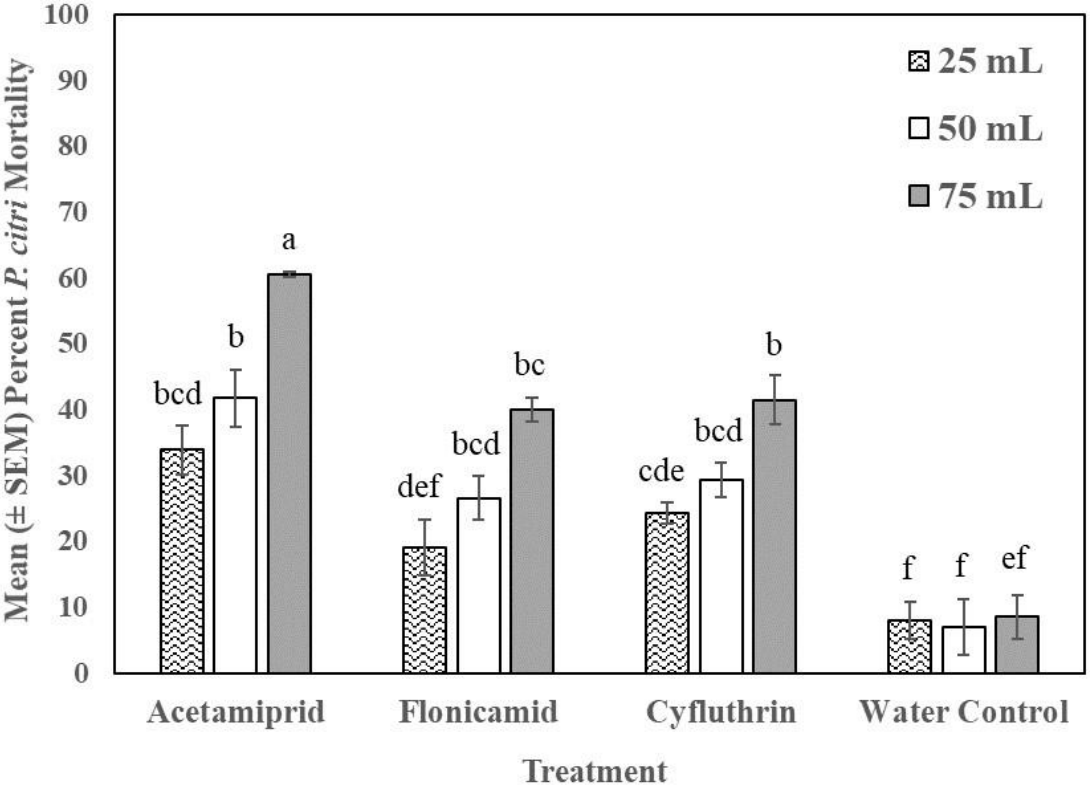

Figure 2.19 Mean (± SEM) percent citrus mealybug, *Planococcus citri*, mortality in experiment 5 after exposure to three weekly applications at spray volumes of 25, 50, or 75 mL associated with the following treatments: 1) acetamiprid (TriStar®) at 0.19 g/946 mL, 2) flonicamid (Aria®) at 0.15 g/946 mL, 3) cyfluthrin (Decathlon®) at 0.13 g/946 mL, and 4) water control. Means with the same letter are not significantly different (*P*>0.05) based on a Tukey's HSD test. Vertical bars represent the standard error of the mean (SEM).

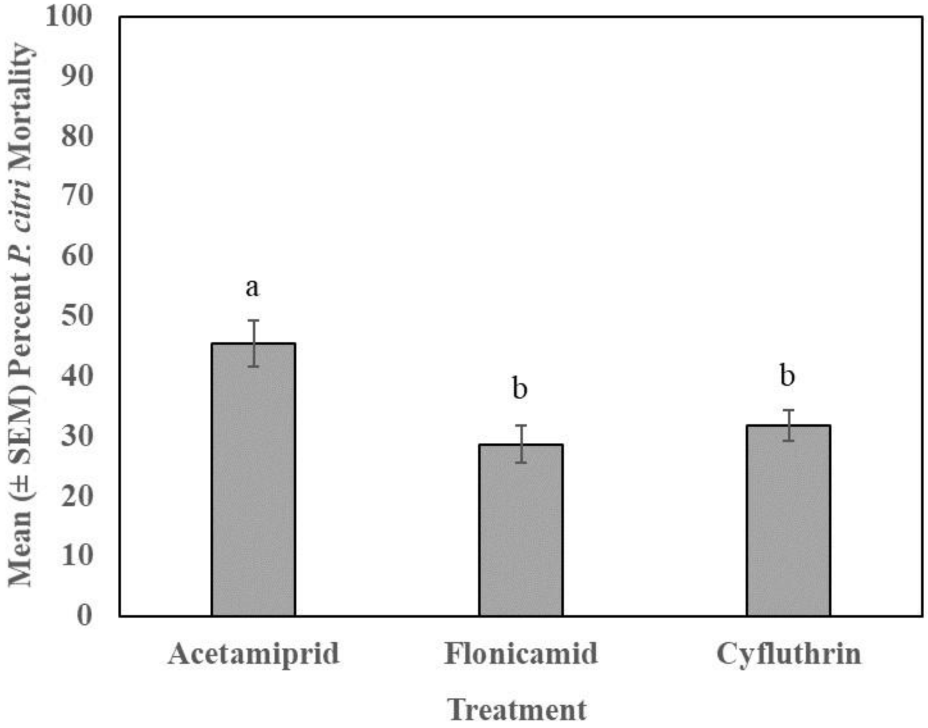

Figure 2.20 Mean (± SEM) percent citrus mealybug, *Planococcus citri*, mortality in experiment 5 after exposure to three weekly applications of the following treatments: 1) acetamiprid (TriStar®) at 0.19 g/946 mL, 2) flonicamid (Aria®) at 0.15 g/946 mL, and 3) cyfluthrin (Decathlon®) at 0.13 g/946 mL. Percent citrus mealybug mortality was averaged across the spray volumes (25, 50, and 75 mL) for each insecticide. Means with the same letter are not significantly different (*P*>0.05) based on a Tukey's HSD test. Vertical bars represent the standard error of the mean (SEM).

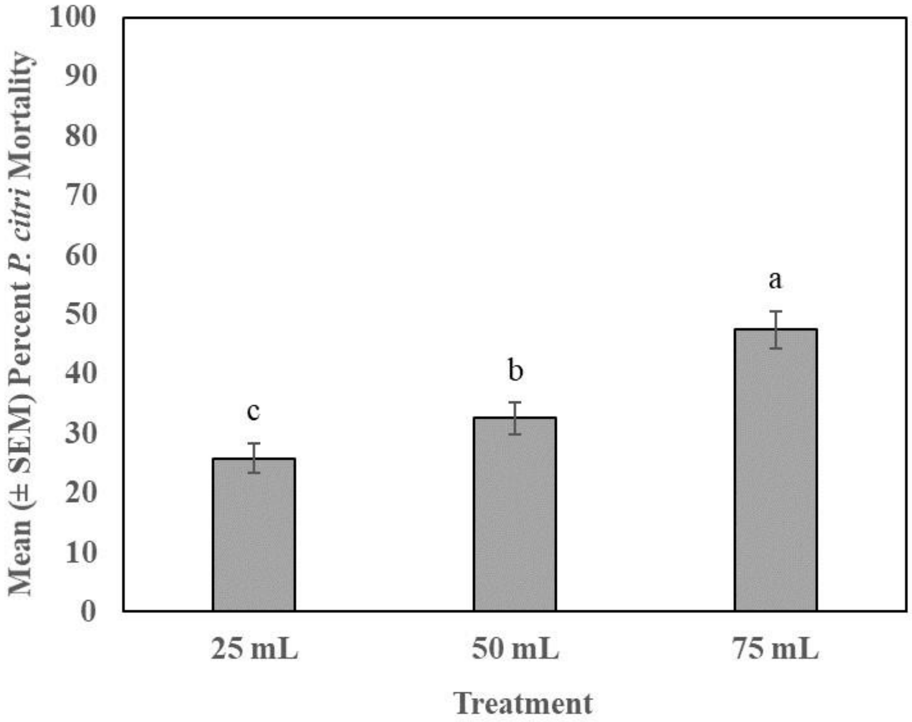

Figure 2.21 Mean (± SEM) percent citrus mealybug, *Planococcus citri*, mortality in experiment 5 after exposure to three weekly applications of the following treatments: 1) acetamiprid (TriStar®) at 0.19 g/946 mL, 2) flonicamid (Aria®) at 0.15 g/946 mL, and 3) cyfluthrin (Decathlon®) at 0.13 g/946 mL. Percent citrus mealybug mortality was averaged across the three insecticides for each spray volume. Means with the same letter are not significantly different (P>0.05) based on a Tukey's HSD test. Vertical bars represent the standard error of the mean (SEM).

Chapter 3 - Effect of spray volume and application frequency on insecticide efficacy against the western flower thrips (*Frankliniella occidentalis*) under greenhouse conditions

Introduction

Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is an insect pest of horticultural crops worldwide (Mouden et al., 2017; Robb and Parrella, 1995; Kirk, 2002; Reitz, 2009). Western flower thrips feeds on over 250 plant species within 60 different families (Tommasini and Maini, 1995). Direct and indirect damage are associated with western flower thrips feeding (Chisholm and Lewis, 1984; Harrewijn et al., 1996; Allen and Broadbent, 1986; Pappu et al., 2009). Adults and larvae cause direct damage by feeding on plants using their piercing-sucking mouthparts (Hunter and Ullman, 1989; Harrewijn et al., 1996). Direct damage is affiliated with leaf, flower, and fruit scarring, distortion and discoloration of flowers, fruit deformation, sunken leaf tissues, and a characteristic "silvering" of leaves and flowers (Cloyd, 2009; Chisholm and Lewis, 1984; Childers, 1997; van Dijken et al., 1994). Indirect damage is attributed to adults vectoring the tospoviruses: *Tomato spotted wilt* virus and *Impatiens necrotic spot* virus (Allen and Broadbent, 1986; Daughtrey et al., 1997; Kirk, 2002; Mound, 1995; Pappu et al., 2009). Direct and indirect damage can result in economic losses to producers of greenhouse-grown horticultural crops (Reitz and Funderburk, 2012; Goldbach and Peters, 1994).

