INTEGRATED MANAGEMENT OF ASIAN CITRUS PSYLLID, *DIAPHORINA CITRI* KUWAYAMA, FOR PROTECTING YOUNG CITRUS TREES FROM HUANGLONGBING

By

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To my parents and my wife

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The Asian citrus psyllid, *Diaphorina citri* Kuwayama, is a serious pest of citrus because it transmits a phloem limited bacterium, *Candidatus* Liberibacter asiaticus (*C.* Las), that putatively causes Huanglongbing (HLB). Also known as citrus greening disease, HLB is considered the most destructive disease of citrus worldwide causing yield loss and decline of the infected trees. There is no cure for the disease, thus current management practices focus on controlling vector populations through frequent use of insecticides. However, the threshold level for vector management is near zero, given the fact that even a single *D. citri* can transmit *C.* Las. Protecting young citrus trees is particularly difficult because *D. citri* reproduce exclusively on soft leaf tissues of newly developing shoots (flushes) and young citrus trees frequently produce flushes throughout the year and thus are attractive to *D. citri*. Furthermore, many of the effective insecticides are of conventional broad spectrum chemistries that entail negative impacts, heavy use of which cannot be sustained. Therefore in this dissertation, the first set of studies investigated kaolin particle film as a potential alternative to broad spectrum insecticides for control of *D. citri*. Second, the efficacy of intensive insecticide

application regimes utilizing currently available insecticides and kaolin particle film were evaluated for mitigating transmission of *C.* Las under commercial citrus growing conditions. Lastly, the impact of recurring infestation and inoculation of *C.* Las by *D. citri* on tree vigor was assessed to determine whether insecticide use for *D. citri* control will be value in maintaining fruit production for already HLB-diseased trees. The results showed that kaolin particle film application deterred psyllid feeding thereby reduce HLB infection in young citrus trees, and that intensive *D. citri* control programs utilizing combination of foliar and soil applied insecticides significantly reduced the *D. citri* infestation and overall infection level in the field compared to the untreated.

CHAPTER 1
INTRODUCTION

Citrus Production in Florida

Citrus is one of the most important fruit crops in the world. It is produced in about 140 countries worldwide and ranks first in the international trade value among fruits (UNCTAD 2011). Sweet oranges account for about 50% of the citrus output and are produced mainly from Brazil (29%), United States (12%), China (9%), and India (7%) (FAOSTAT 2011). In the United States, 65% of the commercial citrus crop is produced in Florida and sweet oranges account for 73% of Florida's citrus production, of which 96% is processed (USDA-NASS 2013). Development of frozen concentrate processing in 1945-46, and more recent introduction of ready-to-serve juice along with publicized health benefits have increased consumer interest and demand for citrus (Davies and Jackson 2009). The economic impact of the citrus industry to the state of Florida is estimated at $9 billion involving nearly 80,000 full time employed jobs. Since the beginning of commercial farming in the mid-1800s, citrus has been a signature crop in Florida.

Although favorable growing conditions in Florida made possible the success of the citrus industry, periodic winter freezes and hurricanes affected the citrus bearing acreage and production. The Great Freeze of 1894-1895 and subsequent winter freezes that happened throughout 1900s resulted in a gradual movement of groves southward to locations less vulnerable to low winter temperatures (Davies and Jackson 2009, USDA-NASS 2013). Four significant hurricanes made landfall in the state of Florida; Charley, Frances, and Jeanne in 2004, and Wilma in 2005. These hurricanes significantly lowered citrus bearing acreage and production in 2004-2005 growing season (USDA-NASS 2013). While periodic winter freezes and hurricanes shaped the development of citrus industry in the past, urbanization and diseases have been the critical factors in recent years. Florida ranked 7th in population growth in the 2000 US census and 4th in population according to the 2008 US national and state population estimates. Citrus bearing acreage in Florida has gradually declined in the past decade from 762,400 acres in 1999-2000 crop year to 530,900 acres in 2008-2009 (USDA-NASS 2013). In recent history prior to citrus canker and huanglongbing, the management of citrus pests in Florida consisted mainly of oil sprays and effective biological control. However, introduction and spread of these two very serious bacterial diseases greatly increased the inputs for pest management particularly for arthropods.

Citrus canker is a leaf, fruit, and stem blemishing disease caused by the bacterium *Xanthomonas citri* subsp. *citri* (Dewdney and Graham 2013). Symptoms are mostly cosmetic with lesions that later develop into crater-like pustules. However, severe infection causes defoliation, shoot die-back and fruit drop. Although citrus leafminer (*Phyllocnistis citrella*) is not a vector, leafminer larvae tunneling produces galleries where increased leaf susceptibility is observed that eventually result in a higher inoculum pressure (Dewdney and Graham 2013, Rogers et al. 2013). Citrus canker was first identified in Florida in 1910 (FDACS-DPI 2012). An intensive eradication program was established and successfully eradicated the disease several years later. The disease was detected again in 1986, when it was eradicated once more through another eradication program (FDACS-DPI 2012). The third detection of canker was in 1995 after which sporadic outbreaks occurred throughout the Florida citrus industry (FDACS-DPI 2012, Davies and Jackson 2009). The eradication efforts were in place until major hurricanes widely spread the disease in 2004 and 2005, which caused withdrawal of the eradication program in 2006 (FDACS-DPI 2012). The impact of citrus canker and its eradication program was a loss of 16 million citrus trees and it still remains as a threat to the growers requiring prevention and management efforts (FDACS-DPI 2012, Dewdney and Graham 2013).

Huanglongbing and Asian Citrus Psyllid

Huanglongbing (HLB), also known as a citrus greening disease, is the most serious and destructive disease of citrus that is estimated to affect about 100 million trees worldwide (NRC 2010). This disease has affected citrus production wherever it occurs because it systemically infects the tree and eventually causes tree decline regardless of the cultivars (Brlansky et al. 2013, Gottwald 2010). The putative causal agent of HLB is a phloem-restricted, gram-negative bacterium of which three species are currently presumed to be the HLB pathogen: *Candidatus* Liberibacter asiaticus (*C.* Las), *Candidatus* Liberibacter africanus (*C.* Laf), and *Candidatus* Liberibacter americanus (*C.*Lam) (Bové 2006). These pathogens can be transmitted by two insect vectors: Asian citrus psyllid, *Diaphorina citri* Kuwayama, and African citrus psyllid, *Trioza erytreae* del Guercio (Bové 2006). *C.* Laf is transmitted by the African citrus psyllid and occurs in Africa, Arabian Peninsula, Mauritius, and Reunion Islands (Bové 2006). *C.* Lam and *C.* Las are transmitted by the Asian citrus psyllid and while *C.* Lam occurs only in Brazil, *C.* Las occurs in Asia, Arabian Peninsula, Brazil, and Florida (Teixeira et al. 2005, Bové 2006). Most recent detections of *C.* Las within the United States include South Carolina, Georgia, Louisiana, Texas, and California (USDA 2012). *D. citri* has been present throughout Florida since its first detection in 1998 and HLB was first detected in 2005 in the south Florida regions of Homestead and Florida City

(Halbert 1998, 2005). Currently, both the disease and the insect vector are found throughout the state of Florida wherever citrus is grown (FDACS-DPI 2011).

HLB symptoms are non specific and may be obscured by other ailments such as nutritional deficiencies. Thus it is generally considered difficult to diagnose the disease based on field observations alone. However, some symptoms are highly characteristic of HLB such as yellow shoots and “blotchy mottle” leaves (Bové 2006). Infected trees develop yellow shoots that distinguishes them from the green canopy, a characteristic that gave its name Huanglongbing, which means “yellow shoot disease” in Chinese. Other conditions such as cool weather and zinc deficiency may cause similar symptoms, but an HLB outbreak is distinguished in that only some trees show symptoms while other conditions affect almost all trees in the grove (Bové 2006). Leaves of the infected tree show a vein yellowing and chlorosis that result in a blend of green and yellow colors with shaded areas where clear limits of two colors are lacking (Bové 2006). Some nutrient deficiencies (zinc, manganese, magnesium, calcium, and iron) may cause similar leaf yellowing symptoms; however, HLB is distinguished in that the yellowing pattern on one side of the leaf midrib does not match or is not symmetrical to that on the other side (Bové 2006). This asymmetric yellowing foliar symptom, termed “blotchy mottle” (McClean and Schwarz 1970), is considered the most characteristic symptom of HLB. Infected trees produce small, asymmetric, lopsided fruits that show color inversion (Bové 2006). Normal fruit changes color from green to yellow/green that starts from the stylar end and moves to the peduncular end. However, fruit from HLB infected trees shows an inversion of this where coloring starts from the peduncular end. Currently, polymerase chain reaction (PCR) is a preferred method for HLB diagnosis.

This molecular technique utilizes primers specific for *Candidatus* Liberibacter species to detect its presence in DNA extracts of psyllids and plant tissues. Quantitative TaqMan PCR using 16S rDNA-based TaqMan primer-probe sets developed by Li et al. (2006) is currently the most widely used technique by laboratories that produces consistent and reproducible results.

D. citri is a Hemipteran pest that feeds on the phloem sap of citrus trees. Severe infestation can damage newly emerging sprouts and cause abscission of leaves (Halbert and Manjunath 2004). The adults are 3-4 mm in length with a mottled brown appearance (Mead 1977). The nymphs are generally yellowish orange in color and pass through five instars (Mead 1977). The eggs are about 0.3 mm long with an elongate and almond-like shape (Mead 1977). Oviposition and nymphal development occur only on young flushes because nymphs feed exclusively on young tender leaves or shoots (Hall and Albrigo 2007). The optimum temperature for *D. citri* development is 25-28ºC and females are capable of laying up to about 750 eggs (Liu and Tsai 2000). The life cycle completes in 15-47 days and the adults may live for several months depending on the season (Mead 1977). In Florida, *D. citri* populations are most abundant during May, June and July but low during the winter (Hall et al. 2008).

D. citri can acquire the *C.* Las through both nymphal (4^{th} and 5^{th} instars) and adult feeding on infected trees or through transmission from parent to offspring (transovarial) (Pelz-Stelinski et al. 2010). The acquisition rate observed from *D. citri* population in Florida was higher in nymphs (60-100%) than in adults (40%), and transovarial transmission occurred at a rate of 2-6% (Pelz-Stelinski et al. 2010). *D. citri* that acquired the pathogen during nymphal stages are able to transmit immediately after emergence while psyllids that acquired the pathogen at adult stage requires a latent period of up to 25 days before successful inoculation (Xu et al. 1988, Innoue et al. 2009). A single *D. citri* infected with *C.* Las is capable of inoculating the pathogen to a healthy plant although low success rate of below 6.3% was observed (Pelz-Stelinski et al. 2010). In the case when a large number (200) of infected *D. citri* was held on a healthy plant for inoculation feeding, the rate of success increased to near 100% (Pez-Stelinski et al. 2010).

Protecting Young Non-Bearing Citrus Trees

Although current estimate of HLB infection in Florida differs greatly by regions with rates ranging from 2% to 100%, every citrus growing region of Florida is considered vulnerable to HLB given the state wide presence of vector and pathogen (FDAC-DPI 2011). Having no cure for the disease, HLB management practices involve inoculum reduction through removal of infected trees, and chemical control of vector populations (Brlansky et al. 2013, Rogers et al. 2013). These strategies are thought to be successful in mitigating the impact of HLB (Belasque et al. 2010). However, many groves with high HLB infection rates considered alternative methods that keep the infected trees by maintaining the productivity through enhanced nutritional programs. A shift in HLB management strategy from inoculum removal to use of nutritional programs to extend the life of diseased citrus trees has occurred recently in the Florida citrus industry. However, the question remains whether intense management of the psyllid vector is necessary if infected trees are not removed. Specific questions will include whether re-inoculation with the bacterium promotes greater tree decline compared with single inoculation event.

