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Aspects of the Biology and the Effects of Traditional and Non-Traditional Insecticides on Citrus Thrips and Avocado Thrips with the Objective of Improving Integrated Pest Management

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Entomology

by

Deane Kathleen Zahn

August 2011

Dissertation Committee:
Dr. Joseph G. Morse, Chairperson
Dr. Michael E. Stanghellini
Dr. John T. Trumble

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The Dissertation of Deane Kathleen Zahn is approved:

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Finally, I thank my husband, Anthony and my critters, Beaux, Jonesy, and Tucker, for their patience, love, support, and encouragement; without them in my life I would not be able to successfully finish this sentence; much love to them now and always.

Dedication

This Dissertation is dedicated to my amazing nieces and nephews: Evelyn, Torrey, and Samantha Zaches and Gregory and Joseph Martinez. They are remarkable, special people who I will always love and cherish.

ABSTRACT OF THE DISSERTATION

Aspects of the Biology and the Effects of Traditional and Non-Traditional Insecticides on Citrus Thrips and Avocado Thrips with the Objective of Improving Integrated Pest Management

by

Deane Kathleen Zahn

Doctor of Philosophy, Graduate Program in Entomology
University of California, Riverside, August 2011
Professor Joseph G. Morse, Chairperson

Citrus thrips, *Scirtothrips citri* (Moulton), is a plant-feeding pest most widely recognized for damage caused to citrus and mango fruits. Citrus thrips have also become a significant pest in California's blueberries. Avocado thrips, *Scirtothrips perseae* Nakahara, is a pest of avocados (*Persea americana* Mill. [Lauraceae]) in California. Pesticides are often used to manage these two species of thrips and therefore, the likelihood of resistance development is high. There is increasing pressure in the United States to move away from broad-spectrum insecticides and focus on alternative methods of control, e.g., genetically modified crop plants expressing *Bt* (*Bacillus thuringiensis*) toxins, use of biorational insecticides such as toxic Bt protein sprays and entomopathogens (such as various strains of *Beauveria bassiana* Balsamo, and other agents). Integrated pest management programs are essential for any agricultural commodity. The goal of the work described here is to add to the foundation of

knowledge to improve the integrated pest management of citrus thrips and avocado thrips. The research conducted for this disseration 1) examined alternatives to traditional insecticides (Bt protein sprays and several strains of *B. bassiana*) to control both avocado and citrus thrips in the laboratory and resulted in no efficacy of the Bts tested but one strain of fungus, the commercially available strain was able to infect citrus thrips at field obtainable levels, 2) tested the commercially available strain in different formulations and water regimes against citrus thrips in blueberry fields which resulted in some control but not enough to strongly recommend this as an alternative to traditional pesticides, 3) evaluated the impact of some of the insecticides registered for avocado thrips management on the beneficial native predaceous mite *Euseius hibisci* Chant in avocado orchards and found that each of the pesticides harmed the mite but at varying levels and durations, 4) assessed citrus thrips oviposition on blueberry varieties with choice and no-choice tests and it was determined that citrus thrips likely oviposit to differing degrees in some plants over others, and finally 5) determined that citrus thrips in the Americas was actually a complex of species that were nearly morphologically identical but molecularly quite distinct.

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Chapter 1

General Introduction

Thrips are members of the order Thysanoptera. This order is subdivided into two suborders, the Terebrantia and the Tubulifera, with about 5,500 described species (Mound and Kibby 1998, Morse and Hoddle 2006) in nine families (Triplehorn and Johnson 2005). The Terebrantia consist of seven families, six of which are present in North America. All members of the Terebrantia have the following in common: the last abdominal segment is rounded or conical, females possess an ovipositor, forewings have veins and setae, fringed cilia of the forewing arise from the basal sockets, and the wing surface typically has numerous microtrichia (Triplehorn and Johnson 2005). The Tubulifera consist of two families, but only one of them is present in North America (Triplehorn and Johnson 2005). The Tubulifera may be distinguished from the Terebrantia by the following characteristics: both males and females have a tubular last abdominal segment, females lack an ovipositor, forewings lack veins and setae except at the base, the fringe cilia lack basal sockets, and the wing surface is bare of microtrichia (Triplehorn and Johnson 2005). The families within the Terebrantia are separated by antennal characters, mainly the number of segments and type of sensoria on the third and fourth segments (Triplehorn and Johnson 2005).

Thrips are tiny, slender, and soft-bodied insects that are 0.5 to 5.0 mm in length. When wings are present, there are four very long and narrow wings that are fringed with long hairs. This fringe, or tassel, gives the order its name, *thysano*, the Greek word for tassel and *ptera* meaning wing. The mouthparts of thrips are unusual and of the

sucking/piercing type. There are two principle structures. The first structure consists of the left mandible, which is modified into a tough, sharp, piercing organ that is hollow but lacking an aperture; the right mandible is reduced and vestigial (Wiesenborn and Morse 1988). The second structure is composed of the paired styliform lacineae of the maxillae, which are interlocked to form a single feeding tube (Wiesenborn and Morse 1988). These structures are contained inside the proboscis that is located opistignagthically on the ventral surface of the head. The labrum forms the front of the proboscis; the basal portions of the maxillae form the sides and the labium forms the rear (Triplehorn and Johnson 2005).

Thrips also exhibit unique metamorphosis, being neither truly hemimetabolous nor holometabolous. The first two instars have no external wings, are referred to as larvae, and are mobile but are relatively slow moving. In many cases, the wings develop internally during these two instars (Triplehorn and Johnson 2005). In the Terebrantia, the third and fourth instars are inactive, unless disturbed, non-feeding, and some lack external wings. The third and fourth instars are referred to as the propupa, and pupa respectively, though they are not 'true pupae' and they are followed by the adult stage. Thrips in the suborder Tubulifera have two pupal stages following the propupal stage. Thrips range in color from translucent white or yellowish to dark brown or blackish depending on the species and life stage (Dreistadt and Phillips 2001). The sexes of thrips are similar in appearance, though the male is often smaller and moves faster than the female. Parthenogenesis can occur in many species of thrips (Triplehorn and Johnson 2005). When an ovipositor is present, phytophagous females ususally oviposit into plant

tissue (Triplehorn and Johnson 2005, Morse and Hoddle 2006). The eggs (0.2 – 0.5 mm in length) are typically oviposited under the cuticle of new leaves, stems and fruit where the larvae feed. In many species, one female may lay as many as 250 eggs. The late second instar of many species drop to the soil or leaf litter or lodge within plant crevices to pupate. However, greenhouse thrips pupate openly on lower leaf surfaces while pupae and eggs of some gall-forming species occur on leaf surfaces but are enclosed within distorted plant tissues. Thrips have several generations per year, some having 8 or more. In some species, the life cycle from egg to adult may be completed in as short as two weeks when environmental conditions are optimal.

Nearly 50% of the known species of thrips feed on fungi, about 40% feed on living tissues of dicotyledonous plants or grasses, and the rest exploit mosses, ferns, gymnosperms, and cycads or are predatory (Morse and Hoddle 2006). Phytophagous thrips mainly feed upon the rapidly growing foliage or 'flush' as well as very small, developing fruits typically not larger than 5 cm in diameter. Because of the preference of thrips for immature fruit and flush foliage, external injury to commercial fruit and plants may be difficult to detect in the early stages of growth. Thrips feeding causes tiny scars on leaves and fruit, often referred to as stippling. Stippled leaves become distorted, colored, rolled, stunted and are often abscised by the plant. Avocado, citrus and greenhouse thrips cause silver to brown scabby scars on avocado and citrus fruit surfaces, but the damage is usually cosmetic (Morse 1995, Hoddle 2002b). Evidence of thrips damage on grapes appears as dark scars surrounded by lighter colored 'halos' (Roditakis and Roditakis 2007). Thrips damage may cause apples, nectarines, onion, pears,

soybean, sugar pea pods, raspberries and tomato, to be deformed, scarred and scabbed (Huckaba and Coble 1991, Pearsall 2000, Maris et al. 2003, Trdan et al. 2005b, Shipp and Wang 2006, Bosco et al. 2008). Some thrips detrimental to crops of economic importance feed on and over-winter in weed hosts and plant material left in growing fields and presumably move into crops when environmental conditions are appropriate (Groves et al. 2001, Matos and Obrycki 2004, Larentzaki et al. 2007).

Like many insects with incomplete metamorphosis, thrips adults and larvae compete for the same food resources. Phytophagous species are often broadly polyphagous, attacking a wide range of host plants representing a spectrum of agricultural crops and non-crop species. For example, western flower thrips, *Frankliniella occidentalis* (Pergande), is an important and polyphagous greenhouse and field pest (Ebssa et al. 2004) with vegetables and ornamental crops being the most important host plants. Western flower thrips cause direct damage on the plants and indirect damage as a vector of tomato spotted wilt and other viruses (Machoux et al. 1991). Tomato spotted wilt virus (TSWV) is one of 14 tospoviruses that are known to infect crops. It infects a wide range of crop and non-crop hosts and causes economic losses worldwide that are estimated at $1 billion per year (Prins and Goldbach 1998, Stumpf and Kennedy 2007). *Thrips tabaci* Lindeman is an important pest of sweet peppers and also vectors a tospovirus (Bosco et al. 2008). The melon thrips, *Thrips palmi* Karny, is a pest of over 60 economically important crops (Cannon et al. 2007) and bean thrips, *Caliothrips fasciatus* (Pergande), is a pest of over 60 plant genera and

approximately 30 economically important crops, although this number may be an overestimation (Bailey 1940, Huckaba and Coble 1991, Harman et al. 2007).

Because thrips populations can build up to injurious levels very quickly if left unchecked in native habitats and in crops where flush is present, colored sticky cards are often used to monitor for thrips levels (Atakan and Canhilal 2004, Joost and Riley 2004, Chen et al. 2006, Chu et al. 2006, McPherson and Riley 2006, Harman et al. 2007). Other monitoring methods include visual inspection, a turpentine funnel wash, and shaking, sweep-net, or beat tray sampling (Gonzalez-Zamora and Garcia-Mari 2003, Joost and Riley 2004, Trdan et al. 2005a, Chu et al. 2006, Boll et al. 2007). Visual inspection and sweep-netting can give some indication as to the presence of thrips, while beating or shaking the plant material and collecting the insects is also sufficient but more labor intensive with extraction efficiency being low. Because thrips can rapidly increase in numbers and move from field to field, it is often necessary to sample thrips frequently.

Many thrips species are key pests of economically important crops and ornamentals, including avocado, citrus, cotton, cowpea, melon, onion, pecan, rose, strawberry and many ornamental flowers (Bailey 1940, Arevalo and Liburd 2007, Boll et al. 2007, Cannon et al. 2007). Historically, chemical controls have been used to combat these pests. The insecticides imidacloprid (Admire), abamectin (Agri-Mek), methomyl (Lannate), spinosad (Success), spinetoram (Delegate) and endosulfan (Thiodan) include only a few of the many insecticides that have been or are currently used to chemically control thrips species (Hare and Morse 1997, Khan and Morse 1997, Byrne et al. 2005, Loughner et al. 2005). However, management of thrips with insecticides can be difficult,

especially when they invade fields and crops when flush is present, which can be at multiple points during the year depending upon the crop. In addition, repeat sprays are often required as thrips populations continue to emigrate into crops from nearby plants in native habitats or other crops. Also, the egg and pupal stages of thrips are often protected from spray impact. The utility of chemical control can change rapidly because of resistance development, environmental contamination, non-target effects on beneficial insects and increased public awareness and concerns, resulting in restrictions or near elimination of several classes of insecticides. In association with integrated pest management, methods that minimize the use of broad-spectrum insecticides are being developed (Dent 1990). For example, increased use of natural enemies, mating disruption with pheromones, use of sterile insects, and genetic engineering of plants are all part of the arsenal being developed to manage insect pests.

Acaricides and insecticides are commonly used for pest suppression in agriculture, forestry and public health. Adverse effects of pesticide use can include the killing of non-target organisms, contamination of water supplies and persistence of unwanted residues on foods and animal feed. Insecticidal effects on non-target organisms have been a concern since the early 1960's (Georghiou 1967, Newsom 1967, Croft 1972, Croft and Brown 1975) and resistance to one or more pesticides has evolved in populations of over 500 insect and mite species (Clark and Yamaguchi 2002), rendering many of those pesticides ineffective against the resistant populations. For these and other reasons, supplementing or replacing pesticides with non-chemical or non-

traditional control tactics, including biological control and biorational insecticides, is a goal in many crop and livestock production systems.

Many researchers have investigated alternatives to conventional insecticides such as biorational insecticides or biopesticides, i.e. natural or organismal methods of controlling pest populations. The utilization of entomopathogens against thrips is not a new concept. For example, entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae are currently used as biological control agents for soil-inhabiting insect pests as these nematodes are lethal insect parasites (Georgis and Manweiler 1994, Chyzik et al. 1996, del Pino and Morton 2008, Toepfer et al. 2008) and nematodes in conjunction with predatory mites have also been used in thrips control (Ebssa et al. 2006). Entomopathogenic fungi, such as *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metchnikoff) Sorokin (Maniania et al. 2003, Iwase and Shimizu 2004, Ansari et al. 2007), *Neozygites parvispora* (MacLeod & Carl) Remaudière & Keller (Grundschober et al. 2001), *Verticillium lecanii* (Zimmerman) Viegas (Abe and Ikegami 2005, Sengonca et al. 2006) and *Paecilomyces fumosoroseus* (Wize) Brown & Smith (Castineiras et al. 1996, Ekesi et al. 1998, Sengonca et al. 2006, Thungrabeab et al. 2006) have also been used in laboratory and greenhouse trials with success (Stanghellini and El-Hamalawi 2005, 2006), whereas field trials have shown limited successes. Various strains of *B. bassiana* have been shown to effectively control western flower thrips (*Frankliniella occidentalis*) on greenhouse ornamentals and peppers (Frantz and Mellinger 1998, Jacobson et al. 2001, Ugine et al. 2005), and several reports indicate that *F. occidentalis*, *Thrips palmi* Karny and *T. tabaci* Lindeman were

successfully controlled under field (Saito 1991, Maniania et al. 2001, Maniania et al. 2003) or laboratory conditions (Makoto and Ikegami 2005).

Evaluation of acaricides and insecticides on non-target organisms is an essential component of any IPM program (Croft 1990) and of particular interest to California avocado growers. California alone grows 95% of the United States avocados on more than 2,500 hectares of land (Hoddle 1998, Hoddle and Morse 2003) and roughly 99% of this land is infested with avocado thrips (Hoddle and Morse 2003). Several species of predaceous insects and mites feed upon avocado thrips and these natural enemies include brown and green lacewing larvae, several predaceous thrips (e.g. *Franklinothrips orizabensis*, *Franklinothrips vespiformis* Crawford, *Leptothrips mali* (Fitch) and several *Aeolothrips* spp.) and the native predaceous mite *Euseius hibisci* (Chant) (Acari: Phytosiidae).

McMurtry and Croft (1997) classify the feeding behavior of predatory Phytosiidae into four groups and Group IV comprises the genus *Euseius*, members of which can subsist on pollen in the absence of prey with minimal reduction in fitness. Species of *Euseius* are the most common phytoseiids on both citrus and avocado. *Euseius hibisci* is known from Santa Barbara County in California to the sate of Oaxaca in southern Mexico (McMurtry et al. 1985). It mainly has a coastal distribution in California and is the dominant phytoseiid on avocados (McMurtry 1989). *Euseius hibisci* is common and abundant in avocado orchards year round, is an important generalist predator, and feeds on pollen and leaf exudates in the absence of prey (McMurtry and Scriven 1964, McMurtry and Johnson 1965, McMurtry et al. 1992). The most studied member of this

genus is probably *Euseius tularensis* Congdon (McMurtry and Scriven 1964, Swirski et al. 1970, Kennett et al. 1979, Jones and Parrella 1983, McMurtry et al. 1992, Ouyang et al. 1992, Grafton-Cardwell et al. 1999, Kahn and Morse 2006) and not nearly as much is known about *E. hibisci* with regards to pesticide exposure. In fact, *E. tularensis* was 'discovered' and described as a new species different from *E. hibisci* based on finding several populations of the former that showed higher tolerance to pesticides (Congdon and McMurtry 1985). Several studies have indicated the relevance of *E. hibisci* as effective biocontrol agents of spider mites and thrips on some crops (Tanigoshi and Nishio-Wong 1981, McMurtry 1985, Tanigoshi et al. 1985, Congdon and McMurtry 1985) and although *E. hibisci* is not a specialized predator, it potentially aids in enhancing control of many different pest mites and thrips (Badii et al. 2004). Evaluating the effects of registered pesticides for avocado thrips management in avocados on *Euseius hibisci* is worthwhile research especially as thrips pressure increases and growers rely more on pesticides for management.

Developments in molecular biology have produced transgenic crops such as cotton, soybeans and corn, which express the *Bacillus thruingiensis* (Bt) endotoxin to protect the plant from primary pests. As a result, the use of insecticides in transgenic crops has declined (Bourguet et al. 2002, Chilcutt 2007). *Bacillus thuringiensis* (Bt) are gram-positive spore-forming bacteria with entomopathogenic properties. Bt produces insecticidal proteins during the sporulation phase as parasporal crystals. These crystals are primarily comprised of one or more proteins, i.e. Crystal (Cry) and Cytolitic (Cyt) toxins, also called δ-endotoxins. Cry proteins are parasporal inclusion (Cry) proteins

from Bt that exhibit experimentally verifiable toxic effects to a target organism or have significant sequence similarity to a known Cry protein (Bravo et al. 2007). Similarly, Cyt proteins are parasporal inclusion proteins from Bt that exhibits hemolytic (Cyt) activity or has obvious sequence similarity to a known Cyt protein. These toxins are highly specific to their target insect, are innocuous to humans, vertebrates and plants, and are completely biodegradable. A major threat to the use of Bt is the appearance of insect resistance, which has been documented in the field with lepidopteran insects (Ferre and van Rie 2002). However, no resistance has been observed in the field to date in mosquito species controlled with Bti (Becker 2000). The lack of resistance to Bti is due to the presence of the Cyt1Aa protein in the crystal (Georghiou and Wirth 1997). It was demonstrated that Cyt1Aa protein synergizes Cry11Aa toxicity by functioning as a receptor molecule (Perez et al. 2005); Cyt1Aa can also extend activity to Cry11Aa within insects that do not posses binding receptors, again by functioning as the receptor (Georghiou and Wirth 1997, Perez et al. 2005). Therefore, Bt is a viable alternative for the control of insect pests in agriculture and of important human disease vectors (Bravo et al. 2005). Cyt1Aa and Cry11Aa would be uncommon Bt protein pairings for agricultural pests to encounter, but because synergism has been shown repeatedly, they are worthy of investigation against the Thysanoptera.

Molecular biology has afforded many researchers the ability to distinguish between seemingly morphologically identical and difficult to identify organisms (Avise 1994, Brunner et al. 2002, Crespi et al. 1998, Feder et al. 1998, Futuyma and Peterson 1985, Kawaski 1990). Larval thrips are often confused for other insects, such as

Collembola (springtails), and adults as Staphylinid beetles (Vierbergen 1995); it is often the case that identification of the larvae is impossible without the presence of adults. The majority of thrips are host-plant specific, but some economically important species are polyphagous and many species are predatory and therefore beneficial for management of immature scale, whiteflies, and mites (Palmer and Mound 1991, Brunner et al. 2002, Brunner et al. 2004, Morse and Hoddle 2006). Predatory thrips may be mistaken for pestiferous thrips but the use of genetic markers represents a valuable addition or alternative (in some cases) to traditional phenotypic methods of species recognition. The development of molecular techniques, PCR in particular, during the last three decades has provided a variety of rapid, simple, sensitive and reliable tools, e.g., PCR-based typing methods, which has revolutionized the genetic understandings in the biological sciences especially when only minimal amounts of template DNA were available (Moritz et al. 1987, Kawaski 1990, Armstrong and Ball 2005). PCR-based DNA technologies such as species-specific PCR (e.g. Kohlmayr et al. 2002, Lu et al. 2002, Liu 2004, Brunner et al. 2002), PCR restriction fragment length polymorphism (PCR–RFLP; e.g., Armstrong et al. 1997, Brunner et al. 2002), multiplex PCR (Kumar et al. 1999, Kengne et al. 2001), DNA sequencing (e.g. Brown et al. 2002, Dugdale et al. 2002) and oligonucleotide array analyses (Naeole and Haymer 2003) are suitable to aid in the development of comprehensive identification methods to differentiate easily between various known species, assist in monitoring for invasive species, and establish and understand species complexes (Armstrong and Ball 2005). These issues are of particular interest as the availability of trained taxonomic experts declines and long-term research strategies are

required to address the deficiencies in existing taxonomic keys to deal with morphologically indistinct immature life stages, cryptic species and damaged specimens (Armstrong and Ball 2005). A number of the most economically significant and global pests morphotaxonomic keys are now supported by molecular diagnostic technology, e.g., fruit flies (Tephritidae; Armstrong et al. 1997), tussock moths (Lymantriidae; Armstrong et al. 2003), leafroller moths (Tortricidae; Dugdale et al. 2002) and some thrips (Thripidae; Toda & Komazaki 2002).

There has been a significant amount of molecular work conducted with the Thysanoptera. The genus *Scitrothrips* Shull, for example, currently includes approximately 100 species (Mound and Palmer 1981, Moritz et al. 2004, Rugman-Jones et al. 2005, Rugman-Jones et al. 2006) throughout the tropics and subtropics and roughly 10 species are economic pests of agricultural commodities such as avocado, citrus, cotton, mango, tea and vegetables (Rugman-Jones et al. 2006). For this genus alone, sufficient molecular data from the conserved 28S-D2 domain of the large subunit rRNA, the cytochrome *c* subunit I (COI) of mitochondrial DNA (mtDNA) and internal transcribed spacer regions 1 and 2 (ITS1 and ITS2) of nuclear ribosomal DNA have been acquired to delineate some of the relationships within the genus (Rugman-Jones et al. 2006, Hoddle et al. 2008a, Hoddle et al. 2008b) but further investigation is required to understand the associations between citrus thrips present in citrus growing regions in North America where citrus is grown and citrus thrips are a major pests but in disparate ways. A personal communication from a collaborator (J. E. Funderburk) in Florida supplied the idea to investigate the differences seen in citrus thrips in Florida and

California. In Florida, citrus thrips are the most abundantly collected thrips on weeds but is not an agricultural pest in citrus, blueberries and mangos. However, in California citrus thrips is a pest of many agricultural crops (Flint et al. 1991, Morse 1995, Morse 1997, Haviland et al. 2009) especially the three listed crops. Thus, it seems prudent to determine if there are genetic differences between citrus thrips populations within North American citrus growing regions.

Biology of the first study species, Citrus Thrips

History. Citrus thrips, *Scirtothrips citri* (Moulton), is a plant-feeding pest most widely recognized for damage caused to citrus and mango fruits (Morse 1997) and has been recognized as a major pest of California citrus since the 1890s (Horton 1918).

Distribution. Citrus thrips appears native to southwestern North America and northwestern Mexico. One of its more common native host plants in this area prior to the introduction of citrus was likely laurel sumac, *Malosma* (=*Rhus*) *laurina* (Nutt.) Abrams (Morse 1997). In the USA, citrus thrips are known from Arizona, California, and Florida (recent appearance in the 1990's), whereas in Mexico they are reported only from northern Mexico (Flowers 1989).

Description. Adult citrus thrips are small, orange-yellow insects with the characteristic fringe wings of Thysanoptera. The females measure 0.60 – 0.90 mm in length while males are similar in appearance, but slightly shorter and narrower. The eggs measure about 0.20 mm and are oviposited under the cuticle of flush leaves, stems and small fruit. Eggs laid in the fall pass the winter and hatch about the time of the spring

foliage flush of citrus. The first and second instar larvae are very active and feed on flush leaves and tender young fruit, typically seeking refuge under the sepals of the young fruit, resulting in a characteristic ring scar around the top of the fruit after it expands. The propupal and pupal stages do not feed or move much unless they are disturbed. These latter two stages complete development on the ground in litter beneath the tree or in crevices in the tree.

Life History. Citrus thrips are multivoltine with 8 – 12 generations per year depending on climate (Tanigoshi and Nishio-Wong 1982, Tanigoshi and Moffitt 1984, Morse 1997, Schweizer and Morse 1997, Khan and Morse 1998). The life history of citrus thrips and a degree-day model has been developed by Rhodes et al. (1989). This model explains the timing of citrus thrips events and is often used to schedule monitoring activities both in the field and laboratory. They reported a developmental threshold of 14.6°C, which to our knowledge, is the highest threshold for any insect species.

Host Plants. Citrus thrips is primarily a pest of citrus in California particularly in the San Joaquin and Coachella valleys. They can have a broad host range, including, but not limited to, alfalfa, rose, grape, laurel, cotton, date, fir, Lucerne and various grasses, pecans and other ornamentals. Citrus thrips have been collected from over 55 different plant species (Flowers 1989). Their native host plant is hypothesized to be *Quercus* (Bailey 1964) or more likely *Malosma laurina* (Morse 1995).

Scirtothrips citri has broadened its known host range and become a significant pest of a relatively new crop to California, blueberries (Haviland et al. 2009). Thrips feeding on blueberry during the middle and late portions of the season cause distorted,

discolored, and stunted flush growth and poor development of fruiting wood for the subsequent crop (Arevalo and Liburd 2007). Thrips pressure of this magnitude, coupled with repeated pesticide applications of the few effective and registered pesticides, poses a concern regarding pesticide resistance management. Currently, there are no integrated pest management plans available for control of citrus thrips in blueberry. This is primarily due to the recent nature of this crop-pest association.

Historically, low-bush varieties of blueberries could only be grown in regions too cold for citrus production. However, the development of heat-tolerant high-bush varieties, which has enabled the development of a blueberry industry in the San Joaquin Valley (Jimenez et al. 2005, Strik and Yarborough 2005), has also caused blueberries to be grown in a region where citrus and citrus thrips flourish. This issue is relevant not only to the blueberry industry, but also for the 108,665 hectares of California citrus (ca. 70% is located in the San Joaquin Valley), which has experienced repeated documented cases of pesticide resistance in citrus thrips populations (Morse and Brawner 1986, Immaraju et al. 1989, Khan and Morse 1998). It is also important to note that not all varieties of high-bush blueberries are fed on equally by citrus thrips; i.e. there is a distinct varietal preference for some hybrids with similar parentage (e.g., the Star variety).

Biology of the second study species, Avocado Thrips

History. Avocado thrips, *Scirtothrips perseae* Nakahara, is a relatively new pest of avocados (*Persea americana* Mill. [Lauraceae]) in California. This species was first noticed in California in June 1996 damaging fruit and foliage in two distant avocado

groves, one each in Irvine, Orange County and Oxnard, Ventura County, CA. By July 1997, infestations of *S. perseae* were spread throughout avocado groves in Ventura and Orange counties (Hoddle 2002b, Hoddle et al. 2003).

Distribution. *Scirtothrips perseae* is native to Mexico and Guatemala and is now present in most avocado growing regions in southern California from San Luis Obispo Country south to San Diego County (Hoddle et al. 2002).

Description. Female avocado thrips lay eggs hidden inside the underside of leaves, in young fruit and stems (Hoddle 2002a). The first instar is white to pale yellow while the second instar is larger, more robust, and bright yellow (Nakahara 1997). Avocado thrips larvae are typically found along major veins on the underside of younger leaves and anywhere on the surface of young fruit (Hoddle 1998, Hoddle and Morse 2003). Although some pupation occurs on the tree in cracks and in crevices, about three-fourths of avocado thrips second instars drop from trees to pupate in the upper layer of dry, un-decomposed leaf litter (Hoddle 2002b). Propupae and pupae are rarely seen and they do not feed and move little unless disturbed. Adults are 0.7 mm (0.03 inch) long and have the typical fringed-tipped wings. Adults are orange-yellow with distinct, thin, brown bands between segments of their abdomen and three small red dots (ocelli) on top of the head (Nakahara 1997).

Life History. Adult avocado thrips resemble citrus thrips to the untrained eye and to an even lesser degree, western flower thrips, which occur on, but do not damage, avocado and citrus. Avocado thrips develop well under cool, humid temperatures (Hoddle 2002b). Populations typically begin increasing in late winter and spring, when

avocado thrips feed on young leaves and fruit. Population abundance peaks in late spring and early summer, when most fruit are young and after the growth flush when hardening of leaves induces thrips to move from foliage to feed on young fruit. Populations are suppressed by warm, dry conditions, but this weather usually occurs later in the season, when most fruit are larger and no longer susceptible to damage by thrips.

Scirtothrips perseae can have 6 or more generations a year. Egg to adult development occurs in about 20 to 30 days when temperatures average 18 to 24°C (Hoddle 2002b). Hoddle (2002b) reported avocado thrips developmental biology and created a developmental degree-day model listing a developmental threshold of 6.9°C, which to our knowledge is the lowest threshold for any insect species. Monitoring temperatures and using degree-day calculations can predict actual development time. Foliar feeding is usually unimportant, except when very high populations cause premature leaf drop (Hoddle and Morse 2003).

Host Plants. Avocado thrips adults can feed on over 11 plant species, however, larvae have only been found on avocados in the field in California suggesting that *S. perseae* has a restricted host range (Hoddle et al. 2002). Although it has little effect on tree health, avocado thrips feed directly on immature fruit (internal fruit quality is not affected), and obvious feeding scars cause severe downgrading or culling damaged fruit (Hoddle 2002b, Hoddle et al. 2003). Moreover, severe scarring when fruit are young can slow and stunt fruit growth. As fruit grow, early feeding by avocado thrips becomes apparent as scabby or leathery brown scars that expand across the skin and is sometimes referred to as "alligator skin" (Hoddle and Morse 2003). Avocado thrips damage is

affected by practices that increase or decrease the abundance of succulent foliage during set and growth of young fruit. Thrips move to young fruit when leaves harden after the growth flush has finished and the most damage occurs when fruit are 5.1 to 15.2 mm long (Hoddle and Morse 2003, Dreistadt et al. 2008). Although Hass fruit are susceptible to feeding until they reach about 51 mm in length, thrips feeding rarely causes scars on fruit larger than about 19.1 mm. This scarring on young fruit may not become obvious until fruit enlarge. In severe cases, all fruit on a tree can have their entire fruit surface scarred by avocado thrips, causing some packinghouses to sell such fruit with the box marked "papacado." The California Avocado Commission estimated a $50 million dollar crop lost in the 2006 due to avocado thrips scarring and the costs of control (Whitney 2009).

Monitoring and Control Methods for Avocado and Citrus Thrips

Monitoring methods. Sticky card and beating tray sampling are research methods used for these two insects but are rarely used by growers or pest control advisors (PCAs). Both PCAs and researchers monitor citrus thrips by counting the percent of fruit infested with immature thrips (adults are ignored because they cause relatively less concentrated damage under the sepal of the fruit which results in a ring scar) and the number of immature thrips per fruit is also indicative of the severity of the infestation. Thresholds in use in the San Joaquin Valley are 20% of Valencia oranges or 10% of navel oranges infested with immature thrips (Haney et al. 1992) until the fruit reaches 20 mm in diameter or more. Thresholds are halved (10% on Valencias, 5% on navels) if *Euseius tularensis* levels are less than 0.2 per leaf (Haney et al. 1992). Avocado thrips

are monitored by counting the number of immature thrips per leaf prior to fruit set or the number of thrips per fruit. No firm economic threshold has yet been developed (Strand et al. 2008) for avocado thrips but PCAs typically treat at 3-5 immature thrips per leaf prior to bloom in San Diego County due to restrictions on use of abamectin during bloom.

Control by natural enemies. The major documented citrus thrips predator is the phytoseiid mite, *E. tularensis* (Grafton-Cardwell and Ouyang 1995), although Jones and Morse (1995) questioned the importance of this predator. Avocado thrips are frequently preyed upon by *Franklinothrips orizabensis* Johansen and *Chrysoperla carnea* (Stevens) and is parasitized by the larval parasitoid *Ceranisus menes* (Walker) (Hoddle et al. 2004, Hoddle and Robinson 2004). *Franklinothrips vespiformis* (Crawford), black hunter thrips (*Leptothrips mali*), and several banded-wing thrips *(Aeolothrips* spp.) also feed on avocado thrips (Hoddle et al. 2002). In many years, natural enemies are unable to suppress avocado and citrus thrips populations below economic thresholds and chemical control is needed to reduce fruit scarring.

