USE OF CURRY LEAF (*Murraya koenigii*) AND VOLKAMER LEMON (*Citrus volkameriana*) AS POTENTIAL TRAP CROPS FOR THE ASIAN CITRUS PSYLLID (*Diaphorina citri)* IN A COMMERCIAL CITRUS GROVE

A Thesis

Presented to the

Faculty of

California State Polytechnic University, Pomona

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science

In

Agriculture Science

By

Amanda S. Marsh

2016

SIGNATURE PAGE

THESIS:	USE OF CURRY LEAF *Murraya KOENIGII* AND *C. VOLKAMARIAN* AS POTENTIAL TRAP CROPS FOR THE ASIAN CITRUS PSYLLID *Diaphorina CITRI* IN COMMERCIAL CITRUS GROVES
AUTHOR:	Amanda S. Marsh
DATE SUBMITTED:	Fall 2016
	College of Agriculture

Valerie Mellano, Ph.D.
Committee Chair
Department of Plant Sciences

____

Eileen Cullen, Ph.D.
Department of Plant Sciences

____

Robert Green, Ph.D.
Department of Plant
Sciences

____

ACKNOWLEDGEMENTS

Psalms 111:2 The works of the Lord are great, studied by all who have pleasure in them.

I want to thank God for giving me the idea for this project. It was a joy to study His creations! It has been my heart to serve the agricultural community through entomology. I was thankful that God gave me the opportunity to do so by further studying how plants and insects work together.

I'd also like to thank my loving husband, Nathan, for encouraging me every step of the way. You're my rock and my best friend.

Thank you to Dr. Valerie Mellano and Dr. Eileen Cullen for providing constructive feedback and mentoring me during the process. Dr. Robert Green, thank you for your patience and thorough attention to detail. I'd also like to acknowledge the support of Dr. Anna Soper and Dr. Kimberley Miller. Each of you gave me support and tools I needed to be successful in this journey. Thank you.

ABSTRACT

Since the introduction of the citrus greening disease *Candidatus* Liberibacter asiaticus Huanglongbing (HLB), from Asian in 2005, Florida citrus production has been in steady decline. Curative treatments for HLB-infected trees are unavailable, and management strategies for the insect vector *Diaphornia citri* (Asian citrus psyllid (ACP)), have been unsuccessful in Florida. With initial HLB detection in Los Angeles California in 2012, and recent detections near the first detection site, and establishment of ACP in San Diego, Orange, Imperial, and Los Angeles counties and in the San Joaquin valley due to greater than 50 ACP detections (University of California, Division of Agriculture and Natural Resources, 2016 c), there is growing concern for movement of the disease throughout the state. Novel ACP and HLB management strategies are needed. Trap cropping is an ecological approach to insect-pest management that utilizes preferred alternative insect host plants to lure the target insect pest away from the harvested agricultural crop. This methodology has not yet been attempted in citrus production with little understanding of trap crop potential for ACP in commercial citrus groves. This experiment applied current knowledge of ACP host plant preferences to evaluate *Murraya koenigi,* (curry leaf) and *Citrus volkamariana* as potential trap crops in and established commercial citrus orchard in Pomona, California.

TABLE OF CONTENTS

SIGNATURE PAGE ii

ACKNOWLEDGEMENTS iii

ABSTRACT iv

LIST OF TABLES vii

LIST OF FIGURES viii

CHAPTER 1 1

INTRODUCTION 1

Objectives 1

CHAPTER 2 2

LITERATURE REVIEW 3

2.1 Background 3

2.2 HLB: History and Biology 3

2.3 ACP primary vector of HLB disease 3

2.4 Economic impact of ACP and HLB 7

2.5 HLB management needed 10

2.6 Integrated pest management for ACP utilizing trap cropping 13

2.7 *M. koenigii* and *C.volkameriana* as potential trap crops for Asian citrus psyllid 16

CHAPTER 3 18

MATERIALS AND METHODS 18

3.1 Trap crop placement and sampling for ACP adults 18

3.2 Monitoring flush and population densities 19

3.3 Monitoring ACP movement 20

3.4 Statistical analysis..20
CHAPTER 4..22

RESULTS AND DISCUSSION..22

4.1 Effects of trap crops on *Diaphorina citri* densities 22
4.2 Adult ACP populations based on sweep sampling...23
4.3 Effects of trap crops on *Diaphorina citri* movement...24
4.4 Effects of flush patterns on *Diaphorina citri* densities.......................................24
4.5 Effect of trap crops on ant populations..224
4.6 Correlations between dependent variables 224
CHAPTER 5..38

CONCLUSION..38

5.1 Curry leaf and Volk as potential trap crops for ACP...38
5.2 Implications of trap cropping for ACP and HLB management...........................39
REFERENCES 42

Definition of Terms 48

LIS OF TABLES

Table 4.1. The effecto fo volk and curry leaf on the average of eggs and the percent branches with flush 25

Table 4.2.The effect of volk and curry leaf on the average of nymphs and adults per branch... 30

Table 4.3.The effect of volk and curry leaf on the percent flush growth and average adults per sweep.. 33

LIST OF FIGURES

Figure 4.1. Percent total branches with flush growth……………………………………6

Figure 4.2. Overall percent total branches with flush new growth……………………….7

Figure 4.3. Average ACP eggs per randomly selected branch……………………………8

Figure 4.4 Overall average ACP eggs per randomly selected branch …………………..29

Figure 4.5 Average number of adult ACP per randomly selected branch ………………30

Figure 4.6 Overall average number of adult ACP per randomly selected branch ………31

Figure 4.7 Average ACP adults per sweep net sample…………………………………..32

Figure 4.8 Overall average adult ACP per sweep net sample …………………………..35

Figure 4.9 Overall yellow panel trap placement effect on adult ACP …………………..37

CHAPTER 1

INTRODUCTION

Since the introduction of the citrus greening disease *Candidatus* Liberibacter asiaticus Huanglongbing (HLB), from Asian in 2005, Florida citrus production has been in steady decline. In 2015, orange production will be the lowest in over 50 years as direct result of the disease. Curative treatments for HLB-infected trees are unavailable, and management strategies for the insect vector *Diaphornia citri* (Asian citrus psyllid (ACP)), have been unsuccessful in Florida. With recent HLB detection in Los Angeles California in 2016 (University of California Agriculture and Natural Resources Statewide Integrated Pest Management Program 2016d) and establishment of ACP in Los Angeles, Orange San Diego, and Imperial counties and San Joaquin valley (University of California, Division of Agriculture and Natural Resources, 2016c), there is growing concern for movement of the disease throughout the state. Novel ACP and HLB management strategies are needed.

Trap cropping is an ecological approach to insect-pest management that utilizes preferred alternative insect host plants to lure the target insect pest away from the harvested agricultural crop. This methodology has not yet been attempted in citrus production with little understanding of trap crop potential for ACP in commercial citrus groves. Additionally, the effect of ACP host plant preferences on population movement within citrus production settings is unknown.

This experiment applied current knowledge of ACP host plant preferences to evaluate *Murraya koenigi*, (curry leaf) and *Citrus volkamariana* as potential trap crops in and established commercial citrus orchard in Pomona, California. Both trap crop species chosen have been shown to be suitable ACP host plants in laboratory settings, and indicate levels of HLB resistance in previous studies. Overall, we can hypothesize that increased plant diversity by adding trap crop plants to an otherwise citrus monoculture will increase invertebrate diversity including predators and parasites of ACP, contributing to overall pest suppression (Michaud 2004; Grafton-Cardwell *et al.* 2006). We hypothesize that a) ACP populations will favor *M. koenigii* over *C. volkamariana* and b), those taxa with greater growth flushes will support higher ACP populations.

Objectives

The experiments herein monitored ACP population movement and densities in an established commercial citrus grove supplanted with potted trap plants of *M koenigii* and *C. volkamariana*. Objectives were to (1) document flushing patterns of new plant growth attractive to ACP for *M. koenigii* and *C. volkamariana* in a commercial field setting, (2) determing ACP density and lifer history parameters on *M. koenigii* and *C. volkamariana* by counting all ACP life stages, (3) determine adult densities on trap plants compared to neighboring citrus grove plants by sweep net sampling (UC, ANR 2014), and (4) monitor ACP movement to trap crops using yellow panel traps.

This thesis follows the format and style of the Journal of Applied Ecology.

CHAPTER 2

LITERATURE REVIEW

2.1 Background

Since the introduction of HLB in Florida in 2005, citrus production has decreased steadily, with orange production especially experiencing losses, the November 2015 harvest season was projected to be lowest production season in over 50 years as direct result of the disease(Beach & Lucie 2015). HLB is a bacterial disease affecting plants in the citrus family, Rutacea. There is no known effective treatment for infected trees, and all commercial citrus genotypes are susceptible (Halbert & Manjunath 2004). Past research indicated that HLB transmission from plant to plant can occur through three possible routes: grafting of infected shoots to healthy trees (Lin, 1956), transmission through the plant parasite dodder (Bové, 2006), or through feeding of the ACP insect (Chen et al., 1973). ACP is the primary vector of HLB (Grafton-Cardwell *et al.* 2006), and establishes and reproduces on citrus and citrus relatives(Pluke, Qureshi & Stansly 2008; Westbrook *et al.* 2011).

2.2 HLB: History and Biology

Thought to have originated in Asia, HLB was first reported from China in 1919 (Bové 2006). From reviews on HLB in Asia and Africa, the name of the disease varied per region and some indicated a symptom exhibited by infected trees. For example, in South Africa, the disease was known as “greening” or “yellow shoot,” and in parts of China “huang long bing” meaning yellow dragon disease due to yellowing of new growth shoots found in infected trees (Bové 2006, da Graca and Korsten, 2004), and in Indonesia

“Vein phloem degeneration” (Bové 2006). Since 1995, the disease is officially designated as huanglongbing, or HLB (Bové 2006). In the 1970’s studies linked the mycoplasma-like organism, later confirmed as the bacterium HLB, to Reunion “greening”, Indian “dieback, and “likubin” in Taiwan and “mottle leaf” in Philippines in 1970’s. Early in the study of HLB, it was believed to be a virus due to its transmission behavior through grafting inoculation as well as through two citrus psyllid species (Bové 2006). It was later thought to be a mycoplasma organism because it could not be obtained in culture. HLB could not be confirmed as a separate pathogen from other citrus diseases and deficiencies until electron microcopy (EM) (Bové 2006).

HLB is a Gram negative bacterium confirmed by a peptidoglycan - containing cell wall similar to *E. coli*, another Gram-negative bacterium (Garnier, et al., 1984). There are two related species of the bacterium, *Candidatus* Liberibacter asiaticus and *Candidatus* Liberibacter africanus (da Graça & Korsten 2004). The bacterium restrict sieve tube function in plants and is found exclusively in this location within the plant (Bové 2006). EM detection is used to confirm the bacterium location in plant sieve tubes and identify the presence of the bacterial cell wall (Bové 2006, p.13). Blotchy mottling of leaves, yellowing shoots, premature fruit drop, and sour and lopsided fruits with aborted seeds are characteristics of the disease within infected plants, but they do not necessarily occur together in the same tree at the same time (Bové 2006).

In South Africa, *Candidatus* Liberibacter *africanus* is vectored by the African psyllid *Trioza erytreae* (Bové 2006), and is found in more temperate climates(Garnier *et al.* 2000; Bové 2006). In Asia, *Candidatus* Liberibacter *asiaticus* and its vector the Asian citrus psyllid (ACP), *Diaphorina citri*, are found in hot low altitude regions (Bové 2006).

Lab experiments and field observations beginning in the late 1960's focused on HLB and vector sensitivities to temperature and humidity. The studies showed that HLB in Africa is heat-sensitive, and occurs in cool areas. The associated African psyllid vector, *T. erytreae*, thrives in cool environments and is also sensitive to high temperatures and low humidity (Bové 2006). HLB in Asia, C. L. *asiaticus*, and its vector the Asian citrus psyllid (ACP), *D. citri*, is more heat-tolerant compared to the African strain, and the associated African vector, *T. erytreae (*Bové *et al.*, 1974, Bové 2006). In experimental research, each of the two vectors can transmit each of the two HLB species (Massonié & Bové 1976; Bové 2006). In 2004 in Brazil, a third species of HLB bacteria, *Candidatus* Liberibacter *americanus* was discovered and reported. This species was the major HLB agent, affecting 92% of infected trees and Ca. L. *asiaticus* affected only 6% of trees. Both HLB species were vectored by the ACP, which had been present within the country since the 1940's(Bové 2006). In 2008, Ca. L. *americanus* has not been confirmed outside of Brazil (Gottwald 2009). In North America, the ACP is considered the primary vector of the HLB strain C. L. *asiaticus* (Grafton-Cardwell *et al.* 2006); *Candidatus* Liberibacter *africanus* and Ca. L. *americanus* have not been detected.