Furthermore, we must determine whether young citrus resets can be brought into production in areas where HLB infection rates are high and inoculum is not removed. Regardless of HLB infection, replacing non-productive trees with new plantings is economically essential for long term viability of a grove. Economic models that project future viability of the citrus industry in Florida are based on the assumption that resets can be protected from *C.* Las transmission by the *D. citri* using currently available tools. However, the survival of young citrus trees to maturity is currently in jeopardy especially in regions with high HLB infection level. Thus, it is imperative to develop intensive *D. citri* control programs that provide comprehensive protection from HLB for resets and new plantings.

Insecticide-based management of *D. citri* populations is currently the most effective tool in HLB management and increased use of insecticides is expected until successful development of transgenic plants resistant to HLB or other therapeutic bactericides is accomplished. Although protecting resets and new plantings is necessary as an intermediate strategy for HLB management, more frequent applications of insecticides are required compared to mature trees, because young trees flush throughout the year and attract *D. citri*. Increased use of insecticides increases the adverse effects on beneficials and the potential for development of insecticide resistance, thus improving current strategies and developing alternatives is recommended. Currently, soil applied systemic neonicotinoids are considered the most important insecticides that provide the longest activity with reduced impact on beneficials. Kaolin particle film applications as a potential alternative to chemical insecticides have shown to suppress *D. citri* field populations. Compared to conventional foliar applied of insecticides, systemic insecticides along with kaolin particle film applications may provide effective and reduced risk management strategies for protecting young non-bearing citrus trees from HLB.

Therefore, the objectives of this dissertation were:

1. To determine potential utility of kaolin particle film applications in *D. citri* management
2. To evaluate intensive *D. citri* control programs utilizing currently available tools to protect young citrus plantings from HLB infection
3. To determine the impact of recurring *D. citri* infestation and *C.* Las inoculation on the vigor of citrus trees

CHAPTER 2
POTENTIAL UTILITY OF KAOLIN PARTICLE FILM APPLICATION IN ASIAN CITRUS PSYLLID (HEMIPTERA: LIVIIDAE) MANAGEMENT

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is the most important pest of citrus because it transmits *Candidatus* Liberibacter asiaticus (*C.* Las), a phloem limited bacterium that putatively causes citrus greening disease (Huanglongbing; HLB) (Bové 2006). Huanglongbing is a devastating disease of citrus worldwide that causes tree decline and yield loss (Gottwald et al. 2007). *D. citri* was first detected in Florida in 1998 followed by the detection of HLB in 2005 in the south Florida regions of Homestead and Florida City (Halbert 1998, 2005). Currently, both the disease and insect vector are found throughout the state of Florida wherever citrus is grown (FDACS-DPI 2011). Having no cure for HLB, current management practices focus on the use of insecticides to suppress vector populations (Rogers et al. 2013). The most effective insecticides are broad spectrum chemistries in the organophosphate, pyrethroid, and neonicotinoid classes. However, frequent use of these insecticides raises environmental concerns, has negative effects on beneficials, and increases the potential for insecticide resistance. In fact, there are already reports of shifts in susceptibility to these insecticides in Florida populations of *D. citri* (Tiwari et al. 2011, 2013).

Covering plant surfaces with kaolin particles to create a physical barrier that prevents access by pests and diseases is a recently introduced technology in agriculture (Glenn et al. 1999). Kaolin is a white, non-porous, non-swelling, low-abrasive, fine-grained, plate-shaped aluminosilicate mineral [$Al_4Si_4O_{10}(OH)_8$] that easily disperses in water and creates a white mineral coating on plant surfaces when sprayed (Glenn and Puterka 2005). A review of choice and no-choice laboratory bioassays revealed that the primary activity of kaolin based particle film is repellence (before contact) or deterrence (after contact) of adult settlement on treated leaf tissues that results in a reduced feeding, and oviposition (Glenn and Puterka 2005). Pear psylla, *Cacopsylla pyricola* Foerster, exposure to kaolin treated pear leaves showed particle attachments to the insect leg, deterred grasping of leaf surfaces, and reduced host selection, oviposition, and feeding behaviors (Puterka et al. 2005). Similar effects of reduced adult settlement and oviposition or suppressive effects in field populations were shown against other psyllid pests including potato psyllid, *Bactericera cockerelli* Sulc, (Peng et al. 2011) and pistachio psyllid, *Agonoscena targionii* Lichtenstein (Saour 2005). *D. citri* exposure to kaolin treated citrus leaves has shown deterred grasping of leaf surfaces and significant suppression of field populations (McKenzie et al. 2002, Hall et al. 2007). Deterred *D. citri* settling might reduce feeding thus lower the acquisition and transmission rates of *C.* Las.

Kaolin showed almost no toxicity against adult *Tamarixia radiata* Waterston (Hymenoptera: Eulophidae) (Hall and Nguyen 2010), a parasitoid of *D. citri* that successfully established in Florida through classical biological control efforts (Hoy et al. 1999, Hoy and Nguyen 2000). Application of kaolin based particle film on citrus trees demonstrated horticultural benefits of enhanced citrus growth (Lapointe et al. 2006) likely due to increased photosynthesis and water use efficiency (Jifon and Syvertsen 2003). Commercialized product of kaolin based particle film is available under the trade name Surround WP Crop Protectant (Tessenderlo Kerley, Inc., Phoenix, AZ) and is certified by the Organic Materials Review Institute (OMRI) for use in organic production. These features of kaolin based particle film may make this product a useful tool in *D.*

citri and HLB management. Therefore studies were conducted to investigate kaolin particle attachment to *D. citri* tarsi, deterrence of feeding behavior, effects on host selection and settling behavior, and deposition characteristics of kaolin residue.

Materials and Methods

Insects and Plants

The insects used in the experiments were obtained from a greenhouse colony located at the University of Florida Citrus Research and Education Center in Lake Alfred, FL. This colony was started with *D. citri* from an in-house source that was free of *C*. Las. Psyllids were reared on potted curry leaf trees, *Murraya koenigii*, inside screen cages (0.6 m × 0.6 m × 0.9 m, Bioquip, Rancho Dominguez, CA). Plants used in the experiments were <3 yr old potted Valencia orange trees, *Citrus sinensis*, on either Carrizo (*Poncirus trifoliate* × *Citrus sinensis*) or Swingle (*Poncirus trifoliate* × *Citrus paradisi*) rootstock.

Kaolin Application

Kaolin particle film was applied as 0.048 kg/L Surround WP suspension unless mentioned otherwise. Surround WP label recommends 0.03-0.06 kg/L as the best concentration range for general application of kaolin based particle film. Similarly for *D. citri* suppression, label recommended rate is 56.04-84.06 kg/ha with specific instruction of using 1870.79 L/ha for mature trees with 3.66 m in height, which calculates to be 0.03-0.06 kg/L. Given that the experimental trees were young potted greenhouse trees with <0.91 m in height, the concentration of kaolin suspension used in this study was label recommended 56.04 kg/ha of Surround WP mixed in a commonly used spray volume of 1169.24 L/ha, which calculated to be 0.048 kg/L. In case of the EPG study, kaolin was applied to Valencia orange trees using an 11.36 L handheld pump sprayer

(997P, Root-Lowel Manufacturing Co. Lowell, MI). For all other experiments kaolin was applied to shoot cuttings using a 946.35 ml generic spray bottle.

Electrical Penetration Graph (EPG)

D. citri feeding behavior on kaolin treated and untreated leaf surfaces was investigated using an EPG monitor as described in Serikawa et al. (2012). Briefly, psyllids were transferred to Valencia orange trees at least 48 h prior to the start of EPG recordings to acclimatize the insects to the citrus cultivar used in the experiment. The Giga-8 EPG system (http://www.epgsystems.eu/products.htm) was setup as following. Gold wire (18.5 µm in diameter, Sigmund Cohn Corp., Mt. Vernon, NY) 1.5 cm in length was attached to dorsal thorax area of *D. citri* using a silver glue. The other end of the gold wire was attached to a copper nail electrode that inserted into EPG probe connected to each of the 8 channels available on Giga-8 control box. Wired *D. citri* were placed on the adaxial side of a mature leaf of either kaolin treated or untreated Valencia orange trees planted in a 3.79 L round plastic pot (18 cm in height and 8 cm in diameter at the top rim). The electrical circuit for each channel was completed by inserting plant electrodes (copper wire, ≈ 2 mm in diameter, 10 cm in length) into the soil, and then the pots were watered for better electrical conductance. The entire circuitry setup was housed within a Faraday cage (1.52 × 0.62 × 1.22 m) for protection from electrical noises, and the Giga-8 control center was connected to a personal computer via analog-to-digital converter (DI-710UHB, DATAQ instruments, Akron, OH). The substrate voltage was set to 75 mV DC and electrical signals resulted from a closed circuit formed when the insect came in contact with the leaf tissue. The electrical signals form patterns characteristic of specific behaviors associated with finding and ingesting phloem or xylem. Such electrical waveforms could be displayed in real time using WinDAQ Pro software (DATAQ Instruments) and recorded for subsequent identification and analysis. Waveform terminology and association to specific behavior of *D. citri* were as described by Bonani et al. (2010). There were 15 EPG recordings per treatment and all recordings were performed for at least 18 h in a closed room. Waveforms C, D, E1, E2, G, and NP that represent stylet pathway, phloem contact, phloem salivation, phloem ingestion, xylem ingestion, and non-probing behaviors respectively were manually identified from EPG recordings using DATAQ Windaq Waveform Browser software, version 2.40 (DATAQ Instruments).

Scanning Electron Microscope

Particle attachment to *D. citri* tarsi after exposure to kaolin treated citrus leaf was investigated using scanning electron microscope (Hitachi S530, Hitachi High Technologies America, Inc., Dallas, TX). Psyllids that were used in the previous EPG experiment, hence been exposed to kaolin treated or untreated leaf surfaces for at least 18 h, were collected and prepared for scanning electron microscope imaging according to the procedure similar to that described in Childers and Achor (1991). The preparation involved air-drying the insect, mounting it on a stub with ventral side up using double sided copper tape, and coating with gold particles using a sputter coater (Ladd Research Industries, Williston, VT). Tarsal areas of the psyllids were observed for visual comparison of particle attachment between kaolin treated and untreated control.

Choice Study

D. citri were released in an arena and given a choice between kaolin treated and untreated shoots to determine how particle film affected host selection and settling behavior. Citrus shoot cuttings pruned to contain only two mature leaves were inserted into wet floral foam (Smithers-Oasis North America, Kent, OH) fitted inside 33 ml clear polystyrene vials (Thornton Plastic Co., Salt Lake City, UT) to maintain the integrity of the shoots. The vials were sealed with parafilm to prevent floral foam desiccation and psyllid access. The choice arena (20 × 13.5 × 7 cm) was designed to house three vial inserts. The center vial contained 20 psyllids for release while two peripheral vials held either kaolin treated or untreated shoot. All choice assays were conducted in screen cages (0.3 × 0.3 × 0.45 m, Bioquip, Rancho Dominguez, CA) inside incubators (Percival Scientific Inc., Perry, IA) setup with 26 ± 1°C temperature and 14L:10D photoperiod. Four different concentrations of kaolin suspension (0.006, 0.012, 0.024, and 0.048 kg/L) were independently compared to untreated control (0 kg/L). For each kaolin concentration, four pairs of kaolin treated and untreated shoots were prepared (four replicates per concentration), and four releases (total 80 psyllids released per replicate) were conducted on the same pair of shoots by rotating the choice arenas 90 degrees clockwise after each release to account for different spatial positions within the incubator. The proportion of psyllids that settled on kaolin treated and untreated shoots were documented 24 h after release.