Insecticides are commonly used to suppress western flower thrips populations because greenhouse producers have zero tolerance for any damage to leaves or flowers (Kontsedalov et al., 1998; Loughner et al., 2005; Cloyd, 2009; Mouden et al., 2017; Reitz and Funderburk, 2012).

However, several factors can influence the effectiveness of insecticide applications, including: spray coverage (Dibble, 1962; McClure, 1977; Shelton et al., 2003; Shelton et al., 2006; Tipping et al., 2003; Martini et al., 2012), frequency of application (Story and Sundstrom, 1986; Ajeigbe and Singh, 2006), and insecticide resistance (Bielza et al., 2007; Cloyd, 2016; Immaraju et al., 1992; Jensen, 2000; Loughner et al., 2005; Zhao et al., 1995). The failure of insecticides to suppress western flower thrips populations may be associated with insecticide resistance, yet operational factors, such as incomplete spray coverage, inappropriate application frequency, or poor application timing, could be responsible for insufficient pest suppression (Cloyd, 2016; Shelton et al., 2006).

Despite the importance of operational factors, no greenhouse studies have been conducted to assess the effect of spray coverage (associated with volume) and application frequency of insecticides against western flower thrips, *F. occidentalis*. Therefore, the objective of the following study was to determine how spray volume and application frequency impact the efficacy of insecticides against western flower thrips under greenhouse conditions. Insecticide efficacy was based on percent mortality of western flower thrips adults.

Materials and Methods

Western flower thrips colony

A laboratory colony of western flower thrips has been maintained for over 10 years inside Glad® (The Glad Products Company; Oakland, CA) plastic containers [20.4 x 14.4 x 9.4 cm (length x width x height)] with No-Thrips insect screening (mesh=150 x 150 microns: Green-Tek; Janesville, WI) at 24 to 27°C, 50 to 60% relative humidity, and constant light in a laboratory in the Department of Entomology at Kansas State University (Manhattan, KS). Green beans (*Phaseolus vulgaris*) were changed every two to three days to provide food for western flower thrips larvae as well as food and oviposition sites for adults.

Plant material and procedures

In the greenhouse experiments, yellow transvaal daisy (*Gerbera jamesonii*) cut flowers were used to test the effects of spray volume and application frequency on insecticide efficacy associated with western flower thrips. Yellow transvaal daisy cut flowers were obtained from a wholesale broker (Koehler & Dramm of Missouri; Kansas City, MO). No pesticides had been applied to the cut flowers before arrival, so the possibility of pesticide residues negatively affecting western flower thrips survival was not an issue. Transvaal daisy has been used as a model host flower in previous studies (Willmott et al., 2013; Cloyd and Raudenbush, 2014) due to their susceptibility to western flower thrips (Daughtrey et al., 1997) and because the disc florets provide a natural habitat for western flower thrips (Cloyd, 2009). Individual transvaal daisy cut flowers, each suitable for development and reproduction of western flower thrips, can be isolated from other flowers to avoid cross-contamination among experimental units (Cloyd and Gillespie, 2012; Willmott et al. 2013).

After arrival, flower stems were excised 5 to 6 cm below the base of the flower and placed into 22 mm low background borosilicate glass vials (Research Products International Corp.; Mt. Prospect, IL) containing tap water. The glass vials were inserted into 250 mL plastic containers with sand (Quikrete® Premium Play Sand). The sand inside the plastic container held the glass vial with tap water in an upright position throughout the course of each experiment. One transvaal daisy cut flower was placed into each glass vial containing tap water. All plastic containers with cut flowers inside the glass vials were positioned on a wire-mesh bench under a

50% black knit shade cloth (Hummert International; Earth City, MO) held in place by an open frame composed of polyvinyl chloride (PVC) piping in a research greenhouse. The black knit shade cloth helped preserve the longevity of the cut flowers by protecting them from direct sunlight. Plastic containers holding a cut flower were placed at least 20.3 cm apart from each other on the wire-mesh bench to mitigate the movement of western flower thrips adults among the cut flowers.

Each cut flower was artificially infested with 15 to 20 western flower thrips adults (about 20 days post-emergence) obtained from the laboratory colony. Western flower thrips adults were allowed to acclimate for one to two days before the designated treatments were applied to the flowers. The environmental conditions inside the greenhouse were 22 to 24°C, relative humidity between 60 and 70%, and natural daylight.

All insecticide treatments were mixed in 946 mL of tap water, and spray applications were made to all cut flowers using a 946 mL plastic spray bottle. One experiment evaluated spray volume with one application of the designated treatment to each flower. The other experiment tested the effect of application frequency (one application or two applications with a consistent spray volume). All flowers were treated with the spray bottle nozzle approximately 25.4 cm away from the flower.

For all experiments, there were four treatments including three insecticides registered for and commonly used against western flower thrips in greenhouses (R. Cloyd: personal communication) and a water control. Experiments were set up as a completely randomized design. One transvaal daisy cut flower was considered an experimental unit. The three insecticides and application rates were: spinosad (Conserve® SC: Dow AgroSciences LLC; Indianapolis, IN) at 11.0 fl oz/100 gallons, flonicamid (Aria®: FMC Corporation; Philadelphia, PA) at 2.1 oz/100 gallons, and chlorfenapyr (Pylon®: BASF Corp.; Research Triangle Park, NC) at 5.2 fl oz/100 gallons. Each insecticide has a different mode of action (IRAC, 2019; Table 3.1).

In the spray volume experiment, treatments were applied once to all flowers. Four or five days after the treatments were applied, flowers were harvested and placed into plastic Petri dishes (14 cm diameter) with lids and then emasculated (destructively sampled) under laboratory conditions. The number of live, dead, and total number of western flower thrips adults associated with each flower were recorded. Percent mortality was calculated by dividing the number of dead western flower thrips adults by the total number recovered from each flower and multiplying by 100.

In the application frequency experiment, all flowers were treated on the same day. Four or five days after the first treatment application, flowers designated to receive one application were harvested and placed into plastic Petri dishes (14 cm diameter) with lids and destructively sampled under laboratory conditions, and the number of live, dead, and total number of western flower thrips adults associated with each flower were recorded. Afterward, the flowers designated to receive two applications were sprayed a second time. Four or five days after the second application, the number of live, dead, and total number of western flower thrips adults associated with the remaining flowers were recorded. Percent mortality was calculated by dividing the number of dead western flower thrips adults by the total number recovered from each flower and multiplying by 100.

Experiment 1: Effect of application frequency on western flower thrips adults on transvaal daisy flowers

On April 8, 2019, transvaal daisy flower stems were excised, placed into glass vials, and positioned on a wire mesh bench in a research greenhouse. The flowers were artificially infested with western flower thrips adults from the laboratory colony (21 days post-emergence) on April 10, 2019. There were five replicates per treatment combination and 40 experimental units. Flowers were exposed to one or two treatment applications with a spray volume of 25 mL per flower for each application. All cut flowers were initially treated on April 12, 2019. Flowers designated to receive one application were destructively sampled on April 17, 2019 while the flowers designated to receive two applications were treated a second time. The remaining flowers were destructively sampled on April 22, 2019.

Experiment 2: Effect of spray volume on western flower thrips adults on transvaal daisy flowers

Transvaal daisy flowers were positioned in a research greenhouse on July 24, 2019. There were five replicates per treatment combination with 60 cut flowers used in the experiment. Each flower was artificially infested with western flower thrips adults from the laboratory colony (16 to 25 days post-emergence) on July 26, 2019. In this experiment, three different spray volumes were used: 5, 12.5, and 25 mL per flower. Each flower was treated on July 28, 2019. All flowers were harvested and destructively sampled on August 2, 2019.

Experiment 3: Effect of spray volume on western flower thrips adults on transvaal daisy flowers

On September 5, 2019, transvaal daisy flowers were positioned on a wire mesh bench in a research greenhouse. Four replicates per treatment combination, with 48 experimental units, were used instead of five replicates because of the limited number of western flower thrips adults available from the laboratory colony (900 western flower thrips adults needed for five replicates versus 720 western flower thrips adults needed for four replicates). Glass vials holding the flowers were filled to approximately 80% with tap water to preserve flower quality. All flowers were artificially infested with western flower thrips adults from the laboratory colony (17 and 19 days post-emergence) on September 7, 2019. Three different spray volumes were used: 2.5, 5, and 10 mL per flower. Treatments were applied once to each flower. All flowers were treated on September 8, 2019 and then destructively sampled on September 12, 2019.

Experiment 4: Effect of application frequency on western flower thrips adults on transvaal daisy flowers

Transvaal daisy flowers were positioned on a wire mesh bench in a research greenhouse on September 23, 2019. There were five replicates per treatment combination with 40 experimental units. Each flower was artificially infested with western flower thrips adults from the laboratory colony (21 days post-emergence) on September 25, 2019. A spray volume of 5 mL per flower was used for each application. All flowers received the first treatment application on September 26, 2019. Flowers designated to receive one application were destructively sampled on September 30, 2019 while the flowers designated to receive two applications were treated a second time. Flowers that received two applications were destructively sampled on October 4, 2019.

Experiment 5: Effect of spray volume on western flower thrips adults on transvaal daisy flowers

There were four replicates per treatment combination and 48 transvaal daisy flowers, which were placed on a wire mesh bench in a research greenhouse on December 4, 2019. Each flower was artificially infested with western flower thrips adults from the laboratory colony (21 days post-emergence) on December 6, 2019. Three different spray volumes were used: 2.5, 5, and 10 mL per flower. The designated treatments were applied to all flowers on December 7, 2019. On December 11, 2019, the flowers were harvested and destructively sampled under laboratory conditions.