2.3 ACP primary vector of HLB disease

The ACP insect has a straw-like mouth part which is inserted into plant phloem during feeding (Rogers & Stansly 2006; Bonani *et al.* 2010). Adults lay eggs on new plant leaf growth referred to as flush (Hall, et al 2013) and nymphs and adults primarily feed on new flush (Grafton-Cardwell *et al.* 2006; Rogers & Stansly 2006). Early instar nymphs settle and feed on lower sides of young leaves probably because they can probe the phloem easier as the thick-walled 'fibrous ring' around the phloem is less prominent in younger leaves compared to older leaves (Ammar, Hall & Jr 2013). Highest concentrations of nitrogen (N) content in plant tissue (3-7% of plant dry weight) occur in young, actively growing tissues, like flush growth in citrus, because these tissues require high N levels to support rapid protein synthesis (Mattson 1980). Phloem sap contains higher percent N and higher amount of sugar than xylem sap (Mattson 1980). This may explain why high ACP population numbers correlate with citrus flush cycles (University of California & Natural Resources 2016 c). Nitrogen levels impact fecundity and survivorship of herbivorous phloem-feeding insects like the Asian citrus psyllid (Awmack & Leather 2002; Tsagkarakis et al 2009)

New growth occurs primarily on outer edges of C. volkamariaana canopy, and proximally to axis of M. koenigii. ACP adult and nymphal stages acquire the HLB bacteria during feeding, where it remains in the gut of the ACP through its lifetime (Capoor, Rao & Viswanath 1970; Rogers & Stansly 2006; Bonani *et al.* 2010). Additionally, adult ACP that acquire HLB pathogen as nymphs are better vectors of the pathogen compared to adults that acquire pathogens only within adulthood (Inoue *et al.* 2009; Pels-Stelinski *et al.* 2010). HLB- positive ACP can easily vector the bacterium to neighboring trees or groves as adults are highly mobile, have wings and can fly or jump to new trees. Therefore, understanding and controlling ACP movement is critical in managing HLB. Because of the relationship between ACP and the HLB citrus disease, changes in ACP population movement to or within a commercial grove will impact the risk of HLB transmission to a commercial orchard (Grafton-Cardwell *et al.* 2006; Slinski, S. Citrus Research and Development Foundation 2016).

Although the psyllid citrus plant host preferences have received substantial attention, less is known about how the host preference may impact ACP movement in field settings. Understanding the effects of psyllid host preferences on population movement and densities in commercial citrus groves represents a critical gap because of the role ACP plays in vectoring the citrus greening disease, Huanglongbing (HLB). Changes in commercial citrus management practices due to ACP infestations may need to integrate trap cropping methodologies utilizing ACP host preferences to deter insect pest movement and disease transmission to commercial citrus crops.

2.4 Economic impact of ACP and HLB

HLB was first documented in China (Bové, 2006), and was first reported in the Americas in 2004, and has since been reported in other citrus-growing regions throughout the world including the United States in 2005 (Halbert& Manjunath 2004; Halbert and Núñez 2004). All commercial U.S. citrus growing regions, Florida, California, Texas, and Arizona have ACP populations (Halbert 2005; Hall & Hentz 2011; Warnet 2012) with HLB disease present in all but Arizona. Further-more, HLB and ACP have been found in Louisiana, South Carolina, Texas, and California (French, et al., 2001, Grafton-Cardwell, 2006,). ACP and HLB continue to impact California with quarantine restrictions due to ACP detection from San Diego north to San Joaquin county ('California Department of Food and Agriculture Plant Quarantine Manual: Asian Citrus Psyllid' 2016), as it is considered an established insect pest meaning no possibility for complete eradication (University of California, 2016a). HLB bacterium was first detected in Southern California in residential neighborhood in Hacienda Heights, CA in 2012. Since then, several trees near the original find site have tested positive for HLB bacteria. All trees determined postivite for HLB bacteria were removed and destroyed (University of California, 2016 c).

The five leading citrus producing counties in California are: Fresno, Kern, Tulare, Ventura and Riverside with most of the acres located in Fresno, Kern, and Tulare (Geisseler & Horwath 2016) ACP has been confirmed in all five counties (‘2016 Asian Citrus Psyllid (ACP) Overview - Grid Pages’ 2016).

The HLB bacterium limits nutrient uptake, directly impacting the vitality of infected trees. Other disease symptoms such as premature fruit drop, and misshapen and bitter fruit reduces production values further. Since introduction of the ACP in 1998 and HLB in 2005, Florida citrus industry has been in steady decline as direct result of HLB. Production particularly in oranges has decreased over past decade, with 27 percent decrease in production tons in 2015/16 from the previous growing season alone (Ferreira & Perez 2016). Overall, the U.S. citrus crop is forecasted for 2015/16 with more than 12 percent decrease in harvested tons from 2014/15 (Ferreira & Perez 2016), will all citrus varieties listed (grapefruit, lemon, oranges, tangelos, tangerines) experiencing decrease except tangerines (Hamer 2016).

Long term solutions for the disease are still unknown. Short term management practices include controlling insect vector populations with insecticide treatments (Belasque Júnior et al., 2010, Grafton-Cardwell, 2013) and removing bacterium inoculum source (Belasque Júnior *et al.* 2010). In compliance with the Florida Citrus Health Response Program, removing HLB-infected trees may be required by the Florida Department of Agriculture at the responsibility of the property owner. More recently because of vast infection incidences throughout Florida tree and/or field removal is voluntary and encouraged cultural practice in Florida (Florida Department of Agriculture 2016). Abandoned or unmanaged fields, an inoculum source for future ACP and HLB, should be registered with the Florida Department of Agriculture. Plants with the bacterium may not show signs or symptoms for at least six months (Bové, 2006), making timely detection of the disease difficult. Because of the severe symptoms HLB causes, it is viewed as the most threatening disease to the world citrus industries (Bove 2006).

ACP management strategies for commercial citrus groves include use of styntheic, broad spectrum insecticeds to target vector populations. Chemical groups of ACP effective synthetic insecticides with longer residual periods include: Organophosphate, carbamate, pyrethroid, neonicotinoids foliar, neonicotinoids systemic, butenolide, pyrethroid + neonicotinoid, neonicotinoid +abamectin, neonicotinoid + chlorantraniliprole. Softer, less residual synthetic insecticide chemical groups include: Spinosyns, avermectins, bezylureas, Meti insecticide, Tetronic acid, Anthranilic Diamide. Organic insecticides provide short-term reduction in ACP populatoins, and require direct contact with the insect body to be effective. Reaching ACP nymphal stages with these chemicals is challenging because nymphs live in small, protected areas within plant flush growth (University of California, 2016 b).

Repeated use of single or related insecticides with similar chemistries can result in insecticide- resistant pest populations (Grafton-cardwell; Coy, Bin & Stelinski 2016, University of California, 2016 b). Rotation of chemicals with different formulations and with increased monitoring practices was shown in 2013 and 2014 survey in Florida to decrease percent populations of insecticide-resistant ACP recorded during 2009 surveys (Coy, Bin & Stelinski 2016).

Classical biological control methods employ the beneficial actions of natural enemies including parasites, predators, and pathogens in managing pests and their damage (University of California Agriculture and Natural Resources Statewide Integrated Pest Management Program 2016 e). Biological control utilizing natural enemies is seen as valuable in ACP pest management practices. In a 2005-2006 Florida study, natural predators, mainly lady bird beetles, significantly contributed to ACP egg and nymph mortality rates (average 36% to 58%), with some mortality due to other natural enemies such as lacewings and the imported parasitoid *Tamarixia radiata* (Qureshi & Stansly 2007). *T. radiata* has been introduced and established in Florida but with limited impact on ACP populations (Monzo, Qureshi & Stansly 2014). Generalist predators were seen to inflict up to 100 percent mortality of ACP nymphs in Florida (Qureshi & Stansly 2009). Full advantage of biological control from natural enemies is only possible with well-timed and minimal insecticidal sprays applied during winter months prior to new flush (Rogers & Dewdney 2015), and when natural enemy activities have declined and there are vulnerable adult populations of ACP (Qureshi & Stansly 2009, 2010).

2.5 HLB management needed

Antibiotics as a curative strategy for HLB-infected trees are a growing area of research. Application of antibiotics to HLB-infected citrus genotypes has shown levels of HLB management by reversing symptoms for months or up to a year in greenhouse settings in Asia (Zhang *et al.* 2014), but is not a widespread practice within North American (Slinski, S. Citrus Research and Development Foundation 2016). In one study comparing injections of tetracycline and penicillin to HLB –infected trees, injected trees had better root systems and produced 'larger symptomless shoots and leaves than untreated controls' (Aubert & Bove 1980; Bové 2006). Penicillin was less persistent in the trees and required repeated injections compared to one injection of tetracycline (Aubert & Bove 1980). In 2011, the combination of penicillin and streptomycin administered to '*Ca*.L.*asiaticus*'- infected citrus and periwinkle plants via root soak or trunk injections was effective in eliminating or suppressing '*Ca*.L. *asiaticus*' bacterium for longer periods of time than either antibiotic administered separately (Zhang *et al.* 2011). In 2014, another study evaluated 31 different antibiotics for effectiveness against '*Ca*.L. *asiaticus*' using optimized graft-based chemotherapy (Zhang *et al.* 2012, 2014). A mixture of penicillin and streptomycin was most effective in eliminating HLB bacterium from infected citrus scions (Zhang *et al.* 2012).

Because HLB is a phloem-inhabiting bacterium, traditional copper-based, foliar-applied bactericides are ineffective at HLB control (Slinski, S. Citrus Research and Development Foundation 2016).There are two main antibiotics labeled for use in agriculture: streptomycin sulfate and oxytetracycline and are mainly used for treating bacterial diseases in pome and stone fruits (Adaskaveg, Gubler & Michailides 2012; Slinski, S. Citrus Research and Development Foundation 2016). Streptomycin is a bactericide, killing fire blight bacteria in apples and pearson contact, whereas oxytetracycline is a bacteriostatic, inhibiting multiplication of streptomycin-resistant strains of blight bacteria (McManus & Jones 1994; Slinski, S. Citrus Research and Development Foundation 2016). As found in these studies, the bacteriostatic bactericides require longer duration of exposure in order to suppress bacterial growth as well as re-applications for continual bacterial suppression. Efficacy of these treatment types comprised by two factors, 1. These compounds are not registered yet for commercial use, and 2. From the HLB studies so far, the main applications is trunk injections, which is not cost effective for large-scale citrus growers especially if these bactericides need re-applications to suppress bacterial growth (Slinski, S. Citrus Research and Development Foundation 2016).

Three-component management programs for HLB in Florida initially included chemical control of ACP, removal of HLB-infected trees, and planting disease-free nursery stock (Gottwald *et al.* 2012). In Florida, ACP population suppression is the focus of management strategies because the HLB disease and psyllid are now considered established with no possibility of complete erradication (Rogers & Stansly 2006). Potential long term solutions to extend life of infected trees by increasing nutritional programs have shown little promise (National Research Council 2010; Gottwald *et al.* 2012). Solutions such as HLB plant resistance via transgenic methods, fast and reliable screening programs, and curative therapies are still in development adding to the need for immediate and novel pest management solutions.

Molecular screening methods have been developed using Polymerase chain reaction (PCR) for tree screening (Li, Hartung & Levy 2006), allowing for quick and accurate detection of the bacterium independent from morpholocigal symptoms and concentration of bacterium within the host plant (Iftikhar *et al.* 2014a). Because trees may live for years harboring the disease without visual signs of symptoms, quick diagnosis through PCR tests will speed up reaction time and tree removal or antibiotic treatment strategies (Iftikhar *et al.* 2014b). Similar screening for psyllids is in development to enable early field detection of HLB bacterium utilizing loop-meditated amplification technology (LAMP) (Keremane *et al.* 2015). Antibiotics, such as penicillin, have eliminated or suppressed the HLB bacterium in trunk injections or root soaking methods in several studies (Aubert & Bove 1980; Bové 2006; Iftikhar *et al.* 2014b), however they are expensive and not widely used at this time (Slinski, S. Citrus Research and Development Foundation 2016). Transgenic varieties resistant to HLB through anti-microbial genes are in development with expectation that production varieties will be available in the next 5 years (Mccollum; National Research Council 2010; Iftikhar *et al.* 2014b).

2.6 Integrated pest management for ACP utilizing trap cropping

Trap cropping is a pest management method for manipulating insect pest movement within an agroecosystem by deploying trap crop plant stands to protect the main cash crop (Shelton & Badenes-Perez 2006). This method may include inter planting (Ichinose *et al.* 2012) or perimeter planting (Boucher & Durgy, 2004; Wallingford *et al.* 2013) of a non-cash crop to lure insect pest out of field or prevent it from accessing cash crop. As a cultural pest management strategy, trap cropping is most successful when utilizing highly attractive and retentive plants as potential trap crop (Hannunen 2005; Holden *et al.* 2012). The trap crop must be more attractive than the cash crop, with higher retention rates of all insect life stages. Successful trap crop systems also depend on the behavior and movement of the insect pest (Shelton & Badenes-Perez 2006).There are no known uses of trap cropping for ACP management in the U.S. at this time.

Intercropping of citrus orchards with guava plants in southern Vietnam was shown to contribute to less cases of HLB for up to one year and a few months (Ichinose *et al.* 2012). Authors suggest confounding factors such as guava plant volatiles, ACP disrupted host- finding abilities, and physical protection by shade provided from guavas while citrus trees were same height contributed to low incidence of HLB. Zaka suggests that guava leaf volatiles possess repellent effect against the adult ACP in a study comparing citrus leaves in the absence and in the presence of guava foliage (Zaka *et al.* 2010). Additionally, volatiles from crushed guava and similar synthetic compounds were found to inhibit responses of ACP in laboratory studies, and contributed to adult ACP population decreases when applied to ACP- infested citrus field plots (Onagbola *et al.* 2011). The use of trap cropping has been studied in control of another piercing-sucking insect crop pest, the Harlequin bug, *Murgantia histrionica*, in collards found to be successful with pest populations in small numbers but not successful when pest levels reach high numbers where the pest moves past the trap crop barrier and into the main crop (Ludwig, Kokt & Station 1998).