No-Choice Study

D. citri were confined to either a kaolin treated or untreated shoot to determine how particle film affected psyllid survival. Citrus shoot cuttings pruned to contain only two mature leaves were treated with four different concentrations of kaolin suspension (0.006, 0.012, 0.024, and 0.048 kg/L) or with just water for controls (0 kg/L). Treated shoots were inserted into wet floral foam (Smithers-Oasis North America, Kent, OH) fitted at the bottom of 960 ml clear polypropylene container (Red Rock Packaging, Kansas City, KS), which served as a confinement cage. A layer of unscented paraffin wax was created to prevent desiccation of the wet floral foam, and this setup kept the integrity of shoots for 7 d of psyllid confinement. Cages were held inside incubators (Percival Scientific Inc., Perry, IA) with 26 ± 1°C temperature and 14 L : 10D photoperiod. Ten psyllids confined per cage was considered a replicate and there were five replicates per treatment. The proportion of psyllids living after 7 d of confinement was documented.

Baseline Residue Level for Repellency

Leaf samples were collected from citrus shoot cuttings used in the Choice Bioassay to determine the baseline kaolin residue level for repellence against *D. citri* settling behavior. Four leaves were randomly collected from shoot cuttings previously treated with various concentrations of kaolin suspension so the mass of dislodgeable kaolin particles could be quantified using a spectrophotometer (Shimadzu UV-2401PC, Shimadzu Scientific Instruments, Inc., Columbia, MD). The quantification procedure was similar to that of Puterka et al. (2000) with some modifications mainly in that the solution used to wash treated leaves and obtain light absorbency level was not MeOH but a dispersant that consisted of 0.00082 mol/L Sodium Hexametaphosphate ($Na_6P_6O_{18}$) and 0.01 mol/L NaOH aqueous solutions per liter (Miller and Miller 1987). The dispersant suspended kaolin particles more uniformly within the solution and slowed the settling, thus allowed consistent light absorbency readings on spectrophotometer. Briefly, sample leaves were placed individually into 50 ml centrifuge tubes (Fisher Scientific, Pittsburgh, PA). Particles were dislodged from the leaf surface by adding 40 ml of dispersant, vortexing for 30 s at max level, and shaking for 10 min at 200 rpm on a reciprocal shaker (New Brunswick Scientific, Edison, NJ). Then, the leaves were taken out for measurement of surface area using an area meter (LI-3000, LI-COR®, Lincoln, NE). For each leaf sample washed, 1 ml of the solution was transferred to a microcuvette to determine its light absorbency at 400 nm wavelength by using the spectrophotometer. The regression equation (y = 6E-10x^3 - 3E-06x^2 + 0.0046x + 0.0276, R^2 = 0.9998) was generated first from absorbency values (y) of a series of known concentrations of kaolin (x), and then it was used to estimate concentrations of sample solutions based on the absorbency values. Given the area of the leaf sample and concentration of washed solution, the mass of dislodgeable particles for each leaf sample could be calculated in a weight of particles per leaf area (μg/ cm^2).

Kaolin Deposition Characteristics

The mass of dislodgeable kaolin particles on citrus leaves of different maturity and treated surfaces was determined to investigate kaolin deposition characteristics. Citrus shoot cuttings containing mature or young leaves were treated with kaolin or with just water. Either the adaxial (upper) or abaxial (lower) surface was treated with kaolin, not both, and kaolin deposits observed on the untreated side were wiped off. Water only controls were not differentiated with respect to the leaf surface. After drying, a single leaf was collected randomly and the mass of dislodgeable kaolin particles was quantified as previously described. There were six treatments based on kaolin spray (treated vs. untreated), leaf maturity (mature vs. young), and treated surface (adaxial vs. abaxial). Each treatment was replicated four times.

Statistical Analysis

Statistical analyses were performed using SAS 9.2 procedures (SAS Institute 2009) under SAS Enterprise Guide® 4.2 (SAS Institute Inc., Cary, NC). For the EPG study, numbers and durations of waveforms from kaolin treated and untreated control were compiled and averaged into non-sequential parameters defined by Backus et al.

(2007). Total waveform duration (TWD) was used to describe the breakdown of how long each behavior was performed at cohort level. Number of Probes per Insect (NPI), and Probing Duration per Insect (PDI) were used to compare the mean number and duration of probing behavior performed by *D. citri*. Probing Duration per Event (PDE) was used to compare duration of each probing event. Waveform Duration per Insect (WDI) was used to compare mean durations of waveforms performed by *D. citri*. For comparison, analysis of variance (ANOVA) (PROC GLIMMIX, SAS Institute 2009) with the least significant difference test (LSMEANS, SAS Institute 2009) was performed for pair wise comparisons between kaolin treated and untreated control. For choice assay, mean proportion of psyllids that settled on kaolin treated and untreated shoots were compared using t-tests (PROC TTEST, SAS Institute 2009). For no-choice assays and residue studies, psyllid survival and kaolin residue level were analyzed using ANOVA (PROC ANOVA, SAS Institute 2009) with Fisher's least significant difference mean comparison test (MEANS / LSD, SAS Institute 2009). For all comparison tests, means were considered significantly different at $\alpha=0.05$.

Results

Electrical Penetration Graph (EPG)

D. citri probing behavior was reduced on kaolin treated leaf tissue compared to the untreated control. A probing behavior is considered insertion of stylets into the leaf tissue and single probing event can contain compilation of C, D, E1, E2, and G waveforms in between NP waveforms. All 15 psyllids on the untreated controls performed probing activity at least once compared to only three psyllids on kaolin treated leaves. Based on the total duration of each waveform, the percentage of time psyllids spent performing probing activity on the adaxial leaf surface of untreated

Valencia orange tree was about 24%, compared to about 3% on kaolin treated leaves (Figure 2-1). The number of probes per insect (NPI) and probing duration per insect (PDI) were significantly lower on kaolin treated leaves compared to untreated control, while there was no significant difference in probing duration per event (PDE) (Table 2-1). Durations of C and G waveforms were significantly lower on kaolin treated leaves compared to untreated control while duration of NP waveform was significantly higher (Table 2-2). For both kaolin treated and untreated control, only one insect out of 15 performed phloem associated behaviors thus precluded statistical analysis of D, E1, and E2 waveforms.

Scanning Electron Microscope

Scanning electron microscope images showed a substantial mass of kaolin particle attachment to tarsi and pulvilli of *D. citri* after exposure to a kaolin treated Valencia orange tree leaves, while no discernible particles were observed from psyllids on untreated control (Figure 2-2).

Choice and No-Choice Bioassays

In choice bioassays, *D. citri* avoided settling on kaolin treated shoots. Psyllid settling was significantly lower on shoots treated with 0.012, 0.024, and 0.048 kg/L kaolin suspension; however, when the concentration was reduced to 0.006, no significant difference was observed (Table 2-3). In no-choice bioassays, a trend of reduced *D. citri* survival was observed as concentration of kaolin suspension applied increased. At 0.048 kg/L concentration, mean survival was significantly lower at 62% on kaolin treated shoots compared to 88% on untreated controls (Figure 2-3). Although statistically non-significant, a slight reduction in psyllid survival was observed at 0.024

and 0.012 kg/L concentrations while there seemed to be no treatment effect at 0.006 kg/L concentration (Figure 2-3).

Baseline Residue Level for Repellency and Deposition Characteristics

Application of lower concentrations of kaolin suspension resulted in a significantly reduced residue level except in case of 0.012 kg/L (Figure 2-4). The lowest application rate of kaolin suspension, at which, repellency was observed from treated shoots in choice bioassay was 0.012 kg/L. Quantified kaolin residue at this concentration was 56.48 $\mu g/cm^2$. The mass of dislodgeable kaolin particles was consistent regardless of leaf tissue or surface except that in case of young leaves, higher deposition was observed in abaxial surface compared to the adaxial (Figure 2-5).

Discussion

The results of the EPG study showed a reduction of *D. citri* stylet-probing behaviors on kaolin treated leaf tissue and demonstrated the potential utility of kaolin in HLB management as feeding deterrent, which may result in reduced pathogen transmission. Probing behavior of Hemipteran phytophagous insects is generally considered as insertion of their stylets into the plant tissue to find a feeding site for phloem or xylem ingestion. Kaolin treatment significantly reduced the number and duration of probes performed by *D. citri*. However, there was no significant difference in the mean duration of each probing event, which suggests that when physical coating is compromised and psyllids succeed in feeding on the leaf tissues, kaolin may not reduce the duration of time psyllid spend with their stylets inserted in the leaf tissue. *D. citri* is generally known to prefer feeding on young feather flushes compared to mature leaf tissue, and abaxial surface of citrus leaf compared to the adaxial side. Although less preferred, uniform coating of particle film could best be achieved on the adaxial surface of mature citrus leaf tissue thus used in this study. Serikawa et al. (2012) reported that psyllids performed probing behavior less often on mature leaves compared to young leaves, and that the number of phloem-associated behaviors was reduced on mature leaves. A low occurrence of phloem-associated behaviors was expected when the adaxial side of mature leaf surface was used (personal communication with Dr. Ebert), but having only one psyllid from each treatment to reach the phloem limited statistical comparison of phloem associated waveforms that are directly related to *C*. Las acquisition and transmission by *D. citri*.

Glenn and Puterka (2005) acknowledged that particle attachment to the insect's body parts is the key mechanism of effects of particle film. Visual confirmation through scanning electron microscope of kaolin particle attachment to *D. citri* legs specifically tarsal claws and pulvilli that are associated with insect grasping of surfaces substantiate the result of inhibited grasping reported by Hall et al. (2007).

Kaolin based particle film deterred *D. citri* host selection, and resulted in significant mortality after 7 d of confinement. In a choice bioassay, psyllids avoided kaolin treated citrus shoots and preferentially settled on untreated shoots. While tactile deterrence is presumably the main activity that contributes to reduced *D. citri* settling behavior, visual repellence may reduce colonization over longer distance. Such activity may be useful to limit psyllid movement between groves and enhance the efficacy of area wide control efforts. In no-choice confinement bioassays, mortality was not comparable to that of conventional insecticides as expected with lack of lethal activity but the deterrence of psyllid feeding likely caused starvation. When confined to untreated shoots, psyllids were observed mostly on leaf tissues. However when confined to kaolin treated shoots, psyllids were observed to have difficulty crawling up the stem or settling on the leaf surface, and ended up aggregating at the base of the shoot. These psyllids were still capable of feeding on the stem, which was not fully coated with particle film, and survive for a 7d confinement period. Better coating of the stem at the base could have resulted in higher mortality. Given the fact that kaolin does not have acute lethality and its activity relies on the persistence of physical coating on the plant surface, kaolin residue profiles during frequent and hard rainfall events of summer season in Florida need to be investigated.