Experiment 6: Effect of application frequency on western flower thrips adults on transvaal daisy flowers

Transvaal daisy flowers were placed on a wire mesh bench in a research greenhouse on February 17, 2020. There were five replicates per treatment combination resulting in 40 experimental units. Flowers were each artificially infested with western flower thrips adults obtained from the laboratory colony (23 days post-emergence) on February 19, 2020. Each treatment application used a spray volume of 5 mL per flower, which was the same spray volume used in experiment 4. All flowers were treated on February 20, 2020. Flowers designated to receive one application were destructively sampled on February 24, 2020 while the flowers designated to receive two applications were treated again. Flowers receiving two applications were destructively sampled on February 28, 2020.

Statistical analysis

There were two treatment factors for each experiment: treatment (three insecticides and a water control) (4 levels) and spray volume (3 levels) or application frequency (2 levels). If model assumptions were met, percent mortality estimates of western flower thrips adults were subject to a two-way analysis of variance (ANOVA) with treatment and spray volume or application frequency as the main effects. Statistical models were fitted using PROC GLIMMIX in a SAS software program (SAS Institute, 2012) with significant treatment means being separated using Tukey's honestly significant difference (HSD) test at *P*=0.05. Pairwise comparisons were conducted using Tukey's HSD adjustment to avoid type I error rate inflation.

Results

Experiment 1: Effect of application frequency on western flower thrips adults on transvaal daisy flowers

In this experiment, transvaal daisy flowers were exposed to one or two treatment applications at a spray volume of 25 mL per flower. A two-way ANOVA was not performed due to a lack of variability among the data for certain treatments. Mean percent western flower thrips adult mortality for spinosad and chlorfenapyr was 100% after one application (n=76 for spinosad and n=107 for chlorfenapyr) and two applications (n=56 for spinosad and n=74 for chlorfenapyr) (Figure 3.1). At the 25 mL spray volume, mean percent western flower thrips adult mortality associated with one flonicamid application was 83.9% (n=85) and 98.3% (n=44) for two applications (Figure 3.1). Live western flower thrips larvae were observed on all flowers, except for those treated with spinosad. The flowers treated with water had more than 20 larvae per flower.

Experiment 2: Effect of spray volume on western flower thrips adults on transvaal daisy flowers

Twenty-five mL was used as the highest volume for experiment 2 because of the high western flower thrips mortality affiliated with this volume in experiment 1. A two-way ANOVA was not performed due to the lack of variability in the data for certain treatments. Mean percent western flower thrips adult mortality was 100% for spinosad applied at 12.5 mL (n=32) and chlorfenapyr applied at 25 mL (n=36) (Figure 3.2). The largest difference in mean percent western flower thrips adult mortality was between flonicamid applied at 12.5 mL (53%; n=16) and at 25 mL (91.4%; n=19) (Figure 3.2). The results obtained may have been due to poor flower quality, which can affect the recovery of western flower thrips adults (D. Radosevich: personal observation). Live western flower thrips larvae were observed on flowers from every treatment, and flowers treated with water had more than 20 larvae per flower.

Experiment 3: Effect of spray volume on western flower thrips adults on transvaal daisy flowers

A two-way ANOVA was performed for experiment 3 because all treatment groups had a variance component. The two-way interaction between treatment and spray volume was not significant (F=2.05; df=6, 36; P=0.084). However, the main effects of spray volume (F=9.83; df=2, 36; P=0.0004) and treatment (F=92.34; df=3, 36; P<0.0001) associated with percent western flower thrips adult mortality were significant. Within each insecticide treatment, an increase in spray volume resulted in a higher percent western flower thrips adult mortality. Mean percent western flower thrips adult mortality on flowers treated with spinosad was similar, regardless of the spray volume (Figure 3.3). For chlorfenapyr and flonicamid, there was a significant increase in percent western flower thrips adult mortality when 10 mL was applied compared to when 2.5 mL was applied (Figure 3.3). Mortality for the water control was 21.9% (n=95), 29.4% (n=86), and 25.9% (n=106) for the 2.5, 5, and 10 mL spray volumes, respectively. Mean percent western flower thrips adult mortality for flonicamid applied at 2.5 mL was not significantly different from the water control (Figure 3.3). No live western flower thrips larvae were observed on flowers treated with spinosad at 10 mL, but live western flower thrips larvae were observed on flowers from all other treatment groups. Seven of the twelve flowers in the water control had more than 50 western flower thrips larvae per flower.

Experiment 4: Effect of application frequency on western flower thrips adults on transvaal daisy flowers

Transvaal daisy flowers were exposed to one or two treatment applications at a spray volume of 5 mL per flower. A two-way ANOVA was not performed due to a lack of variability in the data associated with certain treatments. After two applications, mean percent western flower thrips adult mortality was 100% for spinosad (n=62) and chlorfenapyr (n=56) (Figure 3.4). At the 5 mL spray volume, mean percent western flower thrips adult mortality after one flonicamid application was 83.3% (n=69), and the mortality affiliated with two flonicamid applications was 94% (n=57) (Figure 3.4). For all three insecticides, mean percent western flower thrips adult mortality increased as the number of applications increased (Figure 3.4). However, mean percent western flower thrips adult mortality for flowers that received two water applications was 36% (n=33), which may be associated with poor flower quality at the time of destructive sampling. Ten or more live western flower thrips larvae were observed on all flowers after one application, regardless of treatment. No live western flower thrips larvae were observed on flowers that received two spinosad applications, but there were at least five live western flower thrips larvae on flowers that received two applications of chlorfenapyr, flonicamid, or water. Flowers treated with water had more than 20 western flower thrips larvae per flower.

Experiment 5: Effect of spray volume on western flower thrips adults on transvaal daisy flowers

The spray volumes used in the experiment were the same as those used in experiment 3. A two-way ANOVA was not performed because certain treatments had zero variance. Mean percent western flower thrips adult mortality was 100% for spinosad applied at 5 mL (n=46) and 10 mL (n=62) (Figure 3.5). As spray volume increased within the insecticide treatments, percent western flower thrips adult mortality also increased (Figure 3.5). Mean mortality for all three spray volumes in the water control never exceeded 20% (n=200). Morbid, immobile western flower thrips larvae were observed on all flowers treated with 10 mL of spinosad, chlorfenapyr, or flonicamid. No live western flower thrips larvae were found on flowers treated with spinosad at any spray volume. Less than 10 live western flower thrips larvae were observed on flowers treated with 10 mL of chlorfenapyr or flonicamid. More than 20 western flower thrips larvae were present on flowers associated with the water control.

Experiment 6: Effect of application frequency on western flower thrips adults on transvaal daisy flowers

Data were not subject to a two-way ANOVA due to a lack of variability associated with certain treatments. After two applications, the mean percent western flower thrips adult mortality was 100% (n=64) for spinosad (Figure 3.6). Mean percent western flower thrips adult mortality for all treatments increased as the number of applications increased (Figure 3.6). There was a difference in mean percent western flower thrips adult mortality between one application (40.9%; n=91) and two applications (68.1%; n=50) of flonicamid and between one application (72%; n=102) and two applications (98.8%; n=92) of chlorfenapyr (Figure 3.6). No live western flower thrips larvae were observed on flowers treated with spinosad, regardless of the application frequency. Flowers treated with one water application had less than 50 western flower thrips larvae per flower. However, more than 100 western flower thrips larvae were observed on each flower that received two water applications. There were less than 50 western flower thrips larvae on all flowers treated with chlorfenapyr or flonicamid, and flowers sampled after one chlorfenapyr application had less than 10 live larvae per flower.

Discussion

This is the first study to investigate the effects of spray volume and application frequency on the efficacy of insecticides against western flower thrips under greenhouse conditions. Results from the study emphasize the importance of operational factors, which will allow greenhouse producers to more effectively manage western flower thrips populations with insecticides. The efficacy of three insecticides, each with a different mode of action, were evaluated. It is important to note that the western flower thrips colony used in the study has never been exposed to insecticides, which may explain why the western flower thrips were highly susceptible to certain insecticides.

In the application frequency experiments, two applications resulted in a higher percent mortality of western flower thrips adults than one application for insecticides. After two applications, 100% western flower thrips adult mortality was associated with spinosad and >98% mortality was affiliated with chlorfenapyr. In experiment 6, there was a 27.2% increase in western flower thrips adult mortality between one and two applications of flonicamid and a 26.8% increase in adult mortality between one and two applications of chlorfenapyr. However, increasing the frequency of insecticide applications may promote the development of resistance in western flower thrips populations, especially if insecticides with the same mode of action are used in succession (Bielza, 2008; Loughner et al., 2005; Kontsedalov et al., 1998). Consequently, in order to avoid issues with insecticide resistance, greenhouse producers must develop rotation programs that abstain from using insecticides with the same mode of action (Cloyd, 2016; Gao et al., 2012; Loughner et al., 2005; Mouden et al., 2017). When determining the number of insecticide applications per growing season, greenhouse producers should consider the effect of application frequency on resistance development in western flower thrips populations (Bielza, 2008). In fact, many insecticide labels contain information on the number of applications that are allowed within a growing season or cropping cycle, which is designed to mitigate resistance.

In the spray volume experiments, there was a difference in percent western flower thrips adult mortality between the 2.5 mL spray volume and the 10 mL spray volume for chlorfenapyr and flonicamid. The effect of spray volume on the efficacy of spinosad was less apparent because there was >95% mortality of western flower thrips adults in all experiments, even for the 2.5 mL spray volume. Insect pest mortality can be increased by obtaining thorough spray coverage of plant parts with insecticides (Dibble, 1962; McClure, 1977; Shelton et al., 2003; Tipping et al., 2003; Martini et al., 2012). In general, mean percent western flower thrips adult mortality increased as spray volume increased for each insecticide. Results from these experiments suggest that higher spray volumes of insecticides can increase the mortality of western flower thrips adults. Western flower thrips occupy disc florets (small tubular flowers located in the center of the flower head of certain plants in the family Compositae), unopened buds, and developing leaves, which decreases their exposure to contact insecticides (Cloyd, 2009; Loughner et al., 2005). Therefore, high volume insecticide applications are needed to contact individual western flower thrips inside flowers.