Control with pesticides. By the time damage is noticed on ripening fruit, the thrips that caused the injury are often absent from the fruit. A variety of pesticides are registered for thrips control in different cropping systems (Kahn and Morse 1997). After a number of years of use, pesticides like dimethoate (Cygon), formetante hydrochloride (Carzol), cyfluthrin (Baythroid), and fenpropathrin (Danitol) resulted in failures in citrus thrips control in some regions, along with an increase in resistance confirmed with both laboratory and field bioassays. Also, these materials are detrimental to natural enemies such as *Aphytis melinus* DeBach and other biological control agents important to citrus

pest control. Since it was registered in 1998, spinosad (Success, Entrust is the organically approved formulation of this material) has been the main material used for control of citrus thrips and a related and more effective material, spinetoram (Delegate), was registered late in 2007 and will soon replace spinosad once MRL (maximum residue limit) issues are resolved with export countries. Abamectin (Agri-Mek) is the main material used for avocado thrips control with occasionally rotation with sabadilla (Veratran D). Resistance to sabadilla has been shown with avocado thrips (Morse and Witney 2005) and a similar pattern of resistance development with abamectin is of concern due to the persistence of this material in leaf tissue. To date, citrus thrips resistance to spinosad has not been documented but there is concern that resistance to it or spinetoram may appear soon.

With a limited number of pesticides available for control and the frequency of resistance shown by thrips such as citrus thrips, it is wise to monitor population levels carefully, limit treatments to population levels of concern, and time treatments optimally (Morse and Grafton-Cardwell 2006, Morse and Hoddle 2006). Appropriate cultural practices and conservation of natural enemies should be practiced in concert with the use of pesticides only on an as-needed basis. Thus, the search continues for effective biological and chemical controls useful in citrus and avocado thrips management.

Other Control Tactics. For both species of thrips, some pupation occurs on the tree in cracks and in crevices, however, about three-fourths of avocado thrips drop as late second instars from trees to pupate in the upper layer of dry leaf litter (Schweizer and Morse 1997, Hoddle 1998). Propupae and pupae are rarely seen, move only if disturbed,

and do not feed. This phenomenon of dropping down to the leaf-litter or soil surface for pupation may create the ideal interface for control using the entomopathogenic fungi *B. bassiana*. Adding coarse organic mulch beneath trees and maintaining a mulch layer may reduce survival of thrips that drop from trees to pupate below the tree, especially in avocados, because this is common practice by many growers as a method of *Phytophthora* management. The effectiveness of mulching to control thrips is uncertain and labor costs of adding mulch may not be justified solely for thrips control. However, applying coarse organic material such as composted yard waste beneath trees may help control weeds, and thrips reduction might be an additional benefit, particularly for blueberries. The deep mulch layer that is standard practice with blueberry culture in the San Joaquin Valley may also provide an ideal habitat for *B. bassiana*. It is possible that as citrus thrips are adapted to and evolved in a hot, dry climate, they may be more susceptible to *B. bassiana*, whereas avocado thrips has adapted to and evolved in a wet and cool climate and may be less susceptible to or even tolerant to *B. bassiana*.

Dissertation Goals and Objectives

There is increasing pressure in the United States to move away from broad-spectrum insecticides and focus on alternative methods of control, e.g., genetically-modified crop plants expressing *Bt* toxins, use of entomopathogens, biorational insecticides. Implementation of such methods on avocado and citrus are difficult due to the relatively primitive methods available for thrips sampling, which are labor intensive and rely on experienced and intuitive pest control advisors. The goal of the work

described here is to examine alternatives to traditional insecticides such as Bt proteins and entomopathogenic fungi to control avocado and citrus thrips, with the ultimate target of utilizing entomopathogens to aid in field control, evaluate the insecticides registered for avocado thrips management on the native predaceous mite *Euseius hibisci*, assess citrus thrips oviposition on blueberry varieties, and determine whether citrus thrips is actually a complex of species. The specific objectives are:

1. Determine if *Bacillus thuringiensis* or *Beauveria bassiana* can be utilized effectively against avocado thrips and citrus thrips in the laboratory and ultimately on commercial avocados and blueberries, respectively, in California (Chapter 2),
2. Based on the results from objective one, determine if *B. bassiana* could be utilized effectively against citrus thrips in California blueberries as an alternative to non-traditional insecticides (Chapter 3),
3. Evaluate the currently registered pesticides for avocado thrips management against the native and most abundant predaceous mite, *Euseius hibisci*, in southern California avocados (Chapter 4),
4. Assess female citrus thrips oviposition levels on several blueberry varieties in choice and non-choice oviposition tests (Chapter 5).
5. Determine whether or not citrus thrips in North America is one species or a complex of species using the 28S-D2 domain of the large subunit rRNA and the cytochrome *c* subunit I (COI) of mitochondrial DNA (mtDNA) (Chapter 6).

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Chapter 2

Abstract

Citrus thrips, *Scirtothrips citri* (Moulton), is a plant-feeding pest most widely recognized for causing damage to citrus and mango fruits. This insect has recently broadened its known host range to become a significant pest of California grown blueberries. Avocado thrips, *S. perseae* Nakahara, is a recent, invasive pest of California avocados. Effective alternatives to traditional pesticides are desirable, in general, for both pests to reduce impacts on natural enemies and broaden control options in an effort to minimize pesticide resistance via rotation of control materials. We evaluated *Bacillus thuringiensis* subsp. *israelensis* proteins (Cyt 1A and Cry 11A, activated and inactivated) and multiple strains (GHA, 1741ss, SFBb1, S44ss, NI1ss, and 3769ss) of *Beauveria bassiana* (Balsamo) against both species. Avocado thrips and citrus thrips were not susceptible to either *Bt* protein tested, regardless of activity. All strains of *B. bassiana* were able to infect both avocado thrips and citrus thrips. However, the commercially available strain GHA was the most effective strain against both species and had a faster rate of infection then the other strains tested. Citrus thrips were more susceptible than avocado thrips to all *B. bassiana* strains (LC_{50} and LC_{95} of 8.6 x 10^4 and 4.8 x 10^6 conidia / ml for citrus thrips, respectively). Investigation of field control of citrus thrips using the GHA strain of *B. bassiana* is therefore justified.

Introduction

Citrus thrips, *Scirtothrips citri* (Moulton), is a plant-feeding pest most widely recognized for the damage it causes to citrus and mango fruits (Morse 1997) and has been recognized as a major pest of California citrus since the 1890s (Horton 1918). Recently, its known host range has broadened and they have become a significant pest of a relatively new crop planted in the San Joaquin Valley of California, highbush blueberries (Haviland et al. 2009). Citrus thrips feed on blueberry foliage during the middle and late portions of the season causing distorted, discolored, and stunted flush growth and poor development of fruiting wood required to obtain the subsequent crop (Jimenez et al. 2005, Strik and Yarborough 2005). High numbers of thrips on blueberries (15 thrips or more per leaf; DKZ, unpublished data), coupled with repeated pesticide applications of the few effective and registered pesticides, poses a concern regarding pesticide resistance management (Morse and Grafton-Cardwell 2006, 2009). Currently, there are no integrated pest management plans available for control of citrus thrips in blueberry. This is primarily due to the recent nature of this crop-pest association.

Avocado thrips, *Scirtothrips perseae* Nakahara, is a relatively new pest of avocados in California. It appeared in the state in 1996, and, at the time, was a species new to science (Hoddle 2002). By 1998, crop damage reduced industry revenues by 12% (Hoddle et al. 2003). Avocado thrips adults can feed on over 11 plant species', however, larvae have been found only on avocados in the field in both California and Mexico, suggesting that *S. perseae* has a highly restricted host range (Hoddle et al. 2002).

Although it has little effect on tree health, avocado thrips feed directly on immature fruit (internal fruit quality is not affected), and obvious feeding scars cause severe downgrading and culling of damaged fruit (Hoddle 2002, Hoddle et al. 2003).

With a limited number of pesticides available for thrips control and the propensity with which economically important thrips develop insecticide resistance, it is wise to monitor population levels carefully, limit treatments to population levels of economic concern and time treatments optimally (Morse and Grafton-Cardwell 2006, 2009, Morse and Hoddle 2006). Appropriate cultural practices and conservation of natural enemies should be practiced in concert with the use of pesticides only on an as-needed basis. Thus, continuing the search for effective biological and chemical controls useful in citrus and avocado thrips management is important. For both species of thrips, some pupation occurs on the tree in cracks and in crevices', however, the majority of both species drop as late second instars from trees to pupate in the upper layer of the leaf litter under trees (Schweizer and Morse 1996, Hoddle 1998). Propupae and pupae are rarely seen, move only if disturbed, and do not feed. Thus, pupation in the upper layers of the soil surface may create the ideal interface for control using the entomopathogenic fungi *Beauveria bassiana* (Balsamo). Coarse organic mulch beneath trees and the maintenance of a mulch layer, a common practice by many growers as a method of *Phytophthora* spp. management in avocados (Downer et al. 2002), may reduce survival of thrips that drop from trees to pupate below the tree. The effectiveness of mulching to control thrips is uncertain and labor costs are required to add mulch may not be justified solely for thrips control.

There is increasing pressure in the U.S. to move away from broad-spectrum insecticides and focus on alternative methods of control, e.g., genetically modified crop plants expressing *Bacillus thuringiensis* (Bt) toxins (Gill et al. 1992), use of entomopathogens, and similar approaches. Applications of *B. bassiana* have been reported to decrease populations of thrips in greenhouse cucumbers, chrysanthemums, gerbera daisies, roses, and carnations (Bradley et al., 1998; Jacobson et al., 2001; Ludwig and Oetting, 2002; Murphy et al., 1998; Shipp et al., 2002). Microbial insecticides containing δ-endotoxins (Cry and Cyt proteins) from Bt have been used as alternatives to conventional chemical insecticides for almost 70 years (Gill et al. 1992, Bravo et al. 2007).

Bt produces insecticidal proteins during the sporulation phase as parasporal crystals. These crystals are primarily comprised of one or more proteins, i.e. Crystal (Cry) and Cytolitic (Cyt) toxins, also called δ-endotoxins. From a practical perspective, Cry proteins are parasporal inclusion (Cry) proteins from Bt that exhibit experimentally verifiable toxic effects to a target organism or have significant sequence similarity to a known Cry protein (Bravo et al. 2007). Similarly, Cyt proteins are parasporal inclusion proteins from Bt that exhibit hemolytic (Cyt) activity or has obvious sequence similarity to a known Cyt protein. These toxins are highly specific to their target insect, are innocuous to humans, vertebrates and plants, are regarded as environmentally friendly, are completely biodegradable, and show little adverse effect on non-target species (Gill et al. 1992, Glare and O'Callaghan 2000, Bravo et al. 2005, 2007). The Cyt proteins are significantly different both in their structure and their biological activities from the Cry

proteins. However, Cyt proteins have shown toxicity to non-dipterous insects (Federici and Bauer 1998, Bravo et al. 2007). In fact, Cyt proteins in some cases can extend activity to other *Bacillus* spp. (e.g., *B. sphaericus*) for mosquitoes that lack the proper receptor (Wirth et al. 2000, Georghiou and Wirth 1997, Perez et al. 2005). Many studies with thrips involving Bt proteins have typically evaluated Cry toxins in transgenic crops targeted mainly toward lepidopterous pests (Zwahlen et al. 2000, Reed et al. 2001, Daly and Buntin 2005, Obrist et al. 2005, Parajulee et al. 2006) and there are no published studies we know of representing the impact of Cyt proteins on thrips. Due to the synergism seen between these two Bt proteins and the method of thrips feeding, commonly described as 'punch and suck' (Triplehorn and Johnson 2005), whereby leaf tissue is macerated prior to ingestion, we hypothesized that Cry or Cyt proteins could potentially be useful against thrips pests.

The goal of this investigation was to determine if Cry or Cyt proteins or *B. bassiana* could be used effectively to manage citrus and avocado thrips. Field management of both thrips species is the ultimate goal with these biopesticides but field studies are laborious and expensive. Thus, we evaluated these materials in the laboratory to determine which were sufficiently efficacious to warrant follow-up field studies.

Materials and Methods

Insects. Citrus thrips were collected in Riverside County, Riverside, CA from wild laurel sumac, *Malosma* (=*Rhus*) *laurina* (Nutt.) Abrams, a major host for this

species before citrus was introduced into the state (Morse 1995). Avocado thrips were collected in San Diego County, Fallbrook, CA from non-insecticide treated avocado groves, *Persea americana* Mill. (Lauraceae). Both species of insects were collected via aspiration the morning of the bioassay and held in 15-dram (55 ml) plastic aspiration vials with a copper mesh screened lid. A small leaf, respective to the thrips species collected (sumac and avocado), just large enough to fit in the vial was included to allow the insects to settle on the leaf and feed. For experiments with *B. thuringiensis israelensis,* female and late second instar thrips were used and in experiments with *B. bassiana,* only adult females were used. All bioassay females were of unknown age. Late second instar thrips of both species were classified, for these studies, as thrips that were large and had darkened in color. The abdomens appeared fully distended and the overall color of the thrips was a deep yellow with almost no opalescence. Early second instar thrips show limited abdomen distention and have an overall pearlescent hue.

Screening *Bacillus thuringiensis* endotoxins for activity. Two strains, *B. thuringiensis* subspecies *israelensis* (*Bti*) 4Q7/pWF53 (Cry11Aa) and 4Q7/pWF45 (Cyt1Aa) were grown on 400 ml peptonized milk at 27°C for 5 days (Wu et al. 1994, Park et al. 2001). The two *Bt* proteins, Cyt1Aa and Cry11Aa, were obtained in two forms, activated proteins and non-activated proteins (Wu et al. 1994). Both *Bt* proteins were activated in the following way: 2 mg of each protein was pelleted at 12,000 g for 5 min and the pellet was suspended with 1 ml of 50 mM Na_2CO_3 at pH 10.5 overnight at 37°C to solubilize the crystals. The solubilized crystal solution was adjusted to pH 8.5 using 13N HCl. The solution was centrifuged at 1,000 g for 5 min to pellet any

unsolubilized crystals. The protein concentration of the suspension was determined using the Bradford method (Bradford 1976). The Bt proteins were then used immediately with 50 μg/cm^2 applied to each leaf. The Bt was topically applied to either citrus leaves or avocado leaves by spreading the liquid over the leaf surface with a Teflon policeman (Fischer) and then allowing the liquid to air dry.

Leaves of both avocado and citrus for all bioassays were chosen in observably identical states; young and soft but fully expanded leaves were used as these are the type on which both species of thrips prefer to feed and large leaves were needed to fit in the Munger cell bioassay units that confined the thrips on treated leaves (Munger 1942, Morse and Brawner 1986, Morse et al. 1986). Briefly, Munger cells were constructed by using a Plexiglas sandwich; the middle cell layer was drilled with 3.2-cm diameter bit to provide a circular test arena (0.9 cm high by 3.2 cm diam). The upper (lid) and lower (base) parts of the Plexiglas sandwich were solid and between the lower base and test arena a piece a piece of filter paper was placed to allow moisture exchange and to extend the life of the leaf during the bioassay. Airflow through the test arena was provided through two holes (0.3 cm diam each) drilled through the center cell layer directly opposite one another, with fine-mesh screening melted onto the interior of the test arena to prevent escape. The Plexiglas sandwich was held together with four binder clips positioned such that the airflow was not covered. Once dry, the leaves were placed on the filter paper in Munger cells and the respective thrips species was added. The lid was placed on the cell but leaving the cell arena exposed, so that once the thrips were added, the cells could be closed quickly. Female and late second-instar avocado thrips and citrus

thrips were then placed on treated leaves of their respective host plants inside the Munger cell.

Control leaves for both species were treated with a mixture of the same suspension ingredients minus the protein. Bioassays were conducted concurrently in the following manner for both species: adult female thrips were placed on leaves coated with activated or inactivated forms of both Cyt1A and Cry11A, immature thrips were also placed on leaves coated with activated or inactivated forms of both Cyt1A and Cry11A, and all combinations for adults and immature thrips were carried out along with the corresponding control cells. The Munger cells were closed and placed in an environmental chamber at 28ºC, 55% RH, and long daylight conditions (16:8 L:D). Each cell was carefully removed daily and the filter paper doused with water to prevent leaf desiccation. The bioassay was replicated on two separate dates (2 proteins [activated vs. inactivated] x 2 species x 2 life stages per species x 2 replicates = 16 Munger cells per date). A minimum of 10 individuals was placed into each Munger cell and thrips were checked daily for eight days to assess mortality. Post seven days, the integrity of the leaves was questionable (i.e. rotten or dry and brittle) and in all but one bioassay, mortality was observed before seven days; thus data were analyzed using day 7 mortality. Mortality was determined by lack of movement after gently probing each thrips with a small brush.

Screening *Beauveria bassiana* strains for percent infection. Six strains of *B. bassiana* were obtained from the USDA-ARS Western Integrated Cropping Systems Research Unit located in Shafter, CA. GHA (Laverlam International, Butte, MT) is the

commercially available strain found in the field formulation of *B. bassiana*, Mycotrol O® and the greenhouse formulation BotaniGard ES, (both distributed by BioWorks Inc., Victor, NY for our studies) and each of the other five strains were obtained via isolation from soils in Kern County by USDA-ARS collaborators in 2000. They were stored at -80ºC. Culture methods for the thrips experiments were similar to those described previously for *Lygus hesperus* Knight bioassays (McGuire et al. 2005) and were conducted by collaborators from USDA-ARS, Shafter, CA. Briefly, isolates were grown on SDAY media, or Sabouraud's dextrose agar plus yeast extract (Becton–Dickson, Cockeyesville, MD). The conidia were harvested from culture plates after 10–14 days incubation by scraping with a sterile rubber policeman into a 0.01% solution of Silwet L-77 (GE Silicones, Friendly, WV). The conidia were then enumerated with a hemocytometer. For preservation and storage, glycerol (q.s. 10% v/v) was added to the conidial suspension and stored in aliquots of 2×10^8 in a 2 ml solution at −80°C until needed for bioassays. Conidial viability was assessed following incubation for 16 h in potato dextrose broth (Sigma) just prior to use in experiments. Viability was determined by adding a sample of approximately 10^7 conidia to 20 ml potato dextrose broth and incubating ca. 16 h in a rotary shaker (150 rpm) at 28°C. Conidia germination was examined under a compound microscope at 400× and scored as viable if the germ tube was at least twice the length of the conidium. Percentage viability was measured on 250 conidia of each isolate. All bioassays were conducted on the basis of the number of viable conidia measured after thawing and the desired concentrations were formulated by serial dilution. The strain from Mycotrol (GHA) was isolated and cultured exactly as

above to eliminate possible effects of production methods and formulation ingredients on insecticidal activity. Glycerol was not removed prior to using the conidia in bioassays. All six *B. bassiana* strains (GHA, SFBb1, 1741ss, S44ss, NI1ss, 3769ss) were suspended in 0.01% Silwet (Silwet L-77, Setre Chemical Co. Memphis, TN) in a de-ionized water solution and evaluated on the same date at four concentrations (10^5, 10^6, 10^7, and 10^8 conidia /ml) for each thrips species. The control consisted of 0.01% Silwet in de-ionized water solution. Each of the 25 treatments (6 strains x 4 rates, plus a single control) was evaluated using five Munger cells (see above for cell construction and leaf type used), which contained a minimum of ten (10-13) adult female thrips. These bioassays were repeated on 10 dates with both species tested simultaneously on each date (i.e. n = 5 cells for each thrips species per date per treatment x 10 dates). Groups of thrips (20 - 30) were anesthetized by exposure to CO_2 for 15-30 sec, and each strain was administered to the dorsum of the abdomen of each knocked out thrips quickly and carefully in a 1µl drop with a Burkard Hand Microapplicator (Burkard Manufacturing Co. Ltd., Hertfordshire, England) over filter paper. The droplet spread the length of the thrips immediately and the thrips was then deposited, still knocked out, onto the leaf tissue in the Munger cell. Once a minimum of 10 treated thrips (control thrips were dosed exactly the same way but without *B. bassiana*) were added, Munger cells were closed and sealed with binder clips and placed in an environmental chamber at 28ºC, 55% RH, and long daylight conditions (16:8 L:D). Each cell was checked daily for seven days to observe infection by the fungus. Each cell was carefully removed daily and the filter paper doused with water to prevent leaf desiccation. Individuals infected with *B. bassiana* were defined as those

whose natural activity was retarded and/or showed arrestment and subsequently produced mycelia, which was confirmed post bioassay. Mortality caused by mycosis was confirmed on the basis of visual observation (sporulation on insect cadavers) and then crushing individuals to reveal the presence of mycelial growth. When mycelial growth was not apparent, crushed individual thrips were placed on potato-dextrose agar plates for 5 days and then re-examined for the presence of mycelial growth.

Data were analyzed after Abbott's correction for control mortality (Abbott 1925) using log-probit analysis with PROC PROBIT on SAS 9.2 (SAS Institute 2008) and using the Raymond Statistics package (Raymond 1985). The purpose of the probit analysis was strictly for gross strain comparison. Probit analysis was used to estimate the LC_{50} and LC_{95} levels, confidence intervals, and χ^2 values for each strains. Lethal concentrations with overlapping 95% confidence intervals were not considered significantly different. The daily check data were analyzed as non-cumulative counts per day via the Survival Distribution Function on SAS 9.2 (SAS Institute 2008), where observation *time* represented the probability that the experimental unit from the population would have a lifetime exceeding that *time* (PROC LIFETEST) with the variables *strain* and *concentration*. Assessments for each variable by species were done with Log-rank and Wilcoxon tests and multiple comparisons for the log-rank test were adjusted by using Tukey-Kramer method. Data were then plotted as estimates of the survivor function for the different strains separately for each species.

Results and Discussion

Screening *Bacillus thuringiensis* endotoxins for activity. *Bacillus thuringiensis israelensis* produces two groups of toxic proteins, the Cry and Cyt toxins that have different modes of action. In this investigation, results with Cyt1A and Cry11A were disappointing as both activated and inactivated forms of both proteins showed little effect against adult and second instar citrus thrips and avocado thrips. To our knowledge, there have been no reports of Bt endotoxins with activity against Thysanoptera, although Cyt1Aa was found to be toxic to the non-target species *Chrysomela scripta* Fabricius (Coleoptera: Chrysomelidae) (Federici and Bauer 1998). Many hypothesize that because thrips feed with a punch and suck method, rather than direct chewing and mastication of leaf tissues, they do not receive toxic amounts of the Bt proteins (Zwahlen et al. 2000). Alternatively, they may not possess the proper binding receptors for the Bt proteins tested to date and thus, no pore can be formed in the midgut lining and the Bt proteins are excreted (Obrist et al. 2005). The literature indicates the latter hypothesis is more likely based on findings from life table parameters where development, fecundity, and adult longevity (Obrist et al. 2005) or relative abundance (Reed et al. 2001, Daly and Buntin 2005, Parajulee et al. 2006) are not significantly different from thrips reared on *Bt* positive versus *Bt* negative corn, cotton, or potato plants. The aforementioned studies were not specifically looking at Bt effects on thrips nor were the Bti toxins tested here involved in previous studies involving thrips. The combinations of proteins used in this

study were, to date, unique pairings with thrips. It is indeed possible that there are no Bt endotoxins currently available that cause mortality to Thysanoptera.

Screening *Beauveria bassiana* strains for percent infection. *Beauveria bassiana* was pathogenic to adults of both species of tested thrips.

*Results with citrus thrip*s. The LC_{50} with strain GHA was 8.61 x 10^4 conidia/ ml and was two orders of magnitude lower than for the other five *B. bassiana* strains tested (Table 2-1; Fig. 2-1A). GHA also gave the only statistically valid dose-response values in probit analysis, and provided the only data that fit the probit model. The other *B. bassiana* strains failed to provide a linear relationship based on their *p*-values (data not shown), i.e. the probit regression lines were of poor quality, except for GHA. Therefore, data were evaluated based on line slopes as is commonly seen in the scientific literature with other biological agents where data lines are not straight and do not fit the model (Wirth et al. 1997, Federici et al. 2003, Beckage et al. 2004, Wirth et al. 2005). Strains 1741ss, SFBb1, S44ss, and NI1ss showed a flat dose-response between concentrations, did not fit the model, and LC_{50}'sranged from 2.7 x 10^6 – 9.6 x 10^8.

Assessment of *Beauveria* strain while adjusting for concentration, in both Log-rank (P < 0.0001) and Wilcoxon (P < 0.0001) tests showed that *strain* and *concentration* had a highly significant effect on the infection rate. Multiple comparisons for the Log-rank test (adjusted by the Tukey-Kramer method) to assess the strain effect while adjusting for the concentration differences showed that strains 1741ss, S44ss, 3769ss, and NI1ss infection rates were not distinct from one another. Strain GHA and SFBb1 had infection rates different from each other as well, and GHA had the fastest infection rate

and SFBb1 showed the slowest kill rate (Fig. 2-2). The Survival Distribution Function analysis (see Methods) coupled with the probit analysis clearly shows that GHA would be the best strain choice for citrus thrips control.

Results with avocado thrips. The LC_{50} for strain GHA was 2.2 x 10^6 conidia / ml and was similar to that obtained with the other five *B. bassiana* strains tested (Table 2-2; Fig. 2-1B). Again, because a strong linear response was not observed, the performance between strains was rated based upon the LC_{50} and relative linearity of the response. Based on overlap of confidence intervals, there were no significant differences between any of the strain LC_{50}'s or LC_{95}'s (Table 2-2).

Assessment of *Beauveria* strains while adjusting for the concentration, using both Log-rank ($P < 0.8794$) and Wilcoxon ($P < 0.8601$) analysis showed that strain did not have an effect on the infection rate. The multiple comparisons for the Log-rank test (adjusted by the Tukey-Kramer method) to assess the strain effect while adjusting for the concentration differences showed infection rates for all 5 strains were not distinct from one another (Fig. 2-3). The Survival Distribution Function analysis coupled with probit analysis indicated there was no one best strain to select for avocado thrips management.

Citrus thrips were more susceptible to Beauveria than avocado thrips; citrus thrips LC values were much lower for the most active strain, GHA, indicating that significantly lower dosages of strain GHA were required to infect and kill citrus thrips compared with avocado thrips. The overall survival analysis results showed a similar pattern to the results of the probit analysis; GHA had the fastest infection rate (i.e. performed the best) and SFBb1 had the slowest rate (performed the worst). Infection rates for the other three

strain's (1741ss, S44ss, 3769ss and NI1ss) fit in between the rates for GHA and SFBb1, and 1741ss, S44ss, 3769ss, and NI1ss infection rates were not separable. This low dosage association and having the fastest infection rate suggest GHA is the best candidate for field-testing among the strains examined. Except for the worst performing strain, SFBb1, the performance of all of the strains with avocado thrips were similar. The LC_{50} value for citrus thrips was 8.6×10^4 conidia/ml, which may suggest economical feasibility in some cases, e.g., for use on organic products. The maximum recommended field application rate is 5.0×10^{12} conidia/ha. Therefore, 8.6×10^{11} conidia/ha of GHA is needed based on the estimated LC_{50} of 86 conidia/μl and this amount is reasonable to obtain in a field setting. Conducting the same analysis for avocado thrips control using GHA, with an LC_{50} of 2.2×10^6, 2.2×10^{13} conidia/ha would be required. This is 4.4 times greater than the standard field use rate of GHA.

We hypothesize that differences in susceptibility between citrus and avocado thrips may be due to the different habitats in which they evolved. Citrus thrips are adapted to hot and dry environments and thus, they are less likely to have evolved natural tolerance to fungi, whereas, avocado thrips thrive in a very wet environment where exposure to fungi is more likely. The differences may be due to different habitat adaptations and the different origins of the two thrips species (Morse 1995, Hoddle 2002). We find it interesting that two congenerics have such widely different habitat preferences and this may explain differences in fungal tolerance. Differences were seen when citrus thrips and avocado thrips were placed on leaves of their associated host plants, then placed separately in sealed zip-lock bags (unpublished data) where the

moisture that condensed in the bags was lethal to citrus thrips but not to avocado thrips. Thus, it is possible that avocado thrips, due to their adaptation to living in cool and wet climates (Hoddle 2002), have a higher tolerance to fungal pathogens, as they may encounter them more frequently than citrus thrips, which prefer a hot and drier climate (Morse 1995).

Many researchers have investigated alternatives to traditional insecticides such as biopesticides, i.e. natural or organismal methods of controlling pest populations. The utilization of entomopathogens against thrips is not a new concept; entomopathogenic fungi, such as, *Metarhizium anisopliae* (Metchnikoff) Sorokin (Maniania et al. 2003, Iwase and Shimizu 2004, Ansari et al. 2007), *Neozygites parvispora* (MacLeod & Carl) Remaudière & Keller (Grundschober et al. 2001), *Verticillium lecanii* (Zimmerman) Viegas (Abe and Ikegami 2005, Sengonca et al. 2006), and *Paecilomyces fumosoroseus* (Wize) Brown & Smith (Castineiras et al. 1996, Ekesi et al. 1998, Sengonca et al. 2006, Thungrabeab et al. 2006) have also been used in laboratory and greenhouse trials with much success, whereas field trials have shown limited successes. However, various strains of *B. bassiana* have been shown to effectively control western flower thrips (*Frankliniella occidentalis*) on greenhouse ornamentals and peppers (Frantz and Mellinger 1998, Jacobson et al. 2001, Ugine et al. 2005), and several reports indicated that *F. occidentalis*, *Thrips palmi* Karny and *T. tabaci* Lindeman were successfully controlled under field (Saito 1991, Maniania et al. 2001, Maniania et al. 2003) or laboratory conditions (Makoto and Ikegami 2005).

In conclusion, both citrus and avocado thrips can be infected by *B. bassiana* but high doses may be required, especially for avocado thrips. These high doses are difficult to obtain outside the laboratory and application of such doses would be costly. We believe *B. bassiana* is not a sufficiently effective alternative to traditional insecticides to warrant further study with avocado thrips, particularly because the commercially available strain GHA gave poor control on avocado thrips, but it may have potential against citrus thrips in an integrated pest management program. Further studies are warranted to determine if GHA could be used in field control of citrus thrips.

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Table 2-1. Toxicity of various strains of *B. bassiana* against adult female citrus thrips

Strain	N	Slope (SE)	Lethal concentration in conidia /ml (range) [a]		χ^2	DF
			LC_{50}	LC_{95}		
GHA	828	1.08 (0.11)	8.6 x10^4 (5.8 x10^4 – 1.2 x10^5)	4.8 x10^6 (2.6 x10^6 – 1.1 x10^7)	3.65	2
1741ss	836	0.65 (0.13)	2.7 x10^6 (9.7 x10^4 – 7.7 x10^6)	9.6 x10^8 (1.4 x10^5 – 6.8 x10^{12})	42.07	2
SFBb1	832	0.97 (0.18)	6.3 x10^6 (2.1 x10^5 – 1.9 x10^8)	3.1 x10^8 (2.1 x10^6 – 4.5 x10^{11})	80.31	2
S44ss	834	0.85 (0.19)	4.2 x10^6 (2.8 x10^5 – 6.1 x10^7)	3.5 x10^8 (7.8 x10^5 – 1.6 x10^{11})	41.34	2
NI1ss	854	0.83 (0.16)	3.3 x10^6 (1.1 x10^5 – 9.9 x10^7)	3.1 x10^8 (1.6 x10^6 – 6.3 x10^{11})	65.87	2
3769ss	814	0.93 (0.18)	4.2 x10^6 (7.4 x10^5 – 2.4 x10^7)	2.5 x10^8 (6.1 x10^6 – 1.1 x10^{10})	19.36	2

[a] LC_{50} and LC_{95} values are estimated concentrations required to kill 50 and 95% of the thrips, respectively, based on probit – log dose analysis.

Table 2-2. Toxicity of various strains of *B. bassiana* against adult female avocado thrips

Strain	N	Slope (SE)	Lethal concentration in conidia /ml (range)[a]		χ^2	DF
			LC_{50}	LC_{95}		
GHA	832	0.85 (0.21)	2.2 x10^6 (2.9 x10^5 – 1.7 x10^8)	2.0 x10^8 (2.5 x10^6 – 1.6 x10^{10})	23.88	2
1741ss	815	0.78 (0.14)	4.7 x10^6 (4.0 x10^5 – 5.5 x10^8)	6.2 x10^8 (1.7 x10^6 – 2.3 x10^{11})	29.11	2
SFBb1	819	0.79 (0.23)	1.1 x10^7 (8.7 x10^5 – 1.5 x10^8)	1.4 x10^9 (2.2 x10^6 – 1.1 x10^{13})	29.95	2
S44ss	830	0.86 (0.21)	6.4 x10^6 (3.5 x10^5 – 1.1 x10^9)	5.3 x10^8 (7.3 x10^5 – 9.0 x10^{11})	47.43	2
NI1ss	812	0.87 (0.17)	7.1 x10^6 (9.8 x10^5 – 5.2 x10^8)	5.5 x10^8 (6.2 x10^6 – 5.2 x10^{10})	21.65	2
3769ss	824	0.92 (0.19)	9.7 x10^6 (4.6 x10^5 – 2.1 x10^8)	5.9 x10^8 (5.3 x10^5 – 6.7 x10^{11})	55.53ns	2

[a] LC_{50} and LC_{95} values are estimated concentrations required to kill 50 and 95% of the thrips, respectively, based on probit – log dose analysis.