A series of kaolin concentrations was tested in the choice bioassay to investigate the mass of particles necessary for repellency against *D. citri*. Significant repellency was observed at higher application rates until 0.012 kg/L, of which the dislodgeable kaolin residue was 56 μg/cm^2, and then there was no repellency when the kaolin application rate was reduced to 0.006 kg/L, of which the residue level was 11 μg/cm^2. Although the exact concentration could not be determined, such results suggest that growers can expect significant repellency from kaolin above a 56 μg/cm^2 residue level. Higher concentrations (0.024 and 0.048 kg/L) of kaolin suspension easily clogged the nozzle of spray bottle compared to lower concentrations (0.006 and 0.012 kg/L). The nozzle of spray bottle was adjustable so in case of higher concentrations, it was opened up more to allow higher rate of flow and prevent clogging. The inconsistency in application methods might have resulted in the similar residue level between 0.012 and 0.012 kg/L concentrations. Although the mass of kaolin particles was similar in regards to the leaf tissue and surface, different deposition characteristics were observed visually. Kaolin particles generally deposited on the leaf surface as a thin coating on mature leaves.

However, on young leaves, kaolin spray often stayed in droplets that resulted in islands or spots of particles rather than a thin coating. This was more evident on lower surface of young leaves compared to the upper. Particle film coating on leaf surface that physically deter psyllid settlement is considered the key mechanism of kaolin, thus inconsistent coating of leaf surfaces might reduce efficacy. Furthermore, young leaves expanding in leaf area may expose even more regions of the leaf surface for *D. citri* feeding, thus more frequent applications may be necessary during major flushing periods.

Table 2-1. Mean (±SE) and ANOVA results for non-sequential parameters Number of Probes per Insect, Probing Duration per Insect, and Probing Duration per Event (NPI, PDI, PDE respectively) derived from EPG recordings of *D. citri* on mature leaves of kaolin treated and untreated Valencia orange trees

Parameter	Control			Kaolin			*F*	df	*P*
NPI	6.73	±	1.24	0.53	±	0.35	23.02	28	<0.0001
PDI	15871.30	±	3373.51	2125.67	±	1204.74	14.72	28	0.0006
PDE	2357.12	±	448.24	3985.64	±	1274.05	0.99	107	0.3215

Table 2-2. Mean (±SE) and ANOVA results for parameters Waveform Duration per Insect (WDI), and Proportion of individuals that Produced the specific Waveform type (PPW) derived from EPG recordings of *D. citri* on mature leaves of kaolin treated and untreated Valencia orange trees

Waveform	Control				Kaolin				*F*	df	*P*
	WDI			PPW	WDI			PPW			
NP	50,080.19	±	3362.84	15/15[a]	63893.09	±	1004.4	15/15	15.5	28	0.0005
C	11,353.46	±	3150.27	15/15	1038.21	±	665.59	3/15	10.3	28	0.0034
D	27.69	±	27.69	1/15	1.98	±	1.98	1/15	---	---	---
E1	6.01	±	6.01	1/15	2.25	±	2.25	1/15	---	---	---
E2	412.13	±	412.13	1/15	0	±	0	0/15	---	---	---
G	4,072.01	±	998.49	13/15	1083.23	±	622.72	3/15	6.45	28	0.0169

[a] Number of individuals that produced the waveform type over the total number of individuals recorded

Table 2-3. Mean (±SE) proportion of *D. citri* that settled either on kaolin treated or untreated citrus shoots 24 h after release in a choice arena. The data from kaolin treated and untreated control was compared using t-test (α=0.05)

Concn[a]	Control			Kaolin			*t*	df	*P*
0.006	0.2656	±	0.0521	0.2938	±	0.0413	-0.42	6	0.6871
0.012	0.3469	±	0.0397	0.1281	±	0.0107	5.33	6	0.0018
0.024	0.2375	±	0.0184	0.1438	±	0.0180	3.64	6	0.0109
0.048	0.4156	±	0.0230	0.1219	±	0.0129	11.13	6	<0.0001

[a] Concentration of kaolin suspension in kg/L

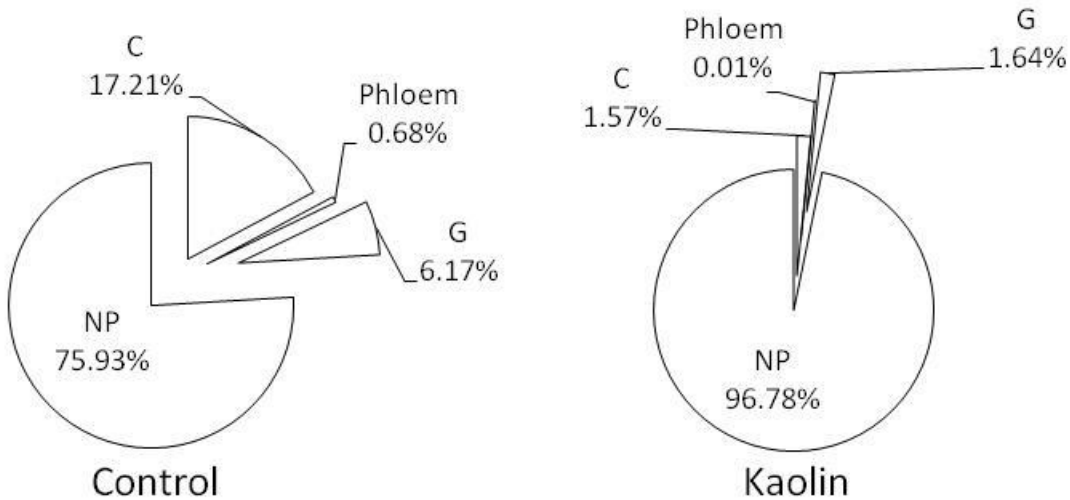

Figure 2-1. Percentage of time *D. citri* spent performing a behavior when placed on the adaxial side of mature leaves from kaolin treated and untreated Valencia orange trees based on the Total Waveform Duration (TWD) derived from EPG recordings of 15 psyllids per treatment (C: stylet pathway, G: xylem ingestion, NP: non-probing). Percentages of D, E1, and E2 waveforms that represent phloem contact, salivation, and ingestion respectively were very low (<1%) so combined percentage is shown as phloem in the figure.

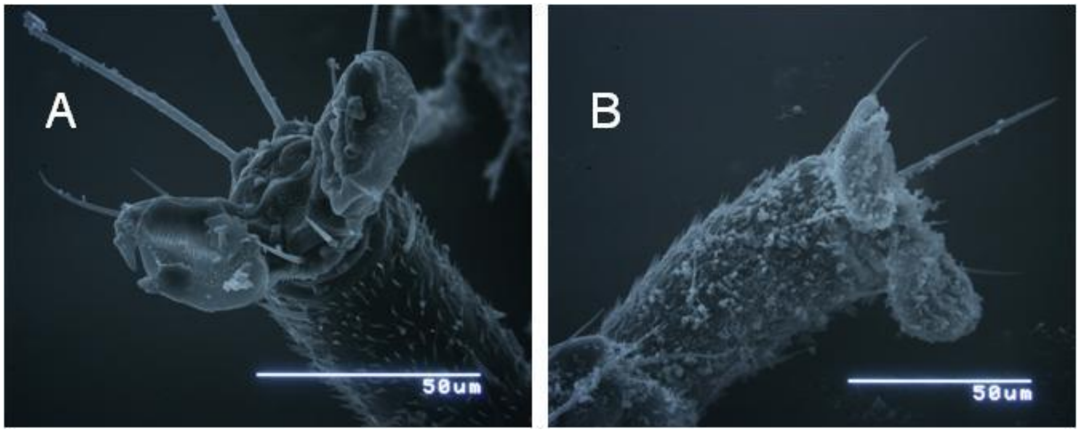

Figure 2-2. Scanning electron microscope images of *D. citri* tarsi after exposure to B) kaolin treated and A) untreated mature leaf of Valencia orange tree.

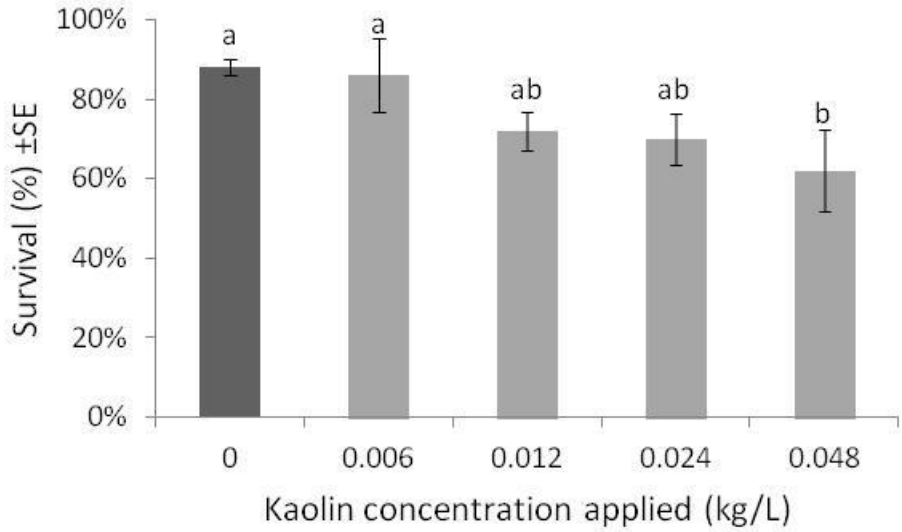

Figure 2-3. Mean (±SE) survival of *D. citri* after 7d of confinement to citrus shoot cuttings treated with a series of kaolin concentrations. Means labeled with the same letter are not significantly different (α=0.05, Fisher's LSD).

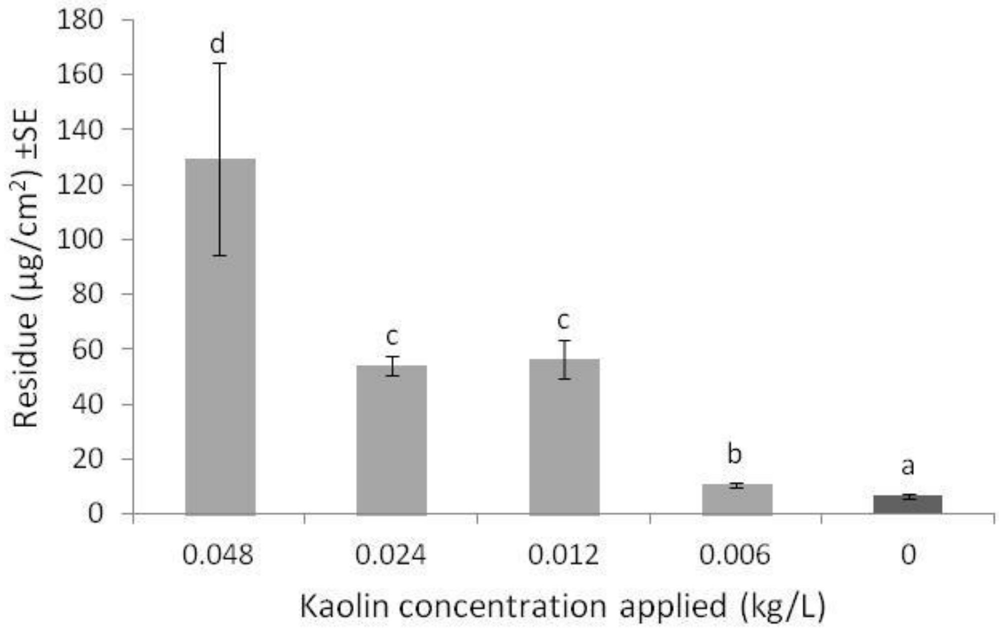

Figure 2-4. Mean (±SE) mass of dislodgeable kaolin residue on citrus leaf surface after application of a series of kaolin concentrations. Means labeled with the same letter are not significantly different (α=0.05, Fisher's LSD).