Based on percent western flower thrips adult mortality, spinosad and chlorfenapyr were generally more effective against western flower thrips adults than flonicamid. The higher efficacy of spinosad and chlorfenapyr may be associated with their modes of action. For example, flonicamid is a selective feeding blocker causing starvation by inhibiting the ingestion of phloem (Cho et al., 2011; Morita et al., 2014). However, this mode of action may increase the time required to kill western flower thrips, especially in flowers. Flowers were destructively sampled four or five days after the last insecticide application, which may not have been enough time for flonicamid to induce sufficient mortality of western flower thrips adults. In contrast, spinosad acts on the nicotinic acetylcholine receptors in the central nervous system (Geng et al., 2013; Watson et al., 2010) whereas chlorfenapyr uncouples oxidative phosphorylation in the mitochondria, which disrupts cellular ATP (adenosine triphosphate) production (Raghavendra et al., 2011; Yu, 2008). The modes of action of spinosad and chlorfenapyr may result in faster mortality of western flower thrips adults.

Spinosad is highly toxic to non-resistant populations of western flower thrips (Jones et al., 2005; Loughner et al., 2005; Warnock and Cloyd, 2005; Willmott et al., 2013). The current study confirms the ability of spinosad to suppress susceptible western flower thrips populations. Spinosad resulted in >95% western flower thrips adult mortality in all experiments, even when applied at 2.5 mL. Spinosad can provide suppression of insect pests for up to 28 days after an application (Tomkins et al., 1999), which may explain why no live western flower thrips larvae were recovered from flowers treated with spinosad at spray volumes between 10 and 25 mL.

Operational factors can influence the success of insect pest management programs. This study has demonstrated that spray volume and application frequency can improve the efficacy of insecticides, based on percent mortality, against western flower thrips. Additional studies should compare the effects of spray volume and application frequency associated with insecticides against laboratory-reared and field populations of western flower thrips. This will determine how insecticide spray volume and application frequency impact the mortality of field populations of western flower thrips that greenhouse producers are more likely to encounter. In addition, future studies are needed to assess the efficacy of other insecticides, such as entomopathogenic organisms (fungi and bacteria), in regards to spray volume and application frequency against western flower thrips.

Insecticides are the primary management strategy used against western flower thrips (Bielza et al., 2007; Kontsedalov et al., 1998; Cloyd, 2009; Mouden et al., 2017; Reitz and Funderburk, 2012). Therefore, improvements in operational factors will benefit greenhouse producers by improving insecticide efficacy against western flower thrips and possibly other insect and mite pests. The implementation of effective operational factors can result in higher mortality of western flower thrips, which will effectively suppress western flower thrips populations, reduce plant damage induced by western flower thrips, and lower inputs and labor associated with insecticides.

Table 3.1 Insecticide treatments (active ingredient, trade name, rate used, and mode of action) used in the spray volume and application frequency experiments associated with western flower thrips, *Frankliniella occidentalis*.

Active Ingredient	Trade Name	Rate Used	Mode of Action*
Spinosad	Conserve®	0.81 mL/946 mL	Nicotinic acetylcholine receptor allosteric modulator-Site 1
Chlorfenapyr	Pylon®	0.38 mL/946 mL	Uncoupler of oxidative phosphorylation via disruption of the proton gradient
Flonicamid	Aria®	0.15 g/946 mL	Chordotonal organ modulator

*Modes of action are based on the classification scheme of the Insecticide Resistance Action Committee (IRAC, 2019).

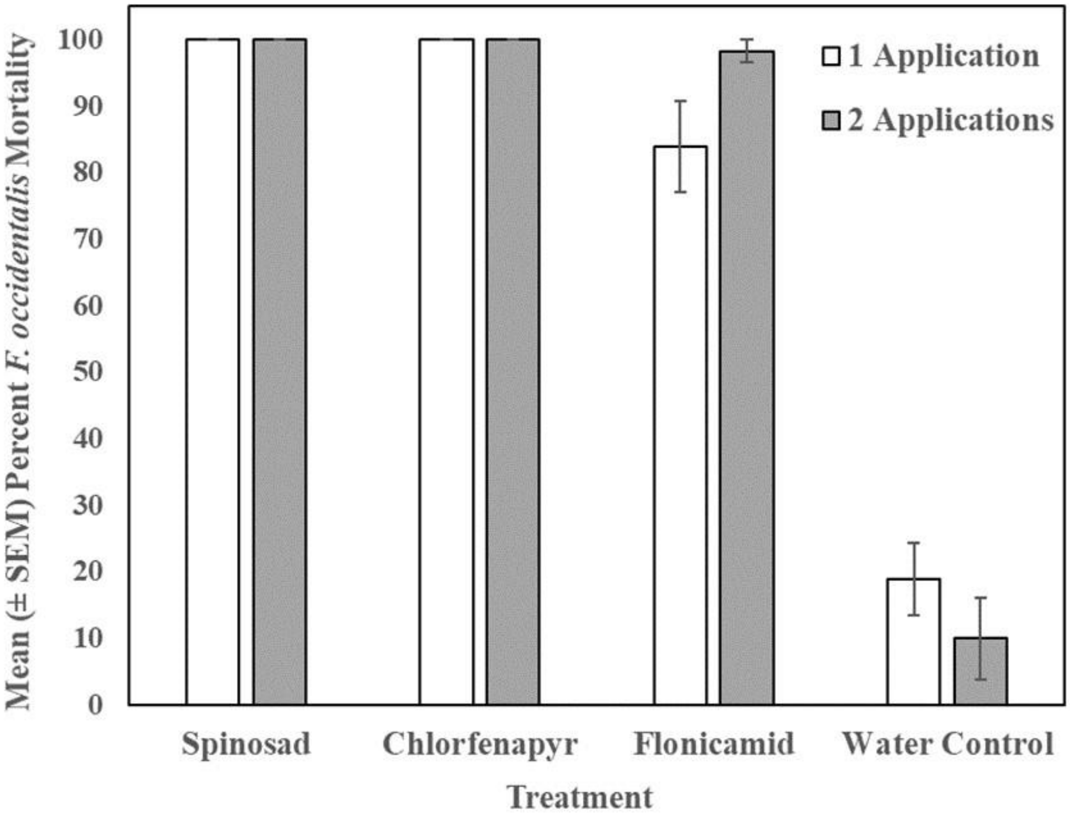

Figure 3.1 Mean (± SEM) percent adult western flower thrips, *Frankliniella occidentalis*, mortality in experiment 1 after exposure to one or two applications at 25 mL of spray solution per flower associated with the following treatments: 1) spinosad (Conserve®) at 0.81 mL/946 mL, 2) chlorfenapyr (Pylon®) at 0.38 mL/946 mL, 3) flonicamid (Aria®) at 0.15 g/946 mL, and 4) water control. Vertical bars represent the standard error of the mean (SEM).

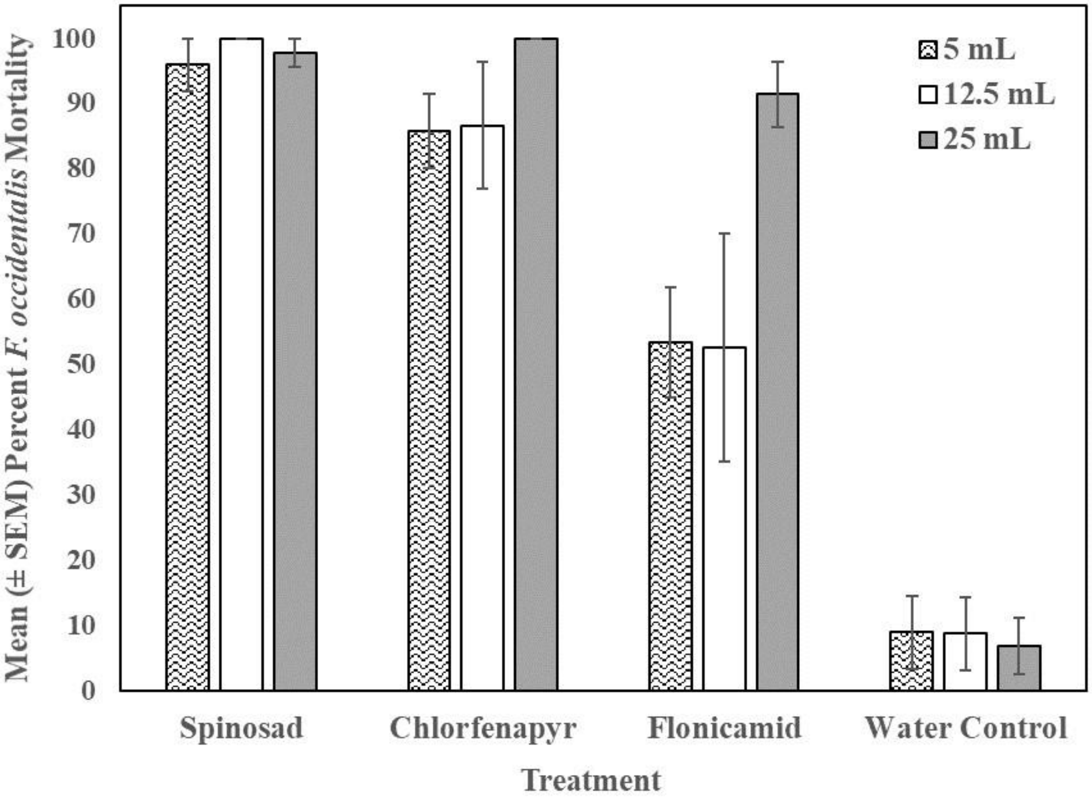

Figure 3.2 Mean (± SEM) percent adult western flower thrips, *Frankliniella occidentalis*, mortality in experiment 2 after exposure to one application at spray volumes of 5, 12.5, or 25 mL associated with the following treatments: 1) spinosad (Conserve®) at 0.81 mL/946 mL, 2) chlorfenapyr (Pylon®) at 0.38 mL/946 mL, 3) flonicamid (Aria®) at 0.15 g/946 mL, and 4) water control. Vertical bars represent the standard error of the mean (SEM).