Successful trap cropping systems deploy crops that attract, trap, or divert insect pests (Shelton & Badenes-Perez 2006). Trap crops may also serve as a ‘sink’ or ‘dead-end’ for insects or pathogens they vector. ‘Dead-end’ trap cropping is ideal because this method utilizes a trap crop that is highly attractive for insect pest feeding or oviposition but does not allow the development of offspring because of plant chemical defenses or applied insecticides (Shelton & Nault 2004; Wallingford *et al.* 2013). From laboratory and screenhouse studies, glossy-type weed *Barbarea vlugaris* was suggested as a ’dead-end’ trap crop for diamondback moths, a collard pest, because it was highly attractive for egg laying by diamondback moth , even in presence of collard cash crop varieties, and with high larvae mortality rates (Shelton & Nault 2004). Insecticide-treated squash trap crop plants, accounting for <1% of total crop area, successfully attracted and killed insect pests squash bug (16-37%) and cucumber beetle (>90%) within seedling plots of cantaloupe, squash, and watermelon (Pair, 1997). In 2002-2003, Connecticut squash farmers utilized "Bluer Hubbard" squash trap crop plants supplemented with border sprays to manage cucumber beetles and the bacterial wilt pathogen vectored by the beetles (Boucher & Durgy 2004). This trap crop variety was highly attractive to the target insect pest and did not serve as a reservoir for the bacterial wilt, minimizing the disease spread to the cash crop. Farmers participating in this study indicated a decrease in average squash damage from both beetle and bacterial wilt compared to previous management strategies of applying full-field sprays, and increased savings in time and money utilizing the perimeter trap crop system.

Trap crop systems, whether treated with insecticides or not, are more commonly seen in annual cash crop management compared to permanent crops (Shelton & Badenes-Perez 2006). There is one commercial use and few field studies of trap cropping in permanent crops (Shelton & Badenes-Perez 2006). In 2010 and 2011, trap cropping of corn and sunflower were found to significantly decrease the populations of yellow peach moth, *C. punctiferalis*, in adjacent peach orchards (Wan *et al.* 2016). Trap cropping leads to more plant biodiversity which may protect or increase populations of natural enemies (Banks & Ekbom 1999; Shelton & Nault 2004; Wan *et al.* 2016). With concern for insecticide resistance and pest resurgence from full-field applications, and side effects against beneficial arthropods (Qureshi & Stansly 2007), trap cropping may be a valuable addition to insecticide-heavy ACP and HLB management strategies.

2.7 *M. koenigii* and *C.volkameriana* as potential trap crops for Asian citrus psyllid

In combination with chemical and biological control practices, trap cropping in commercial citrus groves with suitable hosts of the psyllid that show HLB resistance could suppress ACP populations and HLB movement within groves (Rogers & Stansly 2006). To effectively develop trap cropping to mitigate ACP populations, the host preferences of the insect must be established. Curry leaf, *Murraya koenigii* (Aubert B. 1990), as well as citrus relatives were found to be good host plants for all ACP lifecycle developmental stages in field trials documenting ACP development on curry leaf and citrus taxa (Westbrook *et al.* 2011). One study indicated curry leaf as a non-suitable host for HLB bacterium when compared to other ACP preferred host (Damsteegt *et al.* 2010). In 2009, Volkamer lemon, *C. volkamariana*, citrus root stock was found to have a high bacterial titer count with a moderate tolerance to the disease, exhibiting minimal symptoms (Folimonova *et al.* 2009). Additionally Shokrolla et. Al (2011) showed possible less susceptibility to HLB in trees with *C. volkamariana* used as rootstock or interstock.

Adult ACP that acquire HLB pathogen as nymphs are better vectors of the pathogen compared to adults that acquire pathogens only within adulthood (Inoue *et al.* 2009; Pels-Stelinski *et al.* 2010), therefore the trap crop or crop that maintains the nymphal stage is critical. If curry leaf or *C. volkamariana* are less susceptible to HLB and more attractive to ACP than the commercial citrus genotypes in field settings, and retain all life cycle stages, they could alter the ACP population movement and lead to more integrated pest management programs.

C. volkamariana and curry leaf are currently used in lab rearing facilities for *Tamarixia radiata*, an ACP parasitoid imported from Pakistan to the U.S. This host-specific parasitoid has been released within areas of California with high ACP populations to encourage establishment of the biological control agent as state-wide integrative pest management strategy (Universtiy of California Division of Agriculture and Natural Resources 2016c).

As hosts for ACP with possible resistance to HLB, curry leaf and *C. volkamariana* were tested as potential trap crops in a commercial citrus grove containing mixed citrus genotypes. In addition to the host preference, this study investigated how trap crop growth flushes may act synergistically to alter ACP population movement and dynamics within a commercial citrus grove. The experiments herein monitored ACP population movement and densities in an established commercial citrus grove containing potted trap plants of curry leaf and *C. volkamariana*. Objectives were to (1) document flushing patterns of new plant growth attractive to ACP for *M. koenigii* and *C. volkamariana* in a commercial field setting, (2) determine ACP density and lifer history parameters on *M. koenigii* and *C. volkamariana* by counting all ACP life stages, (3) determine adult densities on trap plants compared to neighboring citrus grove plants by sweep net sampling (UC, ANR 2014), and (4) monitor ACP movement to trap crops using yellow panel traps.

CHAPTER 3

MATERIALS AND METHODS

3.1 Trap crop placement and sampling for ACP adults

This project was conducted within an established commercial citrus grove at California Polytechnic University, Pomona for 10 weeks occurring June through September 2015. The grove has an active ACP population, and consists mainly of 30-year old Valencia oranges (*Citrus sinensis*), mandarins (*C. reticulate*), and grapefruit (*C. paradise*).Trap crop plants of curry leaf, *Murraya koeniggi*, and *C. volkamariana* will be placed in 7 randomized blocks of throughout the perimeter of the grove. Trap crop block placements occur 2m out parallel to citrus grove trees and consist of one row of 3 one-year -old curry leaf plants and 3 one- year -old *C. volkamariana* plants in 5-gallon pot containers spaced one foot apart watered weekly by drip irrigation. Project blocks and adjacent grove trees will be monitored for flush presence and ACP population densities once per week for 10 weeks.

With the exception of four curry leaf plants purchased from a nursery in Los Angeles, trap crop plants were from the California Department of Food and Agriculture *Tamarixia radiata*-rearing program and raised in a greenhouse facility at the university. These plants were transferred from potted 1- gallon pots to 5- gallons within greenhouse at Cal Poly Pomona during May 2015, and then hardened off for two weeks in June 2015 in shade outside the greenhouse before transferring to grove blocks. Soil mix for the 5-gallon trap crop containers includes nitrate at 150ppm nitrogen, with slow release Better Natural® bat guano and 10 lbs, Apex Gold® triple superphosphate micronutrient fertilizer. Pesticides and additional fertilizers are excluded from the trap crop plants during the duration of the project. Pesticides will be omitted from commercial grove during the study, and supplemental nutritional programs will be in place through irrigation lines. No hedging activities will be conducted in grove or trap crop sites prior to or during the study (Hall & Albrigo, 2007).

ACP adult densities will be monitored weekly using sweep net sampling, where the citrus branch is stuffed into the insect sweep net and shaken vigorously (UC, 2014)to loosen and collect feeding adults. Because of their small size, each whole trap crop plant will be ‘swept’ and included in the insect sweep net. Adjacent grove trees will be swept by randomly selecting one branch on each four quadrants for each of three adjacent commercial citrus trees per block. Adults collected from each trap crop type per block will be totaled and averaged. ACP adults from adjacent commercial citrus trees will be totaled and averaged per block.

3.2 Monitoring flush and population densities

To monitor flush growth patterns, 10 branches each per trap crop tree and adjacent commercial citrus trees within each block will be randomly selected and noted for presence of tender flush (Pluke, Qureshi & Stansly 2008). Each branch will also be recorded for presence of flush, number of ACP adults, nymphs, and eggs and presences of ants. Number of ACP eggs, nymphs, and adults per trap crop genotype will be averaged. Number of ACP eggs, nymphs, and adults will be averaged for adjacent commercial trees.

3.3 Monitoring ACP movement

To monitor movement to and from trap crop species, two rows of three Seabright Laboratories' Yellow Panel Traps® (California Department of Food and Agriculture)that are sticky on both sides will be placed on wooden stakes approximately 1 meter above ground in between blocks and citrus grove trees, and in between trap plants and grove street. Trap stakes will be placed one meter in front of and behind trap crop blocks, separating the block from grove trees by 1-2 meters depending on space available. One study showed traps positioned 2 m from citrus trees and positioned 1 m above ground captured significantly greater numbers of ACP than traps placed higher and further from trees (Hall & Hentz 2011). Positioning of traps and blocks will be determined by width of grove access roads. Each block will be positioned in an access road, which varies in width depending on the location. Traps will be checked and replaced weekly for 10 week. ACP adults will be counted and totaled per trap side. Each trap will be labeled indicating sides facing grove, sides facing trap crop, and sides facing grove road.

3.4 Statistical analysis

Analysis of variance (ANOVA) on all dependent variables (percent flush, ACP adults, nymphs, and eggs, and percent ants) was performed using a General Linear Model procedure to test main effects for a randomized complete block design with seven replications of two tree-type treatments (curry leaf and C. volkamariana). The statistical software was SAS 9.4 (SAS Institute, Statistical Analysis System, Cary, North Carolina). Analysis of variance for the overall of dependent variables was performed using a General Linear Model procedure to test main and interaction effects for a repeated-measures design, with date as the repeated-measures factor. Means were compared by using a Fisher's protected LSD Test. Correlations were calculated using Pearson

Correlation Coefficients. The mean and standard error of the mean of grove data were used to compare with the means of two tree types for each date and overall. Analysis of variance on Yellow Panel Trap data was performed using a General Linear Model procedure to test main effects for a randomized complete block design with seven replications of three panel side treatments (block side, grove side, and street side). Analysis of variance for the overall Yellow Panel Trap data was performed using a General Linear Model procedure to test main and interaction effects for a repeated-measures design, with date as the repeated-measures factor. Means were compared by using a Fisher's protected LSD Test.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Effects of trap crops on *Diaphorina citri* densities

Trap crop plants of *C. volkamariana* (volk) and *M. koenigii* (curry leaf) were free of all *Diaphorina citri* population stages prior to field placement. ACP populations were found one week after placement in field (30 June 2015), with presence of eggs and one adult on volk. Population densities for all life stages increased on both trap crop types over the course of the study with highest densities during the second half of the study (Table 4.1 and Table 4.2). Overall data indicates there were more eggs, nymphs, and adult ACP stages found on curry leaf rather than volk during the study but the difference between the trap crop types was not significant for these response variables (Table 4.2, Figures 4.4 and 4.6). There were more of all ACP stages seen in the overall data on both trap crop types compared to grove trees. The overall date effect was significant for eggs (P=0.0157) and adults (P=0.0193) in the study and ranged from 0 eggs (July 14 and August 11)- 4.6 eggs(September 1) (Table 4.1) and 0 adults (June 30, July 7, and August 4)- 0.6 adults (August 18).

Plants were watered weekly via drip irrigation system. All trap crop plants were potted in black 5-gallon containers and set on dirt roads bordering the orchard. All were exposed to direct sunlight during most daylight hours. Heat waves Augusts 16 and August 29 (U.S. Department of Commerce National Oceanic & Atmospheric Administration National Environmental Satellite, 2015) caused drying of both trap crop plants types beginning August 18 through end of study, with more severe drying seen in volk plants than curry. During this time, noticeable dried ACP eggs and nymphs were observed on trap crop plants and grove trees. An influx of leaf miner damage caused drying and curling of volk leaves beginning August 11. Curry leaf plants were unaffected by leaf miner but did succumb to drying, wilting, and leaflet loss during heat waves of over 100° F August 13-16 and August 27-28, 2015 (U.S. Department of Commerce National Oceanic & Atmospheric Administration National Environmental Satellite, 2015).

The results of this study indicate great variability within the data due to high coefficient of variation and low R-Square values per dependent variable.

4.2 Adult ACP populations based on sweep sampling

Sweep sampling (UC, ANR 2014) utilizing an insect net to collect ACP adults was conducted once per week for 10 weeks during the study with the exception of (22 July 2015) when no data were collected. Each trap crop plant per block was shaken within the insect net and ACP adult numbers were recorded per plant. The average was taken for adults per trap crop type, three trees total per block, by summing the adults then dividing by three. The average adults per trap crop types was then compared using ANOVA. The trap crop treatment effect for average number of adults was significant on July 28 and the same was true for percent flush on July 14; however, trap crop means were not significantly different (Table 4.3). The overall date effect for average adult ACP was significant (P=0.0043) and ranged from 0.1 adults (June 30, July 7, July 14, and August 4) to 1.9 adults (August 18). The overall average adults per sweep was higher in both trap crops than grove trees (Figure 4.7, Figure 4.8).