Figure Legends

Fig. 2-1. Dose-response raw data lines for *B. bassiana* tested against (A) avocado thrips, *S. perseae* and (B) citrus thrips, *S. citri*.

Fig. 2-2. Survivor curves of daily mortality data for *B. bassiana* strains infecting citrus thrips. The survivor curves for GHA and SfBb1 are significantly different from all other strains. GHA showed the fastest infection rate.

Fig. 2-3. Survivor curves of daily mortality data for *B. bassiana* strains infecting avocado thrips. The survivor curves for the various strains were not significantly different from one another.

Fig. 2-1

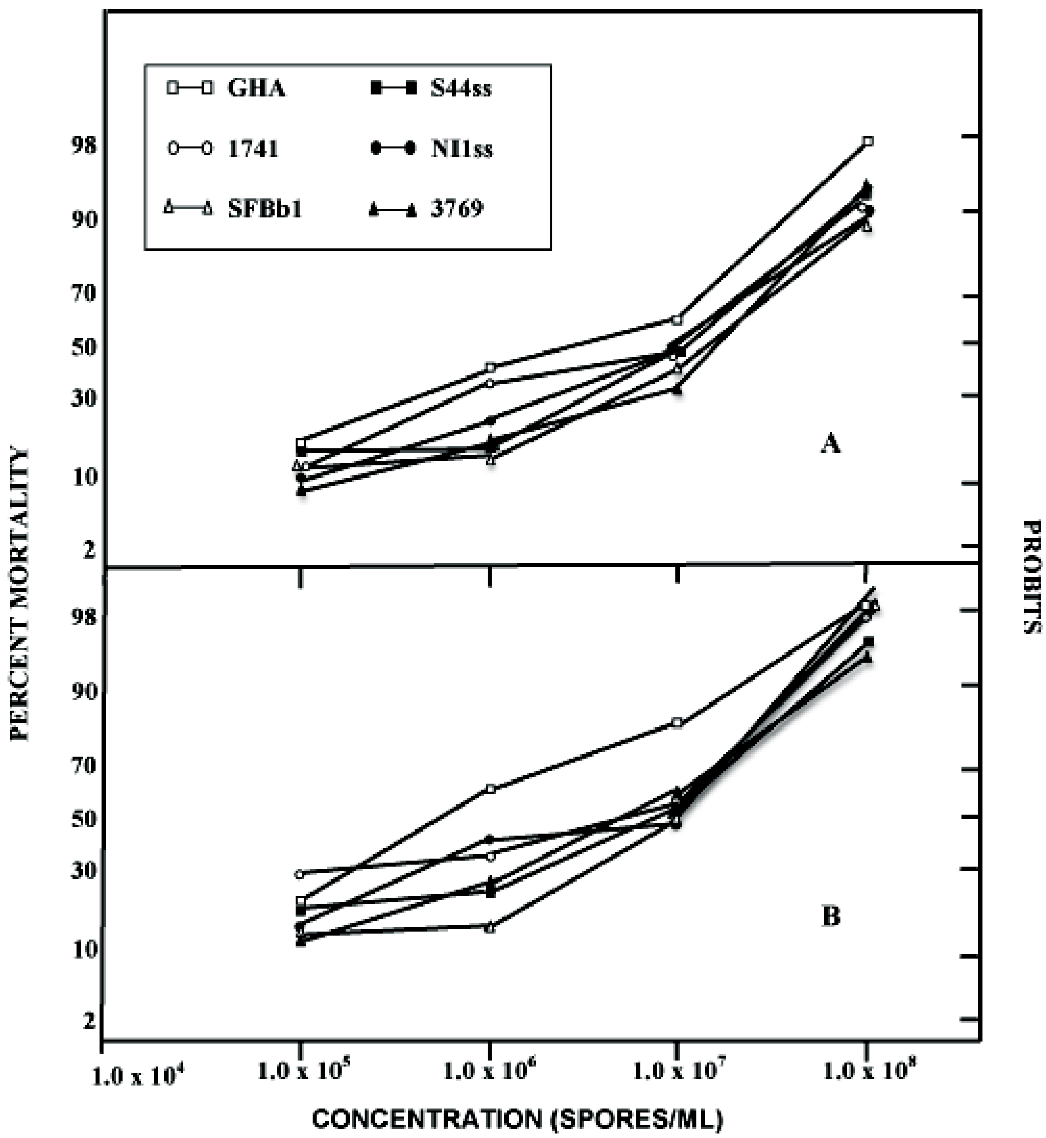


Fig. 2-2.

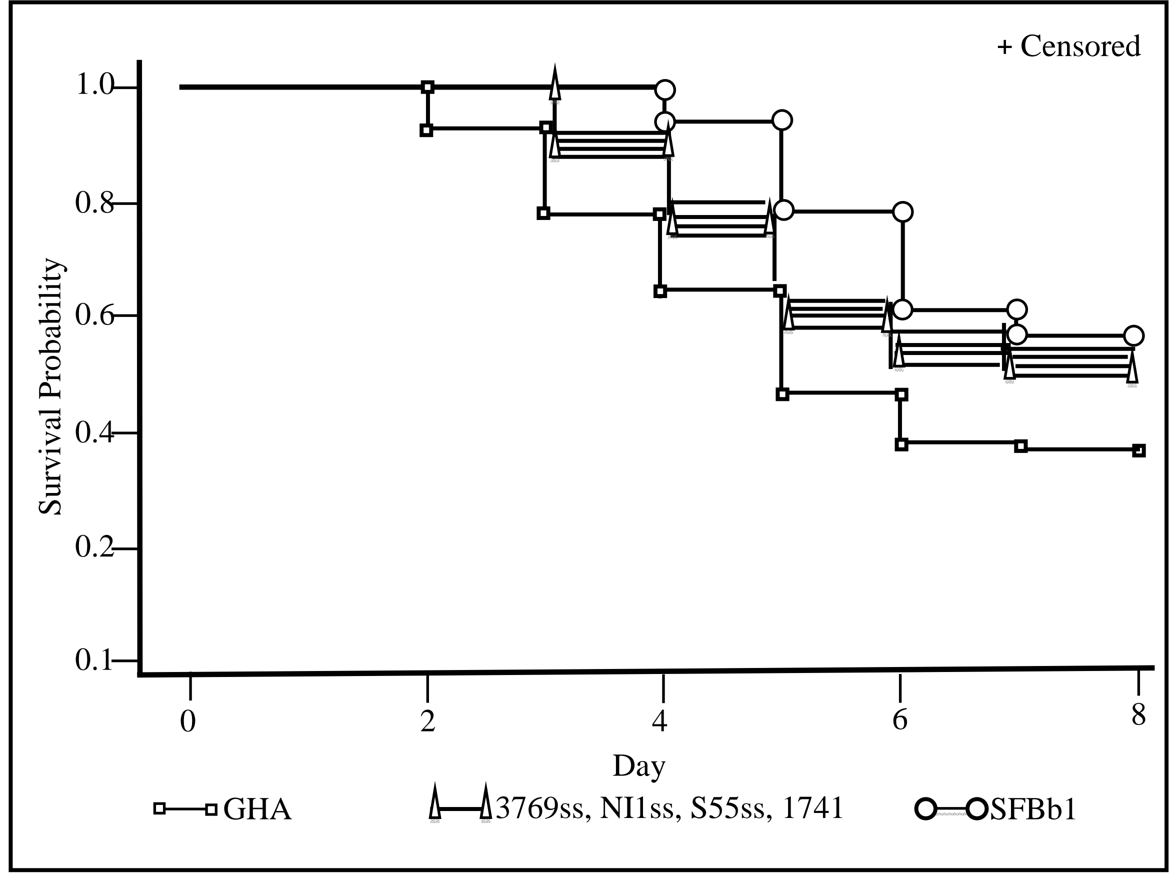
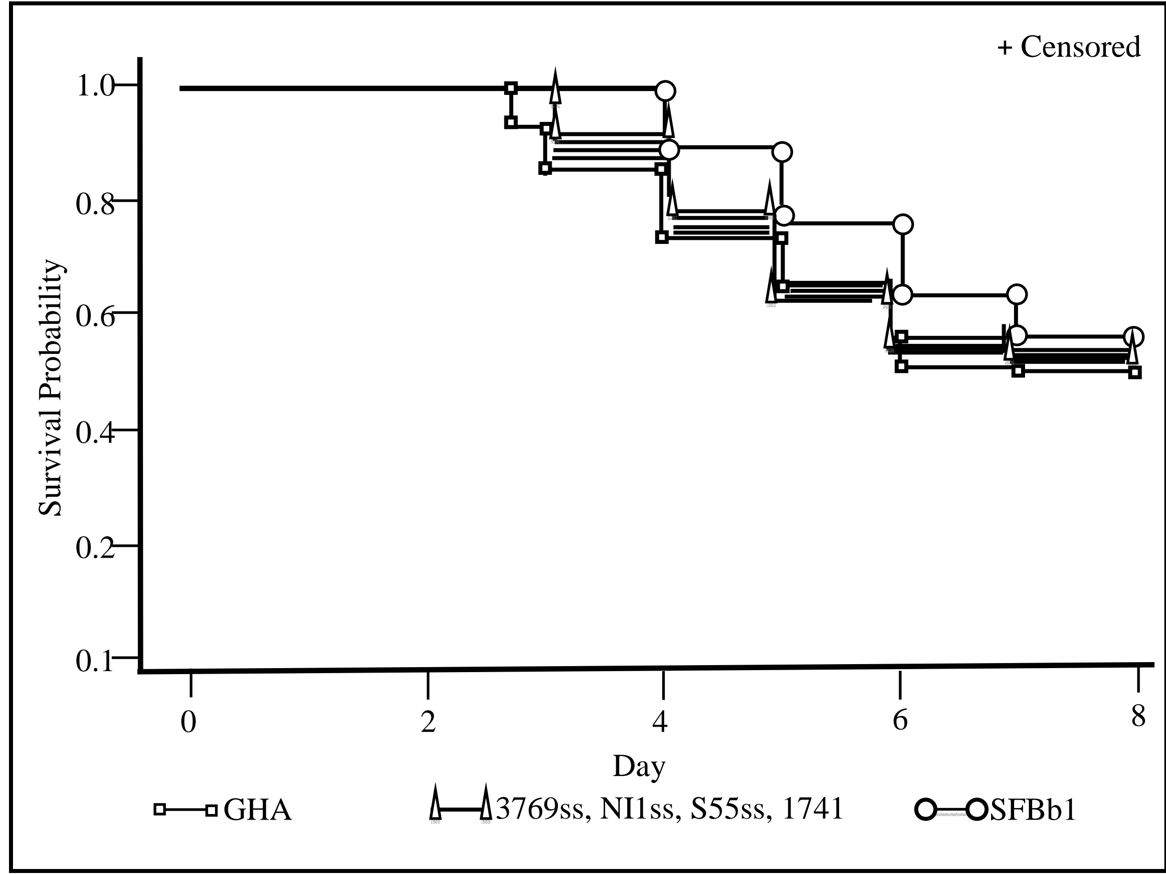


Fig. 2-3



Chapter 3

Abstract

Citrus thrips, *Scirtothrips citri* (Moulton), is a plant-feeding pest most widely recognized for causing damage to citrus and mango fruits. This insect has broadened its host range to become a significant pest of California grown blueberries. Effective alternatives to traditional pesticides are desirable to reduce impacts on natural enemies and increase control options in an effort to minimize pesticide resistance via rotation of control materials with different modes of action. We evaluated *Beauveria bassiana* (Balsamo) as a control agent for citrus thrips in blueberries in California under two water regimes (drip irrigation with and without overhead sprinklers) and using two fungal formulations (commercially available spores in suspension versus colonized seed) over two sampling periods, i.e. for two 3-day periods after treatment. We found significant differences in thrips efficacy as a function of water regime treatment and fungal formulation. Thrips levels were reduced significantly with both fungal treatments at 3 days after treatment, but at 6 days, only results with colonized seed differed from the control treatment. Results suggest entomopathogenic fungi might be useful for control of citrus thrips on blueberries in particular situations (organic production, or as a resistance management option) but that traditional pesticides will still be relied on heavily.

Introduction

Citrus thrips, *Scirtothrips citri* (Moulton), has been recognized as a major pest of California citrus since the 1890s (Horton 1918) and is also known to scar mango fruits (Morse 1997). Historically, highbush varieties of blueberries (*Vaccinium corymbosum* L.) could only be grown in regions too cold for citrus production (Jimenez et al. 2005, Strik and Yarborough 2005). However, breeding efforts to cross the northern highbush blueberries with several other *Vaccinium* species led to the development of heat-tolerant highbush blueberry varieties (*V. corymbosum*). This has enabled the establishment of a blueberry industry in the San Joaquin Valley, a region where both citrus and citrus thrips flourish (Jimenez et al. 2005, Strik and Yarborough 2005). The known host range of citrus thrips has broadened and in recent years, they have become a significant pest of blueberries planted in the San Joaquin Valley of California (Haviland et al. 2009). Citrus thrips feed on blueberry foliage during the middle and late portions of the season causing distorted, discolored, and stunted flush growth and poor development of fruiting wood required to obtain the subsequent crop. Repeated pesticide applications of the few effective and registered pesticides to reduce thrips populations pose a concern regarding pesticide resistance management, and this issue is relevant not only to the blueberry industry but also for the 108,665 ha of California citrus which has experienced repeated documented cases of pesticide resistance in citrus thrips populations (Morse and Grafton-Cardwell 2006, 2009). Currently, there are no integrated pest management plans

available for control of citrus thrips in blueberry, probably due to the recent nature of this crop-pest association.

With a limited number of pesticides available for thrips control and the frequency of insecticide resistance shown by thrips, populations should be monitored carefully, treatments limited to populations of economic concern, and applications timed optimally (Morse and Grafton-Cardwell 2006, 2009, Morse and Hoddle 2006). Appropriate cultural practices and conservation of natural enemies should be practiced in concert with the use of pesticides only on an as-needed basis. Understanding citrus thrips' life history in the blueberry system to determine where and if susceptible stages could be exploited, is one of the first steps in the development of alternative methods to the use of traditional insecticides.

In citrus, citrus thrips pupation occurs on the tree in cracks and in crevices, however, the majority of thrips drop as late second instars from trees to pupate in the upper layer of leaf litter below trees (Grout et al. 1986, Schweizer and Morse 1996) and move upward onto the plant after adult eclosion. Propupae and pupae are rarely seen, move only if disturbed, and do not feed. Pupation in the upper layers of the soil surface may create the ideal interface for control using the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin due to this vertical movement of the citrus thrips. However, blueberry plants have much different plant architecture than citrus trees and citrus thrips pupation behavior has yet to be studied on blueberries.

In the U.S., pressure is increasing to move away from broad-spectrum insecticides and focus on alternative methods of control. Earlier work with *B. bassiana* determined that the commercially available strain, GHA (Laverlam International Butte, MT), was the most effective of six strains tested in laboratory trials against citrus thrips (Zahn and Morse 2011). The goal of this study was to determine if this strain of *B. bassiana* could be utilized effectively against citrus thrips in California blueberry production. To achieve this objective, several factors of importance to fungal efficacy were evaluated before commencement of our field trial: 1) location of citrus thrips pupation in commercial blueberry plantings, 2) field sampling locations and methods, 3) fungal formulation and timing of application, and 4) density of product used and method of thrips infection. We then conducted a field trial evaluating the potential utility of the GHA strain of *Beauveria bassiana* in commercial blueberries for citrus thrips management as a possible alternative to the use of traditional insecticides.

Materials and Methods

Source of insects for greenhouse studies. Citrus thrips were collected in Riverside County, Riverside, CA from wild laurel sumac, *Malsoma* (=*Rhus*) *laurina* (Nutt.), a suspected major host for this species before citrus was introduced into the state (Morse 1995). Thrips were collected via aspiration the morning of the bioassay and held in 15-dram (55 ml) plastic aspiration vials with a copper mesh screened lid. A small

sumac leaf, just large enough to fit in the vial, was included to allow the insects to settle on the leaf and feed.

In experiments where late second instar thrips were needed, i.e. thrips that were close to pupation, selected thrips were large and had darkened in color. Their abdomens appeared plump and the overall color of the thrips was a deep yellow with almost no opalescence. Early to mid second instar thrips show limited abdomen distention and have an overall pearlescent hue. When adult females were used, selected females were of unknown age.

Location of citrus thrips pupation in potted blueberries. Because of the complex arrangement and number of blueberry canes (typically 3-8) arising from the rhizome of commercial blueberry plants, we first evaluated movement of second instar citrus thrips on potted single cane blueberry plants in the laboratory. Known numbers of late second instar citrus thrips were released onto the leaves of potted blueberry plants in the lab. Paper sprayed with Tangle Trap sticky coating (BioQuip Products, Rancho Dominguez, CA) was placed a) at the base of the plants with a ring of sticky tape around the base of the stem and partially on the stem (0-0.1 cm) of the plant to capture any insects crawling down, and b) extending from the base of the plant horizontally outward above the pot surface to ensure complete coverage of the area covered by the plant canopy (thrips numbers were measured at a radius of 0.1 - 12.7 cm and 12.8 - 25.4 cm to split the area under the plant canopy in half). This experiment was replicated on a single potted plant over time on 7 dates (total of 231 thrips, 30 - 38 insects released per pot per

date; a different plant was used each time). Data were analyzed using Fisher's exact test using SAS 9.2 (SAS Institute 2008).

Field sampling of thrips pupation sites. At our planned field trial site (Delano, CA) that would later be used in the *B. bassiana* trial, pupation emergence cages were used to sample insects moving off foliage towards pupation sites and later emerging out of the soil after pupation. Cages were made from Schedule 40 white PVC pipe (Powell Pipe & Supply Co, Riverside, CA) with a diameter of 10.2 cm with cages cut to a height of 5.1 cm. The cage was then topped with a double-sided sticky card cut to fit, which was fixed into place with two elastic bands. Four lines of four cages (16 total per plant) were pushed into the soil to a depth of approximately 1 cm immediately adjacent to each other at the base of a blueberry plant and oriented in a cardinal plane (north, south, east, west) to determine which direction showed the most thrips activity. The four adjacent cages in a particular plane were used to assess thrips movement in the understory of the blueberry plant in each directional. The study was replicated on 5 plants on a single date and conducted just prior to the commencement of the field trial. Data were analyzed with a nested ANOVA using SAS 9.2.

Fungal formulation and timing of application. In a greenhouse trial, Mycotrol O® (BioWorks, Inc., Victor, NY; the label states there are 2 x 10^{10} conidia per ml and the formulated product is 10.9% *Beauveria bassiana* strain GHA) was applied directly to the soil surface as raw spores and compared to the same product colonized onto millet seed, also using soil application. Millet seed colonization used the Stanghellini and El-

Hamalawi (2004, 2005) method as described below. The colonized millet seed, when allowed to imbibe water and incubate in the laboratory, can support 1.0 x 10^6 conidia/seed (Stanghellini and El-Hamalawi 2005).

Based on Stanghellini et al. (2004, 2005) with modification, we held the GHA colonized millet seed in containers such that the seed mat was at a depth of no greater than 2.54 cm. The seeds were wet (but not submerged in water) with the consistency of very thin slurry and were gently stirred three times per day for four days to ensure they imbibed water properly so that mycelial growth and sporulation would occur. Sporulation was confirmed by slide mounting random sections of mycelia and checking for condia formation under the microscope. Once spores were initially observed, the seed was held an additional three days so that sporulation could continue before use of the colonized seed in the field study. Mycotrol O® was applied in the maximum recommended field rate for high thrips levels of 2.84 L of material in 378.5 L of water.

Thrips avoidance of colonized millet seed. The colonized millet seed was tested in the greenhouse (maintained at 27°C, 30% RH, 16L:8D) to determine if late second instar citrus thrips (i.e. those ready to seek a pupation site) would become infected if they crawled over or through the seed when it was placed at the base of a laurel sumac seedling. A single small laurel sumac seedling, about ~10 cm tall, was placed into each of ten, 9.5 x 9.5 x 18 (height) cm styrene cages with 6 cm diam air holes on all four sides that were covered with ultra fine mesh screening (0.015 x 0.0059 mm, Catalog no. 7261 BioQuip Products, Dominguez Hills, CA). Small holes were made in the bottom of the

container and covered with pebbles to allow for drainage, then soil was added to a depth of 7.62 cm and the top of the container was covered with a removable lid. The base of each plant was completely surrounded by either *B. bassiana* colonized millet seed or with uncolonized seed (as a control). A minimum of 20 late second instar thrips were released onto the leaves of each plant, and were left until enough time (average of 5 days) had passed for the thrips to molt to the propupal stage. The seedling was then cut at the soil line and examined for pupating thrips; the removable lid of the cage was sprayed with Tangle Trap sticky coating to collect any emerging adults after 5 days so infection could be measured. The study was replicated on 5 dates (i.e. millet seed with or without *B. bassiana* x 5 replicate plants with 20 thrips per plant x 5 dates). Data were analyzed using 1-way ANOVA with time as a factor and means were separated using Tukey's Least Significant Difference (LSD) test using SAS 9.2.

Density of colonized millet seed to use. To determine the optimum number of colonized millet seeds needed for close to 100% infection when thrips were seeking pupal refuges off the plant, varying amounts of colonized seed were evaluated in a greenhouse trial based on the size of the seed once it had imbibed water and sporulation had occurred. After water inhibition, nine seeds completely filled one square cm of soil surface. A laurel sumac seedling (~10 cm tall) was placed into each of eight styrene cages (same as above) per block. There was a 0.5 cm buffer area around all sides of the cage, which was kept clear of seed to provide a 9 x 9 cm grid of seed on the soil surface below the plant. All but two leaves were plucked from the seedling. Small holes were

made in the bottom of the container, which was covered with pebbles to allow for drainage. The 9 x 9 cm^2 grid was created from wire screen and differing amounts of sporulating seed (0.5, 1 and 2 seeds per cm^2) or seed alone (control) were placed on the light imprint made from the wire screen on the soil surface. Two replicate seedlings per treatment were set up per date in a complete block design (with or without *B. bassiana* colonized seed x 0.5, 1, or 2 seeds per cm^2 x 2 replicate blocks per date x 5 dates). Plants were watered every third day. A minimum of 20 late second instar thrips were placed onto the leaves of the plant, and were left until enough degree-days had passed for the thrips to molt to the propupal stage, typically about 5 days. The seedling was then cut at the soil line and examined for pupating thrips; the removable lid was sprayed with Tangle Trap sticky coating to collect any emerging adults after another 5 days. Data were analyzed using a 3-way ANOVA with density of seed (4 levels including the uncolonized seed control), application of *B. bassiana* (with vs. without), and date as factors (SAS 9.2). Unrecovered insects were counted as missing data and were not included in the analysis.

Field evaluation of *B. bassiana* for citrus thrips management. The commercial blueberry test site selected was located north of Bakersfield in Delano, CA. The trial began in August of 2008 and was conducted post blueberry harvest. The *V. corymbosum* varieties contained within the test area (Fig. 3-1) were, 'Santa Fe', 'Jewel', and 'Star'. The most susceptible variety of blueberry to citrus thrips damage grown at the test site was the ‘Star’ variety (D.K.Z. unpublished data) and ‘Star’ was used consistently for evaluation of thrips numbers for all aspects of the trial (i.e. pupation cage data,

measured shoot growth and thrips beat samples). Our cooperator was interested in alternatives to traditional pesticides as the farm regularly was dealing with extremely high citrus thrips populations. For example, in 2008 the grower sprayed 5-10 times per field (depending on thrips pressure), rotating with traditional chemicals to reduce thrips impact on the subsequent year's fruit set. Irrigation in all fields took place via drip irrigation with one water delivery emitter per line at each plant base (two lines of irrigation tubing positioned with plants in between the two lines of irrigation), but additionally, one portion of the blueberry field was equipped with 360° overhead sprinklers. This irrigation setup provided the ideal situation to test *B. bassiana* under two watering regimes.

The commercially available GHA strain (Mycotrol O®) is formulated to be mixed with water and for application via chemigation or as a foliar spray. The label states that no surfactant is needed to keep the spores in suspension. However, agitation alone in the 1,892.7 L holding tank was not sufficient to keep the material from precipitating, therefore 312.3 ml of Silwet L-77 (GE Silicones, Friendly, WV) was added to the tank mix. Mycotrol O® was applied directly to the soil surface with a gas-powered sprayer with a hand spray gun equipped with an adjustable flow meter. The dimensions of the plots were used to calculate the amount of material needed for both *B. bassiana* formulations (raw spore soil application and colonized millet seed).

Plants in the test field were spaced every 0.92 m down each row, 3.35 m between each row, and each row was about 165 meters in length. Our studies were conducted in

an 18-row section of a 4.04 ha field. The overhead sprinklers were spaced every 7 meters in the row and were located every other row for 12 rows. We chose to investigate the effectiveness of the *B. bassiana* colonized millet seed versus a Mycotrol O® soil application under two watering regimes, (1) drip-line alone versus (2) drip-line with overhead sprinkler, because *B. bassiana* conidia are highly subject to desiccation. Comparing the soil drench in both irrigation types with the colonized millet elucidated the effectiveness of the treatments when compared to the control. The blocks were laid out in a 3 x 2 factorial design, with each block consisting of most of five rows of blueberries (both sides of the inner three rows and only the inner half of the two outer rows), each being 27.4 m long (about 30 plants) (Fig. 3-1).

The berm (raised soil bed) used to grow blueberries at the commercial farm was 1.21 meters wide and each plot was 27.4 meters long. The spacing between adjacent rows was 3.35 m, while the spacing between the plants down a row was approximately 0.92 m with 30 plants per treatment plot (Fig. 3-1). These dimensions result in 0.157 ha treated with raw spores but because the top of the berm (1.21 meters) was where thrips activity was evident and would be sampled, only 36% of the soil surface area was treated. The Mycotrol O® label states that the maximum field rate is 6.9 L/ha mixed in 935.3 L/ha water. We therefore chose to apply the entire 6.9 L of Mycotrol O® in 378.5 L of water per ha directly to the berm with no application between the rows, which resulted in 100% of the per ha rate of product being applied to 36% of the area and allowed the maximum amount of active ingredient to be applied to the area that would have almost all thrips

activity (see the results of the field pupation studies below). Our field trial was intended to determine the extent to which *B. bassiana* might fit into a program projected to both control citrus thrips effectively and provide rotation among available chemistries so as to reduce thrips resistance evolution. Thus, we felt it was important to operate under the best possible conditions for thrips infection by Mycotrol O®, regardless of financial considerations, i.e. application of product at the maximum label rate in the area where thrips were most likely to be active.

The amount of millet seed used in the field trail was calculated based on the area of the berm to be treated and likewise with the Mycotrol O® treatment, only 36% of the total field area was treated. The amount of seed used was one colonized seed/ 2 cm^2 (0.5 seeds per cm^2 determined from greenhouse trials) over an area of 576 m^2; the fact that 0.45 kg of seed was needed per 840 cm^2 resulted in the application of 3.40 kg of colonized millet seed for the 8 treated plots (four each with and without overhead sprinklers).

Every other plant within the middle ten plants of the middle row of each plot ('Star' variety) were sampled with pupation emergence cages (see details above: 10.2-cm diameter Schedule 40 white PVC pipe cut to a height of 5.1 cm, fit with a double sided sticky card on the top, pushed into the soil to a depth of 1 cm). These cages were placed tight against the base of each set of canes on the east side (see Results -- the direction with the most thrips). With 5 cages per block and 4 replicate blocks per treatment, a total of 20 cages sampled thrips pupation per treatment over two sample periods, i.e. for two

consecutive 3-day periods after the Mycotrol O® soil drench. The treatments were: (1, 2) no *B. bassiana* with and without overhead sprinkler; (3, 4) colonized millet seed with and without overhead sprinkler; and (5, 6) a soil drench of Mycotrol O® with and without overhead sprinkler (Fig. 3-1). In total, data were collected from 240 emergence cages over the duration of the trial (irrigation with and without overhead sprinkler x three *B. bassiana* treatments [control, colonized millet, Mycotrol O® soil drench] x four replicate blocks x 5 pupation emergence cages per plot x two consecutive 3-day sampling periods). The colonized millet seed was set to imbibe water and allowed to sporulate for three days before application and was applied using a hand fertilizer applicator (Scott's Handy Green, Model# 71133, Lowe's, Moreno Valley, CA). Four days post application of the millet seed, the soil drench of Mycotrol O® was applied and pupation emergence cages were placed in the field and left out for 3 days (sample period one). After three days, the sticky cards from each emergence cage were collected and replaced with new cards and the traps were switched to the next plant (moving north) on the east side. These traps were left in the field to sample thrips for another 3 days (sample period two). Because the traps were placed out every other plant, this ensured that all of the middle ten plants were sampled over the two, 3-day sampling periods (i.e. blocked through time).

For two weeks before through two weeks after the applications of *B. bassiana* (6 weeks total), counts were taken of thrips levels on plants twice per week. Beat samples were taken by beating random canes of flush foliage such that the thrips would fall onto a 12 x 12 cm black acrylic beat tray. The numbers of thrips on the beat trays were counted

quickly in the field. The counts (leaves beat onto trays, counting larvae and adults) were taken twice per week from each of the 10 central "data plants" from the 'Star' variety of each of the 24 test plots. The new green flush growth was measured on three dates (8/19, 8/29, and 9/4/2011) to record the amount of growth since the beginning of the fungal treatment applications to determine if there were differences based on the treatments and amount of water applied to the different plots. Measurements were made of the average cm of new shoot growth over the 6-week trial period. Due to the complex nature of the experimental design, i.e. treatments nested in a 5-way ANOVA (*B. bassiana* at 3 levels, overhead sprinkler irrigation at 2 levels, 5 data plants within each plot, 'spatial' quadrants at 4 levels and finally, a temporal factor at two levels), beat count data were analyzed using PROC MIXED and means were separated using Tukey's test (SAS 9.2).

Results

Location of citrus thrips pupation in potted blueberries. Figure 3-2 shows the location of late second instar citrus thrips at death in the greenhouse study as well as those that located pupal refuges on the plant. Based on where they dropped off the plant, data indicated that more than 92% of the thrips would have pupated off the plant, likely in the soil near the base of the plant. Numbers did not vary significantly by location over the seven sample dates of this study; therefore data were pooled ($P = 0.587$). A key result was that the proportion of second instar thrips crawling down the base of the plant

was higher (Fig. 3-2, grouping 'a') than the proportion dropping off the plant at distances measured past the base the plant (i.e. in the shadow of the plant a distance 0.1-25.4 cm from the base; grouping 'b') (Fisher's Exact Test, $P = 0.0162$).

Field sampling of thrips pupation sites. The four emergence cages placed under the field blueberry plants in each cardinal direction (16 cages total) provided a means of sampling late second instar thrips moving towards the soil to pupae (Fig. 3-3A) versus adults emerging out of the soil following pupation (Fig. 3-3B). Total numbers of thrips collected were pooled for the four traps in each direction at each respective location to determine which cardinal direction showed the most activity, and therefore was the most appropriate location to sample for citrus thrips in the field trial. Emergence cage data (four lines of four cages placed at the base of the blueberry plant and oriented in cardinal directions) were summarized in two ways; the number of thrips moving off the plant to pupate in the leaf litter (Fig. 3-3A) and the number of thrips emerging from the leaf (Fig. 3-3B). Data from the nested ANOVA generated *p*-values for direction ($P = 0.0217$), as well as distance grouping from the base of the plant ($P < 0.0001$). The cage closest to the base of the plant had significantly higher numbers of thrips emerging from the soil ($P < 0.0001$). Numbers of thrips trapped from the eastern cardinal direction were significantly higher for both mean numbers of thrips moving to and from the soil ($P < 0.0001$), indicating that for the field trial, emergence cages should be placed directly next to the base of the plant on the eastern side to sample the location that would have the most thrips activity.

Fungal formulation and timing of application. Not only did the second instar thrips not avoid the GHA colonized millet seed, they were observed (at irregular intervals) actively walking through it. Mortality was 100% in millet seed treatments across all trials compared to the untreated checks, which ranged from 0% to 8% mortality across these trials.

Thrips avoidance of colonized millet seed. Of the proportion of thrips not finding pupal refuge on the plants with colonized seed (as opposed to controls with seed alone), 100% infection was seen with each of the different quantities of seed, i.e. each of 0.5, 1, or 2 seeds/cm^2 was a sufficient density to infect and kill all late second instar thrips in the greenhouse study. No thrips were infected in the control treatment. There were insufficient data to conduct a 3-way ANOVA because all recovered thrips were infected with the fungus. Because all three densities tested were effective, we chose to utilize the most economical density in the field trial, i.e. 0.5 seeds/cm^2.