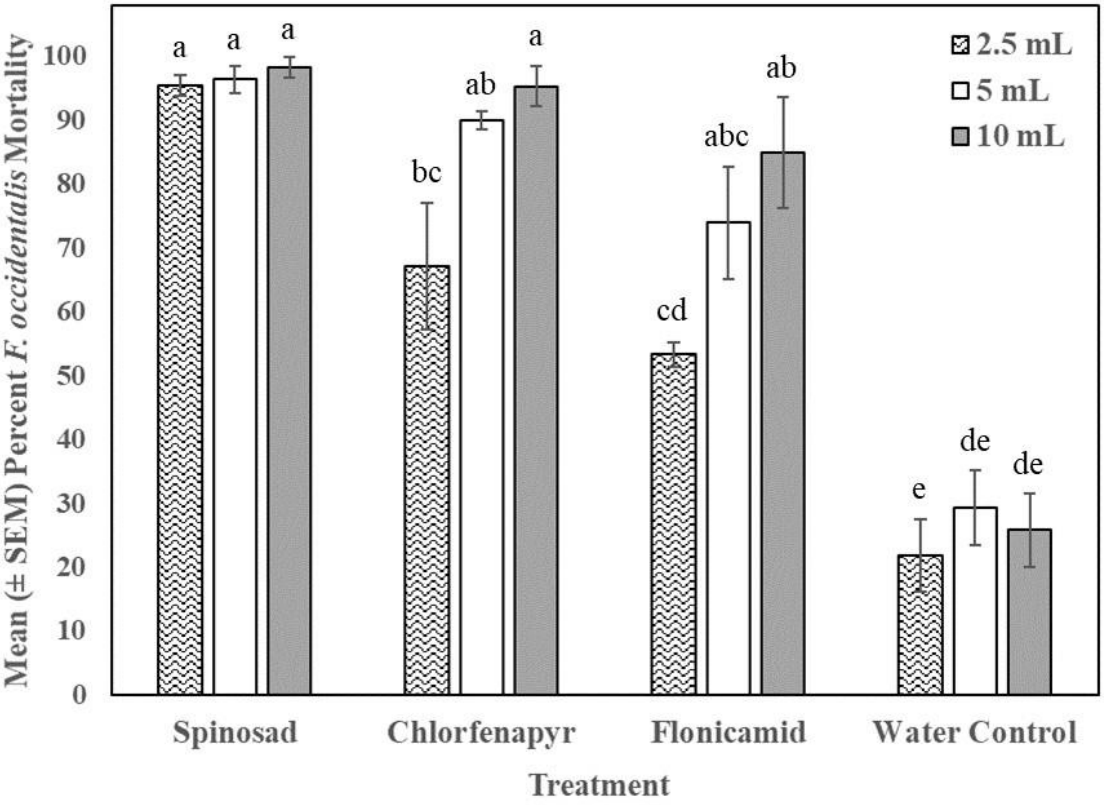

Figure 3.3 Mean (± SEM) percent adult western flower thrips, *Frankliniella occidentalis*, mortality in experiment 3 after exposure to one application at spray volumes of 2.5, 5, or 10 mL associated with the following treatments: 1) spinosad (Conserve®) at 0.81 mL/946 mL, 2) chlorfenapyr (Pylon®) at 0.38 mL/946 mL, 3) flonicamid (Aria®) at 0.15 g/946 mL, and 4) water control. Means with the same letter are not significantly different (*P*>0.05) based on a Tukey's HSD test. Vertical bars represent the standard error of the mean (SEM).

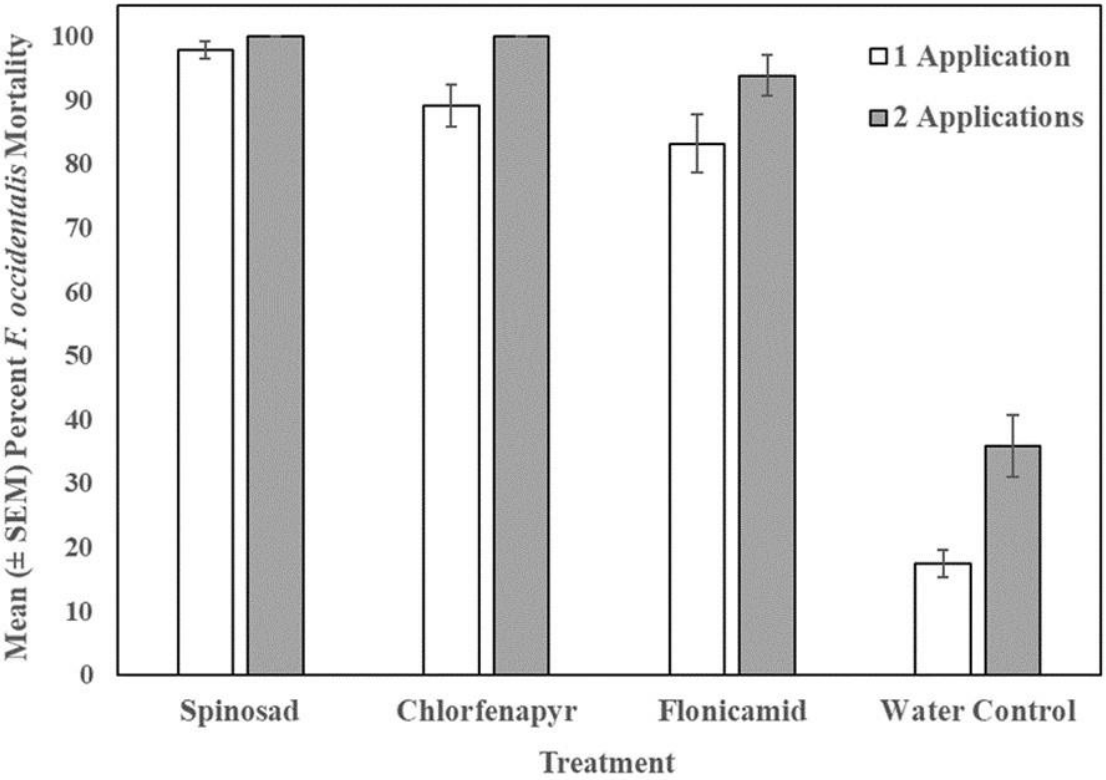

Figure 3.4 Mean (± SEM) percent adult western flower thrips, *Frankliniella occidentalis*, mortality in experiment 4 after exposure to one or two applications at 5 mL of spray solution per flower associated with the following treatments: 1) spinosad (Conserve®) at 0.81 mL/946 mL, 2) chlorfenapyr (Pylon®) at 0.38 mL/946 mL, 3) flonicamid (Aria®) at 0.15 g/946 mL, and 4) water control. Vertical bars represent the standard error of the mean (SEM).

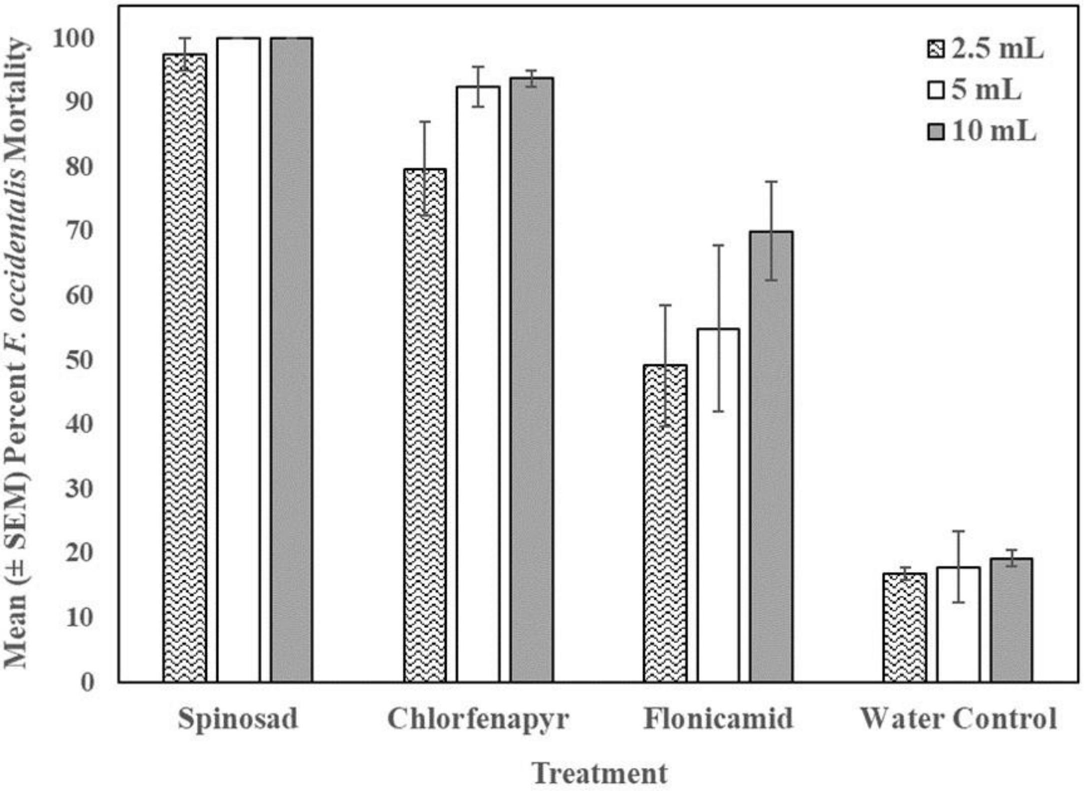

Figure 3.5 Mean (± SEM) percent adult western flower thrips, *Frankliniella occidentalis*, mortality in experiment 5 after exposure to one application at spray volumes of 2.5, 5, or 10 mL associated with the following treatments: 1) spinosad (Conserve®) at 0.81 mL/946 mL, 2) chlorfenapyr (Pylon®) at 0.38 mL/946 mL, 3) flonicamid (Aria®) at 0.15 g/946 mL, and 4) water control. Vertical bars represent the standard error of the mean (SEM).