4.3 Effects of trap crops on *Diaphorina citri* movement

Six yellow panel traps were placed on the perimeter of each block, three on each side of the block. Traps were checked replaced weekly and adult ACP was totaled per trap side per block. The average number of adults was calculated for each side type of the traps by summing the adults then dividing by three per street side, three per grove side, or six per block side. The average adults per trap side were then compared using ANOVA. The overall average of adult ACP trended to be higher on sides of yellow panel traps facing inward (block side) toward the trap crop plants within each block (Table 4.3; Figure 4.9). However, no significant differences were seen between the three yellow panel facing directions: grove side, block side, and street side during the study.

4.4 Effects of flush patterns on *Diaphorina citri* densities

Each trap crop tree was approximately the same size and age per tree type. Each tree type varied in structure: curry leaf with tall, single stalk and leaflets and flush branching from central stalk; volk with short central stalk and larger support branches that varied in new growth appearance. Flush for curry leaf was determined by presence of curled feather growth reaching up to 3.8 cm (1.5 inch). Volk flush was determined by presence of tender new growth (Pluke, Qureshi & Stansly 2008) not exceeding 7.6cm (3 inch) in length. Overall, volk trended to have higher percentages of flush during the study. However, the percent flush difference between the two trap crop types was not significant (Table 4.1). The overall percent flush was higher in trap crop types than in grove trees (Figure 1 and Figure 2).

4.5 Effect of trap crops on ant populations

Probabilities could not be calculated for percent ants in most of the study because there were no ants present except for August 4, 11, 18, and 25 in which the overall date effect for these mean values was not significant (P=0.1018).

4.6 Correlations between dependent variables

Pearson Correlation Coefficients were calculated between all dependent variables in the study (does not include grove nor yellow panel trap data): adults per sweep, percent flush, eggs, nymphs, adults per branch, and ants. The strongest correlations were between: average number of adults per sweep and average number of nymphs ($r = 0.43$; $P < 0.0001$); average number of adults per sweep and average number of adults per branch ($r = 0.71$; $P < 0.0001$); and average number of nymphs and percent ants ($r = 0.39$; $P < 0.0001$).

Table 4.1 The effect of volk and curry leaf on the average of eggs and the percent branches with flush (% flush) during study conducted in Pomona, CA from 30 June through 1 September 2015.

	Date																					
	30-Jun		7-Jul		14-Jul		22-Jul		28-Jul		4-Aug		11-Aug		18-Aug		25-Aug		1-Sep		Overall	
Trap crop treatments	% flush	eggs	% flush	eggs	% flush	eggs	% flush	eggs	% flush	eggs	% flush	eggs	% flush	eggs	% flush	eggs	% flush	eggs	% flush	eggs	% flush	eggs
Volk	88 a*	0.2 a	72 a	0.3 a	55 a	0.0 a	63 a	0.5 a	77 a	4.8 a	71 a	0.4 a	68 a	0.0 a	65 a	0.2 a	75 a	1.6 a	73 a	0.9 a	71 a	0.9 a
Curry leaf	66 a	0.0 a	88 a	0.0 a	82 a	0.0 a	81 a	0.0 a	66 a	0.9 a	64 a	1.3 a	54 a	0.0 a	54 a	0.1 a	58 a	2.1 a	55 a	8.4 a	67 a	1.3 a
Mean % flush	77 A†		82 A		69 A		72 A		71 A		68 A		61 A		60 A		67 A		64 A			
Mean eggs		0.1 BC		0.1 BC		0.0 C		0.3 BC		2.9 AB		0.9 BC		0.0 C		0.1 BC		1.9 AB		4.6 A		
Mean nymphs																						
Mean adults																						
Grove mean	28	1.1	14	0.3	7	0.0	14	0.0	4	0.2	0	0.0	21	0.3	4	0.1	4	0.3	17	0.8	11	0.3
Standard error of grove mean**	4	0.6	3	0.2	3	0.0	5	0.0	1	0.2	0	0.0	13	0.2	2	0.1	2	0.2	3	0.3	2	0.1
Summary of ANOVA effects (*P*)																						
Treatment (T)	0.1262	0.3559	0.1787	0.0966	0.0549	-	0.1326	0.1211	0.3628	0.1960	0.6860	0.4984	0.3680	-	0.5560	0.4491	0.2785	0.8337	0.2927	0.2269	0.6732	0.7149
Date (D)																					0.0793	0.0157
TxD																					0.0048	0.0552

* Means followed by the same lower case letter within the same column are not significantly different, Fisher's protected LSD test, P = 0.05

† Means followed by the same capital case letter within the same row are not significantly different, Fisher's protected LSD test, P = 0.05

‡ (-) probabily of ANOVA effect could not be estimated

** (+/-) interval variability of a sample mean

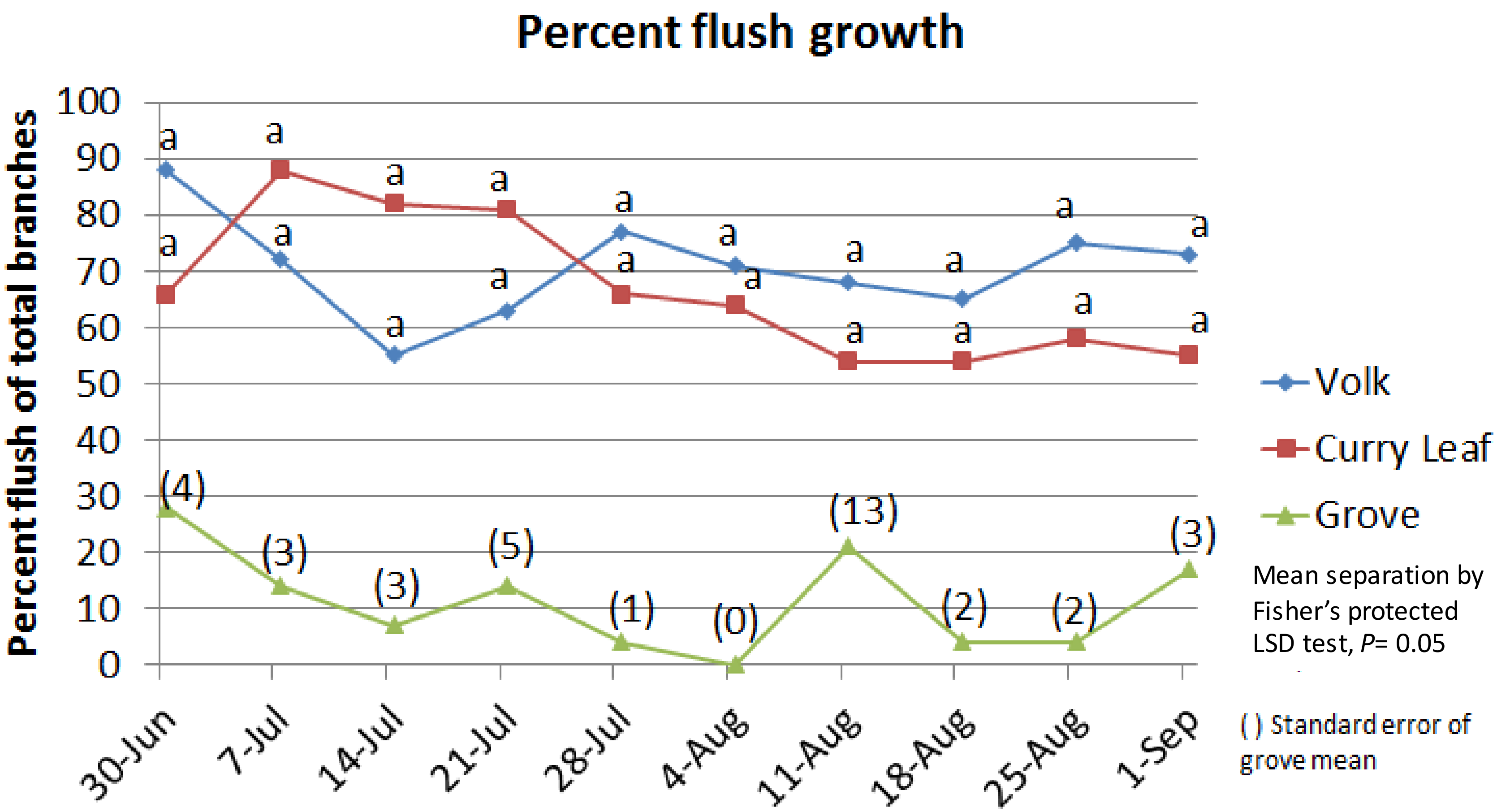

Figure 4.1Percent total branches with flush new growth.

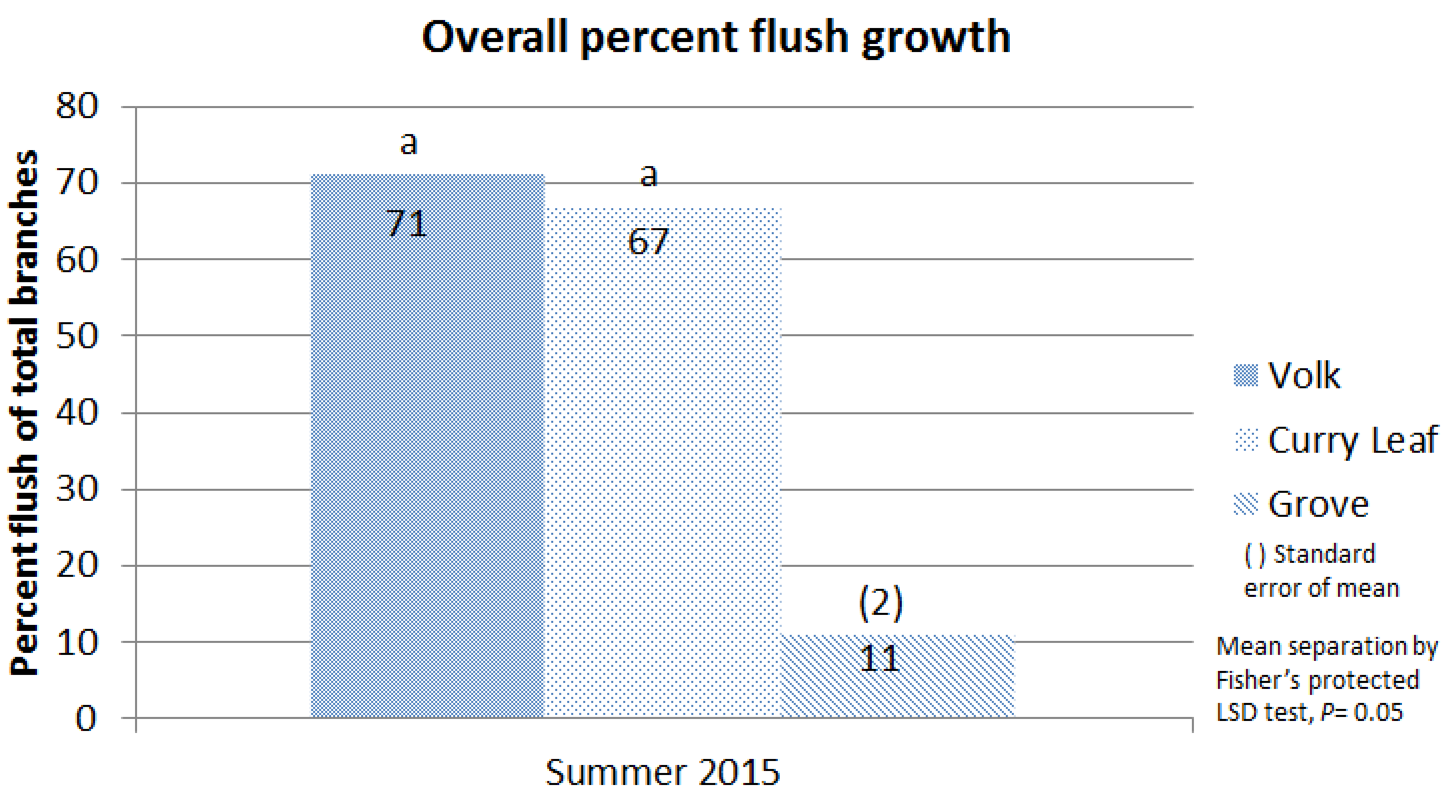

Figure 4.2 Overall percent total branches with flush new growth.