Field evaluation of *B. bassiana* for citrus thrips management. In the split-plot design model, the whole plot factor was water and the split-plot factor was fungus treatment in a type three analysis of variance (Table 3-1). Water, time and treatment were the main effects in the full model. Thrips levels measured on pupation traps at 3 days after treatment were lowest with colonized millet seed, intermediate with Mycotrol O®, and highest in the untreated control (Table 3-2). Additionally, there was fewer thrips counted in the colonized millet seed treatment than in Mycotrol O® treated plots (Table 3-2). However, at time two (emergence cages out for days 3-6 after treatment), thrips levels

with Mycotrol O® were no longer significantly reduced in relation to the control (Table 3-2). While thrips levels measured using pupation traps were significantly less than observed in the control in all plots (Tables 3-2 and 3-3), thrips levels on plants measured using beat samples did not show a significant decrease (Fig. 3-4), although comparing data with no overhead water that with overhead water, there appeared to be fewer numbers of thrips in the overhead sprinkler plots (Fig. 3-4). The measurements from the new green flush growth in the overhead sprinkler treatments showed that those plants had longer growth than those without overhead sprinklers (Fig. 3-5), but thrips numbers were not significantly lower on those plants. At none of the times when foliar beat counts were taken were there significant differences in thrips numbers across any of the three treatments (control, millet seed, Mycotrol O®).

Discussion

The ultimate goal of this work was to determine if the GHA strain of *Beauveria bassiana* could be used effectively as an alternative to traditional insecticides in commercial blueberries in California. Laboratory and greenhouse trials with *Beauveria bassiana* have shown variable success in controlling thrips and several other insect species (Frantz and Mellinger 1998, Murphy et al. 1998, Jacobson et al. 2001, Azaizeh et al. 2002, Stanghellini and El-Hamalawi 2005, Ugine et al. 2005), whereas field trials have shown limited overall success, but very few field trials included Thysanoptera (Saito 1991, Maniania et al. 2001, Maniania et al. 2003). This is mainly due to the fact

that climatic conditions in the laboratory and greenhouse situations are stable and often more humid (optimal conditions for *B. bassiana*, Charnley and Collins 2007) than the ambient field environment in arid areas like most of California. Unfavorable environmental surroundings, including low humidity, high temperature and intense solar radiation are commonly referred to as the principal constraints to the field performance of *B. bassiana* (Hajek 1997, Glare and Milner 1991, Goettel et al. 2000, Inglis et al. 2002, Ugine et al. 2007). Raw fungal spores are prone to desiccation and death if, when sprayed, they do not contact a host immediately (Ignoffo 1992, Hajek and St. Leger 1994, Charnley and Collins 2007). The microclimate around the spore is thought to be primarily responsible for maintaining spore integrity (Fargues and Remaudiere 1977, Goettel and Inglis 1997); temperature, sunlight and ultraviolet light affect spore integrity but humidity, especially the immediate local humidity around the spore, dictates the spore's persistence and germination, particularly when ambient temperatures are high (Ignoffo 1992, Hajek and St. Leger 1994, Azaizeh et al. 2002, Charnley and Collins 2007). Perhaps it is for these reasons, that the water saturated seed was able to provide a suitable microclimate in which strain GHA could better sporulate and persist in comparison with the Mycotrol O® soil drench.

Determining methods of applying the GHA strain of *B. bassiana* so as to optimize field efficacy was one of the more interesting parts of this work. We took advantage of the observation that late second instar larval citrus thrips did not avoid the colonized seed and were able to infect themselves by either walking through or over the colonized seed.

Following the Stanghellini and El-Hamalawi (2004) protocols proved to be an effective method of applying and sustaining strain GHA in the field. Whereas this system is experimental, it provided a more persistent level of citrus thrips control than did the soil application of raw spores.

The foliar beat samples taken before, during and just after the trial did not show significant differences in thrips numbers across any of the treatments, but this could be due to the citrus thrips emigrating and immigrating out of and into the study area. The plots were 27.4 m in length by approximately 5 rows wide (~20 m) and while the samples were taken from the middle plants of the middle row in these plots, flushing blueberry plants were surrounding the area. Citrus thrips adults are highly mobile and search out succulent flush in which to oviposit eggs. The new, green, flush growth was significantly longer in the blocks that had overhead sprinklers, which intuitively is not unexpected, because there was substantially more water available to the plants; this is likely not a positive from a citrus thrips management perspective. If there is an abundance of new plant growth in some areas versus others, thrips likely will move into the parts of the field with more flush, sustaining elevated populations at a time when the other plants becoming less suitable, as their leaves toughen between flushing periods.

Currently, there is no integrated pest management program in place for citrus thrips pests of blueberries in California. The development of economic injury levels, economic thresholds and the optimal timing and rotation of registered insecticides are all essential portions of an IPM program and this information will form the basis of whether

or not the application of *B. bassiana* in any form, e.g., raw spores or colonized seed, would be an effective alternative to rotate with the use of traditional insecticides. There is the possibility of mixing entomopathogenic fungi and insecticide applications and several studies showed a synergistic relationship between the use of insecticides and fungi (Olmert and Kenneth 1974, Anderson et al. 1989, Neves et al. 2001). Possible synergism of strain GHA and insecticides registered for citrus thrips management in blueberries may be worthy of future study.

The costs of various registered pesticides used for citrus thrips management in commercial blueberries in California, including product and application costs but excluding purchase of mechanized equipment, ranges from \$80 – \$138/ ha (unpublished data). The cost of Mycotrol O®, not including application costs, ranges roughly from \$50 - \$120/ L and as mentioned previously, the maximum application rate per ha is approximately 6.5 L of formulated product. The cost of Mycotrol O® at the maximum application rate therefore would be approximately \$325 - \$780/ ha. Biopesticides, such as entomopathogenic fungus, are often higher in price than insecticides because they cost more to produce, are not in widespread use, and thus, are not produced on as large a scale as traditional insecticides. The fermentation process, i.e. submerged liquid fermentation (Romback 1989) or solid state fermentation for production of aerial conidia (Rousson et al. 1983), propagation requirements and storage and shelf life are all important considerations and steps for mass production of entomopathogenic fungi and their successful use. Fungal strain sporulation failure under mass production settings is often

the limiting factor to strain availability and usage and it is currently not well understood why this occurs.

Our results suggest that *B. bassiana* strain GHA can be utilized against citrus thrips on blueberries. We showed that over 0-3 days post-treatment, mean thrips numbers were decreased by 50% in both fungal treatment plots, i.e. with both Mycotrol O® and colonized seed. While this reduction is significant, it may or may not be economically competitive with traditional options. The cost associated with such an application of Mycotrol O®, once registered in blueberries in California, would be nearly triple the cost of a current insecticide treatment (above). Blueberries are a high value crop, estimated at $10 - $17/ kg for low and high fair-market price, respectively. This information, when coupled with the need for insecticide resistance management, indicates that utilizing entomopathogenic fungi could be worthwhile for insecticide resistance management of citrus thrips, as there are repeated documented cases of pesticide resistance in citrus thrips populations (Morse and Brawner 1986, Immaraju et al. 1989, Khan and Morse 1998, Morse and Grafton-Cardwell 2006, Morse and Hoddle 2006). Employing entomopathogenic fungi is costly and based on our data, not as effective as a current insecticide application (i.e. 50% control with fungi compared to much higher levels of control that are typically observed with insecticides). Because Mycotrol O® is an organic formulation (a different surfactant would be needed rather than the synthetic Silwet L-77), its utilization might be of interest to organic growers as an alternative to traditional insecticides.

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Figure Legends

Fig. 3-1. Blueberry field plot arrangement. The symbols S ('Star'), F ('Santa Fe'), and J ('Jewel') at the top of the map indicate the variety of blueberry planted in that row. The solid black lines form four quadrants delineating the four replicate blocks. The rows with dotted headers indicate rows that were saturated with water from the overhead sprinklers. The row bearing a Z indicates rows where the overhead sprinkler line was turned off. The row bearing a W indicates the one row where the overhead sprinkler was fixed to only 180° so that plants on the non-overhead water plots did not receive overhead water.

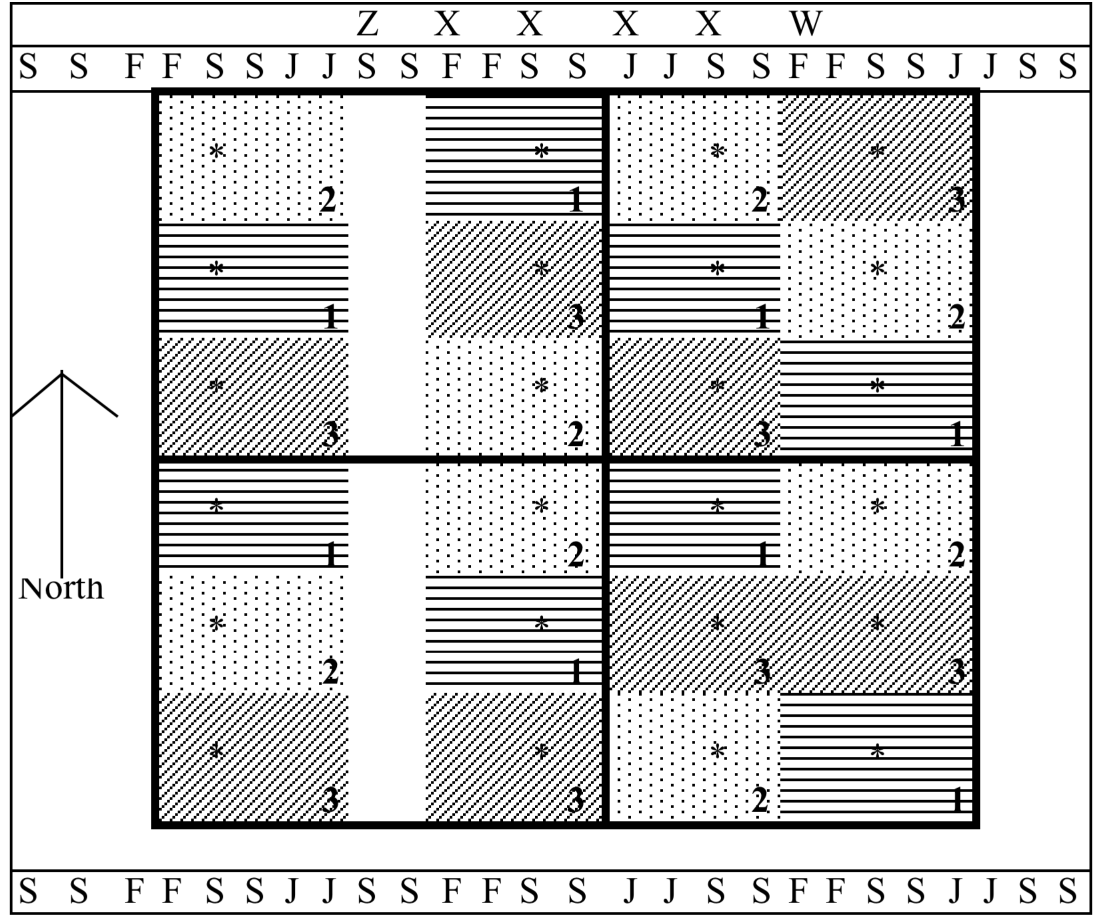
Fig. 3-2. Locations of late second instar citrus thrips at death in the laboratory potted blueberry study; 0-0.1 cm indicates thrips that were found dead on the ring of tape at the base of the blueberry stem. The last bar indicates thrips that were able to complete development by pupating on the blueberry plant and were discovered alive or were found dead (as adults) on the sticky sheet below the plant. Means (SE bars) followed by the same letter are not significantly different.

Fig. 3-3. Preliminary field study showing mean number of (A) late second instar citrus thrips moving off the blueberry plant to pupate and (B) adults emerging out of the soil in a commercial blueberry field. Means followed by the same letter are not significantly different.

Fig. 3-4. Foliar counts of immature and adult citrus thrips taken from beat samples pre-fungal application (7/25, 8/1), during the study (8/8, 8/15), and post-study (8/22, 8/29).

Fig. 3-5. Blueberry shoot measurements taken to observe any differences in plant growth with each treatment for the latter portion of the field study.

Fig. 3-1



No Sprinklers
1 Mycotrol O
2 Millet
3 No *B. bassiana*
* "Data" plant (10 plants)
X Overhead Sprinkler
W Overhead Sprinkler spraying 180° west
Z Off Overhead Sprinkler

Fig. 3-2

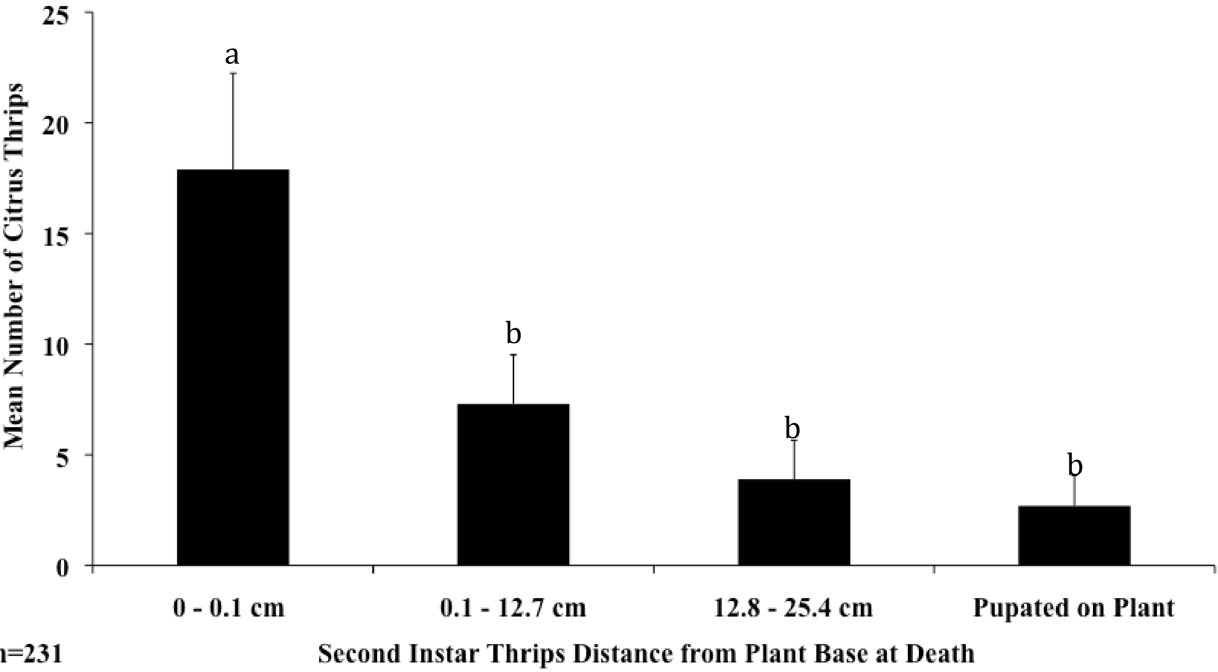


Fig. 3-3

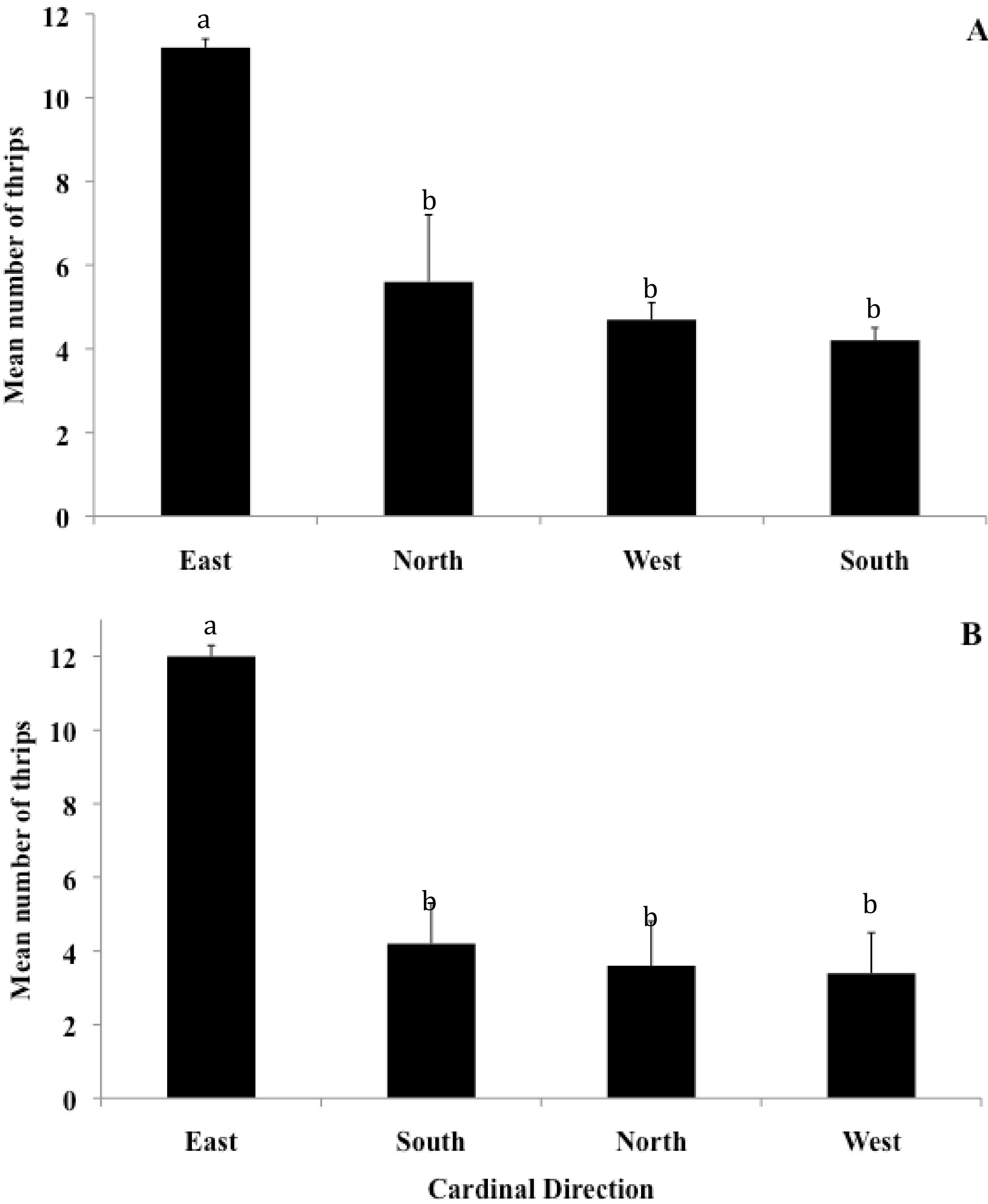


Fig. 3-4

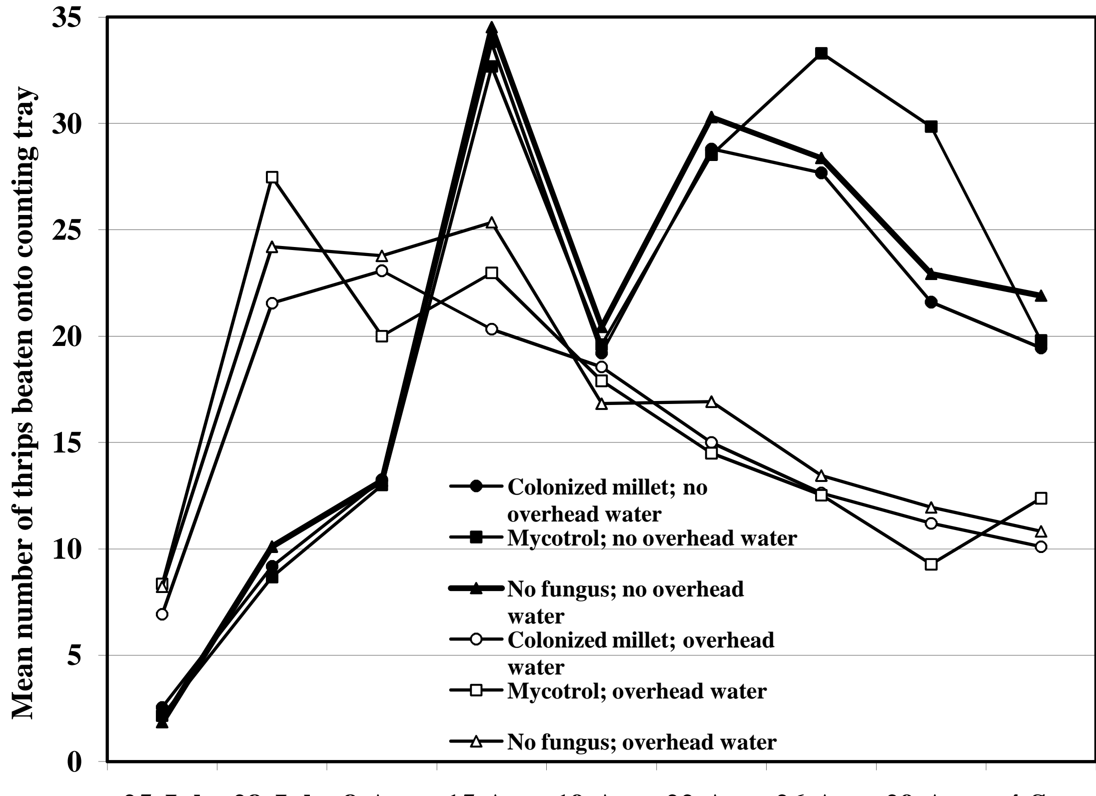


Fig. 3-5

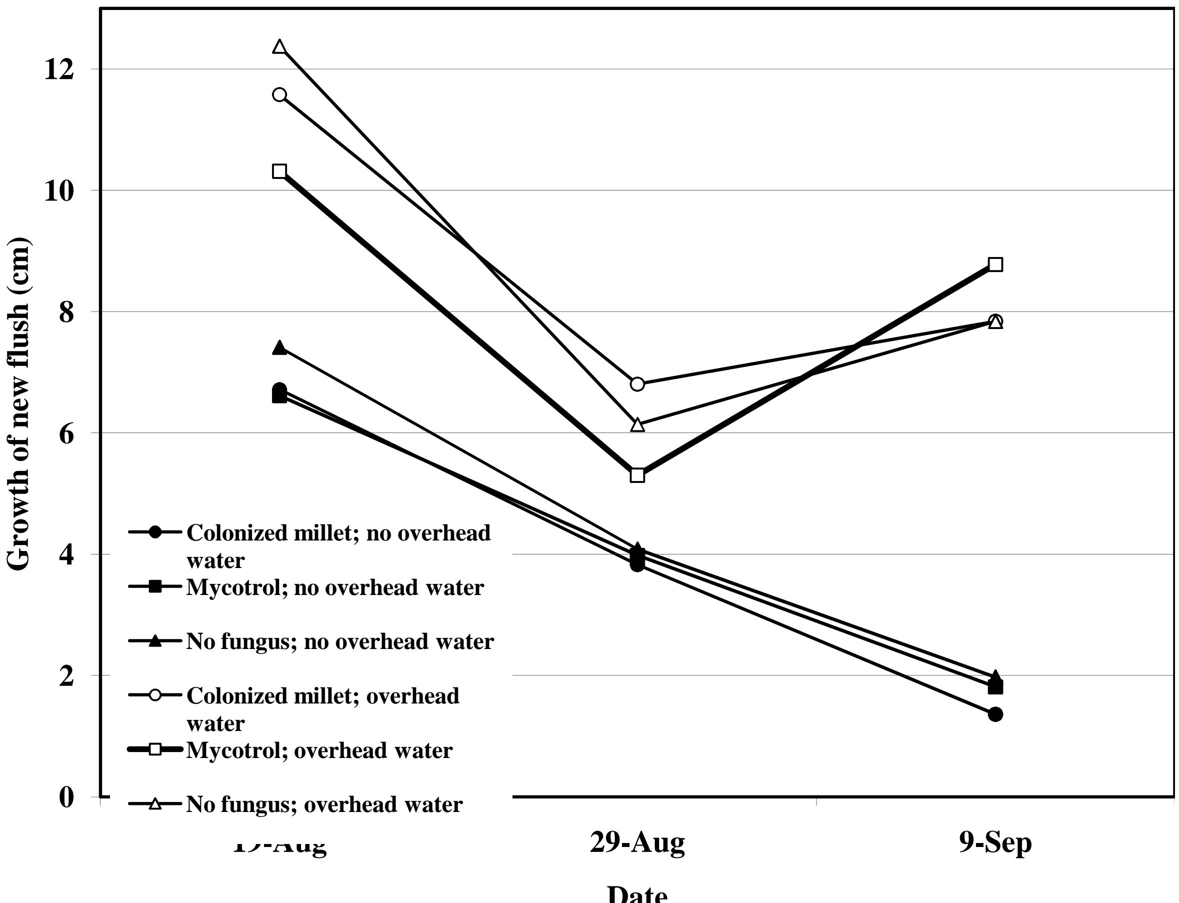


Table 3-1. Statistical model for the field trial pupation cage data showing results from type 3 tests of fixed effects (PROC MIXED, Tukey's Test, $\alpha = 0.05$)

Effect	Numerator DF	Denominator DF	F Value	Prob.>F
Water	1	3	28.52	0.0128
Treatment	2	222	54.23	<0.0001
Time	1	222	68.39	<0.0001
Time*water	1	222	14.95	0.0001
Time*treatment	2	222	2.00	0.1377
Water*treatment	2	222	3.58	0.0294
Time*water*treatment	2	222	0.90	0.1789

Table 3-2. Mean number of citrus thrips emerging from the soil (based on collection of adults from sticky cards) from emergence cages in the blueberry field trial

Treatment	Mean number of citrus thrips (SE) [a]
Sampling time one (0-3 d post-treatment)	
Colonized millet seed	7.4 (1.1) a
Mycotrol O®	10.8 (1.2) b
Control	13.7 (1.1) c
Sampling time two (3-6 d post-treatment)	
Colonized millet seed	3.78 (0.54) a
Mycotrol O®	8.08 (0.90) b
Control	8.78 (0.52) b

[a] Means followed by the same letter within a sampling period are not significantly different ($P < 0.05$, Tukey's test).

Table 3-3. Treatment interactions observed in the blueberry field trial ($\alpha = 0.05$)

Treatment	P-value
Sampling time one (0-3 d post-treatment)	
Colonized millet seed vs. Mycotrol O®	0.0011
Colonized millet seed vs. Control	<0.0001
Mycotrol O® vs. Control	0.0056
Sampling time two (3-6 d post-treatment)	
Colonized millet seed vs. Mycotrol O®	<0.0001
Colonized millet seed vs. Control	<0.0001
Mycotrol O® vs. Control	0.3995

Chapter 4

Abstract

Avocado thrips, *Scirtothrips perseae* Nakahara, is the major arthropod pest attacking avocados in California. Several species of predaceous insects and mites feed on avocado thrips, including the native predatory mite *Euseius hibisci* (Chant). Pesticide impacts on *E. hibisci* had yet to be evaluated and thus, we studied the impacts of abamectin, fenpropathrin, spinetoram, and sabadilla against this mite. Fenpropathrin showed the longest impact (111 d) and mite repellency in the spinetoram treatment was observed (mites drowning in wet felt surrounding the treated leaf). Mites were also confined to the spinetoram treated leaves in Munger cells to measure mortality in the absence of avoidance, which was high. Spinetoram and abamectin treated leaves were exposed to intense ultraviolet radiation to increase photodecomposition and this suggested that impacts may have been due largely to surface pesticide residues because UV exposure decreased repellency with spinetoram and mortality with abamectin. When only half of the surface of the test leaf was treated with spinetoram, *E. hibisci* mortality was reduced and mites gathered on the untreated side of the leaf.

Introduction

Avocado thrips, *Scirtothrips perseae* Nakahara, is the most serious arthropod pest attacking avocados (*Persea americana* Mill. [Lauraceae]) in California (Hoddle and

Morse 2003). They were first noticed in the state in June 1996 damaging fruit and foliage in two distant avocado groves, one each in Irvine, Orange County and Oxnard, Ventura County, CA. By July 1997, infestations of *S. perseae* had spread throughout avocado groves in Ventura and Orange counties (Hoddle 2002, Hoddle et al. 2003). California grows 95% of U.S. avocados on more than 2,500 hectares of land (Hoddle 1998, Hoddle and Morse 2003) and most of this land is infested with avocado thrips (Hoddle and Morse 2003). To date, there are four registered pesticides recommended for avocado thrips management: abamectin, fenpropathrin, spinetoram and sabadilla.

Several species of predaceous insects and mites feed upon avocado thrips and these natural enemies include brown and green lacewing larvae, several predaceous thrips (e.g., *Franklinothrips orizabensis* Johansen, *Franklinothrips vespiformis* Crawford, *Leptothrips mali* (Fitch) and several *Aeolothrips* spp.) and the native predaceous mite *Euseius hibisci* (Chant) (Acari: Phytoseiidae). McMurtry and Croft (1997) classified the feeding behavior of predatory Phytoseiidae into four groups and Group IV comprises the genus *Euseius*, members of which can subsist on pollen in the absence of prey with minimal reduction in fitness. Species of *Euseius* are the most common phytoseiids on both citrus and avocado. *Euseius hibisci* is known from Santa Barbara County in California to the sate of Oaxaca in southern Mexico (McMurtry et al. 1985). It mainly has a coastal distribution in California and is the dominant phytoseiid on avocados (McMurtry 1989). *Euseius hibisci* is common and abundant in avocado orchards year round, is an important generalist predator and feeds on pollen and leaf exudates in the absence of prey (McMurtry and Scriven 1964, McMurtry and Johnson 1965, McMurtry

et al. 1992). The most studied member of this genus is probably *Euseius tularensis* Congdon (McMurtry and Scriven 1964, Swirski et al. 1970, Kennett et al. 1979, Jones and Parrella 1983, McMurtry et al. 1992, Ouyang et al. 1992, Grafton-Cardwell et al. 1999, Khan and Morse 2006) and not nearly as much is known about *E. hibisci* with regards to pesticide exposure. In fact, *E. tularensis* was 'discovered' and described as a new species different from *E. hibisci* based on finding several populations of the former that showed a high tolerance to pesticides (Congdon and McMurtry 1985). Several studies have indicated the relevance of *E. hibisci* as effective biocontrol agents of spider mites and thrips on some crops (Tanigoshi and Nishio-Wong 1981, McMurtry 1985, Tanigoshi et al. 1985, Congdon and McMurtry 1985) and although *E. hibisci* is not a specialized predator, it potentially aids in enhancing the control of many different pest mites and thrips (Badii et al. 2004).

Assessment of acaricides and insecticides on non-target organisms is an essential component of any IPM program (Croft 1990) and is of particular interest to California avocado growers. This study evaluates the non-target impacts of the four pesticides currently recommended for avocado thrips management on *Euseius hibisci*.

Materials and Methods

Mite culture and rearing. *Euseius hibisci* colonies were established from specimens collected from non-insecticide treated avocado groves in San Diego Country, Fallbrook, CA. Modifying McMurtry and Scriven's (1964) rearing technique, we reared

E. hibisci in metal trays (21 by 21 cm), each containing a *Ficus* sp. leaf on a wet sponge surrounded by strips of wet white felt (0.91 m x 9.14 m white Craft Felt, JoAnn's Fabric, Moreno Valley, CA) with plastic cover slips and bits of cotton (pulled apart) placed in the center of the fully expanded leaves to provide shelter. Pollen from ice plant, *Malephora crocea* (Jacq.), was provided as food.

Pesticide application to avocado leaves. Avocado trees for all of these studies were located at the Agricultural Operations Facility at the University of California, Riverside. All avocado leaves used in these studies were fully expanded, mature leaves but not 'hardened' off based on leaf flexibility and color (fully mature leaves are dark green and fairly stiff). Leaves selected for bioassays were hand flagged with color-coded flagging tape the day prior to pesticide application. The four pesticides used in these studies were abamectin (Agri-Mek 0.15 EC [18.0 g AI/liter emulsifiable concentrate, Syngenta Crop Protection, Inc., Wilmington, DE] at 0.936 g AI per 100 liters + 1% NR-415 oil), fenpropathrin (Danitol 2.4 EC [287.6 g AI/liter, Valent BioSciences, Libertyville, IL] at 15.976 g AI per 100 liters), spinetoram (Delegate 25 WDG [25% AI water dispersible granules, Dow AgroSciences, Indianapolis, IN] at 4.369 g AI per 100 liters + 1% NR-415 oil) and sabadilla (Veratran D 0.2% [0.2 % sabadilla alkaloids + 80% sugar, Dunhill Chemical Co., Azusa, CA, acidified to pH 6.0 with citric acid] at 1.598 g AI + 199.7 g additional sugar per 100 liters). All pesticides were applied at their maximum per ha label use rate using a dilution rate of 2,805 L/ha (300 gallons of water per acre). A water only control was also applied. Pesticides were mixed the morning of the application and administered using a hand sprayer (Sprayco 1 L spray bottle, Model

SP32, Lowe's, Moreno Valley, CA). Each flagged leaf was located, the flag was marked with a number and the leaf sprayed one time each on the axial and abaxial sides with a light spray that ensured the whole leaf area was covered. The leaves remained on the avocado trees to weather naturally in the field until they were picked on the day laboratory bioassays were conducted.