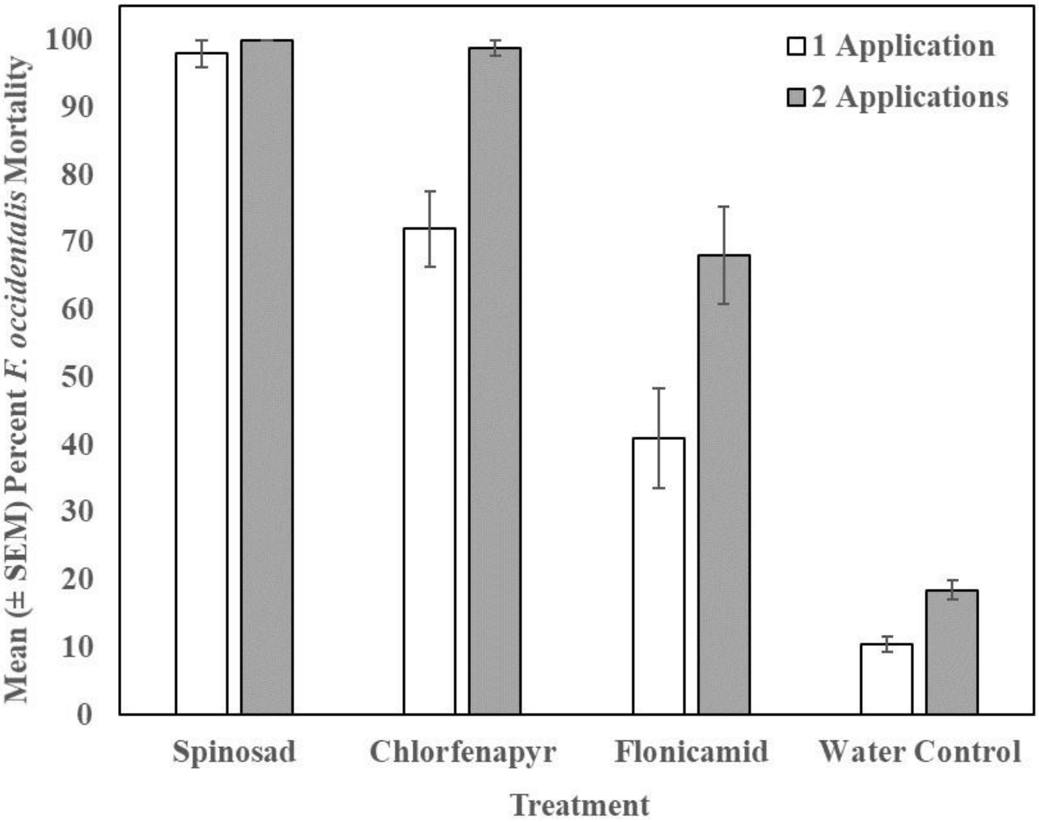

Figure 3.6 Mean (± SEM) percent adult western flower thrips, *Frankliniella occidentalis*, mortality in experiment 6 after exposure to one or two applications at 5 mL of spray solution per flower associated with the following treatments: 1) spinosad (Conserve®) at 0.81 mL/946 mL, 2) chlorfenapyr (Pylon®) at 0.38 mL/946 mL, 3) flonicamid (Aria®) at 0.15 g/946 mL, and 4) water control. Vertical bars represent the standard error of the mean (SEM).

Chapter 4 - Summary and Conclusions

The objectives of the current study were to determine how spray coverage (associated with volume) and application frequency influence insecticide efficacy against the citrus mealybug, *Planococcus citri*, and the western flower thrips, *Frankliniella occidentalis*. Experiments were conducted under greenhouse conditions to evaluate the effect of spray volume and application frequency on insecticide efficacy against citrus mealybug on coleus (*Solenostemon scutellarioides*) and transvaal daisy (*Gerbera jamesonii*) plants and western flower thrips on transvaal daisy cut flowers. For each insect pest, three different spray volumes, two application frequencies (one or two applications), and three insecticides with different modes of action were used.

In the citrus mealybug experiments, the insecticides acetamiprid, flonicamid, and cyfluthrin were used. Results from the spray volume experiments demonstrated that higher spray volumes lead to better coverage of aboveground plant parts (leaves and stems) based on percent citrus mealybug mortality, thus improving insecticide efficacy. Mean citrus mealybug mortality was consistently higher for the 75 mL spray volume than the 15 and 25 mL spray volumes. In addition, citrus mealybug mortalities associated with the insecticides applied at 15 or 25 mL were often similar to the water control mortalities. In the application frequency experiments, mean percent citrus mealybug mortality was significantly higher after two insecticide applications (51.2% in experiment 2 and 42.7% in experiment 3) than after one insecticide application (40.3% in experiment 2 and 36.1% in experiment 3). Overall, acetamiprid was more effective than flonicamid and cyfluthrin against citrus mealybugs, based on percent mortality. For instance, acetamiprid applied at 75 mL resulted in >70% mean citrus mealybug mortality in three of the spray volume experiments. In contrast, mean citrus mealybug mortality was <50%

for flonicamid and cyfluthrin across all experiments. Host plant architecture (height and number of leaves and branches) may have affected spray coverage in the citrus mealybug experiments. For instance, the 25 and 50 mL spray volumes may not have provided sufficient coverage of the aboveground parts (leaves and stems) of coleus and transvaal daisy plants based on percent mortality of citrus mealybugs.

In the western flower thrips experiments, the insecticides spinosad, chlorfenapyr, and flonicamid were used. In general, mean western flower thrips adult mortality increased as the spray volume of each insecticide increased, which suggests that better spray coverage improves insecticide efficacy. Application frequency also affected western flower thrips adult mortality. For example, spinosad resulted in 100% mortality and chlorfenapyr resulted in >98% mortality after two applications. There was a 27.2% increase in western flower thrips mortality between one and two applications of flonicamid and a 26.8% increase in mortality between one and two applications of chlorfenapyr in experiment 6. Spinosad applications resulted in >95% mean western flower thrips adult mortality across all experiments, even when 2.5 mL was applied. Based on percent mortality, flonicamid was less effective against western flower thrips adults than spinosad and chlorfenapyr. The 10 and 25 mL spray volumes resulted in the highest percent mortality of western flower thrips adults on transvaal daisy cut flowers. In addition, two applications resulted in higher mean percent western flower thrips adult mortality than one application for each of the insecticides.

Insecticide type may have influenced the abundance of the immature (nymphs or larvae) stages of citrus mealybugs and western flower thrips. Flowers that received two applications or spray volumes of >10 mL of spinosad had no live western flower thrips larvae. However, live western flower thrips larvae were present on all flowers treated with chlorfenapyr, flonicamid, or the water control. In general, plants treated with acetamiprid or flonicamid had fewer first-instar citrus mealybug nymphs (less than 50 per plant) than plants treated with cyfluthrin or the water control, which had more than 100 first-instar nymphs per plant. No live first-instar citrus mealybug nymphs were observed on plants treated with 75 mL of acetamiprid.

This study has shown that spray volume and application frequency can enhance insecticide efficacy by increasing mortality of citrus mealybugs and western flower thrips. Since insecticides are the primary management strategy used against citrus mealybugs and western flower thrips in greenhouse production systems, greenhouse producers will benefit from improvements in operational factors, which will lead to effective suppression of insect pest populations. Improved insecticide application methods will help greenhouse producers successfully manage insect pest populations, avoid economic losses by preventing plant damage, and reduce inputs and labor costs associated with insecticides.

References

Ahmed, N. H., and S. M. Abd-Rabou. 2010. Host plants, geographical distribution, natural enemies and biological studies of the citrus mealybug, *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae). Egyptian Acad. J. Biol. Sci. 3: 39-47.

Ajeigbe H. A., and B. B. Singh. 2006. Integrated pest management in cowpea: effect of time and frequency of insecticide application on productivity. Crop Protec. 25: 920–925.

Allen, W. R., and A. Broadbent. 1986. Transmission of tomato spotted wilt virus in Ontario greenhouses by *Frankliniella occidentalis*. Can. J. Plant Pathol. 8: 33-38.

Bielza, P. 2008. Insecticide resistance management strategies against the western flower thrips, *Frankliniella occidentalis*. Pest Manag. Sci. 64: 1131-1138.

Bielza, P., V. Quinto, J. Contreras, M. Torné, A. Martín, and P. J. Espinosa. 2007. Resistance to spinosad in the western flower thrips, *Frankliniella occidentalis* (Pergande), in greenhouses of south-eastern Spain. Pest Manag. Sci. 63: 682-687.

Buchholz, A., and R. Nauen. 2002. Translocation and translaminar bioavailability of two neonicotinoid insecticides after foliar application to cabbage and cotton. Pest Manag. Sci. 58: 10-16.

Cabaleiro, C., and A. Segura. 1997. Field transmission of grapevine leafroll associated virus 3 (GLRaV-3) by the mealybug *Planococcus citri*. Plant Dis. 81: 283-287.

Charles, J. G. 1982. Economic damage and preliminary economic thresholds for mealybugs (*Pseudococcus longispinus* TT.) in Auckland vineyards. New Zealand J. Agric. Res. 25: 415-420.

Chen, Y., K. A. Williams, B. K. Harbaugh, and M. L. Bell. 2004. Effects of tissue phosphorus and nitrogen in *Impatiens wallerana* on western flower thrips (*Frankliniella occidentalis*) population levels and plant damage. HortScience 39: 545-550.