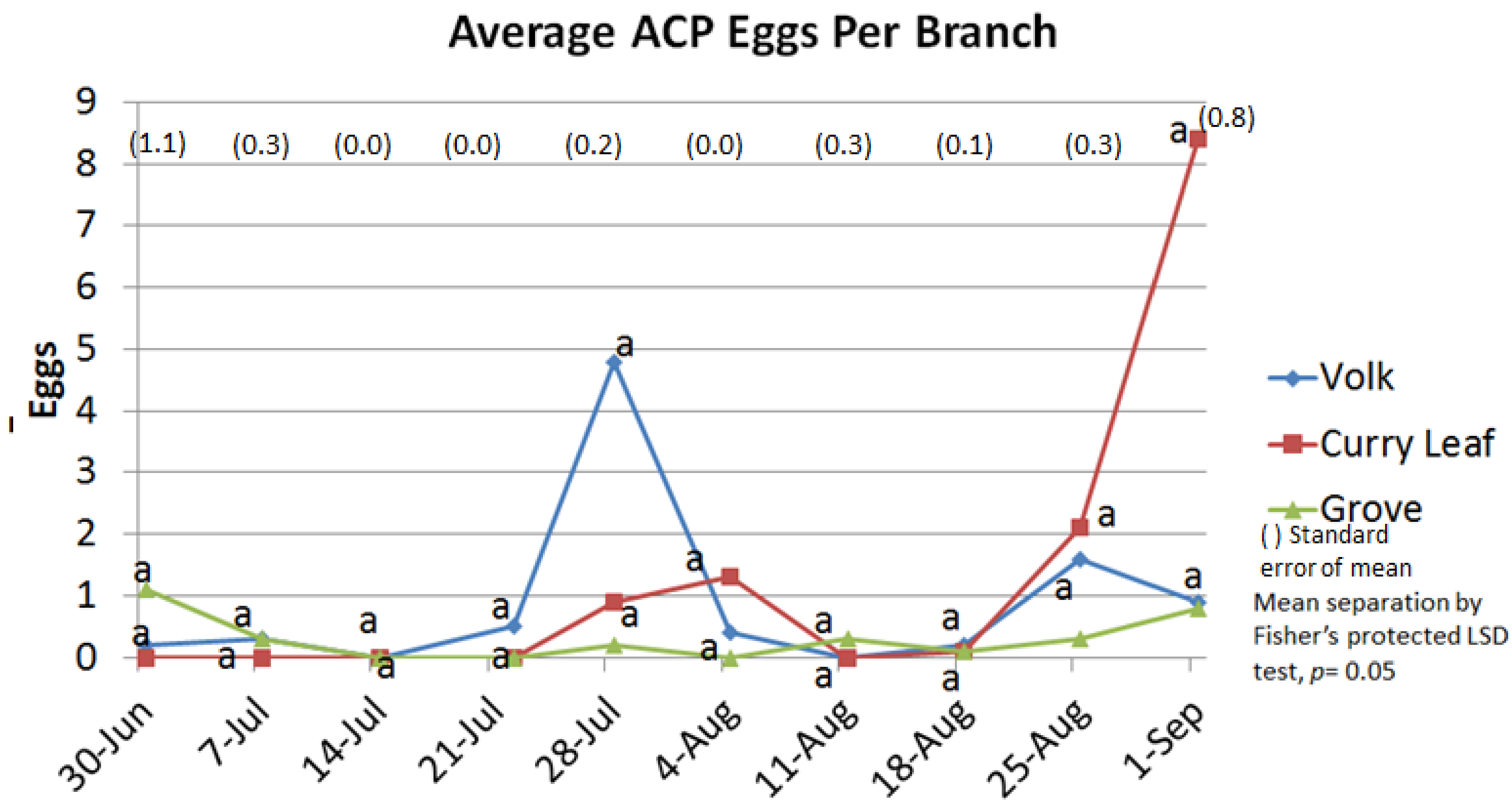

Figure 4.3 Average ACP eggs per randomly selected branch.

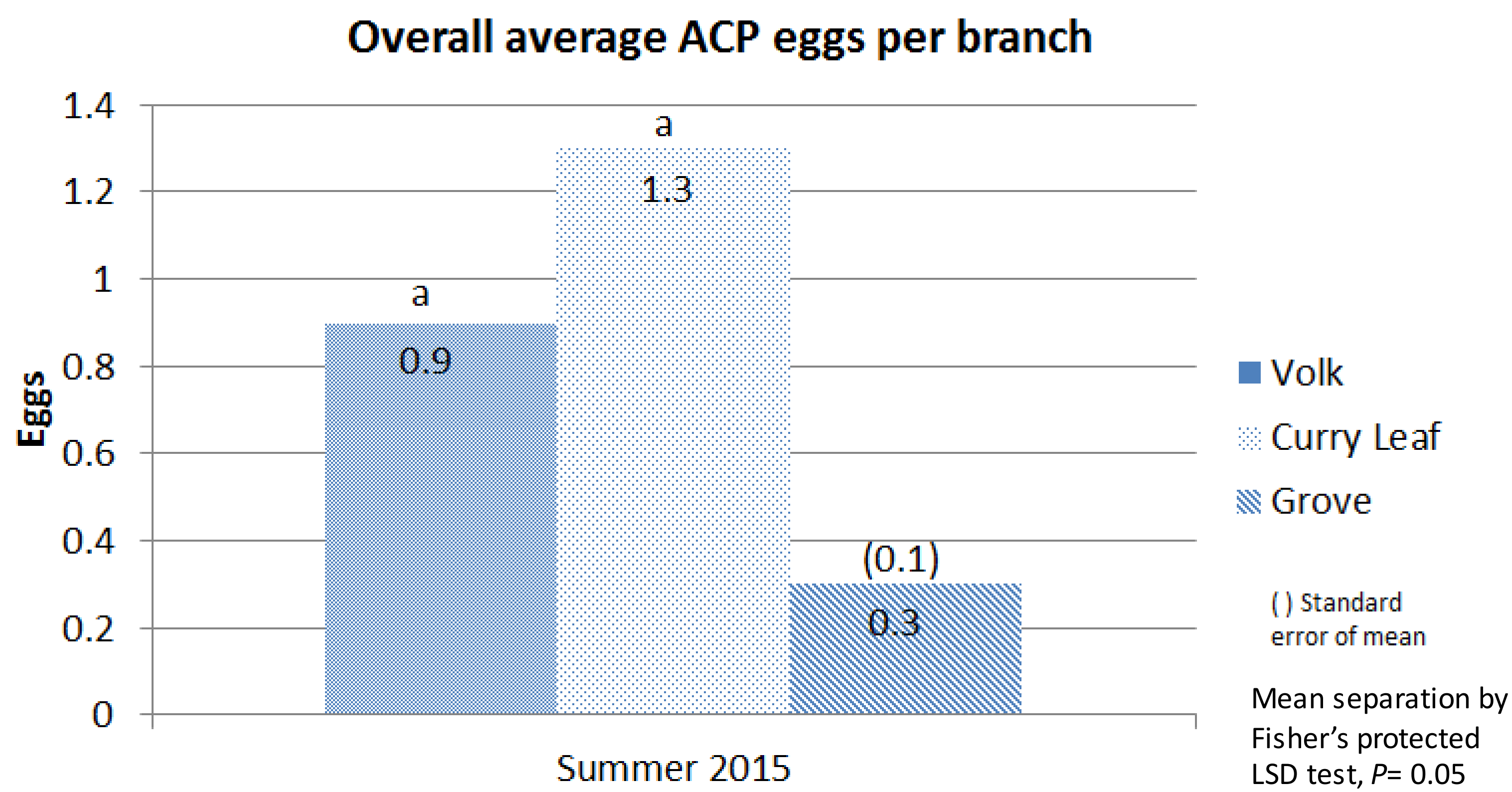

Figure 4.4 Overall average ACP eggs per randomly selected branch.

Table 4.2 The effect of volk and curry leaf on the average of nymphs and adults per branch during study conducted in Pomona, CA from 30, June through 1, September 2015.

	30-Jun		7-Jul		14-Jul		22-Jul		28-Jul		4-Aug		11-Aug		18-Aug		25-Aug		1-Sep		Overall	
Trap crop treatments	nymphs	adults	nymphs	adults	nymphs	adults	nymphs	adults	nymphs	adults	nymphs	adults	nymphs	adults	nymphs	adults	nymphs	adults	nymphs	adults	nymphs	adults
Volk	0.0 a	0.0 a	0.2 a	0.0 a	0.6 a	0.1 a	.2 a	0.1 a	1.2 a	0.1 a	3.2 a	0.0 a	0.7 a	0.0 a	0.1 a	0.2 a	0.1 a	0.1 a	.9 a	0.0 a	0.7 a	0.1 a
Curry leaf	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.1 a	0.0 a	0.1 a	0.6 a	0.0 a	3.0 a	0.1 a	3.4 a	1.0 a	0.7 a	0.5 a	.9 a	0.2 a	0.9 a	0.2 a
Mean % flush																						
Mean eggs																						
Mean nymphs	0.0 A		0.1 A		0.3 A		0.1 A		0.6 A		1.9 A		1.8 A		1.8 A		0.4 A		0.9 A			
Mean adults		0.0 B		0.0 B		0.1 B		0.1 B		0.1 B		0.0 B		0.1 B		0.6 A		0.3 AB		0.1 B		
Grove mean	0.5	0.1	1.1	0.0	0.4	0.0	0.1	0.0	0.1	0.1	0.0	0.0	0.3	0.0	0.3	0.0	0.3	0.0	0.4	0.1	0.4	0.0
Standard error of grove mean**	0.3	0.0	0.8	0.0	0.1	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.2	0.0	0.1	0.0	0.2	0.0	0.3	0.0	0.1	0.0
Summary of ANOVA effects (*P*)																						
Treatment (T)	-‡	0.3559	0.3335	0.1744	0.0980	0.3350	0.2753	0.6511	0.0898	0.9191	0.1283	0.1824	0.2845	0.3758	0.3634	0.2089	0.4446	0.4157	0.9689	0.1805	0.8406	0.1909
Date (D)																					0.1600	0.0193
TxD																					0.0877	0.3146

* Means followed by the same lower case letter within the same column are not significantly different, Fisher's protected LSD test, $P = 0.05$

† Means followed by the same capital case letter within the same row are not significantly different, Fisher's protected LSD test, $P = 0.05$

‡ (-) probabily of ANOVA effect could not be estimated

** (+/-) interval variability of a sample mean

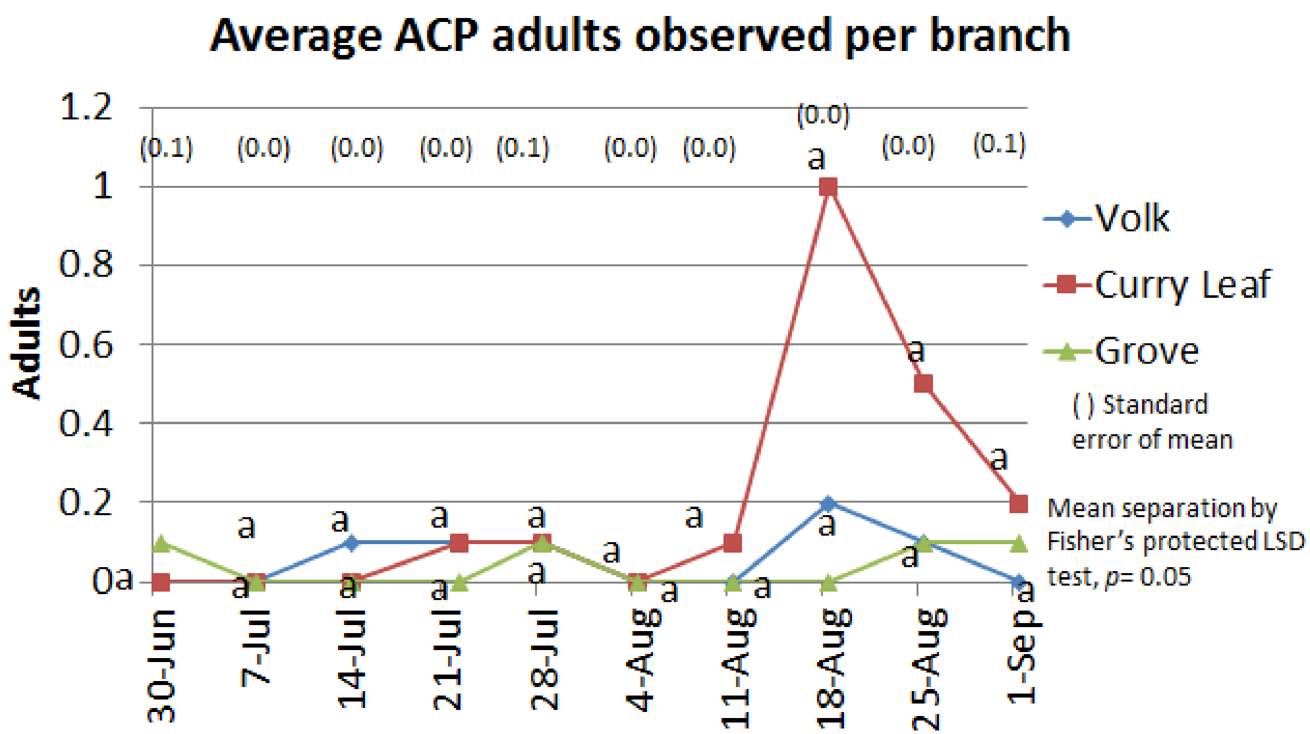

Figure 4.5 Average number of adult ACP per randomly selected branch.