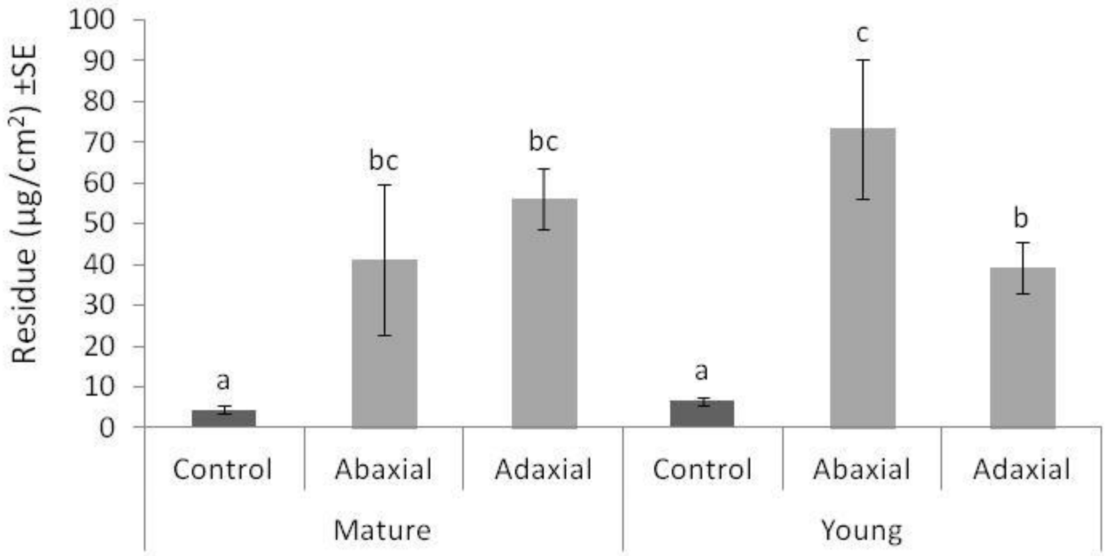

Figure 2-5. Mean (±SE) mass of kaolin particle deposition on citrus leaf with respect to leaf age (mature vs. young) and treated surface (adaxial = upper vs. abaxial = lower). Means labeled with the same letter are not significantly different (α=0.05, Fisher's LSD).

CHAPTER 3
EVALUATION OF INTENSIVE ASIAN CITRUS PSYLLID (HEMIPTERA: LIVIIDAE) CONTROL PROGRAMS FOR PROTECTING YOUNG CITRUS TREES FROM HUANGLONGBING

Huanglongbing (HLB), also known as citrus greening disease is the most devastating disease of citrus worldwide causing canopy thinning, premature fruit drop, and twig dieback that result in a yield loss and eventual death of the infected trees (Gottwald 2007). Since the discovery of HLB in Florida (Halbert 2005), Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), had become the most important pest of citrus because it transmits *Candidatus* Liberibacter asiaticus (*C.* Las), a phloem limited bacterium that putatively causes HLB (Bové 2006). Currently, there is no known cure for HLB and although researches on sustainable long term solution are underway, it may take several years until development and approval for actual implementation in commercial groves. Meanwhile, *D. citri* control through frequent use of insecticides to reduce vector populations and mitigate *C*. Las transmission remains as one of the major parts of HLB management (Rogers et al. 2013).

D. citri reproduce exclusively on soft leaf tissues of newly developing shoot (flush). Compared to mature citrus trees that only have 2-3 major flushing periods per growing season, young trees produce new flushes throughout the year hence much more attractive to *D. citri* and vulnerable to *C*. Las transmission. Such characteristics presumably contributed to faster infection and decline rates in young trees compared to mature trees (Gottawald 2010). Given this continuous flushing pattern of young citrus trees, foliar-applied insecticides alone may not provide sufficient protection because of their relatively short residual activity. Soil-applied systemic insecticides are currently considered the most effective strategy for *D. citri* control that provides the longest lasting protection for young citrus trees (Rogers et al. 2013). A season long application regime can be developed for trees up to 5 ft in height considering the use restrictions per growing season, water solubility of each product, and seasonal likelihood for significant rainfall events (Rogers 2012). However, the active ingredients of soil-applied insecticides are all in a neonicotinoid class of chemistries that share the same mode of action thus additional foliar sprays with different modes of action are recommended to minimize the potential for development of insecticide resistance (Rogers 2012).

The most effective insecticides against *D. citri* are broad spectrum chemistries that entail negative impacts including adverse effects on beneficial insects, potential for insecticide resistance, and environmental concerns (Rogers et al. 2013). Kaolin based particle film (Glenn et al. 1999) is an alternative strategy that showed great potential for incorporation into *D. citri* control programs. Kaolin is certified for organic production by Organic Materials Review Institute (OMRI) and is compatible with *Tamarixia radiata* (Hall and Nguyen 2010), a parasitoid of *D. citri* imported into Florida through classical biological control efforts (Hoy et al. 1999, Hoy and Nguyen 2000). When applied as a suspension in water, kaolin particles create a white coating on plant surface that acts as a physical barrier (Glenn and Puterka 2005). Previous studies have shown deterred *D. citri* grasping behaviors in the laboratory studies, and suppression after field applications (McKenzie et al. 2002, Hall et al. 2007). Interfering with behaviors related to psyllid feeding may have potential utility in mitigating *C*. Las transmission.

As the number of productive trees declines due to the endemic HLB situation in Florida with recent reports of severe fruit drop from declining trees in 2012-2013 growing season, the ability to bring new plantings into production became crucial for continued viability of the Florida citrus industry (Morris and Muraro 2008, Spreen 2012). Given circumstances of Florida where both *D. citri* and HLB had spread throughout the state, abandoned groves are interspersed in the citrus growing regions, and some infected trees are not removed instead managed with enhanced nutritional programs for sustained productivity (Spann et al. 2010), high vector and disease pressure is to be expected for young citrus trees. Furthermore, the fact that a single *D. citri* adult is capable of transmitting *C.* Las (Pelz-Stelinski et al. 2010) requires more intensive control measures to provide a comprehensive protection throughout the growing season. Therefore, intensive season-long *D. citri* control programs exploiting currently available foliar and soil applied insecticides independently or in combination were evaluated for protection of new citrus plantings in high vector and disease pressure setting. Reported here are the results 2 yr after initial planting of Valencia orange trees in Florida.

Materials and Methods

Experimental Site, Planting, and Management

The experimental site was located at the Water Conserv II (http://www.waterconservii.com/index.html) in Winter Garden, FL. A grove of mature trees with a high HLB infection rate was located adjacent to the North of the site. This mature grove served as the primary source of bacterialiferous *D. citri* adult population. There were pine trees to the East of the experimental site and open hay field to the South. Twelve rows of 120 Valencia orange trees, *Citrus sinensis*, (1440 trees total) on Swingle rootstock (*Poncirus trifoliate* × *Citrus paradisi*) were planted in May 2011 with tree spacing of 3.05 m in between trees and 7.62 m in between rows. Trees were irrigated with micro-sprinkler, and received nutrition through slow release granules with occasional foliar sprays. Planted trees were divided into 60 plots consisting of 24 trees (4 rows of 6 trees), of which only the eight center trees were treated with insecticides for *D. citri* control while 16 borderline trees were left untreated to harbor immigrating population of *D. citri*, and maintain high inoculum level to provide constant *C.* Las transmission pressure.

Vector Control Programs

D. citri control programs evaluated in this field study were; 1) Untreated – no insecticide application, 2) Foliar – monthly application of foliar applied insecticides, 3) Kaolin – monthly application of kaolin particle film, 4) Soil – soil drench application of neonicotinoids every 6-8 wk, 5) Soil + Kaolin – combination of programs 3 and 4, and 6) Soil + Foliar – combination of programs 2 and 4. Insecticides utilized in the control programs were selected based on their previously recognized activity against adult psyllids, which is considered the most important stage for HLB management (Rogers et al. 2013). Active ingredients and use rates of insecticides applied as foliar sprays and soil drenches are listed in Table 3-1. Based on the use restrictions, 15 foliar sprays (seven organophosphates, five pyrethroids, and three spinetoram) and 8-10 soil drenches (2-4 imidacloprid according to tree height, two thiamethoxam, and four clothianidin) could be applied per year. Foliar sprays including kaolin were applied monthly using a handgun sprayer (MCCI100SKDS, Chemical Containers, Lake Wales, FL) and soil drenches were applied in 300 ml of total volume per tree using timer controlled electrical pump sprayer (CCIDO35, Chemical Containers) every 8 wk initially, then from November 2011, every 6 wk as trees grew taller. Products used for foliar sprays were rotated based on the three available MOAs to reduce the potential for development of insecticide resistance. Different vector programs (treatments) were set up in a randomized complete block design with 10 replicated plots per treatment.

Vector Population Monitoring

Since the initial planting, the experimental trees were visually inspected every two wk for *D. citri* presence. The first visual detection of *D. citri* did not occur until March 2012 when *D. citri* were seldom observed; however by April 2012, about 11 mo after planting, increases in *D citri* were recorded from a number of plots, hence forth stem-tap sampling (Arevalo et al. 2011) commenced for monitoring the *D. citri* adult population in each plot. Each of the eight center trees in each plot was inspected every 2 wk by tapping 3-4 branches per tree with a stick and quickly counting the number of *D. citri* adults that fell onto a laminated white sheet of paper.

Insecticide Resistance Monitoring

*D. citri s*usceptibility to imidacloprid was evaluated in July 2012 for monitoring resistance to soil applied neonicotinoid systemic insecticides. A dose-response study was conducted on adult *D. citri* collected from plots receiving soil drenches only and untreated control plots using similar methods as described in Tiwari et al. (2011). Briefly, technical grade imidacloprid was dissolved in analytical grade acetone to make a stock solution (500 ppm), which was then diluted into a series of imidacloprid solutions for a topical application. The insects were temporarily anesthetized under CO_2, and a 0.2 µl of the imidacloprid solutions and acetone alone (0 ppm) was applied to the dorsal side of the thorax using a 10 µl Hamilton syringe secured to a microapplicator (PAX 100-3 Automatic Micro-Dispensing System, Burkard Agronomic Instruments, Uxbridge, United Kingdom). Treated insects were placed on a citrus leaf disc fitted to a 35 mm Petri dish layered with 1.5% agarose gel. Each Petri dish containing 10 treated insects was considered a replicate, and there were six replicates per concentration. A total of seven imidacloprid concentrations (0, 0.1, 0.5, 1, 2.5, 5, and 10 ppm) including acetone only as a control, hence 420 psyllids, were tested for each of the *D. citri* populations collected from plots receiving soil drenches only and untreated control plots. The Petri dishes were kept in an incubator (Percival Scientific Inc., Perry, IA) with 25 ± 1°C temperature and 14 L : 10D photoperiod, and mortality was assessed after 24 h. Susceptibility of the field collected *D. citri* to imidacloprid was compared to that of the laboratory susceptible reported previously by Tiwari et al. (2011).