Mite bioassay of field weathered leaves. An initial pesticide application and bioassay were conducted beginning November 9, 2009 to determine a number of factors (quantity of flagged leaves needed, length of bioassay time needed per treatment, suitable bioassay arena set up, etc.) for the subsequent spring pesticide application and bioassay. Late April early May is typically when growers would be treating for avocado thrips in California avocados (Hartill and Sale 1996). Therefore pesticides for the field trial were applied May 4, 2010 and based on the preliminary study, bioassays were conducted, 1, 3, 7, 14, 21, 28, 49, 70 90, 111 and 132 d post pesticide application. From each treatment group (abamectin, fenpropathrin, spinetoram, sabadilla and the water control) five replicate leaves were randomly selected on the morning of each bioassay. If the flagging tape tied to a leaf did not bear a hand written number (to validate it was sprayed and not accidently missed), it was not included in the study. The leaf petioles were placed into 2.4 ml glass jars filled with deionized (DI) water such that the leaf surfaces were not in contact with the water at any time, and transported to the laboratory. A 2.5 cm hole was punched out of the center of each leaf and placed abaxial side up on a wet, white felt covered sponge (15 x 15 cm) in a plastic rectangular Tupperware container (19.5 x 19.5 x 4 cm) with no lid. Each of the five replicate leaf discs was randomly arranged in the tray

with a disc near each corner and one disc in the center. The discs were surrounded with strips of wet white felt to provide a wet border around each disc to keep the mites on the disc. Four to five strands of CelluCotton (no. 44130, Graham Professional, Green Bay, WI) were placed in the middle of each leaf disc and topped with 1/16 of a clear plastic cover slip (no. PCSS18, Fisher Scientific, Pittsburgh, PA). A very small amount of ice plant pollen was carefully placed on the piece of cover slip with a fine tipped paintbrush ensuring no pollen came into contact with the previously treated leaf surface. Once each of the Tupperware trays was set up with the five replicated leaves, a minimum of 20 mature female *E. hibisci* (mature females have a fully distended abdomen and an overall caramel hue) was added to each disc. This was conducted systematically by dipping the tip of the paintbrush into DI water, lightly tapping the brush to remove excess water; then while viewing under a microscope, mature females were selected by lightly and carefully touching the tip of the brush to their dorsal surface, removing the mite from the colony tray and placing it onto the leaf disc such that the mites would grab the disc and pull themselves away from the paintbrush tip.

The trays were then placed in an environmental chamber at 24°C, 50% RH, and long day light conditions (16L:8D). The trays were checked daily for 5 days noting the number of live and dead mites and any mites drowned in the wet felt. Mite mortality was recorded and assessed by lack of movement upon light probing with a fine tipped paintbrush. Drowned mites were recorded as mites stuck in the wet felt, either moving or not moving, and were not ‘rescued’ from the felt if found alive struggling in the felt (it was observed that mites were not able to escape from the felt once they were caught in

it). Data were analyzed by day in two ways: (1) % of mites found trapped in the felt and (2) % dead mites, excluding those trapped in the felt (because it was unknown if they would have died had they not been trapped).

Munger cell trials with spinetoram. Because high numbers of mites were found drowned in the felt after exposure to spinetoram in the preliminary trial, an additional study was conducted (same spinetoram rate as above) beginning at the same time as the May 4, 2010 field trial by confining mites to the leaf disc and not allowing them to suicide in the moist felt. This was done using Munger cell bioassays (Munger 1942, Morse and Brawner 1986). Briefly, Munger cells were constructed using a 3-layer Plexiglas "sandwich"; the middle cell layer was drilled with 3.2-cm diameter bit to provide a circular test arena (0.9 cm high by 3.2 cm diam). The upper (lid) and lower (base) parts of the Plexiglas sandwich were solid and between the lower base and test arena a piece of filter paper was placed under the leaf to allow moisture exchange and to extend the life of the leaf during the bioassay. Airflow through the test arena was provided through two holes (0.3 cm diam) drilled through the center cell layer directly opposite one another, with fine-mesh screening melted onto the interior of the test arena to prevent mite escape. The Plexiglas sandwich was held together with four binder clips positioned such that the passive airflow was not obstructed. The lid of the Munger cell had a 0.5 cm hole that could be plugged and unplugged with a small cork. The mites were transferred into the Munger cell via this hole and the cork remained in place at all times except when probing a mite to evaluate mortality. The control leaves were sprayed with water only. The Munger cells were placed in the same environmental chamber as

the open-faced Tupperware containers described above at 24°C, 50% RH and long day light conditions (16L:8D). The bioassay was conducted 1, 3, 10, 14, 21 and 28 d post pesticide application and mortality readings were taken daily for 5 d after each bioassay was set up.

Evaluation of UV-exposed field-weathered leaves. Two of the pesticides registered for avocado thrips management, abamectin and spinetoram, exhibit translaminar activity. To determine if photodecomposition might affect the impact of these chemicals on *Euseius hibisci*, twice as many control, abamectin and spinetoram leaves were included in the May 4, 2010 field trial and were bioassayed on each date. Half of these field-weathered leaves were randomly selected for exposure to intense ultraviolet light for 120 min after they were picked and before the bioassays were conducted. These leaves were placed perpendicularly into 1-dram vials filled with DI water to prevent desiccation and placed in a hood with a 15-watt UV bulb with a 250-320 nm range (Floesser-Muller and Schwack 2001). After 120 min UV exposure, the leaves were removed and leaf discs were set up in trays as described above with a minimum of 20 mature female *E. hibisci* mites. Data for each treatment (abamectin, abamectin with UV exposure, spinetoram, spinetoram-UV and control) were analyzed by day by calculating: (1) % of mites found trapped in the felt and (2) % dead mites, excluding those trapped in the felt (again, because it was unknown if they would have died had they not been trapped).

Mite detection of spinetoram on a leaf surface. To determine how/if *E. hibisci* females would respond if provided both spinetoram treated and free spaces on a leaf, an

additional study was run in which half of each bioassay leaf was treated with spinetoram. The leaves were selected, flagged and half of each leaf was randomly selected for the field trial on August 23, 2010; the left or right side of the leaf beyond the midrib was randomly selected for treatment on both the axial and abaxial surface, alternating which side was treated. Depending on which side of the leaf was treated, a paper towel cut into approximately 12 x 16 cm rectangles was covered with clear plastic wrap and was paper clipped to the opposite leaf side protecting it from the pesticide spray. Prior to treatment, the leaves were held such that the leaf mid-rib was parallel to the ground with the covered portion above the bare side such that when the pesticide was sprayed on the leaf, run-off fell to the ground and did not contact or accumulate on the covered side of the leaf. The paper towel and plastic wrap covering remained on the leaves for one hour after treatment and were then carefully removed so as to not tear the leaf or drag any remaining wetness across the untreated side of the leaf. The control leaves were covered in exactly the same fashion, but were treated with water. The half-leaf trial bioassay was conducted on 1, 3, 7, 10 and 14 d post pesticide application. As described previously, the leaves used in the bioassays were selected, transported to the laboratory, and the whole leaf (with petiole trimmed off) was placed abaxial side up on wet white felt covered sponge. The whole leaf was rimmed with strips of wet felt and at least 20 mature female *E. hibisci* were hand transferred to each leaf. To account for possible positioning bias, the leaves were treated on the left or right side and the mites were deposited either on the treated or untreated sides so that all combinations were accounted for, with three replicate leaves for each combination. We wanted to determine if the mites could detect the spinetoram on the

leaf surface; therefore on each of the bioassay days, the bioassay was checked every 20 min for the first hour then 3, 5, and 10 h post setup, and once every 24 hours for five days. Mites were scored as being dead, alive or stuck in the felt and which side of the leaf they were found was recorded on at each of the observation time intervals. Data were analyzed by ANOVA with repeated measures using SAS 9.2 (SAS Institute 2008) with the following factors: treatment (spinetoram or control), bioassay day (days post leaf treatment), treatment side (right or left side of the abaxial surface sprayed), initial mite placement location (treated side or untreated side) and observation time (all response variables were measured repeatedly at 20 min, 40 min, 1 h, 3 h, 5 h, 10 h, 1 d, 2 d, 3 d, 4 d, and 5 d after the mites were placed on the leaves).

Results

Mite bioassay of field weathered leaves. Bioassays were conducted, 1, 3, 7, 14, 21, 28, 49, 70 90, 111 and 132 d post pesticide application. Mite mortality and mite repellency were recorded separately because it was unknown if the mites that were found dead (drowned) in the wet felt were dead due to pesticide exposure or from drowning alone. Data were recorded for all bioassay days (1- 132 d) for 5 days post bioassay setup and data from the day 4 count were selected because there was little further activity (mortality or repellency) post 5 days and day 4 counts best represented the data overall. High levels of mite repellency were seen only with the spinetoram treatment (Fig. 4-1) and >20% of the mites in the spinetoram treatment were found drowned in the wet felt

surrounding the leaf discs through 14 days post-treatment. Mite mortality was the highest in the fenpropathrin treatment (Fig. 4-2) and this pesticide showed the most persistent impact (>20% mortality through 90 d). Mite mortality with the spinetoram treatment appeared to increase and then decrease (Fig. 4-2) but this was an artifact of mortality being calculated by excluding mites that drowned. Only moderate mite mortality was observed with the abamectin and sabadilla treatments and this dropped to below 10% on 14 and 21 days post-treatment, respectively (Fig. 4-2).

Munger cell trials with spinetoram. Data from the day 4 count are described (see above). Mortality of mites held in Munger cells (Fig. 4-3) after spinetoram treatment was similar to the combined number of mites dying (Fig. 4-2) and drowning in the wet felt (Fig. 4-1) in the leaf disk bioassays. In both cases, the activity of spinetoram appeared to last for 14 d and then dropped sharply.

Evaluation of UV-exposed field-weathered leaves. Data from the day 4 count are described (see above). Relatively few mites were repelled on abamectin treated leaves and UV exposure showed little impact (Fig. 4-4). After treated leaf exposure to intense UV light for 120 min, high levels (>75%) of mite repellency with the spinetoram treatment (Fig. 4-5) dropped in persistence from 10 d to 3 d. Mite mortality after treated leaf exposure to UV light was reduced to control levels on abamectin treated leaves by the time of the day 1 bioassay (Fig. 4-6). Because mites trapped in the felt were excluded, percent mite mortality on spinetoram versus spinetoram-UV treated leaves must be interpreted carefully in Fig. 4-7. Based on data from Fig. 4-3 (the pattern of mortality + mites trapped in felt on spinetoram discs from Figs. 4-1 and 4-2 was similar

to the mortality seen in Munger cells in Fig. 4-3), Fig. 4-8 depicts a more accurate assessment of the results when mites were placed on UV treated spinetoram leaves because it combines the mortality and repellency (as if assuming mites trapped in the felt likely would have died, which we do not know for sure).

Mite detection of spinetoram on a leaf surface. There was no effect of the leaf side treated (left versus right, P = 0.7788) or leaf side the mites were placed on (treated side on left vs. treated side on right, P = 0.6973) for both treatments (control and spinetoram) and therefore, data were pooled and the new response variable for each treatment became 'mites deposited on treated side' versus 'mites deposited on untreated side'. There was no significant variation observed between the three leaf replicates on any bioassay date (P = 0.9897) and thus, replicate data were pooled. Based upon repeated measures analysis, each response variable (mite survivorship, mite mortality and mite repellency) showed significant impacts by *day* (bioassays at 1, 3, 7, 10 and 14 days post-treatment), *time* (observation at 20 min, 40 min, 1 h, 3 h, 5 h, 10 h, 1 d, 2 d, 3 d, 4 d and 5 d after bioassay setup) and location (mites deposited on the treated versus untreated side of the leaf).

With spinetoram treatment, there were more mites alive on the untreated (Fig. 4-10) side versus the treated (Fig. 4-9) side of the leaf on days 1, 3, 7 and 10 (P = 0.0001). However, on day 14, there was no difference between the numbers of mites on the treated (Fig. 4-9) versus the untreated (Fig. 4-10) side of the leaf (P = 0.5354) for any of the observation *times*. The mites in the control treatment were distributed similarly across both sides of the leaf on all *days* (P = 0.0511) and *times* (P = 0.2132). With spinetoram

treatment, mite mortality was different from the control on both the treated ($P < 0.0001$) and untreated sides ($P < 0.0001$) of the leaves. On the spinetoram-treated side of the leaf, mite mortality by *day* was significantly different from the control treatment for days one through 10 ($P < 0.0001$) but on day 14 mite mortality was no longer different ($P = 1.0000$). The same pattern was observed for the untreated side of the spinetoram leaves, i.e. mites were dying at higher levels versus control leaves on both the untreated and treated sides of the leaf. In the control treatment, mite mortality was not different between the water treated and untreated sides of the leaf ($P = 0.9196$).

Mite repellency (i.e. numbers trapped in the felt) on the spinetoram treated (Fig. 4-11) and untreated (Fig. 4-12) sides of the leaf were different than seen on control leaves ($P < 0.0001$). On the spinetoram treated side, mite repellency by *day* was different from the control for days one and three ($P < 0.0001$) as well as day 7 ($P = 0.006$) and day 10 ($P = 0.027$) but not for day 14 ($P = 1.0000$). Mite repellency on the spinetoram treated side of the leaf by *day* for each level of *time* was not different from the control for *times* 20 min through 10 hours ($P = 0.1770$), but was different for each observation interval from 24 hrs through 5 days ($P < 0.0001$). On the spinetoram untreated side of the leaf, mite repellency by *day* was different from the control for days 1, 3, and 7 ($P < 0.0001$) as well as day 10 ($P = 0.0007$) but not for day 14 ($P = 1.0000$). Mite repellency on the spinetoram treated side of the leaf by *day* for each level of *time* was not different from the control for *times* 20 min through 10 hours ($P = 0.2960$), but was different for observation intervals of 24 hrs through 5 days ($P < 0.0001$).

Discussion

In our assessment of the four pesticides currently recommended for avocado thrips management (abamectin, fenpropathrin, spinetoram and sabadilla), we found that all four products had some negative effects on *E. hibisci*. Mite exposure to abamectin resulted in relatively high mortality within the first two weeks of the bioassay and dropped sharply; presumably as translaminar movement of the material took place and the ultraviolet light rays broke down surface residues (as supported by the abamectin-UV results). Fenpropathrin treatment showed the longest and highest amount of activity. Spinetoram was the only material to which the mites exhibited strong repellency (drowning in the felt) and when mites were bioassayed in the Munger cells with spinetoram, mortality was high and consistent with the pattern observed with the repellency over the first two weeks of the bioassays. Mites exposed to sabadilla, a chemical commonly thought to have little non-target effect (Bellows et al. 1985, Grout 1994, Hare and Morse 1997), showed higher mortality and longer persistence than expected. However, this could be due to the mites feeding on the pesticide-laced sugar on the leaf surface, as sabadilla is formulated with sugar (the formulation is 80% sugar and additional fresh sugar was added as is common practice) (Hare and Morse 1997).

Data clearly showed that exposing the treated, field-weathered leaves to UV light increased the survival of the mites on both abamectin and spinetoram-treated leaves. Mite mortality to the UV-treated abamectin leaves was no different than with control leaves on day one of the bioassay (Fig. 4-6), indicating that surface residual activity had

been eliminated. With spinetoram treatment, mite repellency (Fig. 4-5) and mortality (Fig. 4-8 if one assumes repelled mites would die) were reduced from 14 d to 10 d. The chief differences in spinetoram (spinosyn J and L) from its analog spinosad (spinosyn A and D) are: 1) the addition of the 3'-*O*-ethyl group, which improves potency by altering nicotinic function in the insect nervous system and 2) hydrogenation of the 5,6 double bond, which improves photostability of the molecule and thereby increases residual control (Dripps et al. 2008). Our data show that these modifications increased the longevity of the material on the leaf surface but with intense UV exposure, that activity was broken down to some degree.

The bioassays evaluating mite detection of spinetoram on the leaf surface clearly showed more mites alive on the untreated side of the leaf than the treated side, indicating that the mites were able to detect the material and move away from it. There were fewer mites drowning in the wet felt in the spinetoram detection bioassay on day one than seen in the initial field trial bioassay (whole leaf disk treated) on day one, indicating that the pesticide free leaf surface provided some sort of refuge for the mites. Spinetoram exposure at days one and three ultimately resulted in some mite mortality or mites drowned in the wet felt, but there were fewer overall mites dying and drowning on both sides of the leaf (Fig. 4-11 and Fig. 4-12). Mite repellency was different from the control for both treated and untreated sides of the leaf, but on each subsequent bioassay date, the level of significance dropped until on day 14, there was no statistical separation. Our data suggest that because fewer mites were repelled in the spinetoram detection trial on bioassay days 7 and 10 and because of the pesticide free side of the leaf, more mites were

alive, i.e. fewer picked up a toxic dose or drowned. It remains difficult with our bioassay system to precisely separate whether or not mites received a toxic dose when repellency levels were high.

Our studies were conducted with a conservative dilution rate of 2,843 L of water per ha while the majority of California avocados groves are grown on steep hillsides and utilize helicopter application using 468-935 L of applied water per ha. On these hillside groves, speed sprayers cannot be used and relatively few growers use drag hoses because of the high cost of labor in California. Application by helicopter may not provide complete coverage (Hartill and Sale 1996) and many of the interior portions of the avocado tree (where mite abundance is high) remain untreated. With consideration of the following factors: helicopter application resulting in uneven distribution of pesticide on hillside avocado groves, the conservative dilution rate used in our trial, our containment of mites on the pesticide treated arenas and providing a pesticide treated/ untreated leaf area, our data suggests that in a field setting, mites may not pick up a toxic dose of spinetoram. Those mites that do not pick up a toxic dose will likely be repelled by the spinetoram and this may result in reduced *E. hibisci* mortality. Growers should be aware of the data presented herein when deciding upon a pesticide rotation management plan, which reduces avocado thrips resistance evolution. Each of the four recommended products have different features with respect to the efficacy of thrips control, concurrent control of avocado mite pests, and persistence of impacts on predaceous mites and other natural enemies (the latter being largely unstudied as yet).

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Figure Legends

Fig. 4-1. Repellency of mites (% found in felt) exposed to pesticides on field-weathered leaves in the May 2010 field trial.

Fig 4-2. Mortality of mites (% found in felt) exposed to pesticides on field-weathered leaves in the May 2010 field trial.

Fig 4-3. Mortality of mites confined to Munger cells with leaves during the May 2010 field trial.

Fig 4-4. Repellency of mites (% in felt) exposed to abamectin, UV treated abamectin and two controls during the May 2010 field trial.

Fig 4-5. Repellency of mites (% in felt) exposed to spinetoram, UV treated spinetoram (UV Delegate) and their respective controls during the May 2010 field trial.

Fig 4-6. Mortality of mites exposed to abamectin, UV treated abamectin and their respective controls during the May 2010 field trial.

Fig 4-7. Mortality of mites exposed to spinetoram, UV treated spinetoram, and their respective controls during the May 2010 field trial.

Fig 4-8. Mortality and repellency combined (proxy for total "death") of mites exposed to spinetoram, UV treated spinetoram and their respective controls during the May 2010 field trial.

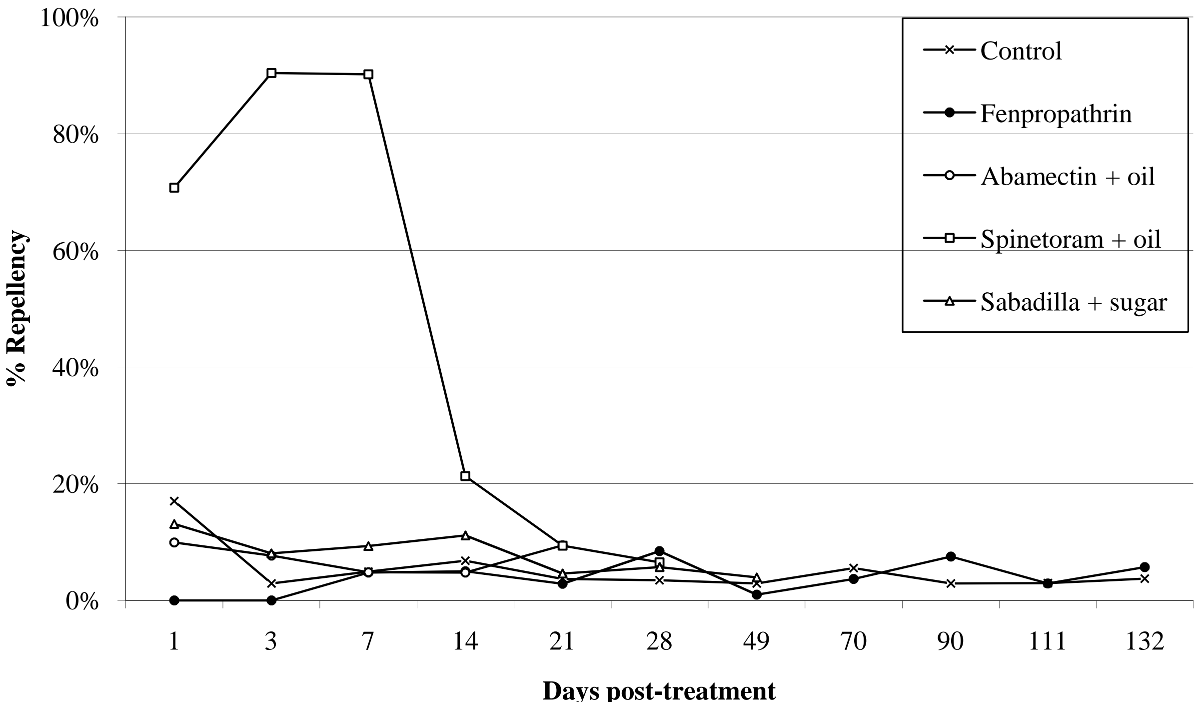
Fig. 4-9. Mean % live mites on the spinetoram treated side of the leaf in the spinetoram detection study conducted August 2010. Each line represents a different bioassay set up 1, 3, 7, 10, and 14 days post-treatment. The X-axis is the observation period (log10 minutes post bioassay setup) over the 5 days post each bioassay was run.

Fig. 4-10. Mean % alive mites on the untreated side of the leaf in the spinetoram detection study conducted August 2010. Each line represents a different bioassay set up 1, 3, 7, 10, and 14 days post-treatment. The X-axis is the observation period (log10 minutes post bioassay setup) over the 5 days post each bioassay was run.

Fig. 4-11. Mean number of mites repelled on the treated side of the leaf in the spinetoram detection study conducted August 2010. Each line represents a different bioassay set up 1, 3, 7, 10, and 14 days post-treatment. The X-axis is the observation period (log10 minutes post bioassay setup) over the 5 days post each bioassay was run.

Fig. 4-12. Mean number of mites repelled on the untreated side of the leaf over time in the spinetoram detection study conducted August 2010. Each line represents a different bioassay set up 1, 3, 7, 10, and 14 days post-treatment. The X-axis is the observation period (log10 minutes post bioassay setup) over the 5 days post each bioassay was run.

Fig 4-1.



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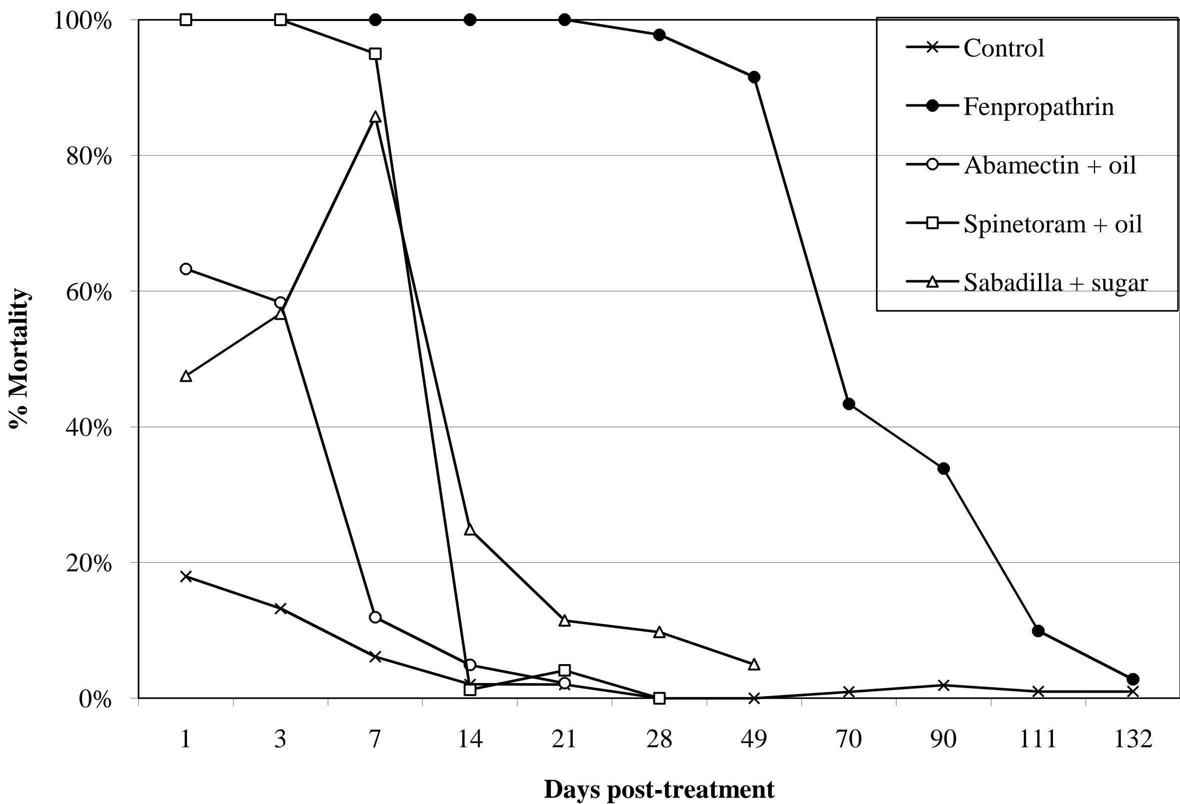

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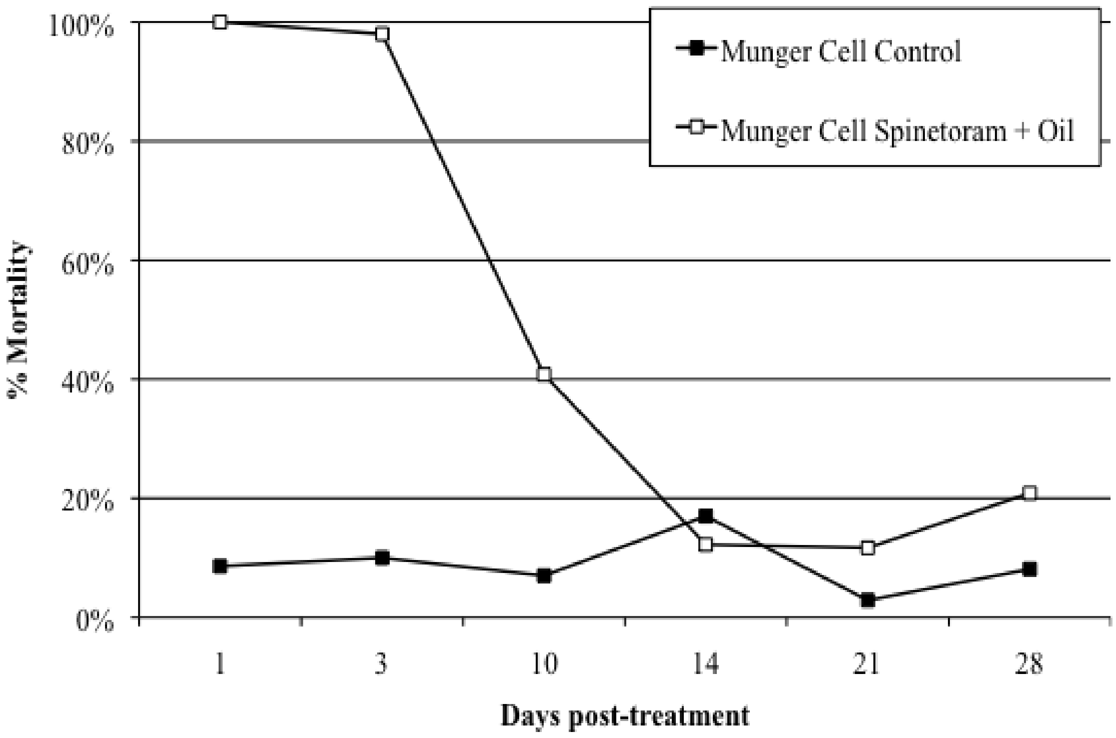

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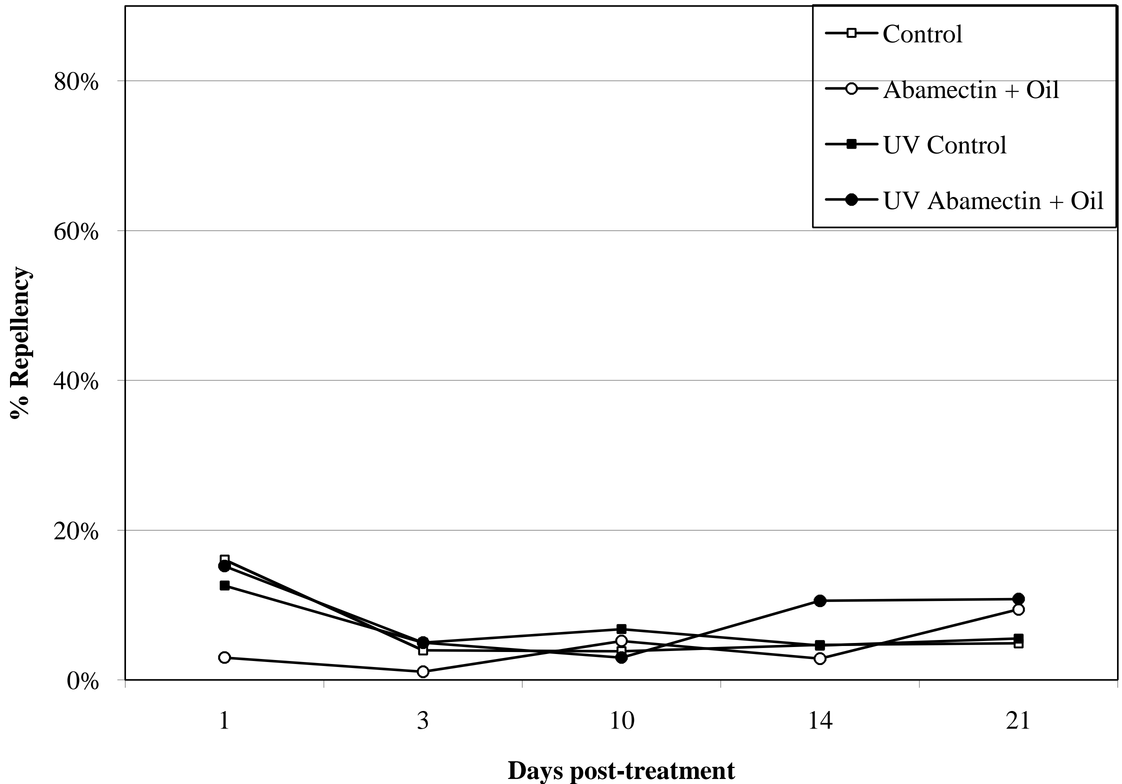