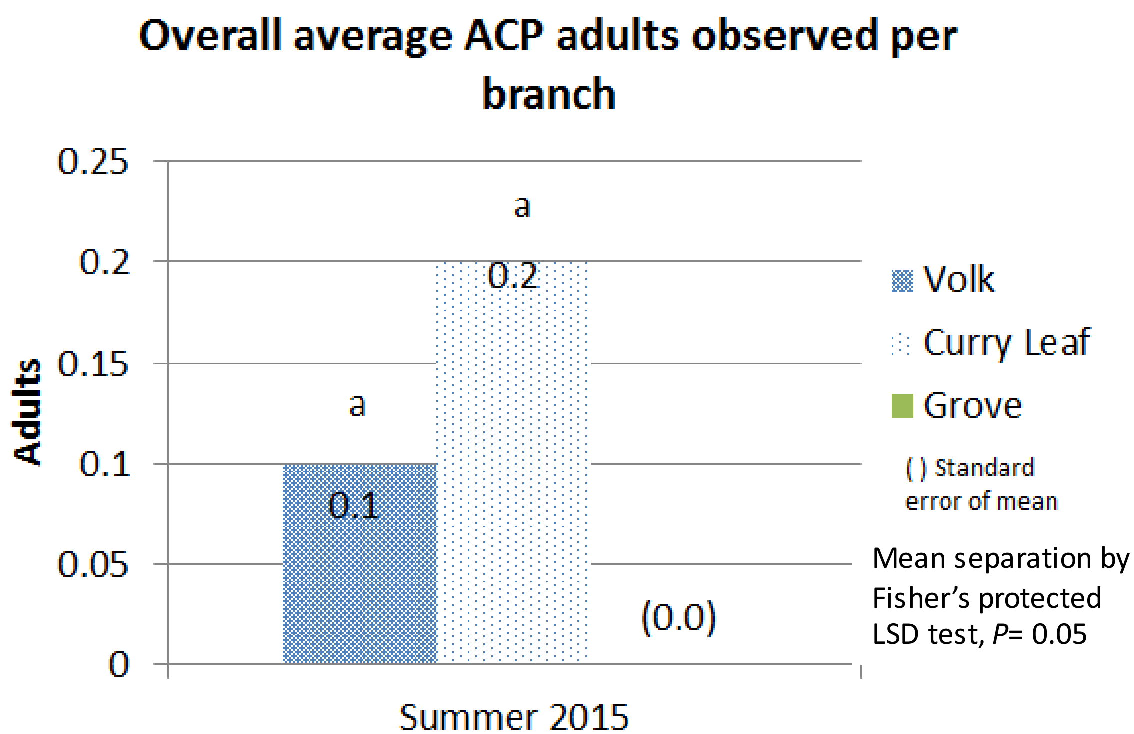

Figure 4.6 Overall average number of adult ACP per randomly selected branch.

Table 4.3 The effect of volk and curry leaf on the percent flush growth (% flush) and average adults per sweep (adults/sw) during study conducted in Pomona, CA from 30, June through 1, September 2015.

	Date																					
	30-Jun		7-Jul		14-Jul		22-Jul		28-Jul		4-Aug		11-Aug		18-Aug		25-Aug		1-Sep		Overall	
Trap crop treatments	% flush	adults/sw	% flush	adults/sw	% flush	adults/sw	% flush	adults/sw	% flush	adults/sw	% flush	adults/sw	% flush	adults/sw	% flush	adults/sw	% flush	adults/sw	% flush	adults/sw	% flush	adults/sw
Volk	88 a*	0.1 a	72 a	0.1 a	55 a	0.1 a	63 a	-‡	76 a	0.0 a	71 a	0.1 a	68 a	0.6 a	65 a	1.8 a	75 a	0.9 a	73 a	0.3 a	71 a	0.5 a
Curry leaf	66 a	0.1 a	88 a	0.0a	82 a	0.1 a	81 a	-	65 a	0.4 a	63 a	0.2 a	54 a	0.3 a	54 a	1.9 a	57 a	2.1 a	54 a	0.3 a	67 a	0.6 a
Mean % flush	77 A†		82 A		69 A		72 A		71 A		68 A		61 A		60 A		67 A		64 A			
Mean adult/sweep		0.1 C		0.1 C		0.1 C		-		0.2 C		0.1 C		0.5 BC		1.9 A		1.5 AB		0.3 C		
Grove mean	28	0.3	14	0.4	7	0.4	14	-	4	0.2	0	0.1	21	0.1	4	0.1	4	0.3	17	0.2	11	0.2
Standard error of grove mean**	4	0.1	3	0.1	3	0.1	5	-	1	0.0	0	0.1	13	0.0	2	0.0	2	0.1	3	0.1	2	0.0
Summary of ANOVA effects (*P*)																						
Treatment (T)	0.1262	0.8565	0.1787	0.1723	0.0549	0.8046	0.1326	-	0.3628	0.0474	0.686	0.3632	0.368	0.4605	0.556	0.9919	0.2785	0.601	0.2927	0.9116	0.6732	0.9132
Date (D)																					0.0793	0.0043
TxD																					0.0048	0.9625

* Means followed by the same lower case letter within the same column are not significantly different, Fisher's protected LSD test, *P* = 0.05.
† Means followed by the same capital case letter within the same row are not significantly different, Fisher's protected LSD test, *P* = 0.05.
‡ (-) data not collected.
** (+/-) interval variability of a sample mean

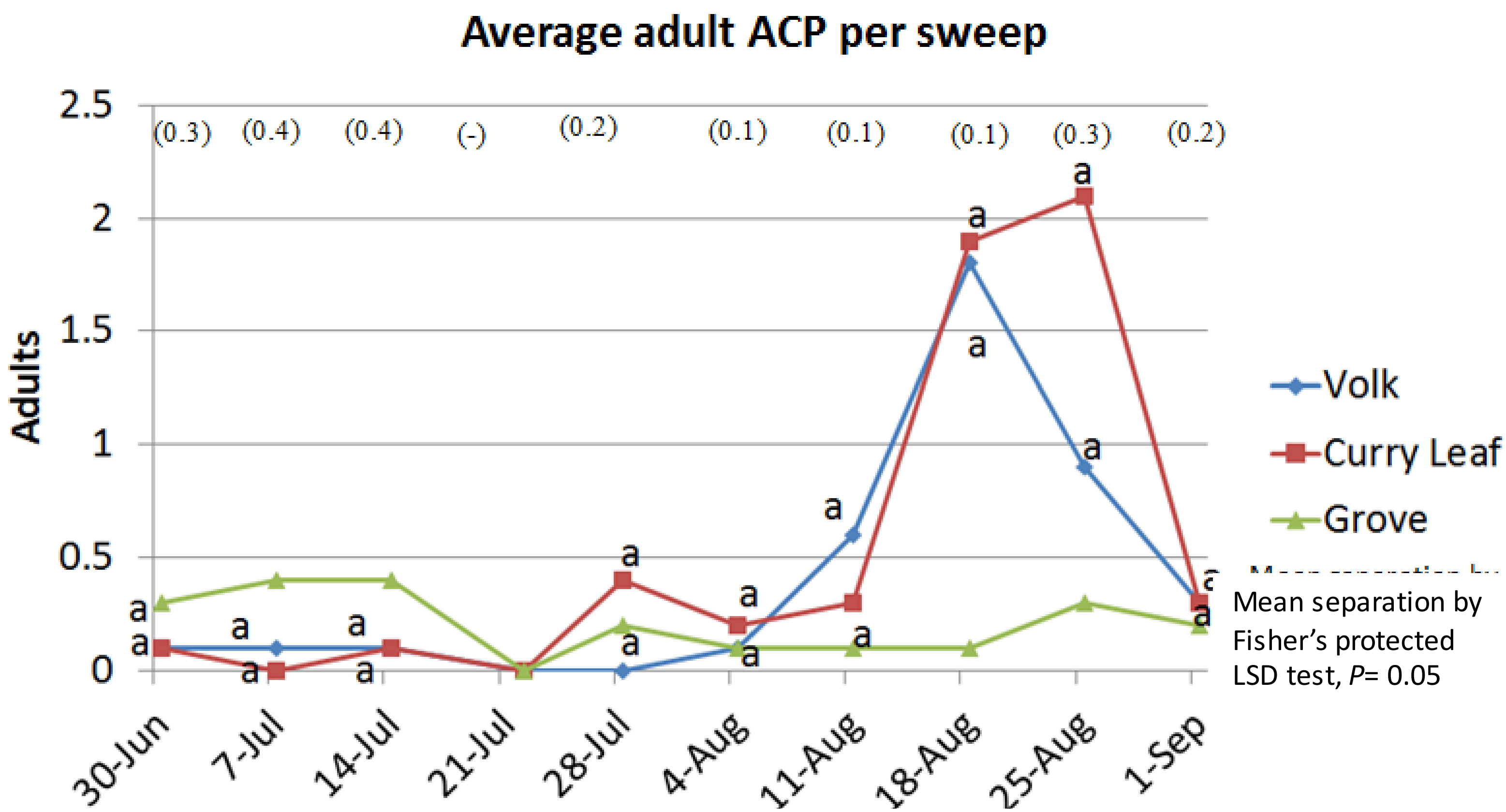

Figure 4.7 Average ACP adults per sweep net sample.

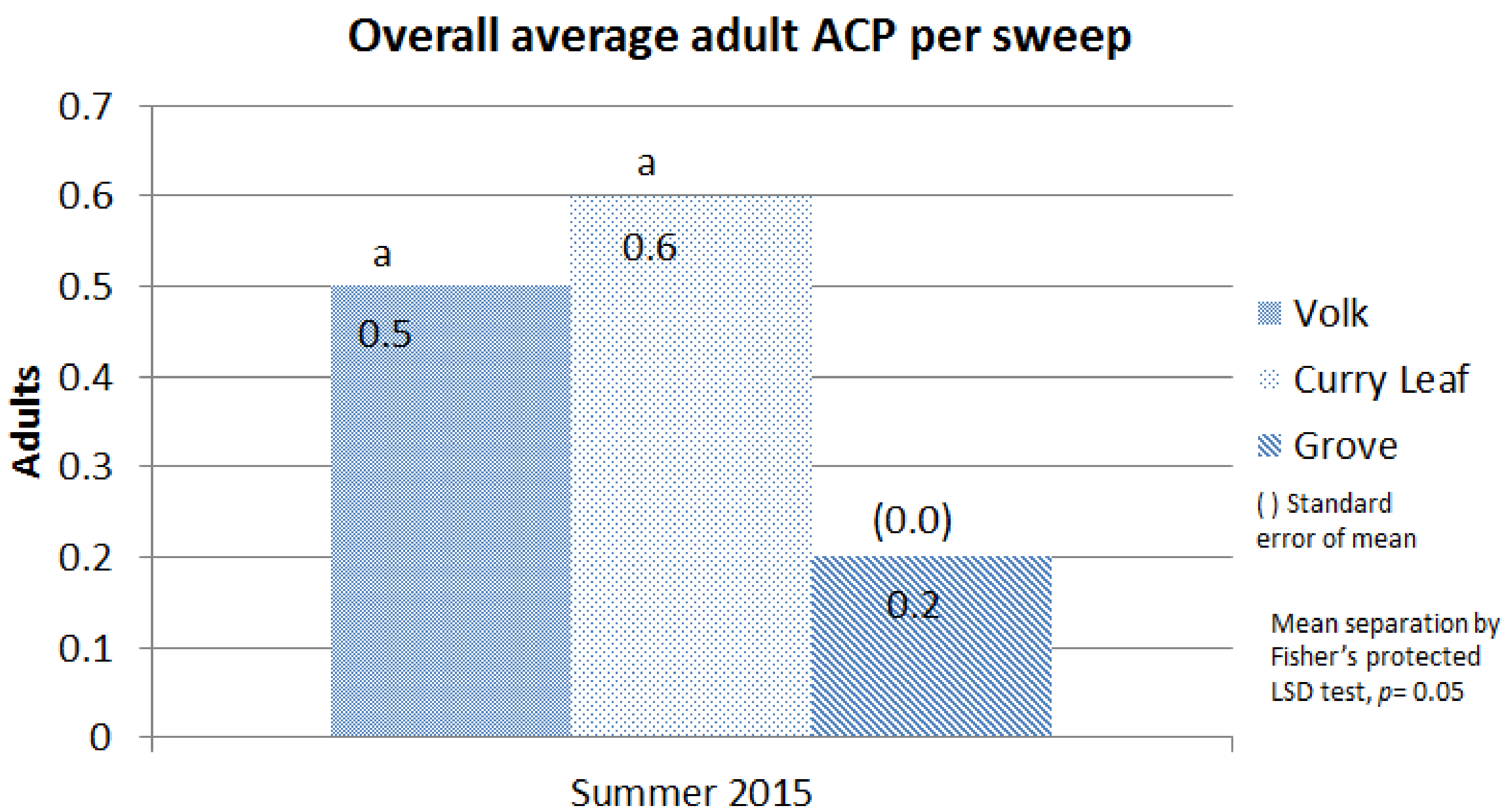

Figure 4.8 Overall average adult ACP per sweep net sample.

Table 4.4 The effect of trap placement within trap crop blocks on average adult ACP.

Table 4.4 The effect of trap placement within trap crop blocks on average adult ACP.