HLB Incidence

The experimental trees were sampled for PCR detection of *C.* Las immediately after planting to ensure that the trees were in fact pathogen free, thereafter, trees were sampled every 3 mo to monitor HLB incidence in each plot. The center eight trees in each plot were first divided into east and west four trees, then composite sample of four leaves were collected from each direction by taking one leaf per tree. The presence of *C.* Las in sampled leaf tissue was determined by DNA extraction and PCR detection methods as described in Pelz-Stelinski et al. (2010), which utilize 16S rDNA-based TaqMan primer-probe sets designed by Li et al. (2006). Briefly, leaf petiole and midvein were separated from collected samples, and cut into small pieces. Then, subsamples of 100 mg were placed in 1.5-ml microcentrifuge tubes containing a 5-mm stainless steel bead, and ground using a bead mill (Tissue-Lyzer II, QIAGEN, Valencia, CA) under liquid nitrogen for 1 min at 30 Hz/s. Plant DNA was extracted from the ground leaf tissues using DNeasy Plant kit (QIAGEN) according to manufacturer’s Mini protocol. The PCR amplification of *C.* Las-specific sequence was conducted using ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA) to determine its presence in the extracted plant DNA. In conjunction, amplification reactions also contained primer and probe sets targeting citrus cytochrome oxidase gene regions for internal control (Li et al. 2006). Duplicate reactions were conducted per DNA sample, and each PCR run included positive and negative controls. When a composite sample tested positive, relevant trees were individually sampled and PCR tested again, this time by collecting four leaves per tree. By August 2012, composite samples from most plots tested positive, hence starting November 2012, every tree was sampled individually.

Tree Growth Measurement

Tree height and trunk diameter were measured to compare the effects of different *D. citri* control programs on tree growth. Tree height was measured using a 1.52 m measuring stick, and trunk diameter was measured using a dial caliper (SPI 31-415, Swiss Precision Instruments Inc., Garden Grove, CA) 35.56 cm above ground at the top end of a sprout guard. Measurements were taken from each of the eight center trees in November 2011 for the first time, then after at every 3 mo.

Field-Aged Kaolin Residue Analysis

Kaolin residue analysis was conducted during July-August 2012 to investigate the persistence of the kaolin particle film after heavy rainfall events. Leaf samples were collected at 4 hr, 7 d, 14 d, and 21 d after spray from 10 plots that were under Soil+Kaolin *D. citri* control program by taking one random leaf per plot, and then the mass of dislodgeable kaolin particles on the sampled leaf surfaces were quantified using methods described in the previous chapter. Briefly, collected leaves were placed individually into 50 ml centrifuge tubes (Fisher Scientific, Pittsburgh, PA). Particles were dislodged from the leaf surface by adding 40 ml of a dispersant (Miller and Miller 1987), vortexing for 30 s at max level, and shaking for 10 min at 200 rpm on a reciprocal shaker (New Brunswick Scientific, Edison, NJ). Then, the leaves were taken out for measurement of surface area using an area meter (LI-3000, LI-COR®, Lincoln, NE). For each leaf sample washed, 1 ml of the solution was transferred to a microcuvette to determine its light absorbency at 400 nm wavelength by using a spectrophotometer (Shimadzu UV-2401PC, Shimadzu Scientific Instruments, Inc., Columbia, MD). A regression equation ($y = 6E\text{-}10x^3 - 3E\text{-}06x^2 + 0.0046x + 0.0276$, $R^2 = 0.9998$) was generated using absorbency values (y) of a series of known concentrations (x), which was then used to estimate concentrations of sample solutions based on the absorbency values. Given the area of the leaf sample and concentration of washed solution, the mass of dislodgeable particles for each leaf sample could be calculated in a weight of particles per leaf area ($\mu g / cm^2$). Also, weekly cumulative rainfall during July-August 2012 period was calculated based on the data retrieved from Florida Automated Weather Network (FAWN, location: Avalon) database.

Statistical Anlysis

Statistical analyses were performed using SAS 9.2 procedures (SAS Institute 2009) under SAS Enterprise Guide 4.2 (SAS Institute Inc., Cary, NC). For vector monitoring, the insect count data documented for 1 yr from April 2012 to April 2013 were log(x+1) transformed, then the mean values for different *D. citri* control programs were compared using analysis of variance (ANOVA) (PROC ANOVA, SAS Institute 2009) with Fisher's least significant difference (LSD) test (MEANS / LSD, SAS Institute 2009). For insecticide resistance monitoring, mortality after topical application of a series of imidacloprid concentrations were submitted to PROBIT analysis (PROC PROBIT, SAS Institute 2009) to determine LD_{50}, and LD_{95} values. For HLB incidence, the proportion of infected trees in each plot at final sampling in February 2013 was arcsine square root transformed, and then the means for different *D. citri* control programs were compared using ANOVA with Fisher's LSD test. Area under the disease progress curve (AUDPC) analysis (Madden et al. 2007) and logistic regression analysis were performed to compare the rate of HLB increase in plots that received different *D. citri* control programs. For AUDPC analysis, the HLB incidence curve (percent infected over time) of each plot was broken into a series of segments between sampling periods, then the underlying area of each segment was calculated and added up to determine the total area under the entire curve. Mean values of AUDPC for different *D. citri* control programs were compared using ANOVA with Fisher's LSD. Further analysis on mean AUDPC values was conducted after dividing the data set into northern and southern half of the grove to account for relatively high infection level observed in the north compared to the south. For logistic regression analysis, HLB incidence in each plot was fitted to the logistic model [ln(y/1-y)=a+bt] (PROC LOGISTIC, SAS Institute 2009), then the mean values of resulting parameter estimates for different *D. citri* programs were compared using ANOVA with Fisher's LSD. Also, the collective incidences of HLB for each *D. citri* control program were fitted to logistic model to produce single regression curve per program, then ellipse-shaped joint confidence regions of two parameters a and b were constructed (von Luxburg and Franz 2009), and examined for overlapping. For tree growth measurement, mean values of final measurements taken in March 2013 for different *D. citri* control programs were compared using ANOVA with Fisher's LSD. For field-aged kaolin residue analysis, kaolin residue level at 4 hr, 7 d, 14 d, and 21 d after initial spray was compared using ANOVA with Fisher's LSD. All mean comparison tests were performed with α=0.05 error rate.

Results

Vector Population

The *D. citri* adult population was monitored using stem-tap sampling for 1 yr since the first visual detection in April 2012, and is shown in Figure 3-1. *D. citri* numbers increased quickly for the untreated trees reaching five psyllids per tree in May 2012, and 10 psyllids in July 2012, and then the population peaked at over 20 psyllids per tree in August 2012. A general trend of knockdown effect right after insecticide application followed by quick recovery was observed for all *D. citri* control programs during summer months, except that in soil drench only program, *D. citri* numbers increased gradually to reach three psyllids per tree in June 2012, then sustained at similar level until August 2012. After a grove-wide insecticide spray in late August 2012, *D. citri* numbers decreased to below one per tree level in all treatments. *D. citri* levels then remained low level for all treatments except the control, in which *D. citri* populations quickly increased to an average three per tree a month later (September 2012). In general, *D. citri* numbers stayed low during winter months from late 2012 to early 2013 then started to increase again in spring 2013. As for the efficacy of different *D. citri* control programs in reducing vector population, every program provided significant reductions in overall psyllid numbers with the Soil+Kaolin combination providing the greatest control whereas the Soil-application only treatments provided the least amount of control overall (Figure 3-2).

Susceptibility to Imidacloprid

The PROBIT analysis results from field collected *D. citri*, and that from the laboratory susceptible previously reported by Tiwari et al. (2011) are shown in Table 3-2. Also shown in the table are the resistance ratio values (LD_{50} of field population / LD_{50} of laboratory susceptible). A reduced susceptibility to imidacloprid with significantly higher LD_{50} values compared to the laboratory susceptible was observed in both *D. citri* field populations collected from plots receiving soil applied neonicotinoid insecticides only and untreated control plots. Resistance ratio value was as high as 127.5.

HLB Incidence

First detection of HLB infected trees occurred in May 2012 for Untreated, Kaolin, Foliar and Soil programs, which was 12 mo after initial planting. For Soil+Kaolin and Soil+Foliar combination programs, the first detection occurred in August 2012, 15 mo after initial planting. According to the final sampling in February 2013, HLB incidence was significantly lower for Kaolin, Soil, Soil+Kaolin programs with 36%, 29% and 42% reductions respectively compared to untreated (Figure 3-3.). Although Foliar and Soil+Foliar programs also showed 4% and 22% reductions respectively compared to untreated, there was no significant difference (Figure 3-3). Area under the disease progress curve analysis conducted on the entire data set did not show any significant differences; however, when just the northern half was analyzed separately, Kaolin and Soil+Kaolin programs showed significant reduction in mean AUDPC values compared to untreated (Figure 3-4). For logistic regression by plot and comparison of mean estimates of parameters a and b, all *D. citri* control programs showed significant differences compared to untreated, but no significant differences between programs (Figure 3-5). For logistic regression by treatment and evaluation of joint confidence regions of parameters a and b, Kaolin and Soil+Kaolin programs did not overlap with untreated while other programs did (Figure 3-6).

Tree Growth

Generally, trees that received any form of insecticide treatments grew significantly taller in height and had thicker trunk diameter compared to untreated (Figure 3-7). Among different *D. citri* control programs, Soil+Kaolin and Soil+Foliar showed the best growth level, then Soil, Kaolin, Foliar, Untreated in that order (Figure 3-7).

Kaolin Field Residue

The mass of kaolin particles on leaf surfaces decreased significantly at every 7 d sampling period (Figure 3-8). Kaolin residue level was 202 μg/ cm^2 at 4 h after spray however as more rainfall events occurred, it was reduced to 65, 48, and 36 μg / cm^2 at 7, 14, and 21 d after spray respectively (Figure 3-8). The cumulative amount of rainfall was 0, 4.5, 6.6, and 8.9 cm at 4 h, 7 d, 14 d, and 21 d respectively (Figure 3-8).

Discussion

Given the endemic HLB situation in Florida with high inoculum level and transmission potential, *D. citri* control programs evaluated in this study were intensive regimes that were designed to provide a season-long protection for young trees in extremely high vector and disease pressure surroundings. As discussed by Hall et al. (2013), *D. citri* was slow to infest the newly planted trees. The first detection of *D. citri* occurred nearly one year after initial planting. However since the establishment of vector population, the HLB infection level increased rapidly. The average infection level reached near 70% for untreated plots in just a year after the first detection of *D. citri* suggesting that leaving borderline trees untreated resulted in a quick build up of high vector population and inoculum level that provided a constant *C.* Las transmission potential.

The *D. citri* control program consisting of only foliar applied insecticides provided significant reduction in vector population; in fact, the overall psyllid numbers observed was one of the lowest among all 5 treatments tested in the study. However, as expected, foliar sprays alone did not provide effective protection, and showed the highest HLB infection level comparable to that of untreated. In contrast, the *D. citri* control program consisting of only kaolin sprays provided a reduced level of control compared to the foliar applied insecticides, yet significantly mitigated HLB spread compared to untreated resulting in one of the lowest HLB infection levels. Such results suggest utility of kaolin's feeding deterrent activity in mitigating *C.* Las transmission. Although Soil+Kaolin combination program provided the best control of vector population and the lowest level of HLB infection, it would probably be the best to exploit all three (Foliar, Kaolin, and Soil) strategies in combination for young tree protection, given that kaolin does not provide a lethal activity to reduce the potential for development of resistance to soil applied neonicotinoids.