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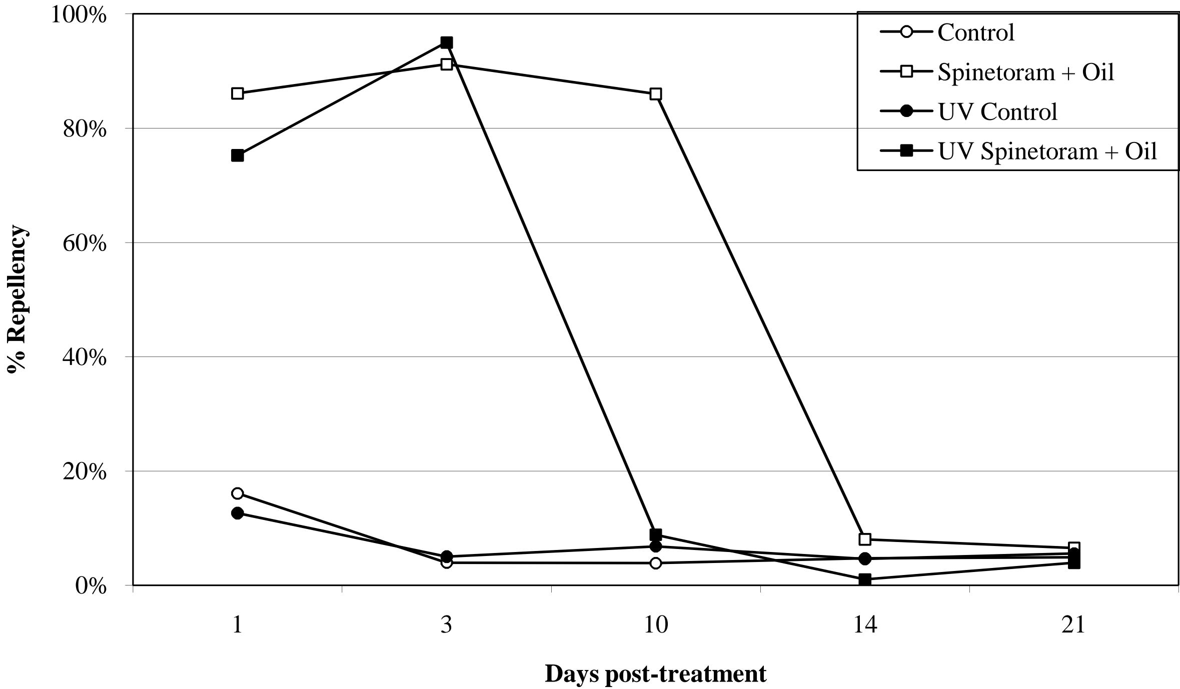

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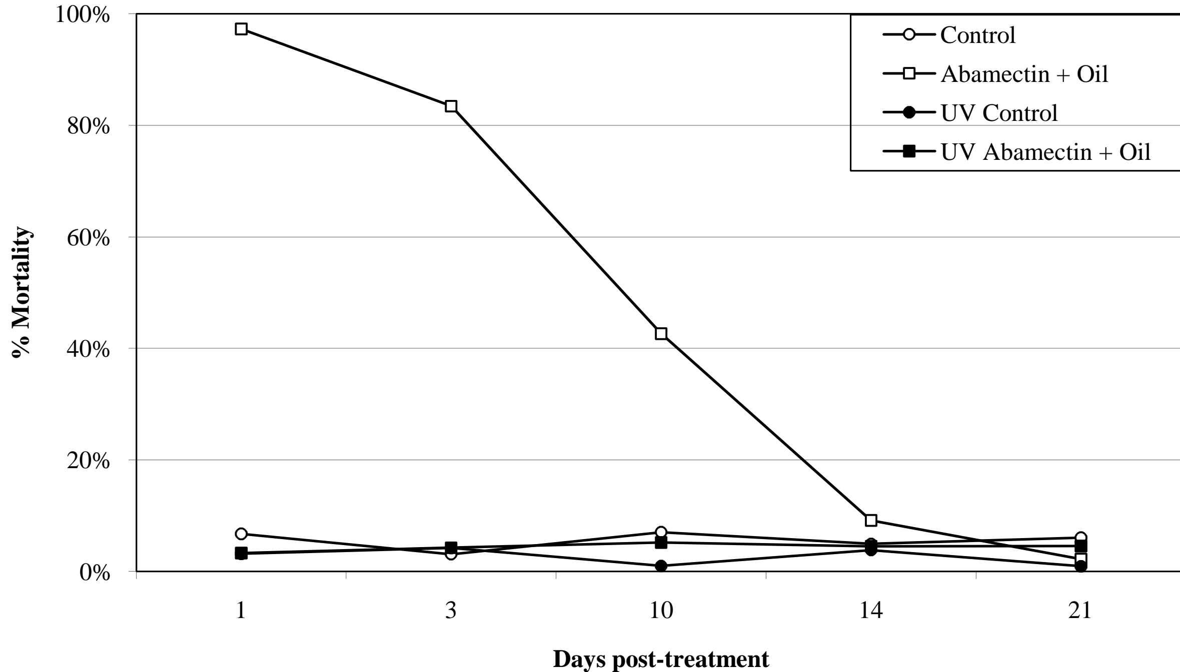

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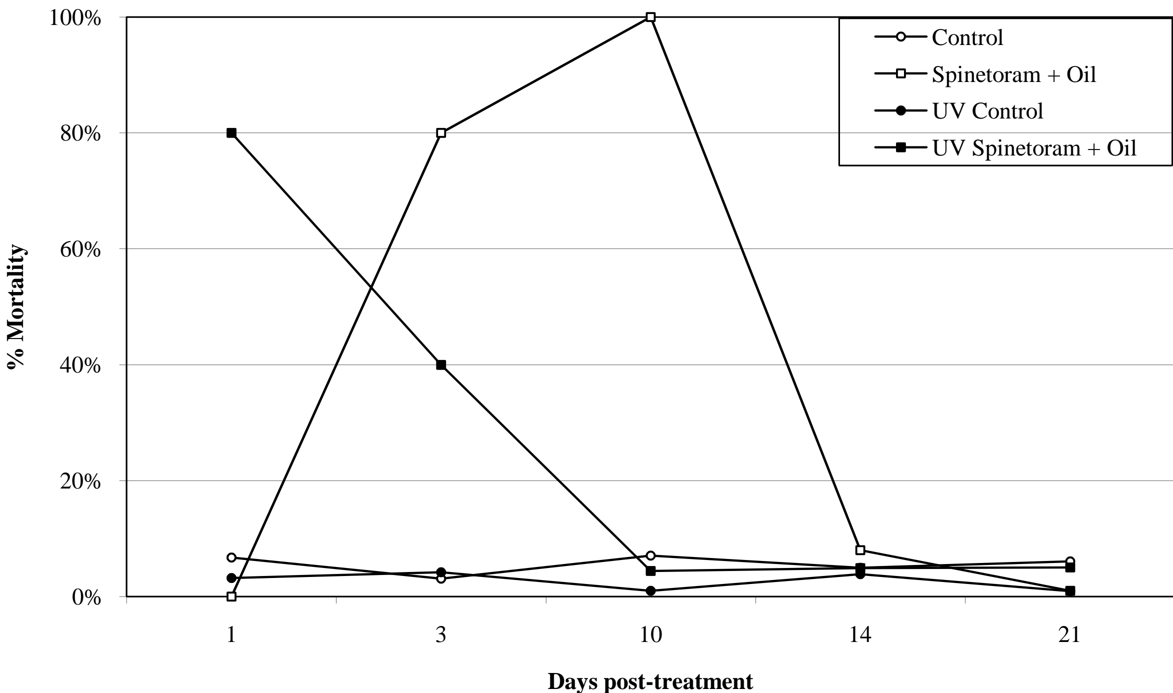

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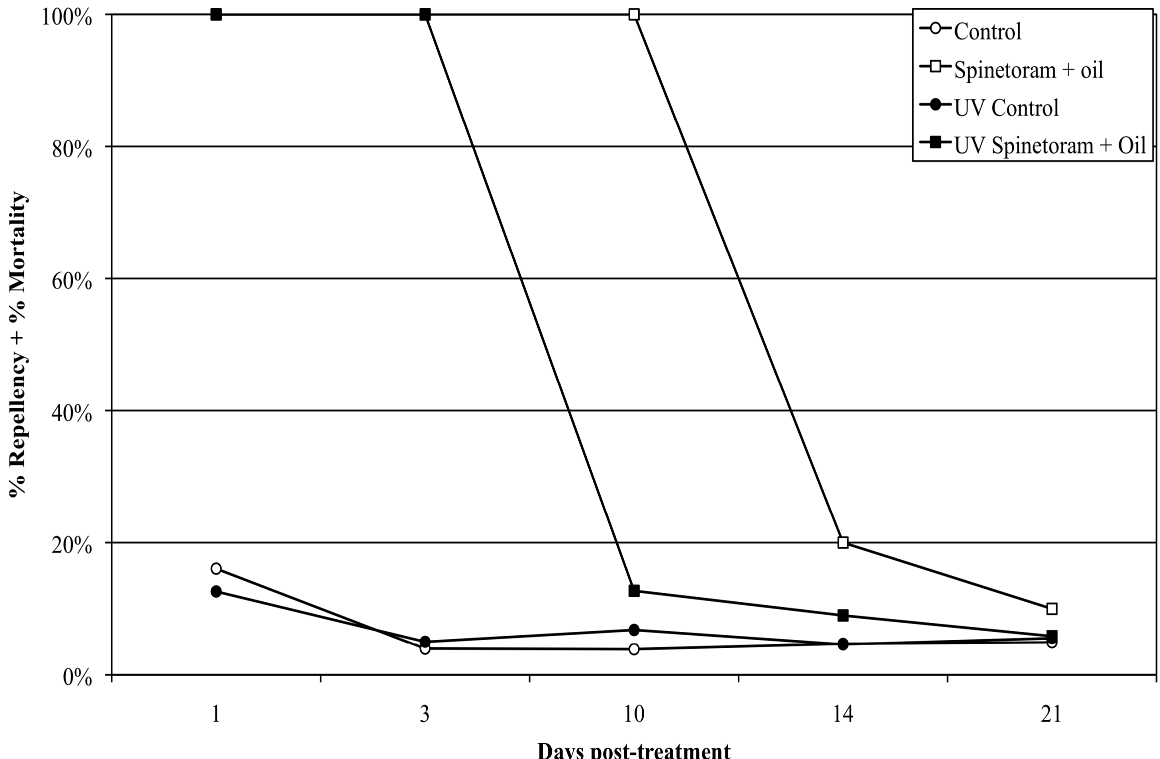

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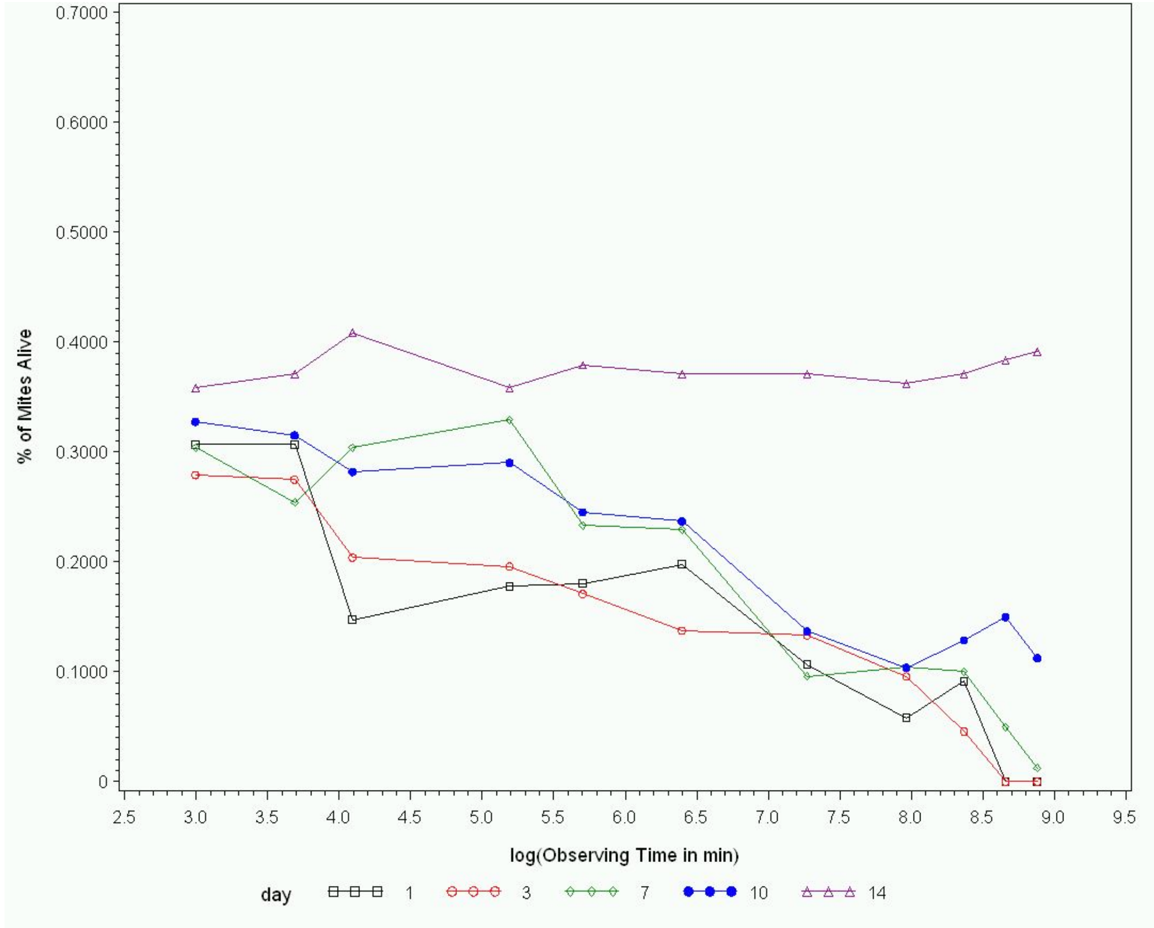


Fig. 4-10.

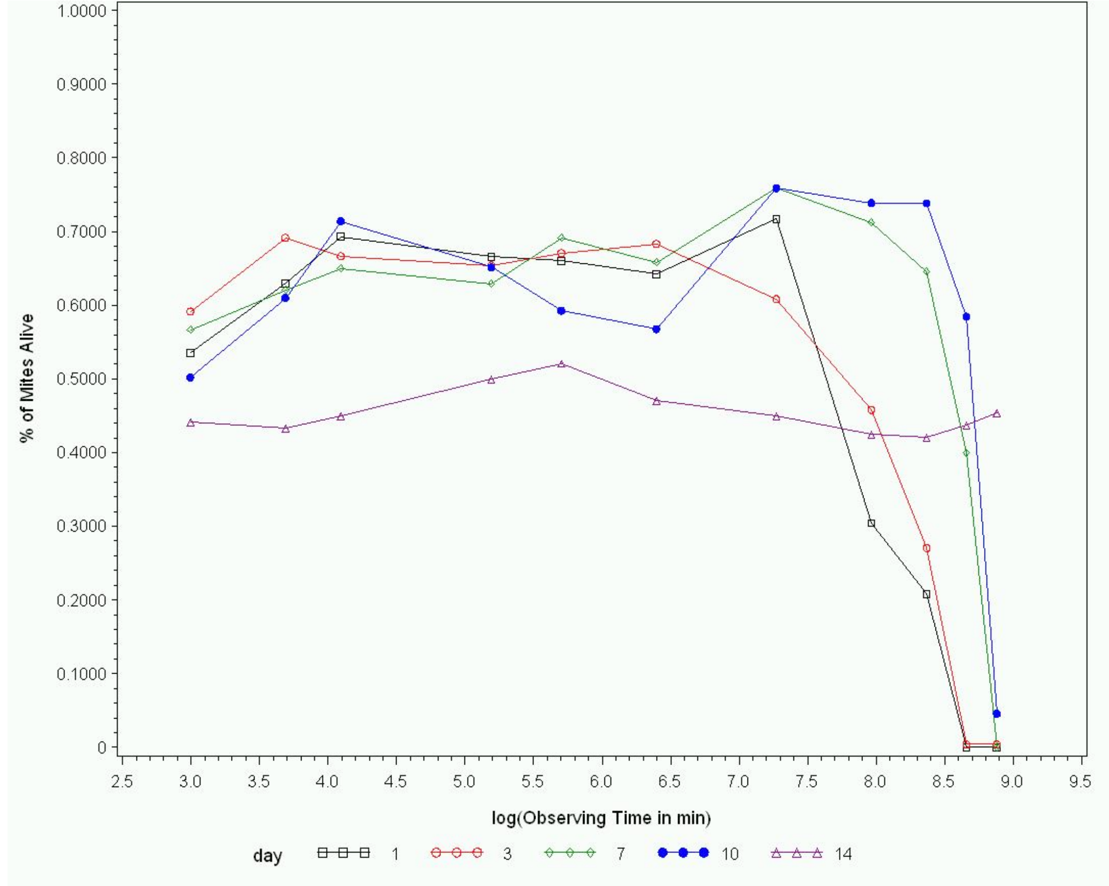


Fig. 4-11.

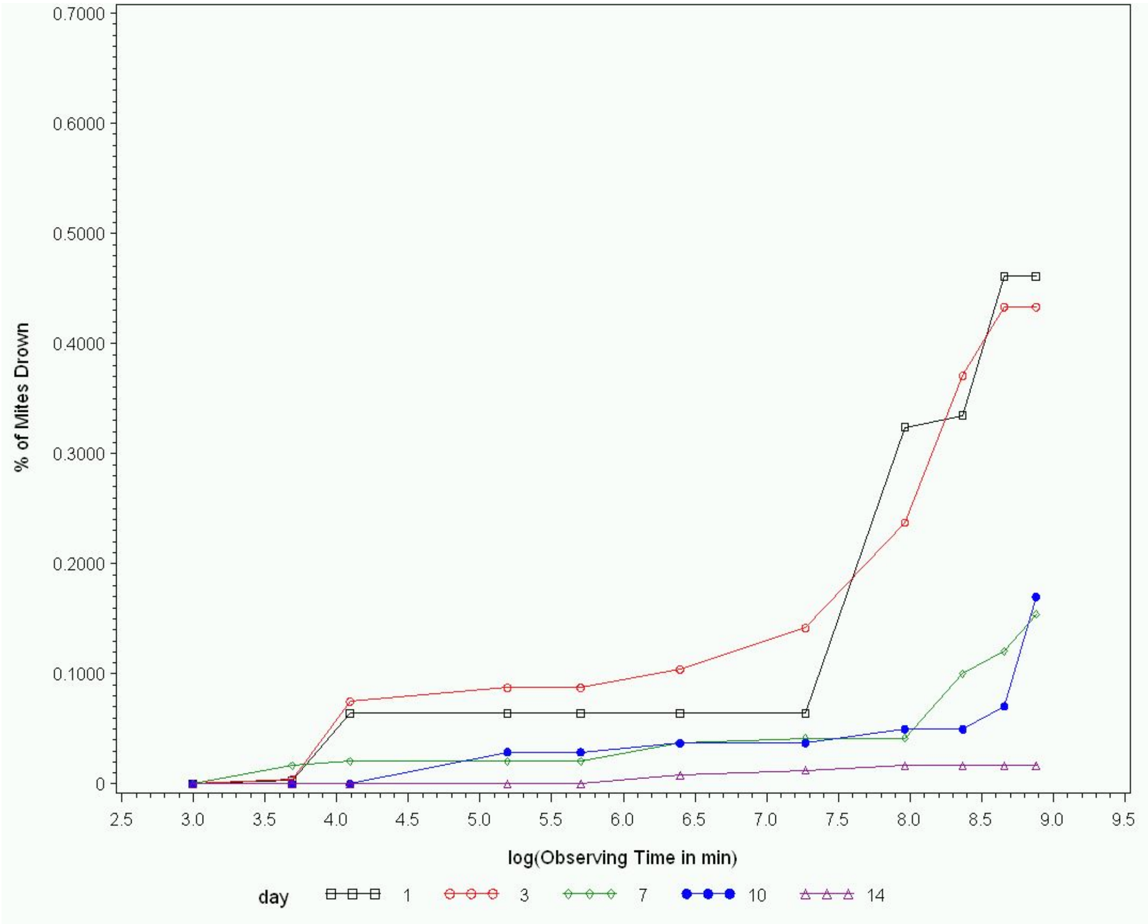
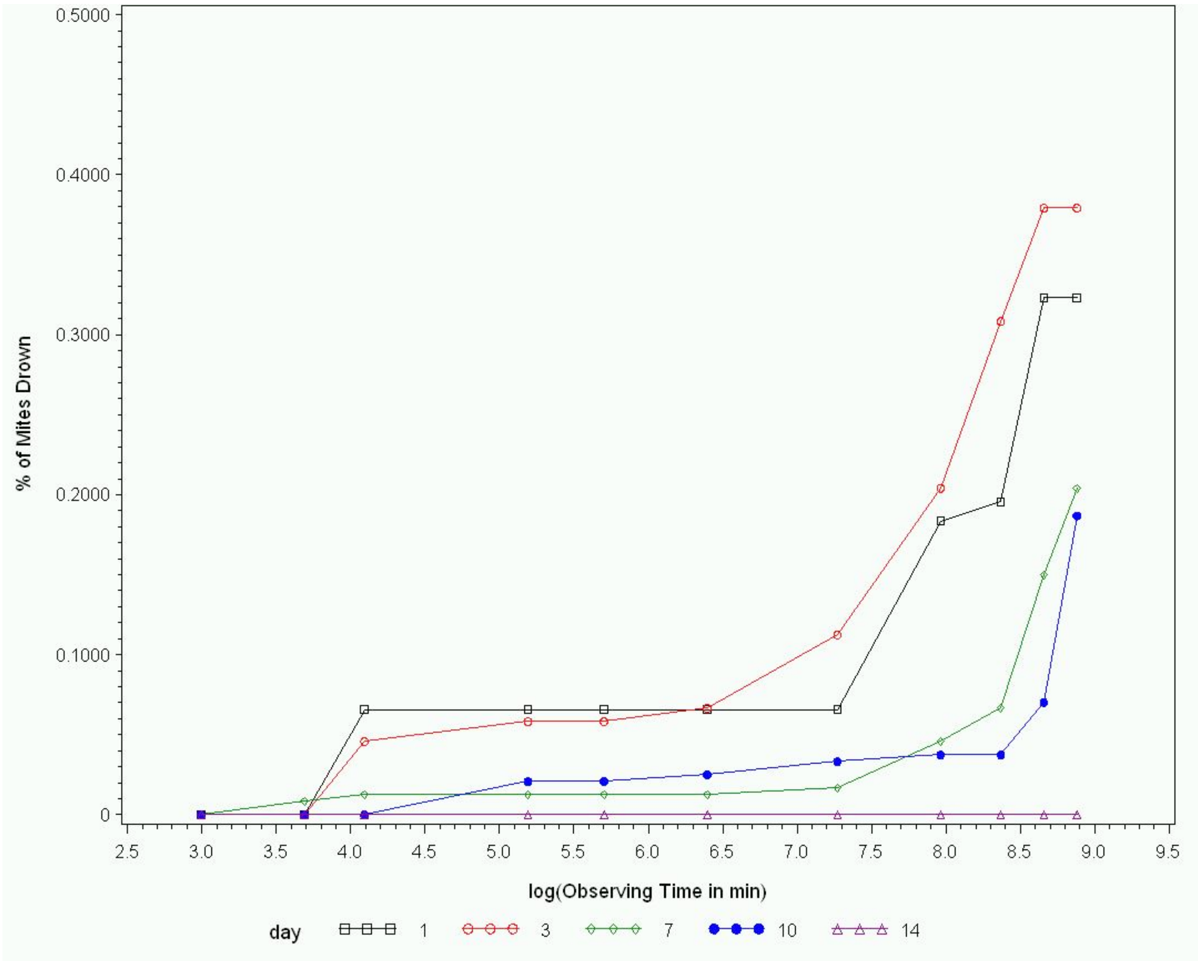


Fig. 4-12.



Chapter 5

Introduction

Thrips are common pests in most blueberry growing regions of the United States. These insects feed on the blueberry leaves, flowers and fruit and cause distorted, discolored, and stunted flush growth and poor development of fruiting wood for the subsequent crop (Polavarapu 2001, Arévalo and Liburd 2007, Haviland et al. 2009). Many thrips preferentially feed on the styles and ovules, as well as the surrounding new green tissue, or 'flush growth' (Arévalo and Liburd 2007). Some studies have shown that thrips can feed on blueberry pollen and, under severe infestations, cause dimpling on the fruit, which can severely affect marketable yields (Horton and Sampson 2001, England et al. 2006). In Georgia and Mississippi, a 60% reduction in fruit set has been attributed to thrips injury in southern highbush blueberries (Horton and Sampson 2001).

There is substantial variability in the thrips species complex that attacks blueberries across geographic regions of North America (Rodriguez-Saoma et al. 2010). The eastern flower thrips, *Frankliniella tritici* (Fitch), and *Scirtothrips ruthveni* Shull infest northern highbush blueberries (*Vaccinium corymbosum* L.) in New Jersey (Polavarapu, 2001). *Frankliniella vaccinii* Morgan, *Catinathrips vaccinophilus* (Hood) and *Catinathrips kainos* O'Neil are the main thrips species infesting lowbush blueberries (*Vaccinium angustifolium* Aiton) in Canada and Maine (Langille and Forsythe 1972). In contrast, the Florida flower thrips, *F. bispinosa* (Morgan), and *F. tritici* are the most abundant thrips species in Florida and Georgia, respectively, where southern highbush (*V.*

corymbosum x *V. darrowi* Camp) and rabbiteye blueberries (*Vaccinium virgatum* Aiton) are grown (Arévalo et al. 2006). In the San Joaquin Valley (SJV) of California, the southern highbush blueberry is a somewhat new host for citrus thrips, *Scirtothrips citri* (Moulton) (Haviland et al. 2009). One of its more common native host plants in this area prior to the introduction of citrus was likely laurel sumac, *Malosma* (=*Rhus*) *laurina* (Nutt.) Abrams (Morse 1997). In Florida however, *S. citri* have been reported on grapes but is rarely found on blueberries (Flowers 1989).

Historically, lowbush varieties of blueberries could only be grown in regions too cold for citrus production. However, the development of heat-tolerant highbush varieties, which has enabled the development of a blueberry industry in the San Joaquin Valley (Jimenez et al. 2005, Strik and Yarborough 2005), has also caused blueberries to be grown in a region where citrus and citrus thrips flourish. Other, less common thrips infesting blueberries include tobacco thrips, *Frankliniella fusca* (Hinds), *Frankliniella hawaiiensis* (Morgan) and western flower thrips, *Frankliniella occidentalis* (Pergande) (Arévalo and Liburd, 2007). Little is known about the biology, ecology (Rodriguez-Saona et al. 2010) and host plant selection of thrips in southern highbush blueberries, particularly in California and not all varieties are fed on equally by citrus thrips; i.e. there is a distinct varietal preference for some hybrids (e.g., the Star variety based on grower reports).

Thrips preferences for tender, young flush foliage are well documented (Chisholm and Lewis 1984, Agrawal et al. 1999, Leiss et al. 2009, Hoddle 2002, Hoddle and Morse 2003) and determining the oviposition rates in the varieties of blueberries grown in

California will begin to assist researchers in understanding thrips host plant preferences. Based on a survey of growers, the varieties of blueberry predominately planted in California's SJV are Emerald, Jewel, Misty, O'Neal, Santa Fe and Star (D. K. Zahn, unpublished). The goal of this research was to determine if oviposition preferences differed between these varieties of blueberries using choice and no-choice oviposition tests and to document which varieties grown in the SJV of California are preferred.

Materials and Methods

Insects. Citrus thrips were collected in Delano, Kern County, California from southern highbush blueberries varieties Star, Santa Fe, Jewel, Misty and O'Neal. Thrips were collected on blueberry canes by placing a brown paper bag over the flush growth and clipping the cane off into the bag with shears. This was done approximately 10 times per blueberry variety. The brown paper bags were carefully placed into a cooler with ice packs and brought to a greenhouse (environmental conditions: 24-34°C, ca. 20% RH, and long-day light conditions 16L:8D) located at the University of California, Riverside, Agricultural Operations Field 16 in Riverside, California. Once in the greenhouse, the bags were opened, and canes gently placed on top of 40 potted Misty variety blueberry plants (2 L sized pots) with a large amount of flush growth. The Misty variety was selected for rearing because growers reported it to be of intermediate preference to citrus thrips versus two preferred varieties (Emerald and Star) versus two varieties that thrips appear to build up on to a lesser degree (O'Neal and Jewel). The brown paper bags that

transported the citrus thrips were gently placed on the greenhouse bench with the opening pointed toward the potted plants to entice any remaining thrips to leave the bags and move to the plant foliage. The thrips colony was established and maintained in the greenhouse for 1.3 years before used in the oviposition trials beginning on June 6, 2011 and were augmented with additional thrips in exactly the same manner as described above an additional three times over that 1.3 years.

Blueberry, citrus and sumac plants. All blueberry plants were grown in 2 L pots and were watered 3-4 days per week and fertilized as needed with azalea/camellia/ rhododendron acid fertilizer (Scott's 4-8-5, Lowe's, Moreno Valley, CA). The potted Misty plants were pruned in the citrus thrips colony greenhouse room and rotated out once only hardened leaves were present on the plants, at which point, new flushing Misty plants were rotated into the colony cyclically with the flushing and pruning of the colony plants. Fall Creek Farm and Nursery, Lowell, Oregon, USA supplied all potted blueberry plants used for the colony and in oviposition trials. Varieties provided were Emerald, Jewel, Misty, O'Neal, Snowchaser and Star. Snowchaser was a fairly new variety at the time we obtained the plants and was used in our studies in lieu of Santa Fe (grown in California), which was unavailable. Snowchaser has similar parentage to Santa Fe. *Citrus volkameriana* V. Ten. & Pasq. and laurel sumac plants were maintained in the same fashion as the blueberry plants but were fertilized with Miracle Grow All-Purpose plant fertilizer (Scotts 2.3 kg box, Lowe's, Moreno Valley, CA) as needed. Plants used in the oviposition trials were held in a separate greenhouse from the citrus thrips colony (environmental conditions: 24-34°C, 20% RH, and long-day light conditions 16L:8D).

No-choice oviposition tests. Potted blueberry (two L pots) with the varieties Emerald, Jewel, Misty, O'Neal, Snowchaser and Star, as well as, flushing citrus and laurel sumac potted plants (also two L sized pots, used as positive controls) were placed in random order on a greenhouse bench in 5 replicate blocks of the 8 plants (6 blueberry plants and two positive controls, thus 40 plants total). Two leaves from each plant that were flushing and nearly fully expanded were selected and clip-cages were clipped to the leaves while still attached to the plant such that the experimental arena was located on the abaxial surface of the leaf. Circular clip-cages (diam 2.0 cm) consisted of two plastic rings covered on one side with a 1 cm thick foam pad enclosing a leaf surface area of 3.1 cm^2. The foam-plastic rings, one of which was covered by ultra fine nylon mesh, were connected by a coiled length of spring steel wire glued to the plastic which allowed the clip cages to attach to the leaf like a clothes pin without visibly damaging the leaves. Two adult female and two male citrus thrips of unknown age were carefully added to the clip cage and left in the cage for 5 days after which the leaf was excised from the plant, with the cage still attached, and was carefully transported to the laboratory. Once in the laboratory, leaves were thoroughly examined for any first instar larvae and adults. The leaves were then boiled in deionized (DI) water for 3 minutes in the microwave (700 watt) and stained with methyl red (de Kogel et al. 1997, Rahmen et al. 2010). Eggs were clearly visible using a binocular microscope with transmitted light. This experiment was repeated on two dates total and total counts of eggs and larvae combined were square root transformed pooled data (2 dates x 8 plant types x 5 replicate plants x 2 clip cages per plant with 2 females per cage left in the cage to oviposit for 5 days with 2 males) were

analyzed using a two-way ANOVA with count data and developmental stage (eggs *versus* larvae) as main treatment factors and means were separated by Tukey's HSD test. The correlation coefficient between numbers of hatched larvae and eggs was also calculated.