	Date										
	30-Jun	7-Jul	14-Jul	22-Jul	28-Jul	4-Aug	11-Aug	18-Aug	25-Aug	1-Sep	Overall
Trap crop treatments											
Grove Side	0.2 a*	0.2 a	0.0 a	0.0 a	0.1 a	0.1 a	0.1 a	0.1 a	0.3 a	0.3 a	0.1a
Block Side	0.0 a	0.1 a	0.0 a	0.1 a	0.1 a	0.1 a	0.1 a	0.1 a	0.6 a	0.5 a	0.2a
Street Side	0.0 a	0.1 a	0.0 a	0.0 a	0.0 a	0.1 a	0.0 a	0.1 a	0.2 a	0.1 a	0.1a
Mean adult ACP	0.1 B†	0.1 B	0.0 B	0.0 B	0.1 B	0.1 B	0.1 B	0.1 B	0.4 A	0.3 A	
Summary of ANOVA effects (*P*)											
Treatment (T)	0.0573	0.7595	0.3966	0.4972	0.1681	0.7608	0.2500	0.9995	0.3486	0.1721	0.1652
Date (D)											<.0001
TxD											0.616

* Means followed by the same lower case letter within the same column are not significantly different, Fisher's protected LSD test, $P = 0.05$

† Means followed by the same capital case letter within the same row are not significantly different, Fisher's protected LSD test, $P = 0.05$

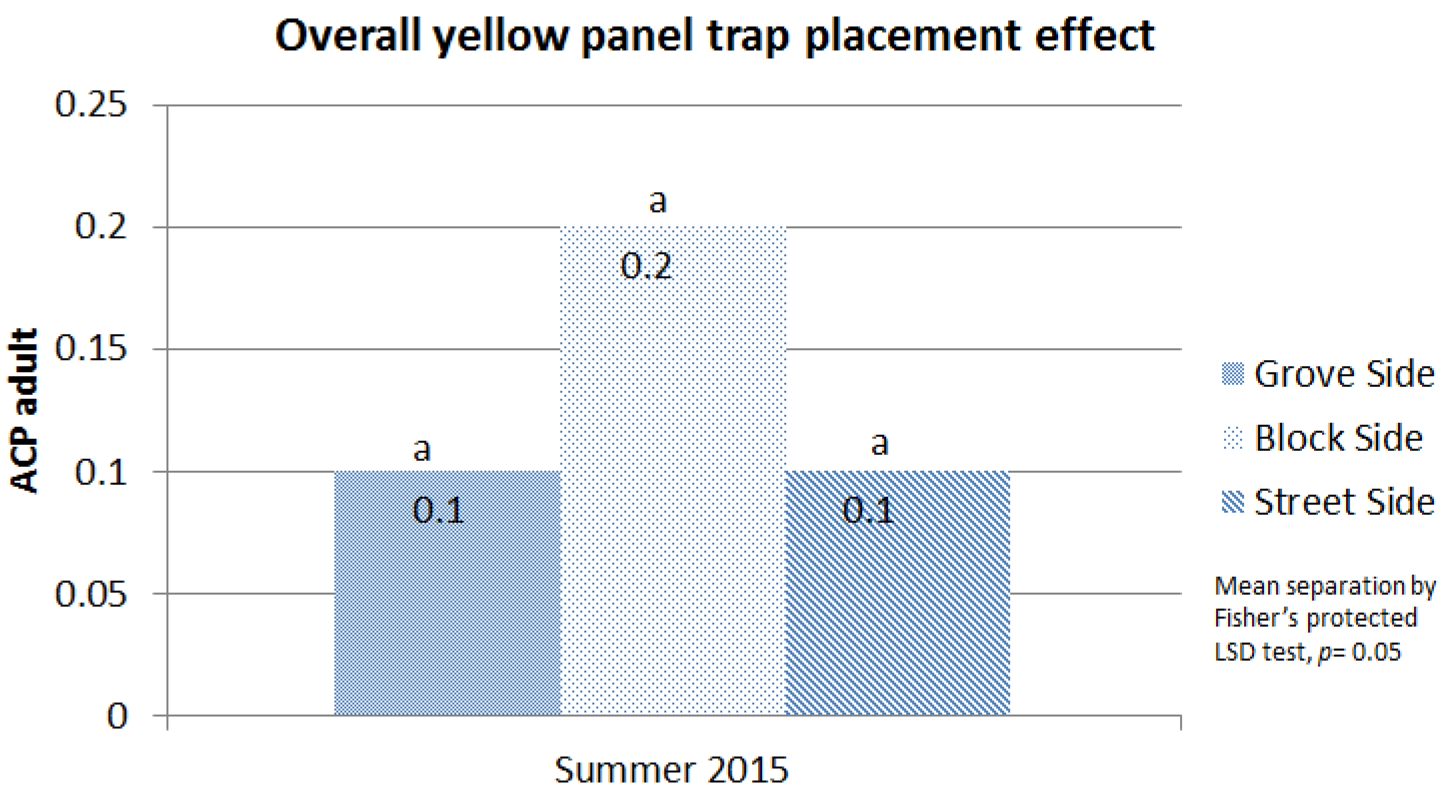

Figure 4.9 Overall yellow panel trap placement effect on adult ACP

CHAPTER 5

CONCLUSION

5.1 Curry leaf and Volk as potential trap crops for ACP

Results of this study indicate both Citrus *volkamariana* and curry leaf attract and maintain ACP insect populations in commercial citrus grove settings. Additionally, there were more of all ACP stages seen on both trap crops compared to grove trees. The data did not indicate that C. *volkamariana* or curry leaf as trap crops under a commercial orchard situation to have significantly different impact on ACP populations. There were fluctuations in when ACP population concentrations appeared on either curry or volk, and because the study was one season long no patterns in population densities or flushing patterns were observed. This is supported by basically no correlation between percent flush and number of adults per sweep nor number of adults per branch: ($r = -0.11$; $P = 0.2370$) and ($r = -0.18$; $P = 0.0341$), respectively. Continuing the study for one or more complete years will help identify trap crop flushing patterns in comparison with orchard plants. Flushing periods provide ACP with resources for product, and play a critical role in biological control efforts (Monzo, Qureshi & Stansly 2014). In our study older grove trees may have had more accurate flushing patterns because of their mature age compared to younger curry leaf and volk trap plants. The percent flush for grove trees was considerably less than for both trap crops on all dates during the study which may be explained by younger trap plants experiencing more growth because of their earlier developmental stage (Mattson 1980).

Relative attractiveness and size of trap crop are characteristics of trap crops that need considering before implementing in an integrated management program. In this study, the trap crop treatment plants were considerably smaller (5 gal) than the adjacent full-grown commercial citrus orchard trees. We did not have access to full grown curry leaf or volk trees or resources to plant them permanently in field. Future studies should consider using larger plants for trap crop species. Our study used trap crops potted in 5-gallon containers placed in short lines on streets bordering the field. Future studies utilizing these crops may need larger plants in larger containers or planted in permanent rows outfitted with automatic watering systems to prevent drying. Having the plants potted could allow for flexibility for the grower to remove the trap crop, however utilizing in-ground trap crops more comparable in size or age to grove trees may produce more consistent results. Trap crops require resources that would normally be used for cash crop production, so economic and agronomic analysis would need to be done before implementing trap cropping in citrus production (Shelton & Nault 2004).

5.2 Implications of trap cropping for ACP and HLB management

Trap crops may not be perfect for all situations, especially when pest populations become attracted to trap crop but increase in number so much that it damages cash crop (Ludwig, Kokt & Station 1998) or creates a disease reservoir to continue spread of plant pathogen (Ratnadass *et al.* 2012).These questions were not addressed in this study. Curry leaf had been found to be highly attractive to ACP and has been documented as a suitable host supporting all life stages (Aubert B. 1990, Westbrook *et al.* 2011), with indications of HLB resistance (Damsteegt *et al.* 2010). Attractive host plant may encourage ACP to remain on curry leaf or volk to complete its lifecycle (Van den Berg 2006) but may also be possible source for pathogen in-take rather than a sink. It is unknown at this time if the attraction and retention values of curry leaf or volk are high enough to compete with the values of citrus grove plants in commercial grove settings. If the trap crop was, in fact, found to be highly attractive and retentive, this strategy could concentrate ACP populations creating more accurate targets during insecticide spray programs (Shelton & Badenes-Perez 2006). Timing should also be considered in application of pesticides to trap cropping in effort to augment trap cropping efficacy (Wallingford *et al.* 2013). Well-timed and targeted sprays may further decrease and prevent insecticide-resistant ACP populations (Rogers & Dewdney 2015).

Trap crop guidelines recommend about 10% of total crop area be planted with the trap crop (Hokkanen 1991). Our study was limited to planting the trap crops in specific areas of the field to access available water supply independent of the orchard. In future studies, trap crops should be placed more uniformly around the perimeter of the grove to create a boarder effect. ACP populations have been documented as entering fields from the perimeters, and with a perimeter trap crop of highly attractive and retentive host plants ACP infestation of the cash crop may be minimized. Planting a perimeter crop has been found to be effective at limiting pest movement into annual cash crop fields (Boucher & Durgy, 2004; Wallingford *et al.* 2013). Minimal planting of trap crops, even in permanent crops, also increases biodiversity which could contribute to increase in natural predators and parasitoids. Field experiments in China found significant reduction in peach orchard insect pest due to trap cropping methods (Wan *et al.* 2016). The benefits of increasing crop diversity may lead to increase in natural predators and parasitoids (Qureshi & Stansly 2007; Qureshi *et al.* 2013), further contributing to ACP control especially in the absence of insecticides. In our study we observed some parasitized nymphs on trap crops and grove plants indicated by exit holes from *T. radiata* parasitism, and possible native Syrphid fly larva actively consuming ACP nymphs on heavily infested branches of grove trees and volks (3 August 2015).

REFERENCES

2016 Asian Citrus Psyllid (ACP) Overview - Grid Pages. (2016) , 2016.

Adaskaveg, J. (U. C.R., Gubler, D. (U. C.D. & Michailides, T. (U. S.D.A.C. (2012) FOR DECIDUOUS TREE FRUIT , NUT , STRAWBERRY , AND VINE CROPS.

Ammar, E., Hall, D.G. & Jr, R.G.S. (2013) Stylet Morphometrics and Citrus Leaf Vein Structure in Relation to Feeding Behavior of the Asian Citrus Psyllid Diaphorina citri , Vector of Citrus Huanglongbing Bacterium. , **8**.

Aubert, B. & Bove, J.M. (1980) Effect of Penicillin or Tetracycline Injections of Citrus Trees Affected by Greening Disease Under Field Conditions in Reunion Island.

Awmack, C.S. & Leather, S.R. (2002) Host Plant Quality and Fecundity in Herbivorous Insects. *Annual review of entomology*, 817–44.

Banks, J.E. & Ekbom, B. (1999) management potential of intercropping and trap cropping.

Beach, P. & Lucie, S. (2015) C Itrus. , **32751**, 1–3.

Belasque Júnior, J., Bassanezi, R.B., Yamamoto, P.T., Ayres, A.J., Tachibana, a., Violante, a. R., Tank Jr., a., Di Giorgi, F., Tersi, F.E. a., Menezes, G.M., Dragone, J., Jank Jr., R.H. & Bové, J.M. (2010) Lessons from huanglongbing management in São Paulo state, Brazil. *Journal of Plant Pathology*, **92**, 285–302.

Van den Berg, J. (2006) Vetiver grass (Vetiveria zizanioides (L.) Nash) as trap plant for Chilo partellus (Swinhoe) (Lepidoptera: Pyralidae) and Busseola fusca (Fuller) (Lepidoptera: Noctuidae). , **9271**.

Bonani, J.P., Fereres, a., Garzo, E., Miranda, M.P., Appezzato-Da-Gloria, B. & Lopes, J.R.S. (2010) Characterization of electrical penetration graphs of the Asian citrus psyllid, Diaphorina citri, in sweet orange seedlings. *Entomologia Experimentalis et Applicata*, **134**, 35–49.

Bové, J.M. (2006) I NVITED R EVIEW HUANGLONGBING : A DESTRUCTIVE , NEWLY-EMERGING , CENTURY-OLD DISEASE OF CITRUS 1. , **88**, 7–37.

California Department of Food and Agriculture. *Asian Citrus Psyllid (ACP) Trapping*.

California Department of Food and Agriculture Plant Quarantine Manual: Asian Citrus Psyllid. (2016) , 1–13.

Capoor, S.P., Rao, D.G. & Viswanath, S.M. (1970) Greening Disease of Citrus in the Deccan Trap Country and its Relationship with the Vector , Diaphorina citri Kuwayama. *Sixth IOCV Conference*, 43–49.

Coy, M.R., Bin, L. & Stelinski, L.L. (2016) Reversal of insecticide resistance in Florida populations of Diaphorina citri (Hemiptera : Liviidae). , **99**, 26–32.

Damsteegt, V.D., Postnikova, E.N., Stone, a L., Kuhlmann, M., Wilson, C., Sechler, a, Schaad, N.W., Brlansky, R.H., Schneider, W.L., Science, F.D., Maryland, U., Park, C., Alfred, L. & Disease-weed, F. (2010) Murraya paniculata and related species as potential hosts and inoculum reservoirs of 'Candidatus Liberibacter asiaticus', causal agent of Huanglongbing. *Plant Disease*, **94**, 528–533.

Ferreira, G. & Perez, A. (2016) Fruit and Tree Nuts Outlook.

Florida Department of Agriculture. (2016) Citrus Health Response Program.

Folimonova, S.Y., Robertson, C.J., Garnsey, S.M., Gowda, S. & Dawson, W.O. (2009) Examination of the Responses of Different Genotypes of Citrus to Huanglongbing (Citrus Greening) Under Different Conditions.