Although, the size of each experimental plot was small (24 trees), different statistical analyses were conducted to be consistent with previously published studies (Bassanezi et al. 2013, Hall et al. 2013, Gatineau et al. 2010) that evaluated the efficacy of vector control in mitigating the HLB disease progression. Comparing the mean estimates of parameters of the logistic model showed significance of any form of *D. citri* control in mitigating HLB disease progression compared to untreated, while comparison of AUDPC values and evaluation of joint confidence regions showed the utility of feeding deterrent activity by kaolin particle film in HLB management. Furthermore, one of the valuable findings of this study was how quickly the resistance had developed in the absence of insecticide rotation. Development of resistance to neonicotinoids was presumably the main reason for soil drenches to show such a low efficacy in reducing *C.* Las transmission. If the resistance had been prevented, soil drenches would have reduced HLB infection level significantly more given its effects on *D. citri* feeding behavior (Serikawa et al. 2012).

In order to discuss the results of this study in full scope, a systems approach in IPM (Wise and Whalon 2009) was considered that takes into account the relationships of key components of a system. Plant Insect Chemistry-Triad designed by Wise and Hoffman (Wise et al. 2007) is a good example of the systems approach employed in IPM. Similarly, relationships of four key components identified in this case as Environment (rainfall events), Plant (growth, flush production), Insect (host preference, insecticide resistance), and Chemistry (residue) were discussed in an attempt to elucidate the circumstances of different *D. citri* control programs. For convenience, each period between sampling dates for PCR detection of *C.* Las was discussed separately focusing on the important events or factors to consider in HLB management.

Feb-May 2012. Trees receiving neonicotinoid soil drenches were already in a much better growth condition compared to untreated (Figure 3-7). Consequently, these trees were more abundant in flushes, hence more attractive of *D. citri* compared to the borderline trees or those in untreated plots as psyllids started migrating into the experimental grove from outer sources. While other combination treatments received foliar applied insecticides or kaolin applications that provided an additional protection to account for this higher attraction for *D. citri*, trees receiving soil drenches only did not have any additional protection. This could have resulted in a lengthy duration of *D. citri* exposure to a sub-lethal level, a condition that would increase the potential for Las transmission as well as development of insecticide resistance.

May-August 2012. June through August is generally considered the wettest period of a year in Florida, and likewise in 2012, there were frequent heavy rainfall events during this period (Figure 3-9). The residues of foliar applied insecticides and physical coating of kaolin particles probably would have degraded much faster in such conditions, thus the expected duration of activity against *D. citri* would have decreased significantly. A quick recovery in tap count numbers after initial knock down by foliar sprays evidently showed this reduced residual efficacy. A sustained number of three psyllids per tree shown during June-August 2012 in the soil only treatment was considered a product failure. In response to speculated resistance development, a dose response bioassay was conducted using a micro applicator similar to methods described in Tiwari et al. (2011). Significantly higher LD_{50} value had resulted from *D. citri* collected from the experimental grove compared to the previously published baseline LD_{50} value from laboratory susceptible population by Tiwari et al. (2011). Because of the confirmed resistance, a grove-wide insecticide spray was conducted to kill off the resistant population in late August 2012. These two conditions with respect to the rainfall and resistance development explain why HLB infection level in all treatments increased so rapidly during this period. Such conditions were especially evident in the result that showed the greatly increased HLB infection level for Soil+Foliar treatment, which did not have any HLB infection at all up until this period.

August-Novembert 2012 and on. According to the tap count numbers, the efficacy of foliar insecticides seemed restored as rainfall started to ease off, and also the efficacy of neonicotinoids seemed restored after a grove-wide insecticide application. The HLB infection level was about 70% for untreated and seemed to be nearing the asymptotic level.

Table 3-1. List of insecticides used in *D. citri* control programs

Application Method	Product	Active Ingredient	MOA[a]	Use Rate/ha	Restriction[b]
Foliar Spray	Lorsban 4E	Chlorpyrifos	1B	5.85 L	17.54 L
	Dimethoate 4E	Dimethoate	1B	1.17 L	2.34 L
	Imidan 70-W	Phosmet	1B	1.12 kg	2 applications
	Danitol 2.4 EC	Fenpropathrin	3A	1.17 L	3.12 L
	Mustang	Zeta-cypermethrin	3A	0.31 L	0.94 L
	Delegate WG	Spinetoram	5	0.28 kg	0.84 kg
	Surround WP	Kaolin	N/A	56.04 kg	N/A
Soil Drench	Admire Pro	Imidacloprid	4A	0.51 L	1.02 L
	Platinum 75SG	Thiamethoxam	4A	0.13 kg	0.26 kg
	Belay 50WDG	Clothianidin	4A	0.22 kg	0.90 kg

[a] Insecticide Mode of Action (MOA) classification according to Insecticide Resistance Action Committee (IRAC)
[b] Use restriction per hectare per year

Table 3-2. Susceptibility of field collected *D. citri* populations to imidacloprid

	LS[a]	Untreated	Soil drenches only
LD_{50}[b]	0.004 a[c]	0.51 b	0.25 b
(95% CL)	(5×10^{-4}-0.05)	(0.43-0.60)	(0.08-0.57)
LD_{95}	2:00 AM	1.97 a	2.57 a
(95% CL)	(0.11-2.9×10^{5})	(1.53-2.82)	(0.93-123.91)
x^2	18.05	19.2	95.87
Slope ± SE	0.62 ± 0.12	1.61 ± 0.37	2.82 ± 0.29
RR_{50}[d]	-	127.5	62.5
RR_{95}	-	0.985	1.285

[a] PROBIT analysis results on the laboratory susceptible (LS) *D. citri* population reported previously by Tiwari et al. (2011)
[b] LD_{50} and LD_{95} values are in (ng AI / insect)
[c] LD_{50} and LD_{95} values followed by different letters within each row were significantly different from one another, based on non-overlap of 95% confidence intervals
[d] Resistance ratio (LD_{50} of field population / LD_{50} of laboratory susceptible)

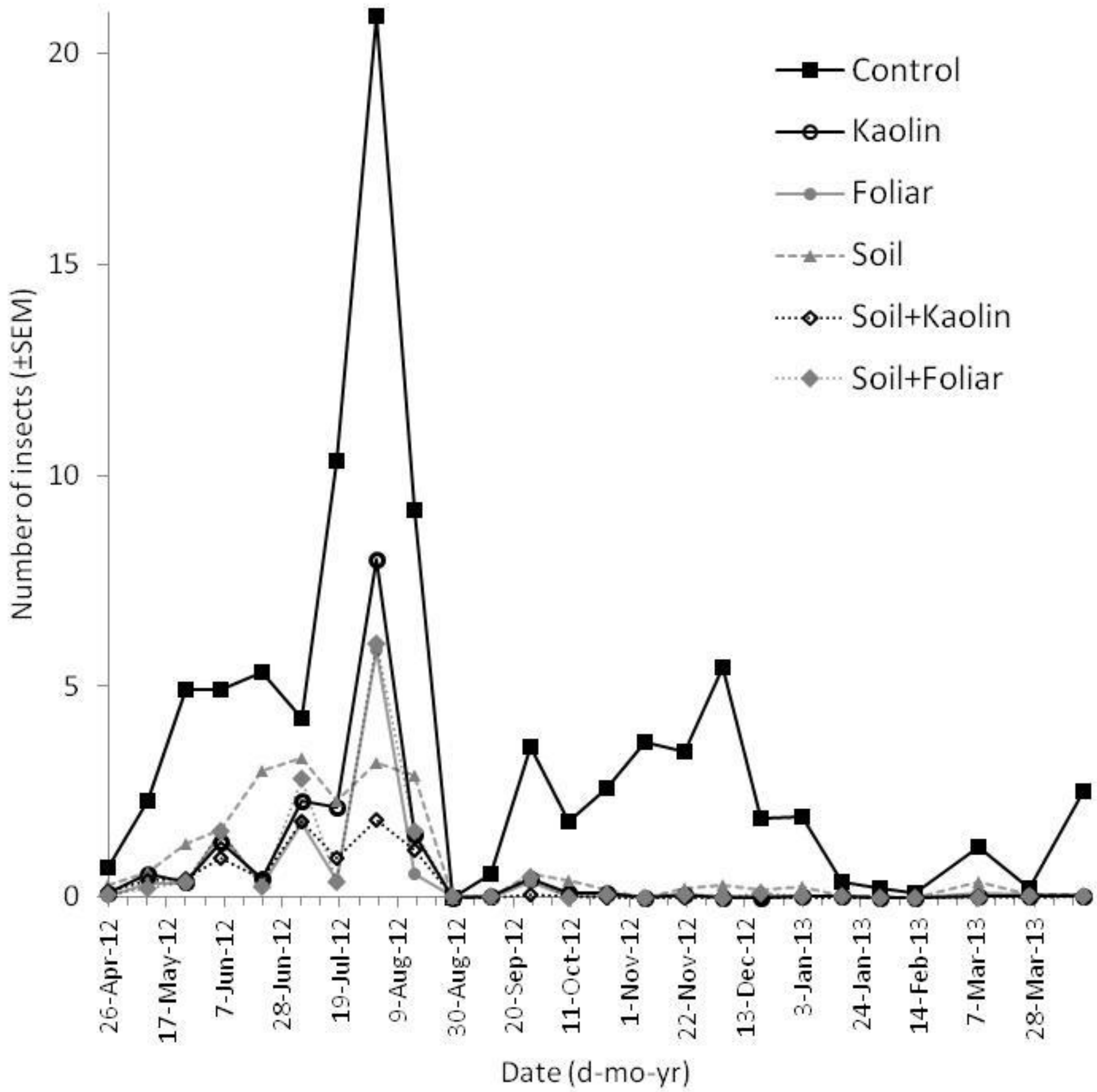

Figure 3-1. Mean (±SEM) number of *D. citri* adults observed after stem-tap sampling of 3-4 branches per tree.

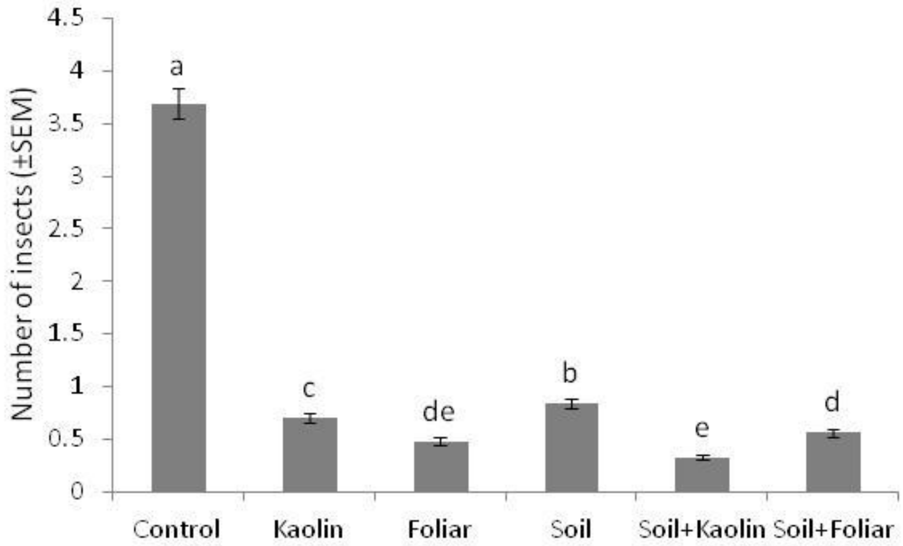

Figure 3-2. Mean (±SEM) number of *D. citri* adults observed after tapping 3-4 branches per tree. Combined data of stem-tap sampling conducted biweekly for a 1 yr period from April 2012 to April 2013. Means labeled with same letter are not significantly different (α=0.05, Fisher's LSD).