Choice oviposition tests. Five two L potted blueberry plants, one each of the Emerald, Jewel, O'Neal, Snowchaser and Star varieties were placed inside five replicate "No-thrips" screened bug dorms (60 x 60 x 60 cm with 150 x 150 fine mesh; BioQuip Products, Rancho Dominguez, CA, USA). Plants were arranged in a circle with the position of a variety randomized in the 5 bug dorms, ensuring that each variety occupied each position in the rotation. For example, positions one through five contained the varieties in order, Star, Jewel, Snowchaser, Emerald, and O'Neal and each subsequent cage's plants rotated those varieties in that order one position clockwise. This arrangement was conducted twice on the same date (10 bug dorms total, thus two replicate cages of 5 plant arrangements). Each pot was reduced to one blueberry cane with approximately the same numbers of leaves (25), similar cane lengths (25 cm) and leaf flush and canes did not remain in contact with each other. The plants were placed in square foil pans (18 x 18 x 2.5 cm) to collect excess water or spilt water after plants were watered. Ten groups of approximately 60 male and 60 female citrus thrips each were collected from the colony the morning of the test and held in 15-dram (55 ml) plastic aspiration vials with a copper mesh screened lid. Adult thrips were of unknown age. The vials were then placed vertically into the center of the bug dorms and the lid removed to allow the thrips to escape. The plants were watered every third day, carefully through the

mesh sleeve on the bug dorm door with an adjustable watering wand taking care to not fill the foil trays with water. Thrips were left with the plants for 14 days, after which time the cages were carefully opened, and a "No-thrips sleeve" open at one end (90 cm with 150 x 150 fine mesh; BioQuip Products, Rancho Dominguez, CA, USA) was quickly placed over each plant and secured at the base of the cane with double-sided sticky tape. Once the sleeve was securely fastened to the cane, the cane was trimmed at the soil line. The sleeved canes were then transported to the laboratory where the adults were lightly shaken off into the sleeve and leaves quickly examined for remaining adults. If adults were found on the leaves, they were picked up with a paintbrush and added to the respective sleeve. The sleeves no longer containing the canes were placed in a freezer for 10 min and adults were counted. The leaves on the canes were broken into thirds, with each third about 8 cm in length. The canes were examined and any larvae found were counted. Once all live thrips were removed, the leaves were stripped from the respective third of each cane, boiled in deionized (DI) water for a minimum of 3 minutes in the microwave (700 watt) and stained with methyl red. Older leaf tissue (lowest third) had to be boiled for 5 minutes to clearly see eggs in the leaf. Subsequently, eggs were clearly visible using a binocular microscope using transmitted light. This experiment was replicated twice on one date (2 cages x 5 plant arrangements) and data were analyzed using a G-test of goodness-of-fit (with a likelihood Chi-square ratio test) with plant, location on the plant (top third versus bottom two-thirds), larval count and egg count as the main operators in the test. The first and second instar larvae were grouped into a

single ‘larvae’ observation and recovered males and females were counted separately (data not shown) as well as grouped into an ‘adults’ observation (Fig. 5-2).

Results

No-choice oviposition tests. There were clear differences among the mean number of eggs and larvae found across the different plant types (Fig. 5-1). ANOVA indicated a significant interaction between the plant and the developmental stage present on the respective plant (stage: df = 1, MS = 897.8, $P < 0.001$; developmental stage: df = 7, MS = 89.4, $P < 0.001$; interaction: df = 7, MS = 22.5, $P < 0.001$). The interaction indicates there was a differential treatment impact with respect to the number of eggs laid and the number of larvae found. The presence of a significant effect of developmental stage indicates that the number of eggs and larvae in clip cages were not similar, i.e. egg mortality was higher with some plants versus others (Fig. 5-1). A correlation coefficient (r) of 0.75 ($n = 3.78$, $P < 0.001$) was calculated between the mean numbers of eggs and hatched larvae. Tukey’s HSD rendered a significant plant effect for the total number of eggs and larvae present in the clip cages (Fig 5-1). More eggs and larvae were found on four types of plants, i.e. citrus (5.9), sumac (5.4), Star (5.1) and Jewel (4.6). The blueberry varieties Showchaser and O’Neal had significantly lower total number of eggs and larvae (4.25 and 4.45, respectively, Tukey Grouping “c”) and numbers on Emerald (6.4) and O’Neal (4.3) were intermediate (Tukey Grouping “b”). The Jewel variety data were slightly skewed, however, as the mean number of eggs and hatched larvae were

negatively correlated (i.e., not as many larvae were present as eggs laid when compared to the other plant varieties in the Tukey Grouping “a”).

Choice oviposition tests. Roughly 25% of males and less than 50% of female citrus thrips were recovered from the plants in the bug dorms 14 days post release. The between replicate bug dorm adult survivorship was relatively low. Of ca. 60 adults of each sex released, male survivors per bug dorm ranged from 13-21 with a mean of 15.4 ± 3.3 [SE]; female counts ranged from 20-37 with a mean of 28.2 ± 6.4 [SE]. The Star variety had the highest mean numbers of adults collected in comparison with other varieties (Fig. 5-2).

Data from the bottom two thirds of the plant were pooled together, as there were few thrips collected and eggs found on the lowest third. There were more eggs located on the top third of the plant than the bottom two thirds of the plant across all plant varieties. There were also more larvae found on the top third of the plant than the bottom two thirds, but no differences in larvae found on the bottom two thirds of the plant than eggs present in those areas, for all plant varieties (Fig. 5-2). The Star variety had the numerically highest numbers of total eggs and larvae per plant compared with any other variety. The likelihood ratio chi-square test indicated a significant effect of egg count on the top third of the plant (top third versus bottom two thirds; Chi-square = 5.65, P = 0.2270) as well as a significant effect of larval count on the two plant locations, i.e. the top third also having the highest count (top third versus bottom two thirds; Chi-square = 1.62, P = 0.2025). More eggs were found on the top third of the blueberry plants and there was a higher percent of larvae present on the top third of the plant (Table 5-1),

regardless of plant variety. However, in the choise test, the Star variety had the highest percent of eggs found and larvae present. The Snowchaser variety had the lowest numbers of thrips counted overall, with fewer eggs, larvae and adults collected on that variety. The Misty variety had fewer larvae present than eggs laid and the number eggs laid were comparable to levels on Emerald.

Discussion

Under the conditions of these trials (24-34°C), citrus thrips egg hatch typically would take place within 4-9 days (Rhodes et al. 1989), therefore numbers of motile insects counted in both studies would be larvae from the first generation and surviving adults. The primary focus of this work was to determine if there was a difference in the number of eggs laid on the different types plants and our data clearly show this was the case (Fig. 5-1). The no-choice oviposition test showed that the highest number of eggs and larvae were found on citrus and sumac (positive controls) and the blueberry varieties Star and Jewel. Intermediate levels were found on Emerald and Misty, and the lowest numbers on Snowchaser and O'Neal. The highest numbers of larvae present in clip-cages (based on counts) were on the citrus, sumac and Star blueberry variety. The Tukey HSD separation (Fig. 5-1) for total counts of eggs and larvae group Jewel with the citrus, sumac, and Star, but these data may be skewed due to the negative correlation between the number of eggs laid on the Jewel variety and the number of larvae present.

The choice oviposition test also showed a correlation between the number of eggs located and the number of larvae present on the plant. Evaluation of count means (Fig. 5-2) showed a difference in citrus thrips numbers on the Star variety over the other blueberry varieties in the test. When given the choice to move about the plants, the citrus thrips' activities at the time of isolation and counting (egg laying, larval and adult distributions) appear to be similar for Emerald and O'Neal with a mean of less than 10 adults recovered from those plants. In the choice oviposition tests, the negative correlation between egg and larval counts is also apparent for the Jewel variety. Some explanations for this negative correlation could be: first instars eclosing from the egg suffered high mortality, the larvae did not or cannot feed and/or develop well on this variety, and/or the larvae dispersed away from the plant onto other plants. If the latter were the case, then one would expect to see one or more other blueberry varieties with higher numbers of larvae present, but that was not the case (Fig. 5-2, Table 5-1) and given the fact the canes did not remain in contact with one another, it is unlikely that high levels of larval dispersion to different pots took place. There are several possibilities which explain the results: 1) females chose to lay eggs preferentially in some varieties over others, 2) egg survivorship influenced the numbers counted/found, 3) first instar egg eclosion varied across variety, 4) differential larval survivorship and development on the plants, and 5) larval dispersion, possibly to other plants, but possibly off the plants with mortality ensuing in unevaluated portions of the bug dorm.

The Chi-square values for plant location (top third versus bottom two thirds) were significant (Chi-square = 5.65 and 1.62 respectively) indicating that there were disparate

numbers of eggs laid and larvae counted between the two locations, with the top third of the plants, regardless of variety, preferentially chosen over the bottom two-thirds. This was likely due to the amount of flush growth present on the top third of the plants. Also, given data in our studies, thrips activities appear higher on the top third of the plant; this is likely a good area to sample when looking for thrips on blueberries.

Combining all the data, our studies clearly show that the Star variety had more citrus thrips (totals of eggs, larvae and adults) than the other blueberry varieties, and numbers of oviposited eggs found on Star, Jewel, citrus and sumac were similar. This quite possibly is due to Star's parentage. The Star variety is a variety that produces flowers the earliest and for the longest period during the season and while yield may not be as high as some of the other varieties, it is planted as a season long fruit bearer. Because the highbush blueberries (like most blueberries) are hybrids of one another and the nurseries have proprietary rights to the genetics of these varieties, we are therefore unsure how closely related the varieties we tested are. Snowchaser was the variety least preferred by the citrus thrips based on our data, however, to our knowledge it is not a variety currently grown commercially in California.

There are countless factors that need to be considered to fully understand insect host choice, including but not limited to, host plant finding, host plant acceptance, host plant relatedness, resource concentration, resource availability and host use (Bernays and Chapman 1994, Mayhew 1997, Awmack and Leather 2002). The insect's ability to locate the host plant, settle, feed, reproduce and successfully produce the next generation that achieves reproductive maturity involves countless steps and subtle interactions that

are beyond the scope of the work presented here. We provide a platform for further work on the distribution, abundance and avenues for research related to citrus thrips as a pest of blueberries in California. We anticipate citrus thrips will remain a pest of concern to the blueberry industry of California as the industry continues to expand.

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Figure Legends

Fig. 5-1. Results of no-choice oviposition tests. Mean counts of eggs, larvae and total counts (egg + larvae). Total count bars bearing the same letter indicate means were not significantly different from one another based on the Tukey HSD test ($\alpha = 0.05$) (no statistical separation was found in analysis of egg and larval counts).

Fig 5-2. Results of choice oviposition tests. Mean number of eggs, larvae and adults found on each of five varieties of highbush blueberries.

Fig. 5-1.

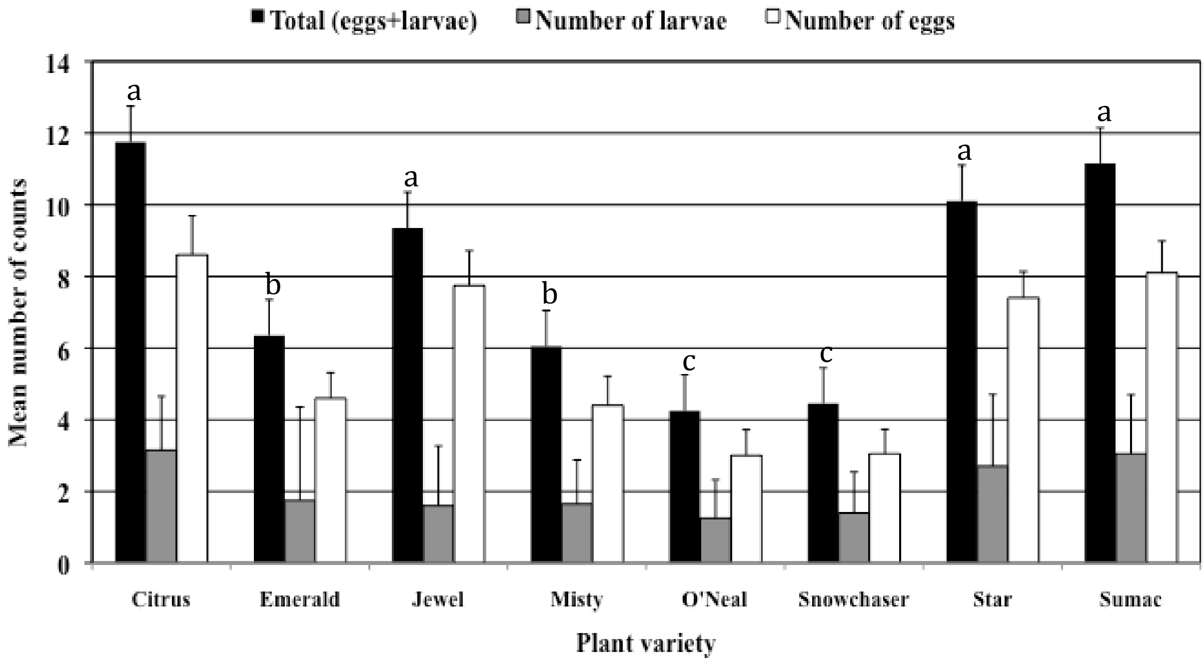

Fig. 5-2.

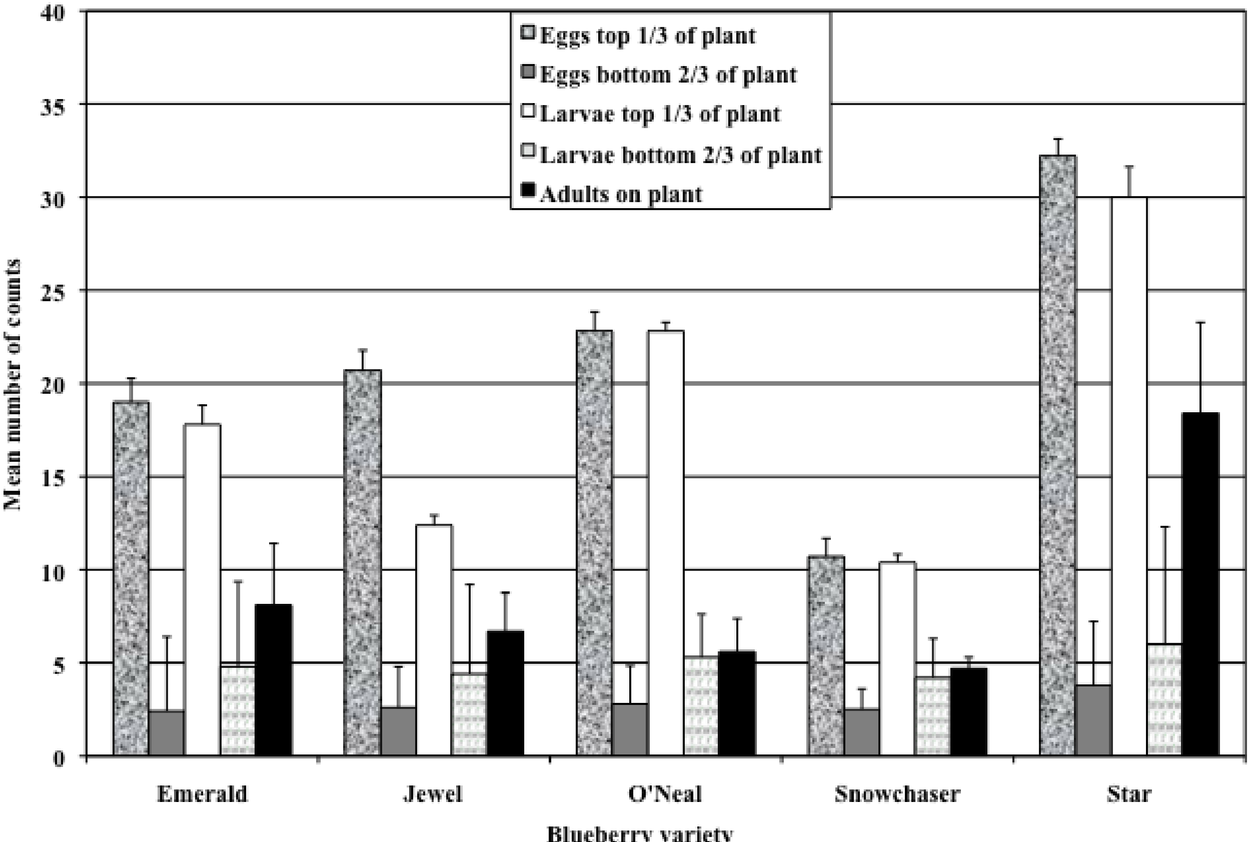

Table 5-1. Percentage of citrus thrips eggs and larvae found in each location in choice tests on five varieties of highbush blueberries (total larvae, n = 1,237; eggs, n = 1,195).

Plant Variety	Top third of plant		Bottom two-thirds of plant	
	% eggs	% larvae	% eggs	% larvae
Emerald	16	15	2	4
Jewel	17	12	2	4
O'Neal	19	18	2	4
Snowchaser	9	9	2	3
Star	27	26	4	5
% Total	88	80	12	20

Chapter 6

Introduction

Citrus thrips, *Scirtothrips citri* (Moulton), is a significant insect pest of citrus and mango fruits (Morse 1997) and has been recognized as a major pest of California citrus since the 1890s (Horton 1918). In the USA, citrus thrips are known from Arizona, California, Texas and somewhat recently, possibly Florida (Peairs and Davidson 1956, Childers and Nakahara 2006), whereas in Mexico they are reported only from northern Mexico (Flowers 1989, Johansen and Mojica-Guzman 1998). Based on its past distribution, several authors have reported that citrus thrips is native to southwestern North America and northwestern Mexico (Bailey 1964, Flowers 1989, Morse 1997).

Citrus thrips is primarily a pest of citrus in California, particularly in the San Joaquin (SJV) and Coachella valleys. They can have a broad host range, including, but not limited to, alfalfa, rose, grape, laurel, cotton, date, fir, lucerne and various grasses, pecans, and other ornamentals. Citrus thrips have been collected from over 55 different plant species (Bailey 1964, Flowers 1989). Their native host plant is hypothesized to be *Quercus* (Bailey 1964) or more likely *Malosma* (=Rhus) *laurina* (Nutt.) Abrams (Morse 1995) which was likely one of citrus thrips more common native host plants in southern California and northwestern Mexico prior to the introduction of citrus. In the SJV of California, *S. citri* has recently broadened its known host range and become a significant pest of high-bush blueberries (Haviland et al. 2009).

Scirtothrips citri was not known in Florida until 1986 where it was first detected in grape surveys (Flowers 1989, Frank and McCoy 1992). A collaborator (J. E. Funderburk, personal communication) was aware that in Florida, *S. citri* is not often collected from or abundant in several crops it is notorious for attacking in other regions of the Americas (i.e. blueberry, citrus, mango), but it is the most common thrips species he has collected from native vegetation and weeds.

Species identifications from slide-mounted specimens can be unreliable or inconsistent (Mound and zur Strassen 2001) and alternative or additional methods of identification may be necessary. Morphological identification suggests that *S. citri* is present in California, Arizona and Florida, but given that it is not a pest on several crops one might expect in Florida, further investigation is necessary to determine if *S. citri* is actually a cryptic species complex. The development of molecular genetic techniques (Saiki et al. 1988), predominantly analysis of mitochondrial DNA (mtDNA), has significantly contributed to an understanding of natural genetic diversity and speciation (Moritz et al. 1987, Avise 1994, Brunner et al. 2004, Rugman-Jones et al. 2005, 2006; Hoddle et al. 2008). Genetic markers offer additional methods of species determination and delineation, especially when coupled with morphological identifications (Stouthamer et al. 1999, Moritz et al. 2004, Rugman-Jones et al. 2006). These approaches are especially useful in groups that demonstrate a mixture of diverse ecological traits coupled with a conserved morphology.

Given the distribution of *S. citri* in major citrus growing regions of North America and the level of its pest status in those regions, re-evaluation of morphological and

molecular identifications was deemed necessary. The goals of this work were to investigate the haplotypic variation among *S. citri* populations based on phylogenetic analysis of the mitochondrial and ribosomal DNA, and to identify possible cryptic species complexes within the *Scirtothrips* attacking citrus.

Materials and Methods

Collection. The collection records for all specimens used in this study are listed in Table 1. Specimens were collected from various parts of California, Arizona, Texas, Florida (Quincy and Miami), Mexico, Nicaragua and Turkey. Specimens from Turkey were included in this analysis as it is an under-represented area of the world and at the time of collection from citrus, the collector believed the specimens to be citrus thrips. Specimens were collected into 95% ethanol by beating the live thrips onto a white piece of paper, touching a clean 5/0 Princeton paint brush (Princeton Artist Brush Co., Princeton, NJ) into the ethanol filled collection vial, touching the ethanol imbibed paint brush tip to the live insect so that the insect stuck to the paint brush tip and then depositing it passively into the collection vial. After collection, all specimens were stored at -20°C until analysis. Some of the collections contained *Frankliniella occidentalis* (Pergande) and *Neohydatothrips burungae* (Hood) but these collected groups were not included in our analysis.

DNA Extraction, Amplification, and Sequencing. Thrips were removed from ethanol and allowed to air dry on filter paper for 2 min. Total DNA was isolated using an

EDNA HiSpEx Tissue Kit (Saturn Biotech, Perth, Australia), following the manufacturer's protocol. This method is non-destructive, allowing slide mounting and morphological examination of the specimen after extraction. After DNA extraction, two separate gene regions were amplified using PCR: the conserved 28S-D2 domain of the large rRNA subunit and the cytochrome *c* subunit I (COI) of mitochondrial DNA (mtDNA) (Rugman-Jones et al. 2007). A ~553-bp section of the 28S-D2 domain was amplified in 25-µl reactions containing 2 µl of DNA template (concentration not determined), 2.5 × PCR buffer (containing 2.5 mM $MgSO_4$), 1.0 µl of $MgCl_2$, 5 µM dUTP, 0.5 µM each of the primers CF and CR (Campbell et al. 1993, 2000), 2 µl of bovine serum albumin (BSA) (New England Biolabs, Ipswich, MA) and 0.2 µl of *Taq* polymerase (New England Biolabs). Amplification was performed in a Mastercycler 5331 (Eppendorf, Hamburg, Germany) programmed for an initial denaturing step of 3 min at 94°C, followed by 30 cycles of 45 s at 94°C, 30 s at 50°C, 1.5 min at 72°C and a final extension of 30 min at 72°C. A ~663-bp segment of the COI gene also was amplified in 25-µl reactions containing 1 µl of DNA template (concentration undetermined), 2.5 × PCR buffer (containing 2 mM $MgSO_4$), 20 µM each dUTP, 2 mM $MgCl_2$, 0.2 µM each of the primers LCO1490 and HCO2198 (Folmer et al. 1994), 1.25 µl of BSA and 0.2 U of *Taq* polymerase. The thermocycler was programmed for an initial denaturing step of 1 min at 94°C; followed by five cycles of 30 s at 94°C, 1.5 min at 45°C and 1 min at 72°C; followed by a further 35 cycles of 30 s at 94°C, 1.5 min at 51°C and 1 min at 72°C; and a final extension of 5 min at 72°C. PCR products were visualized

on a 1% agarose gel stained with ethidium bromide, cleaned using the Wizard PCR Preps DNA purification system (Promega, Madison, WI) and direct sequenced in both directions at the UC Riverside Genomics Institute Core Instrumentation Facility using an Applied Biosystems 3730 DNA analyzer with a Big-Dye version 3.1 kit (Applied Biosystems, Foster City, CA).

Morphological Data. Post DNA extraction, specimens were transferred into 70% ethyl alcohol to prepare for slide mounting. Specimens were cleared in 5% NaOH for 12 h, processed through an alcohol dehydration series, placed in clove oil, and slide mounted in balsam (Mound and Marullo 1996). Specimens were identified to species (or closest matching species) using the online and interactive key of the "Thrips of California" (Hoddle et al. 2008). A representative from each haplotype generated from DNA analysis for this work was slide mounted and placed at the University of California, Riverside Entomology Research Museum as vouchers, and their collection numbers are included in Table 6-1.

Alignment and Phylogenetic Analyses. Representative species-specific sequences are deposited in GenBank. A BLASTN 2.2.19 search (Zhang et al. 2000) was used to compare sequences to existing *Scirtothrips* and *Neohydatothrips* sequences deposited in GenBank. Sequences were initially aligned manually in Bio-Edit version 6.0.7 (Hall 1999). The COI sequences were all of equal length and contained no stop codons, with the first position equal to codon position one, making alignment simple. The 28S-D2 (655 aligned bases) included six ambiguous regions that were variable enough to make alignment difficult. The six regions were coded as single multistate

characters (Table 6-2) in the analysis, or INNASE coding (Lutzoni et al. 2000, Hoddle et al. 2008). This mixed alphabetical and numeric multistate coding (maximum of 15 states) was treated as unordered and combined with the other data.

Parsimony analyses using 1,000 random addition sequences and random starting trees were done on 1) the complete molecular data set (28S-D2 and COI), 2) molecular data with only 28S-D2 (ambiguous region data included) and 3) molecular data with only COI. All analyses were performed using PAUP 4.0* (Swofford 2002), with the complete matrix verified using TNT with a new technology search (Goloboff et al. 2003, 2008). Bootstrap (BS) values were generated in PAUP 4.0* using 1,000 BS replicates with two random heuristic searches for each replicate.

Maximum Likelihood (ML) analyses and associated bootstrapping, were conducted on the complete molecular data set with RAxML v.7.2.7 via the CIPRES Science Gateway (http://www.phylo.org) (Miller et al. 2010, Stamatakis 2006, Stamatakis et al. 2008). Two gene partitions were included (one for COI and one for 28S-D2), and data were analyzed with 1000 rapid bootstrap replicates.

Results

Morphological Data. None of the specimens collected from citrus in Texas were *Scirtothrips*; all thrips in the vials collected from multiple types of citrus were *Frankliniella occidentalis* (Pergande) and were therefore not included in any analyses for *Scirtothrips citri* or near *citri*. All specimens from California and Arizona keyed to

Scirtothrips citri. The distinguishing features for *S. citri* include: 1) both sexes fully winged, 2) body mainly yellow without dark markings, 3) antennae 8-segmented with segments III – IV having forked sensorium and segments III-VIII grey, 4) head wider than long with ocellar triangle and postocular region having closely spaced sculpture lines, 5) three pairs of ocellar setae present with pair III close together between the anterior margins of the hind ocelli, 6) pronotum with closely spaced sculpture lines and the posterior margin with four pairs of setae, 7) metanotal posterior half with irregular longitudinal reticulations and median setae originating behind the anterior margin, 8) first vein of the forewing with three setae on the distal half (incomplete first vein setal row), the second vein with three widely spaced setae (incomplete second vein setal row) and the posteromarginal cilia wavy, 9) abdominal tergites III – VI with median setae close together, tergites II – VIII with lateral thirds covered in closely spaced rows of fine microtrichia, these microtrichial fields had four discal setae, the posterior margin had a fine comb (tergite VII posteromarginal comb incomplete medially and tergite VIII comb complete) and the lateral discal microtrichia extended medially and lastly 10) abdominal sternites were without discal setae and the posterior margins were without a comb of microtrichia (Hoddle et al. 2008).

Morphological differences from the above on specimens from Florida (Quincy), Mexico and Nicaragua could not be found and all were keyed to *S. citri*. The Florida citrus thrips collected from *Mimosa* sp. did not fit well into the Thrips of California key. The metanotal median setae arising at the anterior margin are in contrast to that of *S. citri,* where the metanotal median setae arise behind the anterior margin. All other characters

appeared to be in congruence with *S. citri*. The thrips collected from *Mimosa* in Miami, Florida were keyed to *Scirtothrips* near *citri*. The specimens from Turkey were keyed to *Scirtothrips citri* using the Thrips of California key.

Phylogenetic Analyses. Relationships were largely congruent among all analyses with the following exceptions: the group 'Turkey_Hatey_citrus1_5' in the combined tree (both 28S-D2 and COI; Fig. 6-1) and in the 28S-D2 alone tree (Fig. 6-2) as a sister group to the *S. citri* clade but these were placed without support along with *S.* aff *dorsalis* (Fig. 6-3). This group switched positions, depending on the type of analysis. Results regarding *S. citri* will be discussed in terms of the combined parsimony analysis.

The parsimony analysis of the complete molecular data set, including ambiguous region coding, resulted in 114 most parsimonious trees (length 1985, r.i. 0.71; Fig. 6-1). The overall = consensus solution of all of the trees had no significant areas of conflict and collapsed with strong support for branches and monophyly of the groups. Further, analyses based upon exclusion of one gene region at a time (28S-D2, length 349, r.i. 0.828, Fig. 6-2; and COI, length 1521, r.i. 0.69, Fig. 6-3) resulted in trees with the following monophyletic groupings 1) Arizona and California, 2) Mexico-Nicaragua and 3) Florida (Quincy). The group collected from Miami, Florida was sister to *Scirtothrips bounites* Mound & Marullo in all analyses. The RAxML analysis resulted in a single tree (not shown) with a final Ln likelihood of -9,623.28. The parsimony and RAxML analyses both produced similar trees for the specimens collected in Arizona, California, Florida (Quincy and Miami), Mexico and Nicaragua. Clades Arizona and California,

Mexico-Nicaragua, Florida (Quincy) and Florida (Miami), while morphologically indistinguishable, are highly molecularly divergent differing by 10-40 base pairs.

Discussion

The analyses conducted on molecular data confirmed that *Scirtothrips citri* in the Americas is not a single species but a complex of at least three molecularly divergent groups (Fig. 6-1). The parsimony and maximum likelihood analyses both produced similar trees for the specimens collected in Arizona, California, Florida (Quincy and Miami), Mexico and Nicaragua with the specimens collected from Turkey switching positions, depending on the type of analysis. Arizona and California specimens were morphologically identified using the Thrips of California key as 'California' citrus thrips and the Quincy (Florida), Mexico and Nicaragua specimens also keyed out as 'California' citrus thrips but this is not surprising as the key was developed for the known thrips found in California. The Miami (Florida) citrus thrips collected from *Mimosa* sp. did not fit well into the Thrips of California key, which is again not surprising. Our molecular results suggested the specimens collected from *Mimosa* in Miami are *S. bounites*, although these specimens were identified as *S. citri* by a collaborator (J. E. Funderburk). To our knowledge, *S. bounites* has not been found in California to date but was collected from mango in Mexico (Rugman-Jones et al. 2006). This suggests that without any consistent morphological differences, a cryptic species complex is likely present.

The specimens collected from Turkey were morphologically keyed to *S. citri* but were grouped differently based on parsimony analyses of the two gene regions separately. The Turkey specimens in the 28S-D2 alone analysis were placed as the sister group to the overall '*Scirtothrips citri*' clade (Fig. 6-2) however, in the COI alone analysis, the Turkey specimens are grouped within the *Scirtothrips dorsalis* Hood clade. These specimens do not share *S. dorsalis* specific characters such as microtrichia covering the sternites and straight wing cilia. In the parsimony and maximum likelihood combined gene region analyses, the Turkey specimens are placed as the sister group to the overall '*Scirtothrips citri*' clade. The Turkey specimens did not appear to be different than *S. citri* morphologically and were included in the analysis because the group may be a new/related species to the '*Scirtothrips citri*' clade. A comparison of bootstrap values between the individual gene trees suggests that 28S-D2 is driving the backbone of the combined tree. This is expected as the 28S-D2 gene region is generally more highly conserved than the COI region (Rugman-Jones et al. 2007).

There is a need for further research into the relationships between the groups of *S. citri* presented here. Based on our molecular data, citrus thrips in California and Arizona are clearly different than those found in Quincy (Florida) and Mexico-Nicaragua but morphological data suggests they are the same. These conflicting pieces of information illustrate the fact that morphological identifications may not be accurate enough especially when dealing with some organisms of economic importance. For example in a hypothetical situation, if specimens were detected and incorrectly morphologically identified as a species of economic concern but actually are a part of a non-economic

clade, not distinguishing these groups could have serious import/export implications. Utilizing molecular markers for species identification and separation could be an immensely useful tool if/when morphological identifications are unclear. There are a few instances of cryptic species investigations with thrips (Brunner et al. 2004, Morris and Mound 2004, McLeish et al. 2005) and the development of molecular keys for many different types of thrips pests (Brunner et al. 2002, Toda and Komazaki 2002, Rugman-Jones et al. 2006) have aided in our understanding that thrips populations from various areas, or thrips collected from different host plants, are not necessarily all the same species, even if morphological analysis suggests this is the case.

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Figure Legends

Fig. 6-1. Single most parsimonious tree from combined the 28S-D2 and COI analysis with ambiguous multistate coding.

Fig 6-2. Single most parsimonious tree from the 28S-D2 analysis with ambiguous multistate coding.

Fig. 6-3. Single most parsimonious tree from the COI analysis.

Fig. 6-1.