Childers, C. C. 1997. Feeding and oviposition injuries to plants, pp. 505-537. In: T. Lewis [ed.]. Thrips as Crop Pests. CAB International, New York, NY.

Chisholm, I., and T. Lewis. 1984. A new look at thrips (Thysanoptera) mouthparts, their action and effects of feeding on plant tissue. Bull. Entomol. Res. 74: 663-675.

Cho, S. R., H. N. Koo, C. Yoon, and G. H. Kim. 2011. Sublethal effects of flonicamid and thiamethoxam on green peach aphid, *Myzus persicae* and feeding behavior analysis. J. Korean Soc. App. Biol. Chem. 54: 889-898.

Cloyd, R. A. 2009. Western flower thrips (*Frankliniella occidentalis*) management on ornamental crops grown in greenhouses: have we reached an impasse? Pest Tech. 3: 1-9.

Cloyd, R. A. 2015. Western flower thrips management in greenhouse production systems in the 21st century: alternative strategies need to be considered. Acta Hortic. 1104: 381-394.

Cloyd, R. A. 2016. Western flower thrips (Thysanoptera: Thripidae) and insecticide resistance: an overview and strategies to mitigate insecticide resistance development. J. Entomol. Sci. 51: 257-273.

Cloyd, R. A., and A. L. Raudenbush. 2014. Efficacy of binary pesticide mixtures against western flower thrips. HortTech. 24: 449-456.

Cloyd, R. A., and C. S. Sadof. 2000. Effects of plant architecture on the attack rate of *Leptomastix dactylopii* (Hymenoptera: Encyrtidae), a parasitoid of the citrus mealybug (Homoptera: Pseudococcidae). Environ. Entomol. 29: 535-541.

Cloyd, R. A., and J. D. Gillespie. 2012. Effect of sugar-based compounds in enhancing the efficacy of insecticides against the western flower thrips. HortTech. 22: 177-184.

Copeland, M. J. W., C. C. D. Tingle, M. Saynor, and A. Panis. 1985. Biology of glasshouse mealybugs and their predators and parasitoids, pp. 82-86. In: Hussey, N. W., and N. Scopes [eds.]. Biological Control: The Glasshouse Experience. Cornell Univ. Press, Ithaca, NY.

Cox, J. M., and M. J. Pearce. 1983. Wax produced by dermal pores in three species of mealybug (Homoptera: Pseudococcidae). Intl. J. Insect Morphol. Embryol. 12: 235-248.

Daughtrey, M. L., R. K. Jones, J. W. Moyer, M. E. Daub, and J. R. Baker. 1997. Tospoviruses strike the greenhouse industry: INSV has become a major pathogen on flower crops. Plant Dis. 81: 1220-1230.

Denholm, I., M. Cahill, T. J. Dennehy, and A. R. Horowitz. 1998. Challenges with managing insecticide resistance in agricultural pests, exemplified by the whitefly *Bemisia tabaci*. Phil. Trans. Royal Soc. London. Series B: Biol. Sci. 353: 1757-1767.

Dibble, J. 1962. Insecticide application and coverage. Calif. Agric. 16: 8-9.

Dole, J. M., and H. F. Wilkins. 2005. Gerbera, pp. 545-552. Floriculture: Principles and Species, 2nd edition. Pearson/Prentice Hall, Upper Saddle River, NJ.

Flaherty, D., W. Peacock, L. Bettiga, and G. Leavitt. 1982. Chemicals losing effect against grape mealybug. Calif. Agric. 36: 15-16.

Fondren, K. M., and D. G. McCullough. 2005. Phenology, natural enemies, and efficacy of horticultural oil for control of *Chionaspis heterophyllae* (Homoptera: Diaspididae) on Christmas tree plantations. J. Econ. Entomol. 98: 1603-1613.

Franco, J. C., A. Zada, and Z. Mendel. 2009. Novel approaches for the management of mealybug pests, pp. 233-278. In: Ishaaya, I., and A. R. Horowitz [eds.]. Biorational Control of

Arthropod Pests: Application and Resistance Management. Springer Science and Business Media B. V., New York, NY.

Furlan, L., A. Pozzebon, C. Duso, N. Simon-Delso, F. Sánchez-Bayo, P. A. Marchand, F. Codato, M. Bijleveld van Lexmond, and J. M. Bonmatin. 2018. An update of the Worldwide Integrated Assessment (WIA) on systemic insecticides. Part 3: alternatives to systemic insecticides. Environ. Sci. Poll. Res. 1-23.

Gao, Y., Z. Lei, and S. R. Reitz. 2012. Western flower thrips resistance to insecticides: detection, mechanisms and management strategies. Pest Manag. Sci. 68: 1111-1121.

Gaum, W. G., J. H. Giliomee, and K. L. Pringle. 1994. Life history and life tables of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae) on English cucumbers. Bull. Entomol. Res. 84: 219-224.

Geng, C., G. B. Watson, and T. C. Sparks. 2013. Nicotinic acetylcholine receptors as spinosyn targets for insect pest management. Advan. Insect Physiol. 44: 101-210.

Goldbach, R., and D. Peters. 1994. Possible causes of the emergence of tospovirus diseases. Sem. Virol. 5: 113-120.

Gullan, P. J., and M. Kosztarab. 1997. Adaptations in scale insects. Ann. Rev. Entomol. 42: 23-50.

Harrewijn, P., W. F. Tjallingll, and C. Mollema. 1996. Electrical recording of plant penetration by western flower thrips. Entomol. Exp. Appl. 79: 345-353.

Herrick, N. J., and R. A. Cloyd. 2017. Effect of systemic insecticides on the citrus mealybug (Hemiptera: Pseudococcidae) feeding on coleus. J. Entomol. Sci. 52: 104-118.

Herrick, N. J., R. A. Cloyd, and A. L. Raudenbush 2019. Systemic insecticide applications: effects on citrus mealybug (Hemiptera: Pseudococcidae) populations under greenhouse conditions. J. Econ. Entomol. 112: 266-276.

Hogendorp, B. K., and R. A. Cloyd. 2013. Effect of potassium bicarbonate (MilStop®) and insecticides on the citrus mealybug, *Planococcus citri* (Risso), and the natural enemies *Leptomastix dactylopii* (Howard) and *Cryptolaemus montrouzieri* (Mulsant). HortScience 48: 1513-1517.

Hogendorp, B. K., R. A. Cloyd, and J. M. Swiader. 2006. Effect of nitrogen fertility on reproduction and development of citrus mealybug, *Planococcus citri* Risso (Homoptera: Pseudococcidae), feeding on two colors of coleus, *Solenostemon scutellarioides* L. Codd. Environ. Entomol. 35: 201-211.

Holmes, N., J. Bennison, K. Maulden, and W. Kirk. 2012. The pupation behaviour of the western flower thrips, *Frankliniella occidentalis* (Pergande). Acta Phytopathol. Entomol. Hung. 47: 87-96.

Horowitz, A. R., Z. Mendelson, P. G. Weintraub, and I. Ishaaya. 1998. Comparative toxicity of foliar and systemic applications of acetamiprid and imidacloprid against the cotton whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae). Bull. Entomol. Res. 88: 437-442.

Hunter, W. B., and D. E. Ullman. 1989. Analysis of mouthpart movements during feeding of *Frankliniella occidentalis* (Pergande) and *F. schultzei* (Trybom) (Thysanoptera: Thripidae). Intl. J. Insect Morphol. Embryol. 18: 161-171.

Immaraju, J. A., T. D. Paine, J. A. Bethke, K. L. Robb, and J. P. Newman. 1992. Western flower thrips (Thysanoptera: Thripidae) resistance to insecticides in coastal California greenhouses. J. Econ. Entomol. 85: 9-14.

Insecticide Resistance Action Committee (IRAC). June, 2019 (Version 9.3). Accessed: 25 July 2019. IRAC Mode of Action Classification Scheme. www.irac-online.org

Jensen, S. E. 2000. Insecticide resistance in the western flower thrips, *Frankliniella occidentalis*. Integ. Pest Manag. Rev. 5: 131-146.

Jones, T., C. Scott-Dupree, R. Harris, L. Shipp, B. Harris. 2005. The efficacy of spinosad against the western flower thrips, *Frankliniella occidentalis*, and its impact on associated biological control agents on greenhouse cucumbers in southern Ontario. Pest Manag. Sci. 61: 179-185.

Kirk, W. D. J. 2002. The pest of the west: *Frankliniella occidentalis*. Thrips and Tospoviruses: Proc. 7th Intl. Symp. Thysanoptera 2: 33-42.

Kontsedalov, S., P. G. Weintraub, A. R. Horowitz, and I. Ishaaya. 1998. Effects of insecticides on immature and adult western flower thrips (Thysanoptera: Thripidae) in Israel. J. Econ. Entomol. 91: 1067-1071.

Laflin, H. M., and M. P. Parrella. 2004. Developmental biology of citrus mealybug under conditions typical of California rose production. Ann. Entomol. Soc. Amer. 97: 982-988.

Li, Y. 2019. Effects of rove beetle, *Dalotia coriaria*, on western flower thrips, *Frankliniella occidentalis*, under laboratory conditions; and integrating the entomopathogenic fungus, *Beauveria bassiana*, with *D. coriaria* to suppress western flower thrips populations under greenhouse conditions. PhD dissertation: Department of Entomology; Kansas State University, Manhattan, KS. 176 pp.

Loughner, R. L., D. F. Warnock, and R. A. Cloyd. 2005. Resistance of greenhouse, laboratory, and native populations of western flower thrips to spinosad. HortSci. 40: 146-149.

Lublinkhof, J., and D. E. Foster. 1977. Development and reproductive capacity of *Frankliniella occidentalis* (Thysanoptera: Thripidae) reared at three temperatures. J. Kans. Entomol. Soc. 50: 313-316.