Garnier, M., Bové, J.M., Cronje, C.P.R., Sanders, G.M., Korsten, L. & Roux, H.F. Le. (2000) Presence of ' Candidatus Liberibacter africanus ' in the Western Cape Province of South Africa.

Geisseler, D. & Horwath, W.R. (2016) Citrus Production in California. , 1–4.

Gottwald, T. (2009) P r o c e e d i n g s o f t h e M e e t i n g. *International Research Conference on Huanglongbing* pp. 1–480.

Gottwald, T.R., Graham, J.H., Irey, M.S., McCollum, T.G. & Wood, B.W. (2012) Inconsequential effect of nutritional treatments on huanglongbing control, fruit quality, bacterial titer and disease progress. *Crop Protection*, **36**, 73–82.

Grafton-cardwell, B. ACP Update and Overview of Treatment Protocol for Commercial Citrus.

Grafton-Cardwell, E.E., Godfrey, K.E., Rogers, M.E., Childers, C.C. & Stansly, P. a. (2006) Asian Citrus Psyllid. *Small*, 1–8.

Halbert, S. (2005) Second International Citrus Canker and Huanglongbing Research Workshop 50. *Proceedings of Second International Citrus Canker and Huanglongbing Research Workshop*, H–3.

Halbert, S.E. & Manjunath, K.L. (2004) Asian Citrus Psyllids (Sternorrhyncha: Psyllidae) and Greening Disease of Citrus: a Literature Review and Assessment of Risk in

Florida. *Florida Entomologist*, **87**, 330–353.

Hall, D.G. & Hentz, M.G. (2011) Seasonal flight activity by the Asian citrus psyllid in east central Florida. *Entomologia Experimentalis et Applicata*, **139**, 75–85.

Hamer, H. (2016) USDA NASS Crop Production Report June 2016. , 1–33.

Hannunen, S. (2005) Modelling the interplay between pest movement and the physical design of trap crop systems. *Agricultural and Forest Entomology*, **7**, 11–20.

Holden, M.H., Ellner, S.P., Lee, D.H., Nyrop, J.P. & Sanderson, J.P. (2012) Designing an effective trap cropping strategy: The effects of attraction, retention and plant spatial distribution. *Journal of Applied Ecology*, **49**, 715–722.

Ichinose, K., Hoa, N. V., Bang, D. V., Tuan, D.H. & Dien, L.Q. (2012) Limited efficacy of guava interplanting on citrus greening disease: Effectiveness of protection against disease invasion breaks down after one year. *Crop Protection*, **34**, 119–126.

Iftikhar, Y., Rauf, S., Shahzad, U. & Zahid, M.A. (2014a) Huanglongbing: Pathogen detection system for integrated disease management – A review. *Journal of the Saudi Society of Agricultural Sciences*, 0–10.

Iftikhar, Y., Rauf, S., Shahzad, U. & Zahid, M.A. (2014b) Huanglongbing: Pathogen detection system for integrated disease management – A review. *Journal of the Saudi Society of Agricultural Sciences*, 0–10.

Keremane, M.L., Ramadugu, C., Rodriguez, E., Kubota, R., Shibata, S., Hall, D.G., Roose, M.L., Jenkins, D. & Lee, R.F. (2015) A rapid field detection system for citrus huanglongbing associated 'Candidatus Liberibacter asiaticus' from the psyllid vector, Diaphorina citri Kuwayama and its implications in disease management. *Crop Protection*, **68**, 41–48.

Li, W., Hartung, J.S. & Levy, L. (2006) Quantitative real-time PCR for detection and identification of Candidatus Liberibacter species associated with citrus huanglongbing. , **66**, 104–115.

Ludwig, S.W., Kokt, L.T. & Station, G.E. (1998) Evaluation of trap crops to manage harlequin. , **17**, 123–128.

Mattson, W.J. (1980) Herbivory in Relation to Plant Nitrogen Content. *Ecological Systems, Annual Review*, 119–61.

Mccollum, G. Mechanism for Release of Genetically-modified Citrus.

McManus, P.S. & Jones, A.L. (1994) Epidemiology and Genetic Analysis of Streptomycin-Resistant Erwinia amylovora from Michigan and Evaluation of Oxytetracycline for Control.

Michaud, J.. (2004) Natural mortality of Asian citrus psyllid (Homoptera: Psyllidae) in central Florida. *Biological Control*, **29**, 260–269.

Monzo, C., Qureshi, J. a & Stansly, P. a. (2014) Insecticide sprays, natural enemy assemblages and predation on Asian citrus psyllid, Diaphorina citri (Hemiptera: Psyllidae). *Bulletin of entomological research*, **104**, 576–85.

National Research Council. (2010) *Strategic Planning for the Florida Citrus Industry: Addressing Citrus Greening Disease.*

Onagbola, E.O., Rouseff, R.L., Smoot, J.M. & Stelinski, L.L. (2011) Guava leaf volatiles and dimethyl disulphide inhibit response of Diaphorina citri Kuwayama to host plant volatiles. *Journal of Applied Entomology*, **135**, 404–414.

Pluke, R.W.H., Qureshi, J. a. & Stansly, P. a. (2008) Citrus Flushing Patterns, Diaphorina Citri (Hemiptera: Psyllidae) Populations and Parasitism By Tamarixia Radiata (Hymenoptera: Eulophidae) in Puerto Rico. *Florida Entomologist*, **91**, 36–42.

Qureshi, B.J.A., Khan, A.A., Jones, M., Stansly, P.A., Is, W., Production, O., States, U., Standards, N., Production, O.A., Ser-, A.M. & Materi-, T.O. (2013) Management of Asian citrus psyllid in organic citrus groves. , 8–10.

Qureshi, J. a & Stansly, P. a. (2007) Integrated Approaches for Managing the Asian Citrus Psyllid Diaphorina citri (Homoptera : Psyllidae) in Florida. *Proceedings of the Florida State Horticultural Society*, 110–115.

Qureshi, J.A. & Stansly, P.A. (2009) Exclusion techniques reveal significant biotic mortality suffered by Asian citrus psyllid Diaphorina citri (Hemiptera : Psyllidae) populations in Florida citrus. *Biological Control*, **50**, 129–136.

Qureshi, J.A. & Stansly, P.A. (2010) Dormant season foliar sprays of broad-spectrum insecticides : An effective component of integrated management for Diaphorina citri (Hemiptera : Psyllidae) in citrus orchards. *Crop Protection*, **29**, 860–866.

Ratnadass, A., Fernandes, P., Avelino, J. & Habib, R. (2012) *Plant Species Diversity for Sustainable Management of Crop Pests and Diseases in Agroecosystems : A Review*.

Rogers, M.E. & Dewdney, M.M. (2015) Florida Citrus Pest Management Guide.

Rogers, M.E. & Stansly, P. a. (2006) Biology and Management of the Asian Citrus Psyllid , Diaphorina citri Kuwayama , in Florida Citrus. *University of Florida. IFAS Extensión.*, 1–6.

Shelton, a M. & Badenes-Perez, F.R. (2006) Concepts and applications of trap cropping in pest management. *Annual review of entomology*, **51**, 285–308.

Shelton, A.M. & Nault, B.A. (2004) Dead-end trap cropping: a technique to improve management of the diamondback moth , Plutella xylostella (Lepidoptera : Plutellidae). , **23**, 497–503.
Slinski, S. Citrus Research and Development Foundation, I. (2016) The Use of Bactericides in Plant Agriculture with Reference to Use in Citrus to Mitigate HLB.

Tsagkarakis, A., Schumann, A, & Rogers, M (2009) Effects of nutrition on the biology of the Asian citrus psyllid, Diaphorina citri. Conference paper presented at the Entomological Society of America Annual Meeting 2009.

U.S. Department of Commerce National Oceanic & Atmospheric Administration National Environmental Satellite, Data, and I. *Record of Climatological Observations*.

University of California Agriculture and Natural Resources Statewide Integrated Pest Management Program. (2016) *Asian Citrus Psyllid and Huanglongbing Disease*.

University of California, Division of Agriculture and Natural Resources (2016, a). Asian Citrus Psyllid Distribution and Management, Grower Management. Retrieved from http://ucanr.edu/sites/ACP/Grower_Options/Grower_Management/

University of California, Division of Agriculture and Natural Resources (2016, b). Asian Citrus Psyllid Distribution and Management, ACP Effective Insecticides. Retrieved from http://ucanr.edu/sites/ACP/Grower_Options/Grower_Management/ACP_Effective_Insecticides/

University of California, Division of Agriculture and Natural Resources (2016, c). Asian Citrus Psyllid Distribution and Management, Asian Citrus Psyllid distribution and Management. Retrieved from http://ucanr.edu/sites/ACP/Distribution_of_ACP_in_California/

University of California, Division of Agriculture and Natural Resources (2016, d). Asian Citrus Psyllid Distribution and Management, Asian Citrus Psyllid and Huanglongbing Disease. Retrieved from http://ipm.ucanr.edu/PMG/PESTNOTES/pn74155.html

University of California, Division of Agriculture and Natural Resources (2014).Biological Control and Natural Enemies of Invertebrates Retrieved from http://ipm.ucanr.edu/PMG/PESTNOTES/pn74140.html

Wallingford, A.K., Kuhar, T.P., Pfeiffer, D.G., Tholl, D.B., Freeman, J.H., Doughty, H.B. & Schultz, P.B. (2013) Host plant preference of harlequin bug (Hemiptera: Pentatomidae), and evaluation of a trap cropping strategy for its control in collard. *Journal of economic entomology*, **106**, 283–8.

Wan, N., Zhang, Y., Huang, K., Ji, X. & Jiang, J. (2016) Ecological engineering of trap cropping promotes biocontrol services in peach orchard ecosystems. *Ecological Engineering*, **90**, 427–430.

Westbrook, C.J., Hall, D.G., Stover, E. & Duan, Y.P. (2011) Colonization of Citrus and Citrus- related Germplasm by Diaphorina citri (Hemiptera: Psyllidae). *Hortscience*, **46**, 997–1005.

Zaka, S.M., Zeng, X.N., Holford, P. & Beattie, G.A.C. (2010) Repellent effect of guava leaf volatiles on settlement of adults of citrus psylla, Diaphorina citri Kuwayama, on citrus. *Insect Science*, **17**, 39–45.

Zhang, M., Guo, Y., Powell, C.A., Doud, M.S., Yang, C. & Duan, Y. (2014) Effective Antibiotics against ' Candidatus Liberibacter asiaticus ' in HLB-Affected Citrus Plants Identified via the Graft-Based Evaluation. , **9**, 17–21.

Zhang, M., Powell, C.A., Guo, Y., Doud, M.S. & Duan, Y. (2012) A Graft-Based Chemotherapy Method for Screening Effective Molecules and Rescuing Huanglongbing-Affected Citrus Plants. , **102**, 567–574.

Zhang, M., Powell, C.A., Zhou, L., He, Z., Stover, E. & Duan, Y. (2011) Chemical Compounds Effective Against the Citrus Huanglongbing Bacterium ' Candidatus Liberibacter asiaticus ' In Planta. , **101**, 1097–1103.

Definition of Terms

Asian citrus psyllid (ACP): *Diaphorina citri*. This insect belongs to the order of arthropods, Homoptera. It has a piercing-sucking mouth part that allows it to puncture tender plant tissue and extract plant sap. While feeding, it is known that the ACP can ingest the plant pathogen *Candidatus* Liberibacter asiaticus, a bacteria that causes citrus greening disease.

Cash crop: The main crop planted with the intent to harvest for profit.

Curry leaf: *Murraya koenigii*. A citrus relative in the Rutaceae plant family. This plant is native to south Asia. Its leaves are harvested for their flavor, and are used for cooking and medicinal purposes in Asian countries.

Dead-end trap crop: A particular species of trap crop that attracts the target insect pest as well as kills it. An example would be a genetically modified species of trap crop. The GMO trap crop attracts the insect pest and has a modification to kill the insect once the plant material is ingested.

Host: A plant species that an insect feeds and/or develops on.

Huanglongbing (HLB): Synonymous with citrus greening disease, HLB is a bacterial disease effecting species of citrus and citrus relatives. It is harmless to humans, but causes serious symptoms in citrus plants. Some symptoms include yellowing of new shoots, mottled yellow pattern on leaves, unripened fruit, fruit souring, premature fruit drop, and plant death. The bacterium clogs the phloem on the infective plant which leads to symptoms. There is no cure for the disease. One of the main vectors of the disease is the ACP, *Diaphorina citri*.

Intercropping: Planting more than one species of cash crop within a field location. The biodiversity of intercropping creates a more ecological setting that potentially decreases concentrations of insect pests. This is to be compared with mono-cropping.

Monoculture/Mono-cropping: Planting one species of cash crop in a given area. The lack of bio-diverse plant material may increase pest and pathogen abundance.

Retention: The potential of a trap crop to retain an insect pest population; the likelihood of the insect choosing the trap crop over the cash crop.

Trap crop/Trap cropping: Planting one or more species of plant that is not the cash crop, within an agriculture field for the sole purpose of deterring an insect pest from reaching the cash crop. The trap crop may also lure the target insect pest species away or out of the agriculture cash crop.