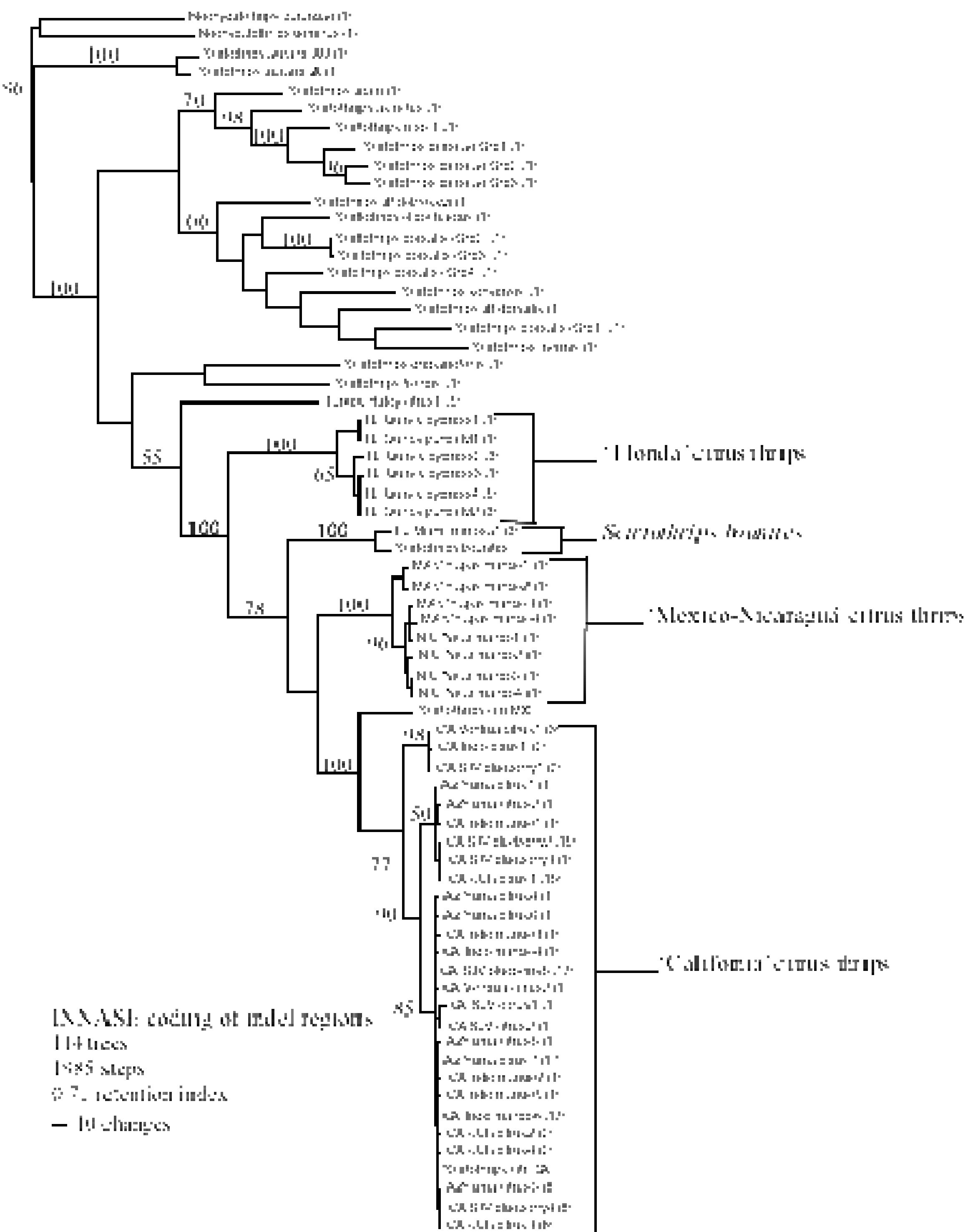

Fig. 6-2. Single most parsimonious tree from the 28S-D2 analysis with ambiguous multistate coding.

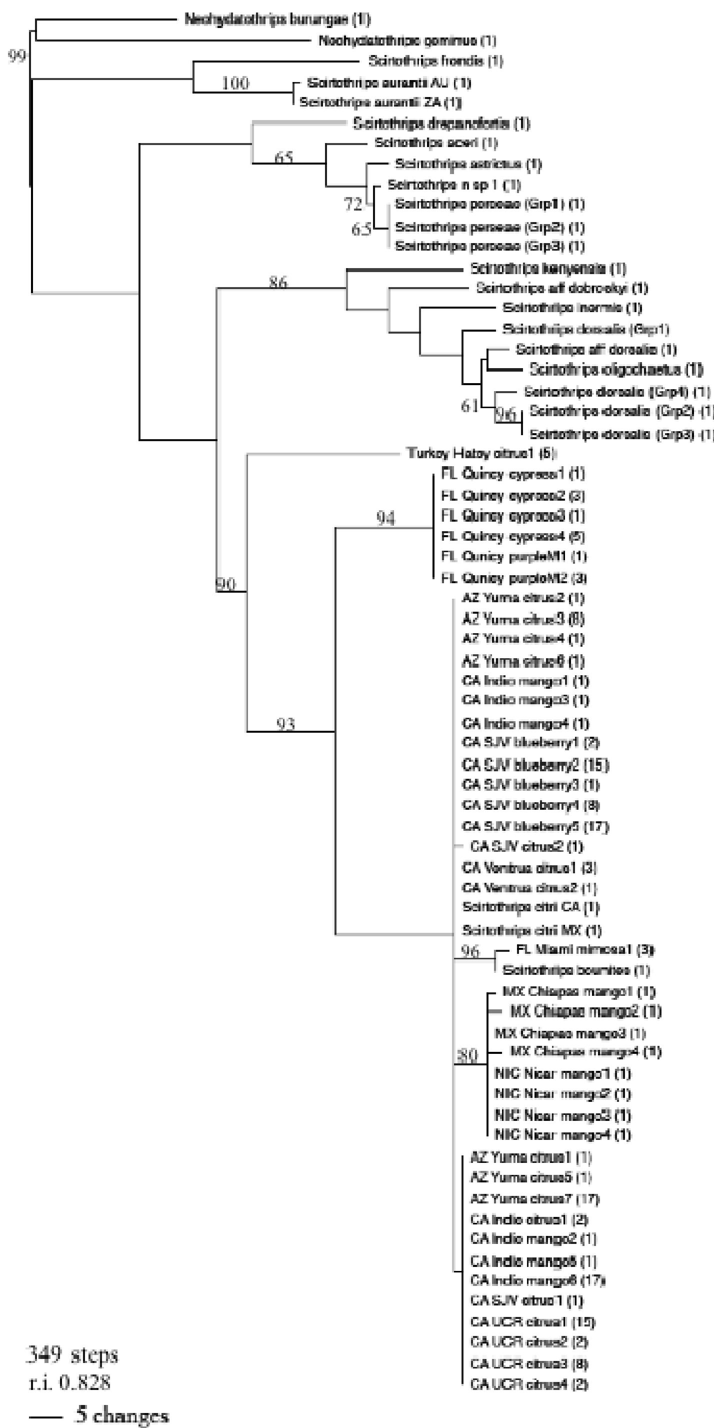

Fig. 6-3. Single most parsimonious tree from the COI analysis.

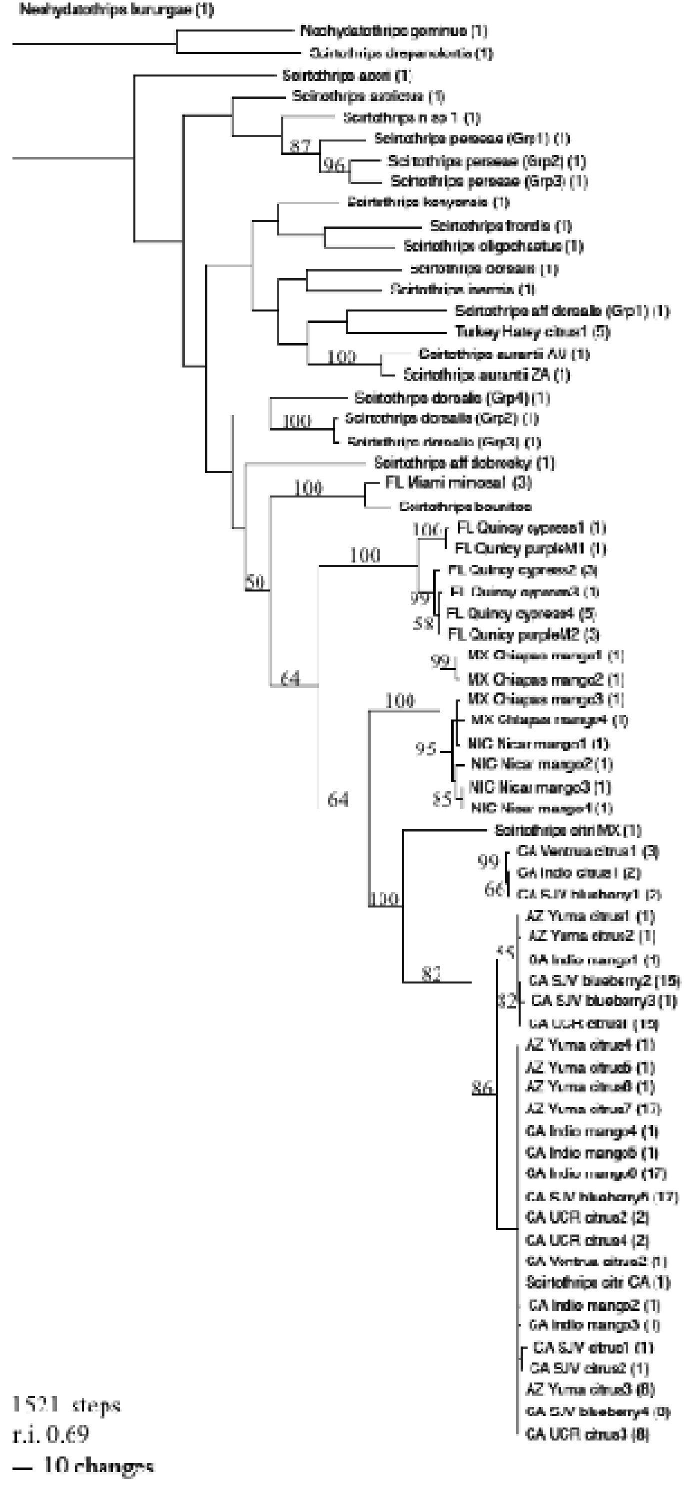

Table 6-1. Collection records of *S. citri* used in molecular and morphological phylogenetic analyses.

Grouping	Name	Location	Host Plant	Collector	Date	UCRC ENT	GPS
Citrus thrips California	AZ_Yuma_citrus1_1	Yuma, AZ	Citrus, lemons	T. Tellez	3-May-11	297142	N32° 36’ 43” W114° 38’ 02”
	AZ_Yuma_citrus2_1	Yuma, AZ	Citrus, lemons	T. Tellez	3-May-11	297143	N32° 36’ 43” W114° 38’ 02”
	AZ_Yuma_citrus3_8	Yuma, AZ	Citrus, lemons	T. Tellez	3-May-11	297144	N32° 36’ 43” W114° 38’ 02”
	AZ_Yuma_citrus4_1	Yuma, AZ	Citrus, lemons	T. Tellez	3-May-11	297145	N32° 36’ 43” W114° 38’ 02”
	AZ_Yuma_citrus5_1	Yuma, AZ	Citrus, lemons	T. Tellez	3-May-11	297146	N32° 36’ 43” W114° 38’ 02”
	AZ_Yuma_citrus6_1	Yuma, AZ	Citrus, lemons	T. Tellez	3-May-11	297147	N32° 36’ 43” W114° 38’ 02”
	AZ_Yuma_citrus7_17	Yuma, AZ	Citrus, lemons	T. Tellez	3-May-11	297148	N32° 36’ 43” W114° 38’ 02”
	CA_Indio_citrus1_2	Indio, California	Citrus, Valencia	D. Zahn	11-Oct-10	297149	N35° 33’ 16” W116° 10’ 08”
	CA_Indio_mango1_1	Indio, California	Mango	D. Zahn	11-Oct-10	297150	N33° 55’ 43” W117° 09’ 34”
	CA_Indio_mango2_1	Indio, California	Mango	D. Zahn	11-Oct-10	297151	N33° 55’ 43” W117° 09’ 34”
	CA_Indio_mango3_1	Indio, California	Mango	D. Zahn	11-Oct-10	297152	N33° 55’ 43” W117° 09’ 34”
	CA_Indio_mango4_1	Indio, California	Mango	D. Zahn	11-Oct-10	297153	N33° 55’ 43” W117° 09’ 34”
	CA_Indio_mango5_1	Indio, California	Mango	D. Zahn	11-Oct-10	297154	N33° 55’ 43” W117° 09’ 34”
	CA_Indio_mango6_17	Indio, California	Mango	D. Zahn	15-Jun-10	297155	N33° 55’ 43” W117° 09’ 34”
	CA_SJV_blueberry1_2	Delano, California	Blueberry, 1	D. Zahn	15-Jun-10	297156	N35° 57’ 44” W119° 02’ 45”
	CA_SJV_blueberry2_15	Delano, California	Blackberries	D. Zahn	15-Jun-10	297157	N354° 7’ 56” W119° 6’ 22”
	CA_SJV_blueberry3_1	Delano, California	Organic Blueberries	D. Zahn	15-Jun-10	297158	N35° 57’ 44” W119° 02’ 45”
	CA_SJV_blueberry4_8	Delano, California	Organic Blueberries	D. Zahn	15-Jun-10	297159	N35° 57’ 44” W119° 02’ 45”
	CA_SJV_blueberry5_17	Delano, California	Blueberries	D. Zahn	15-Jun-10	297160	N35° 57’ 44” W119° 02’ 45”
	CA_SJV_citrus1_1	Delano, California	Lemons	D. Zahn	15-Jun-10	297161	N35° 57’ 44” W119° 02’ 45”
	CA_SJV_citrus2_1	Delano, California	Lemons	D. Zahn	15-Jun-10	297162	N35° 57’ 44” W119° 02’ 45”
	CA_UCR_citrus1_15	Riverside, California	Citrus, lemons	D. Zahn	4-Jun-10	297163	N33° 58’ 15” W117° 19’ 34”
	CA_UCR_citrus2_2	Riverside, California	Citrus, lemons	D. Zahn	4-Jun-10	297164	N33° 58’ 15” W117° 19’ 34”
	CA_UCR_citrus3_8	Riverside, California	Citrus, lemons	D. Zahn	4-Jun-10	297165	N33° 58’ 15” W117° 19’ 34”

	CA_UCR_citrus4_2	Riverside, California	Citrus, lemons	D. Zahn	4-Jun-10	297166	N33° 58’ 15” W117° 19’ 34”
	CA_Ventrua_citrus1_3	Ventura Co., California	Citrus, lemons	M. Hoddle	27-Aug-08	297167	N34°15’ 59” W119°06’ 05”
	CA_Ventrua_citrus2_1	Ventura Co., California	Citrus, lemons	M. Hoddle	6-Jun-08	297168	N34°16.320 W119°04.034
Citrus thrips FL	FL__Qunicy_purpleM1_1	Quincy, Florida	Purple Mulchy	J. Funderburk	19-May-10	297169	N30° 32’ 54” W84° 35’ 07”
	FL__Qunicy_purpleM2_3	Quincy, Florida	Purple Mulchy	J. Funderburk	19-May-10	297170	N30° 32’ 54” W84° 35’ 07”
	FL_Quincy_cypress1_1	Quincy, Florida	Bald Cypress, 1	J. Funderburk	29-Apr-10	297171	N30° 32’ 54” W84° 35’ 07”
	FL_Quincy_cypress2_3	Quincy, Florida	Bald Cypress, 1	J. Funderburk	29-Apr-10	297172	N30° 32’ 54” W84° 35’ 07”
	FL_Quincy_cypress3_1	Quincy, Florida	Bald Cypress, 2	J. Funderburk	19-May-10	297173	N30° 32’ 54” W84° 35’ 07”
	FL_Quincy_cypress4_5	Quincy, Florida	Bald Cypress, 1	J. Funderburk	29-Apr-10	297174	N30° 32’ 54” W84° 35’ 07”
Citrus thrips FL	FL_Miami_mimosa1_3	Miami, Florida	Mimosa	T. Skarlinsky	5-Apr-11	297175	N25° 79’ 09” W80° 30’ 07”
Citrus thrips Central America	MX_Chiapas_mango1_1	Chiapas, Mexico	Mango	J. Funderburk	1-Mar-11	297176	N14° 44’ 07” W92° 21’ 28”
	MX_Chiapas_mango2_1	Chiapas, Mexico	Mango	J. Funderburk	1-Mar-11	297177	N14° 48’ 14” W92° 20’ 52”
	MX_Chiapas_mango3_1	Chiapas, Mexico	Mango	J. Funderburk	1-Mar-11	297178	N14° 44’ 07” W92° 21’ 28”
	MX_Chiapas_mango4_1	Chiapas, Mexico	Mango	J. Funderburk	1-Mar-11	297179	N14° 48’ 14” W92° 20’ 52”
	NIC_Nicar_mango1_1	Leon, Nicaragua	Mango	J. Funderburk	15-Mar-11	297180	N12° 35’ 20” W86° 21’ 46”
	NIC_Nicar_mango2_1	Leon, Nicaragua	Mango	J. Funderburk	15-Mar-11	297181	N12° 35’ 20” W86° 21’ 46”
	NIC_Nicar_mango3_1	Leon, Nicaragua	Mango	J. Funderburk	15-Mar-11	297182	N12° 35’ 20” W86° 21’ 46”
	NIC_Nicar_mango4_1	Leon, Nicaragua	Mango	J. Funderburk	15-Mar-11	297183	N12° 35’ 20” W86° 21’ 46”
Citrus thrips Turkey	Turkey_Hatey_citrus1_5	Hatey Prov. Turkey	Citrus, meyers lemons	M. Hoddle	20-Sep-10	297184	N36° 26’ 32” E35° 55’ 78”

Table 6-2. Ambiguous region coding for the 28S-D2 region in *S. citri* and related specimens used in the molecular and morphological phylogenetic analyses. Ambiguous regions, in brackets, correspond to the following 28S-D2 sit positions: Ambig-1 (88-93), Ambig-2 (226-233), Ambig-3 (239-242), Ambig-4 (271-288), Ambig-5 (342-345) and Ambig-6 (380-397), which are each followed by the multistate codes.

Species or ID	Ambig-1	Ambig-2	Ambig-3	Ambig-4	Ambig-5	Ambig-6
Neohydatothrips_geminus	[CTTTT] 0	[TGGTC] 0	[TGAA] 0	[TCGCTTCGGTGGC] 0	[ATT] 0	[TGTAGCAGGCTACA] 0
Neohydatothrips_burungae	[TTTT] 1	[TGTTC] 1	[TTAT] 1	[TCGTTTCGGCGGC] 1	[TT] 1	[CGTAACGGGCTACA] 1
Scirtothrips_frondis	[CGTT] 2	[GTTGTTC] 2	[AA] 2	[GATTGGTTTCGGCCTTCC] 2	[ATTT] 2	[CGCCCGCGGGCTACG] 2
Scirtothrips_drepanofortis	[CAGTTC] 3	[TGGTC] 0	[TCAT] 3	[GCAGGCTTGCTTGCC] 3	[ATTT] 2	[CTCAATTGGGCTACA] 3
Scirtothrips_bounites	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
Scirtothrips_citri_CA	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
Scirtothrips_citri_MX	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
Scirtothrips_aurantii_ZA	[TGTT] 5	[GGTTC] 4	[ATAT] 5	[CAGGCTTGTCTTGC] 5	[TT] 1	[CGCATCGGGCTACG] 5
Scirtothrips_aurantii_AU	[TGTT] 5	[GGTTC] 4	[ATAT] 5	[CAGGCTTGTCTTGC] 5	[TT] 1	[CGCATCGGGCTACG] 5
Scirtothrips_aceri	[CGGG] 6	[GGTTC] 4	[TTAT] 1	[GTAGGCTTGCTTGCC] 6	[TT] 1	[CGTATCGGGCTATA] 6
Scirtothrips_kenyensis	[CGAC] 7	[GGTTC] 4	[TGT] 6	[GTGGCTTTCGGGCCGCC] 7	[TT] 1	[TGATTTCATCGGGCTACA] 7
Scirtothrips_inermis	[CGGC] 4	[TCGAC] 5	[GT] 7	[GTGGGCTCGTCTCGCC] 8	[TT] 1	[CGCAACGGGCTGTA] 8
Scirtothrips_astrictus	[CGGC] 4	[GGTTC] 4	[TCAT] 3	[GTAGGCTTGTCTTGCC] 9	[TT] 1	[CGTACCGGGCTACA] 4
Scirtothrips_n_sp_1	[CGGC] 4	[GGTTC] 4	[TTAT] 1	[GTAGGCTTGTCTTGCC] 9	[TT] 1	[CGCACCGGGCTACA] 9
Scirtothrips_aff_dobroskyi	[CGGC] 4	[TCGTTC] 6	[GA] 8	[GTGGGCTTGCCTGCC] A	[TT] 1	[CGTATCGGGCTACA] A
Scirtothrips_oligochaetus	[CGGC] 4	[GGTTC] 4	[GT] 7	[GTGGGCTTGCCCGCC] B	[ATT] 0	[CGTTTCGGGCTGTT] B
Scirtothrips_aff_dorsalis	[CGGC] 4	[AGTTC] 7	[GT] 7	[GTGGGCTTGCCCGCC] B	[ATT] 0	[CGTTTCGGGCTGCT] C
Scirtothrips_perseae_Grp1	[CGGC] 4	[GGTTC] 4	[TTAT] 1	[GTAGGCTCGTCTTGCC] C	[TT] 1	[CGCACCGGGCTACA] 9
Scirtothrips_perseae_Grp2	[CGGC] 4	[GGTTC] 4	[TTAT] 1	[GTAGGCTCGTCTTGCC] C	[TT] 1	[CGCACCGGGCTACA] 9
Scirtothrips_perseae_Grp3	[CGGC] 4	[GGTTC] 4	[TTAT] 1	[GTAGGCTCGTCTTGCC] C	[TT] 1	[CGCACCGGGCTACA] 9
Scirtothrips_dorsalis_1	[CGGC] 4	[GGTTC] 4	[GT] 7	[GTGGGCTTGCTCGCC] D	[ATT] 0	[CGTTTCGGGCTGTT] B
Scirtothrips_dorsalis_2	[CGGC] 4	[TTGTTC] 8	[GT] 7	[GTGGGCTTGCTCGCT] E	[ATT] 0	[CGTTTCGGGCTGTT] B

Scirtothrips_dorsalis_3	[CGGC] 4	[TTGTTC] 8	[GT] 7	[GTGGGCTTGCTCGCT] E	[ATT] 0	[CGTTTCGGGCTGTT] B
Scirtothrips_dorsalis_4	[CGGC] 4	[GGTTC] 4	[GT] 7	[GTGGGCTTGCYCGCT] F	[CAT] 4	[CGTTTCGGGCTGTT] B
Turkey_Hatey_citrus1_5	[CTGTC] 8	[GGTTTC] 3	[GAT] 4	[GTGGCTTGCTGCC] G	[ATT] 0	[CGTTTATCGGGCTATA] D
FL_Quincy_cypress1_1	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
FL__Qunicy_purpleM1_1	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
FL_Quincy_cypress2_3	[CGGC] 4	[GGTTTTTC] 9	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
FL_Quincy_cypress3_1	[CGGC] 4	[GGTTTTTC] 9	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
FL_Quincy_cypress4_5	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
FL__Qunicy_purpleM2_3	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
MX_Chiapas_mango1_1	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
MX_Chiapas_mango2_1	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
MX_Chiapas_mango3_1	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
NIC_Nicar_mango1_1	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
MX_Chiapas_mango4_1	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
NIC_Nicar_mango2_1	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
NIC_Nicar_mango3_1	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
NIC_Nicar_mango4_1	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
CA_SJV_blueberry1_2	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
CA_Indio_citrus1_2	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
CA_Ventrua_citrus1_3	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
CA_SJV_citrus1_1	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
CA_SJV_citrus2_1	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
CA_UCR_citrus1_15	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
CA_SJV_blueberry2_15	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
CA_SJV_blueberry3_1	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
CA_Indio_mango1_1	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
AZ_Yuma_citrus1_1	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
AZ_Yuma_citrus2_1	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4

CA_Indio_mango2_1	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
CA_Indio_mango3_1	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
CA_Indio_mango4_1	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
CA_UCR_citrus2_2	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
CA_UCR_citrus3_8	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
CA_SJV_blueberry4_8	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
AZ_Yuma_citrus3_8	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
CA_UCR_citrus4_2	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
CA_Indio_mango5_1	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
CA_Ventrua_citrus2_1	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
AZ_Yuma_citrus4_1	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
AZ_Yuma_citrus5_1	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
AZ_Yuma_citrus6_1	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
CA_SJV_blueberry5_17	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
CA_Indio_mango6_17	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
AZ_Yuma_citrus7_17	[CGGC] 4	[GGTTTC] 3	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4
FL_Miami_mimosa1_3	[CGGC] 4	[GGTC] A	[GAT] 4	[GTGGTTCGCCGCC] 4	[TCA] 3	[CGTACCGGGCTACA] 4

Chapter 7

The goal of my dissertation was to add to the body of knowledge regarding citrus thrips [*Scirtothrips citri* (Moulton)] and avocado thrips (*Scirtothrips perseae* Nakahara), improve how they are managed at the commercial level, and better understand portions of their biology that have not been investigated to date.

In chapter one, I conducted a thorough review of thrips pest management focusing on avocado thrips in avocados and citrus thrips in citrus and blueberries. I discussed what is known about both species' origins, host plants, methods of sampling and control and the economic damage they cause. I developed each of the data chapters of my dissertation by discussing what is known in the literature about each subject on other species of thrips. I also discussed some of the non-target effects of pesticide control options for thrips and the relevance of this issue to thrips resistance development.

In chapter two, I began investigating alternatives to traditional pesticides for avocado thrips and citrus thrips pest management with the long-term goal of controlling these two species of thrips in the crops they affect. I screened two *Bacillus thuringiensis israelensis* (Bti) proteins in activated and inactivated forms against larvae and adults and six stains of *Beauveria bassiana* (Balsamo) Vuillemin (against adults only), including the commercially available strain (GHA), in the laboratory to determine mortality and infection rates in both avocado thrips and citrus thrips. I found that none of the Bti proteins tested were able to kill either species of thrips in either activated or inactivated forms. The *B. bassiana* strains tested were able to infect females of both species of thrips but to varying degrees. None of the tested strains was able to infect avocado thrips

females more effectively than another, however, the commercially available strain infected citrus thrips females faster and at lower dosages than any of the other strains tested in the laboratory. Therefore the commercially available strain (GHA) was the 'best' strain and was selected for the next set of studies to examine field control of citrus thrips in the blueberry system.

In chapter three, I conducted a field trial of *B. bassiana* strain GHA in blueberries for citrus thrips management as an alternative to traditional insecticides. However, prior to the field study, there were a number of preliminary studies to conduct and information to obtain such as, amounts and formulation of GHA to use (commercially available and experimental colonized millet seed), timing, sampling location, duration of sampling and planning the trial with both drip irrigation and overhead water. The development of a 3x2 factorial strip plot design with 4 replicate blocks was constructed in the blueberry fields and multiple types of thrips samples were taken. Overall, data gathered from my sampling cages showed the commercially available formulation of GHA and the colonized millet seed treatments decreased the number of citrus thrips in blueberries by 50% relative to the control at the first sampling time (3 days post application), but at the second sampling time (6 days post application), only the colonized millet seed treatment had a significant impact relative to the control. This level of control is not effective enough to highly recommend entomopathogenic fungi as an alternative to traditional insecticides, but they could possibly be used in conjunction with pesticides early on in the season before thrips populations grow or as a treatment on organic blueberries. Based on the different types of data gathered, we decided additional fieldwork with *B. bassiana*

was not warranted. This chapter concluded with the idea that, based on our data, it is up to the grower to decide if they want to include entomopathogenic fungus in their citrus thrips control programs; while somewhat effective it is a quite costly control tactic.

Chapter four covers several aspects of the impacts of pesticides used for avocado thrips management on a non-target predaceous mite. The native predatory mite found in southern California avocados is *Eusieus hibisci* Chant and I tested four of the currently five recommended pesticides (abamectin, fenpropathrin, spinetoram and sabadilla) for avocado thrips management in avocados on these mites by placing the mites on pesticide treated leaves and measured mite mortality and repellency. The pesticides were applied at a conservative dilution rate of 2,805 L/ha (typical applications are 468-935 L/ha). Each of the four pesticides caused mite mortality; fenpropathrin had the longest activity, abamectin, sabadilla, and spinetoram caused mite mortality, but one of the pesticides, spinetoram, caused the mites to be repelled by the material. Therefore further studies were conducted to investigate the repellency observed to spinetoram. First I wanted to know if exposing spinetoram and abamectin (they have similar types of chemistry) treated leaves to UV light would cause enough photodegradation to the surface residues to change the repellency observed with spinetoram and possibly reduce levels of mortality. The UV light shifted the repellency observed with spinetoram from 14 days post treatment to 10 days post treatment and completely knocked out mortality to abamectin. I also wanted to know what level of mortality would take place if the mites were confined (not able to 'run away') on the treated leaves in Munger cells (i.e. bioassay units that would allow escape, see ch 2 for details) and I found a similar pattern of

mortality to that of the observed repellency. The last set of experiments in this chapter addressed a more 'natural' field situation, i.e. where mites might be exposed to partial leaf coverage or parts of a tree with pesticide residues. Most pesticide applications in avocados are made via helicopter (typically ca. 467 liters per ha) and coverage is not complete on/within the trees. I treated half of each bioassay leaf, wanted to know if the mites would be able to detect the spinetoram on the leaf surface and if mites were provided a pesticide free area, whether or not they would survive exposure to the spinetoram. Overall, I found more mites alive on the untreated side of the leaf, less repellency (less mite suicides into wet felt) and that at 14 days, there were no differences observed on treated versus control leaves (sprayed with water only) with respect to mite mortality, repellency and the number of females alive at the end of the 4 day bioassay period. This chapter concluded with the ideas that avocado growers should be aware of the effects that pesticides used for avocado thrips management have on *E. hibisci* and if predaceous mites are important to them might plan on using materials such as fenpropathrin sparingly, focusing instead on materials such as abamectin and spinetoram, which given field circumstances (UV sunlight breakdown of surface residues and most likely refuge from the materials with lack of complete tree coverage) may have less of an impact on the mites.

In chapter five I evaluated the oviposition of citrus thrips in blueberries via choice and no-choice tests because preliminary field observations indicated that citrus thrips shows a preferences for certain varieties of blueberries such as Star. In the no-choice tests I evaluated female oviposition in 6 blueberry varieties (Emerald, Jewel, Misty,

O'Neal, Snowchaser, and Star) and had one each citrus and laurel sumac (*Malosma laurina* (Nutt.) Abrams) as positive controls. I counted more eggs and hatched larvae in the citrus, sumac, Star and Jewel varieties, an intermediate number in Emerald and Misty and the fewest eggs and larvae in O'Neal and Snowchaser. These differences in the no-choice tests indicated that females laid differing numbers of eggs depending on plant variety. In the choice study, I evaluated Emerald, Jewel, O'Neal, Snowchaser and Star in the same cage with males and females and after 14 days, I counted how many adults, eggs and larvae were found on each variety in each cage. In this study I observed more eggs on the Star and Jewel varieties and more larvae on Star variety over any of the others and I also measured the most thrips in the top third of all the plants, indicating that if one is looking for citrus thrips on blueberries, the top third is where one should look. The data were consistent between the two tests in that there was more eggs laid on the Jewel variety than larvae found on that variety possibly suggesting that larval survivorship is hindered on Misty. In addition, the Star variety had more levels of thrips at the end of the experiment than any of the other plants tested, consistent with field observations. The major conclusion of this chapter was that citrus thrips females oviposit quite differently on different blueberry varieties.

In chapter six, I investigated the molecular genetics of citrus thrips populations in the Americas based on collections from citrus and mangos. There is a distinct difference in host plant selection between the populations found in Florida from other areas; citrus thrips in Florida are not pests on citrus and blueberry grown there but they are quite pestiferous on citrus, mango and blueberry where they are present in the other parts of the

Americas. I compared the DNA of "*Scirtothrips citri*" and related specimen samples from Arizona, California, Florida, Mexico, Nicaragua and Turkey (because we had *Scirtothrips* spp. samples in hand from several under-investigated portions of the world and thought we should include them). I sequenced gene regions 28S-D2 (highly conserved region of ribosomal DNA) and COI (less conserved cytochrome *c* subunit I of mitochondrial DNA) and compared these sequences to sequences in GenBank. I also identified the thrips specimens morphologically and tried to determine if they looked like different species. Other thrips collaborators also evaluated some of these specimens and could not distinguish most of them. The morphological identifications indicated that they were all *Scirtothrips citri* or near *citri* but molecular evidence suggested that all specimens from California and Arizona were genetically the same as one another (while different from the other populations), those from Mexico and Nicaragua were genetically the same as one another (while different from the other populations), and those from Turkey and Florida were distinctly different from each of the other populations. This evidence suggested that while they may all appear morphologically very similar and are currently called *Scirtothrips citri,* based on molecular evidence they are different monophyletic groups. This work points to the fact that molecular genetics are quite important for many small and difficult to identify organisms and further work is needed to determine what these 'near *citri*' organisms actually are.

In conclusion, my dissertation contributes information and knowledge aimed at improving citrus thrips and avocado thrips management.