Mani, M., and C. Shivaraju. 2016. Damage, pp. 117-122. In: Mani, M., and C. Shivaraju [eds.]. Mealybugs and Their Management in Agricultural and Horticultural Crops. Springer (India) Pvt. Ltd. Springer, New Deli, India.

Martini, X., N. Kincy, C. Nansen. 2012. Quantitative impact assessment of spray coverage and pest behavior on contact pesticide performance. Pest Manag. Sci. 68: 1471-1477.

McClure, M. S. 1977. Resurgence of the scale, *Fiorinia externa* (Homoptera: Diaspididae), on hemlock following insecticide application. Environ. Entomol. 6: 480-484.

McKenzie, H. L. 1967. Mealybugs of California: With Taxonomy, Biology, and Control of North American Species. Univ. California Press, Berkeley and Los Angeles, CA.

Meyer, J. B., G. G. F. Kasdorf, L. H. Nel, and G. Pietersen. 2008. Transmission of activated-episomal *Banana streak OL (badna) virus* (BSOLV) to cv. Williams banana (*Musa* sp.) by three mealybug species. Plant Dis. 92: 1158-1163.

Moreno, D. S., J. Fargerlund, and W. H. Ewart. 1984. Citrus mealybug (Homoptera: Pseudococcidae) behavior of males in response to sex-pheromone in laboratory and field. Annals Entomol. Soc. Amer. 77: 32-38.

Morita, M., T. Yoneda, and N. Akiyoshi. 2014. Research and development of a novel insecticide, flonicamid. J. Pestic. Sci. 39: 179-180.

Moritz, G. 1997. Structure, growth and development, pp. 15-63. In: Lewis, T. [ed.]. Thrips as Crop Pests. CAB International, New York, NY.

Mouden, S., K. F. Sarmiento, P. G. Klinkhamer, and K. A. Leiss. 2017. Integrated pest management in western flower thrips: past, present and future. Pest Manag. Sci. 73: 813-822.

Mound, L. A. 1995. The thysanoptera vector species of tospoviruses. Acta Hortic. 431: 298-309.

Murphy, B. C., T. A. Morisawa, J. P. Newman, S. A. Tjosvold, and M. P. Parrella. 1998. Fungal pathogens control thrips in greenhouse flowers. Calif. Agric. 52: 32-36.

Pappu, H., R. A. C. Jones, and R. K. Jain. 2009. Global status of tospovirus epidemics in diverse cropping systems: successes achieved and challenges ahead. Virus Res. 141: 219-236.

Parrella, M. P. 1999. Arthropod fauna, pp. 213-250. In: Stanhill, G., and H. Zvi Enoch [eds.]. Ecosystems of the World 20. Greenhouse Ecosystems, Elsevier, New York, NY.

Parrella, M. P., and V. P. Jones. 1987. Development of integrated pest management strategies in floricultural crops. Bull. Entomol. Soc. Amer. 33: 28-34.

Passaro, L. C., and F. X. Webster. 2004. Synthesis of the female sex pheromone of the citrus mealybug, *Planococcus citri*. J. Agric. Food Chem. 52: 2896-2899.

Raghavendra, K., T. K. Barik, P. Sharma, R. M. Bhatt, H. C. Srivastava, U. Sreehari, and A. D. Dash. 2011. Chlorfenapyr: a new insecticide with novel mode of action can control pyrethroid resistant malaria vectors. Malaria J. 10: 16.

Reitz, S. R. 2009. Biology and ecology of western flower thrips (Thysanoptera: Thripidae): The making of a pest. Fla. Entomol. 92: 7-13.

Reitz, S. R. and J. Funderburk. 2012. Management strategies for western flower thrips and the role of insecticides, pp. 355-384. In: Perveen, F. [ed.]. Agric. Biol. Sci. Insecticides – Pest Engineering. InTech, Rijeka, Croatia.

Robb K. L., and M. P. Parrella. 1995. IPM of western flower thrips, pp. 365-370. In: Parker, B. L., M. Skinner, and T. Lewis [eds.]. Thrips Biology and Management. Plenum Press, New York, NY.

SAS Institute. 2012. SAS/STAT user's guide, version 9.4. SAS Institute, Cary, NC.

Seaton, K. A., D. F. Cook, and D. C. Hardie. 1997. The effectiveness of a range of insecticides against western flower thrips (*Frankliniella occidentalis*) (Thysanoptera: Thripidae) on cut flowers. Austral. J. Agric. Res. 48: 781-788.

Shelton, A. M., B. A. Nault, J. Plate, and J. Z. Zhao. 2003. Regional and temporal variation in susceptibility to λ-cyhalothrin in onion thrips, *Thrips tabaci* (Thysanoptera: Thripidae), in onion fields in New York. J. Econ. Entomol. 96: 1843-1848.

Shelton, A. M., J. Z. Zhao, B. A. Nault, J. Plate, F. R. Musser, and E. Larentzaki. 2006. Patterns of insecticide resistance in onion thrips (Thysanoptera: Thripidae) in onion fields in New York. J. Econ. Entomol. 99: 1798-1804.

Sparks, T. C. 2013. Insecticide discovery: an evaluation and analysis. Pestic. Biochem. Physiol. 107: 8-17.

Spear, R. C., D. L. Jenkins, and T. H. Milby. 1975. Pesticide residues and field workers. Environ. Sci. Tech. 9: 308-313.

Story, R. N., and F. J. Sundstrom. 1986. Influence of cabbage cultivar and frequency of insecticide application on damage by the cabbage looper (Lepidoptera: Noctuidae). Fla. Entomol. 69: 174–179.

Tanwar, R. K., P. Jeyakumar, D. Monga. 2007. Mealybugs and their management. Technical Bulletin: 19 September. National Centre for Integrated Pest Management, LBS Building, Pusa Campus, New Delhi, India.

Tipping, C., V. Bikoba, G. J. Chander, E. J. Mitcham. 2003. Efficacy of Silwet L-77 against several arthropod pests of table grape. J. Econ. Entomol. 96: 246-250.

Tomkins, A. R., P. T. Holland, C. Thomson, D. J. Wilson, and C. P. Malcolm. 1999. Residual life of spinosad on kiwifruit-Biological and chemical studies. Proc. 52nd N. Z. Plant Protection Conf. 52: 94-97.

Tommasini, M., and S. Maini. 1995. *Frankliniella occidentalis* and other thrips harmful to vegetable and ornamental crops in Europe. Wageningen Agric. Univ. Papers 95: 1-42.

Trichilo, P. J., and T. F. Leigh. 1988. Influence of resource quality on the reproductive fitness of flower thrips (Thysanoptera: Thripidae). Ann. Entomol. Soc. Amer. 81: 64-70.

Tsuda, S., I. Fujisawa, J. Ohnishi, D. Hosokawa, and K. Tomaru. 1996. Localization of tomato spotted wilt tospovirus in larvae and pupae of the insect vector *Thrips setosus*. Phytopathol. 86: 1199-1203.

Van de Wetering, F., R. Goldbach, and D. Peters. 1996. Tomato spotted wilt tospovirus ingestion by first instar larvae of *Frankliniella occidentalis* is a prerequisite for transmission. Phytopathol. 86: 900-905.

van Dijken, F. R., M. T. A. Dik, B. Gebala, J. de Jong, C. Mollema. 1994. Western flower thrips (Thysanoptera: Thripidae) effects on chrysanthemum cultivars: plant growth and leaf scarring in nonflowering plants. J. Econ. Entomol. 87: 1312-1317.

Venkatesan, T., S. K. Jalali, S. L. Ramya, and M. Prathibha. 2016. Insecticide resistance and its management in mealybugs, pp. 223-229. In: Mani, M. and C. Shivaraju [eds.]. Mealybugs and Their Management in Agricultural and Horticultural Crops. Springer (India) Pvt. Ltd. Springer, New Delhi, India.

Wang, C. J., and Z.Q. Liu. 2007. Foliar uptake of pesticides—present status and future challenge. Pestic. Biochem. Physiol. 87: 1-8.

Warnock, D. F., and R. A. Cloyd. 2005. Effect of pesticide mixtures in controlling western flower thrips (Thysanoptera: Thripidae). J. Entomol. Sci. 40: 54-66.

Watson, G. B., S. W. Chouinard, K. R. Cook, C. Geng, J. M. Gifford, G. D. Gustafson, J. M. Hasler, I. M. Larrinua, T. J. Letherer, J. C. Mitchell, W. L. Pak, V. L. Salgado, T. C. Sparks, and G. E. Stilwell. 2010. A spinosyn-sensitive *Drosophila melanogaster* nicotinic acetylcholine receptor identified through chemically induced target site resistance, resistance gene identification, and heterologous expression. Insect Biochem. Mol. Biol. 40: 376-384.

Willmott, A. L., R. A. Cloyd, and K. Y. Zhu. 2013. Efficacy of pesticide mixtures against the western flower thrips (Thysanoptera: Thripidae) under laboratory and greenhouse conditions. J. Econ. Entomol. 106: 247-256.

Yu, S. J. 2008. The mode of action of insecticides, pp. 115-142. Toxicology and Biochemistry of Insecticides. CRC Press, Taylor & Francis Group, Boca Raton, FL.

Zhang, A., H. Kaiser, P. Maienfisch, and J. E. Casida. 2000. Insect nicotinic acetylcholine receptor: Conserved neonicotinoid specificity of [3H] imidacloprid binding site. J. Neurochem. 75: 1294-1303.

Zhang, Z. J., Q. J. Wu, X. F. Li, Y. J. Zhang, B. Y. Xu, and G. R. Zhu. 2007. Life history of western flower thrips, *Frankliniella occidentalis* (Thysan., Thripae), on five different vegetable leaves. J. Appl. Entomol. 131: 347-354.

Zhao, G., W. Liu, J. M. Brown, and C. O. Knowles. 1995. Insecticide resistance in field and laboratory strains of western flower thrips (Thysanoptera: Thripidae). J. Econ. Entomol. 88: 1164-1170.