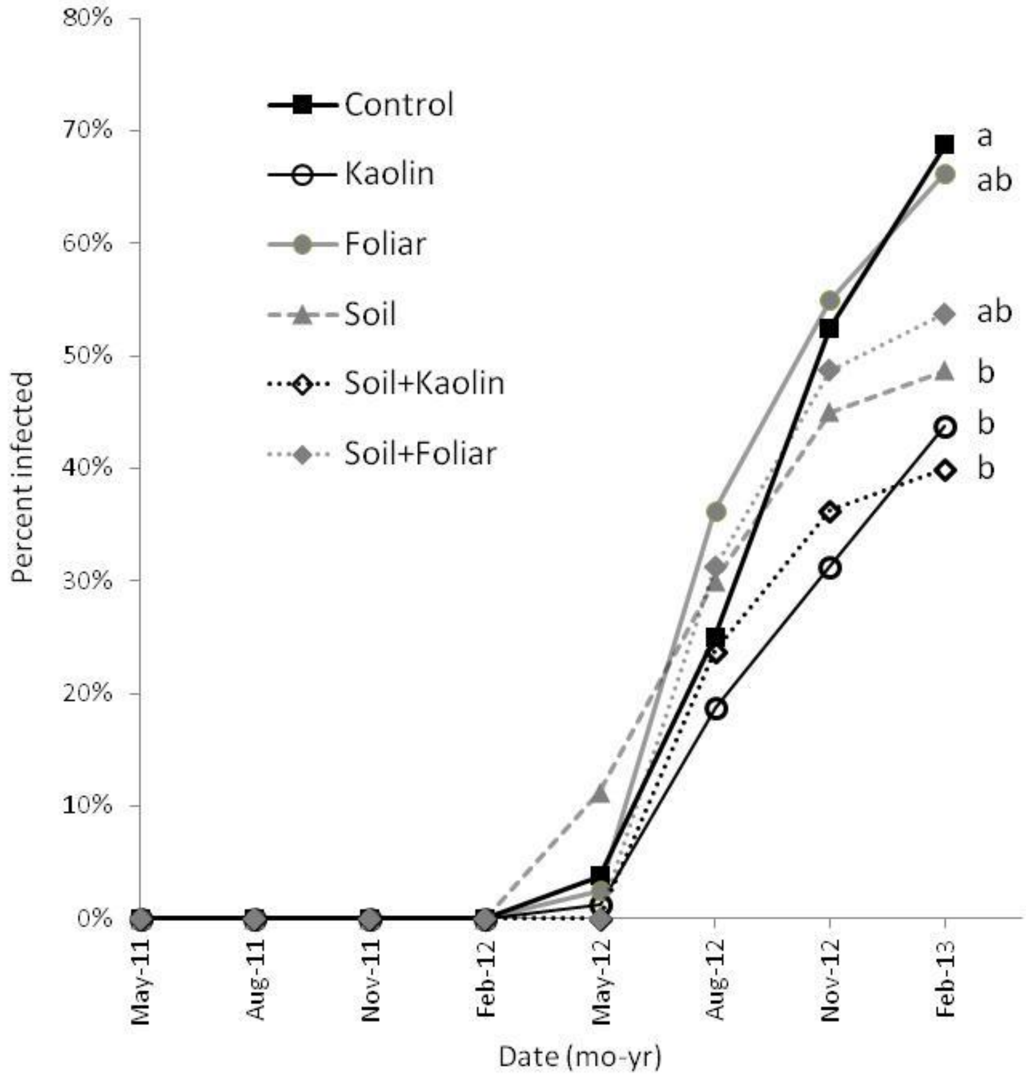

Figure 3-3. Mean proportion of HLB infected trees in each plot over time. Means at the final sampling period labeled with same letter are not significantly different (α=0.05, Fisher's LSD).

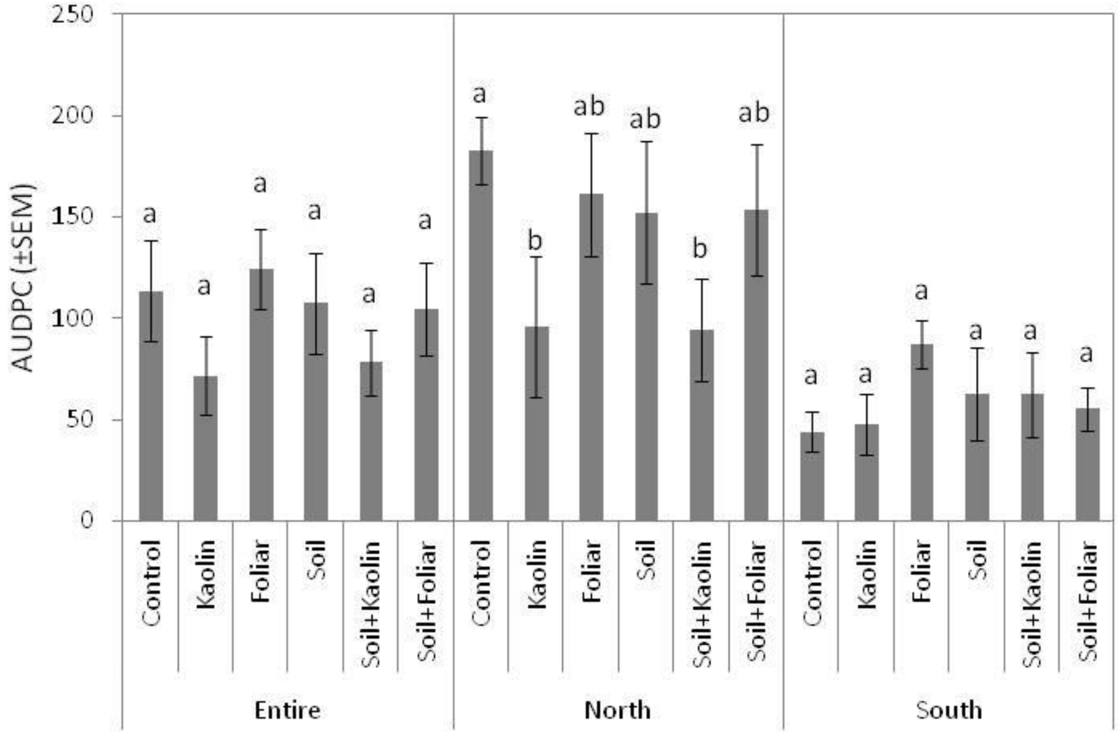

Figure 3-4. Mean (±SEM) values of area under the disease progress curve for different *D. citri* control programs. The data set was divided into northern and southern half of the grove, and then separately analyzed. Means labeled with same letter are not significantly different (α=0.05, Fisher's LSD).

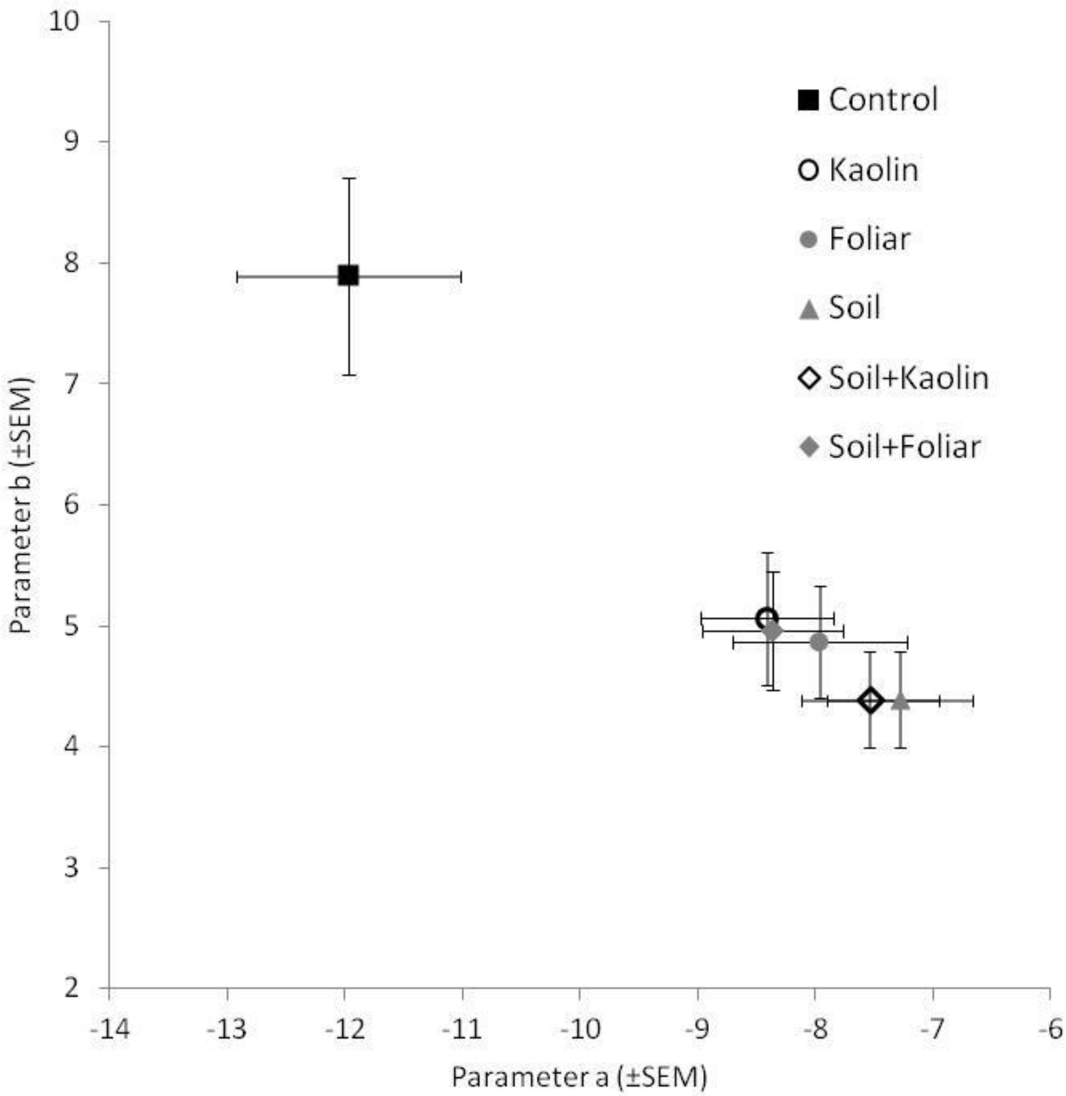

Figure 3-5. Mean (±SEM) estimates of parameters a and b of logistic model fitted to the HLB incidence data from each plot. Means labeled with same letter are not significantly different (α=0.05, Fisher's LSD).

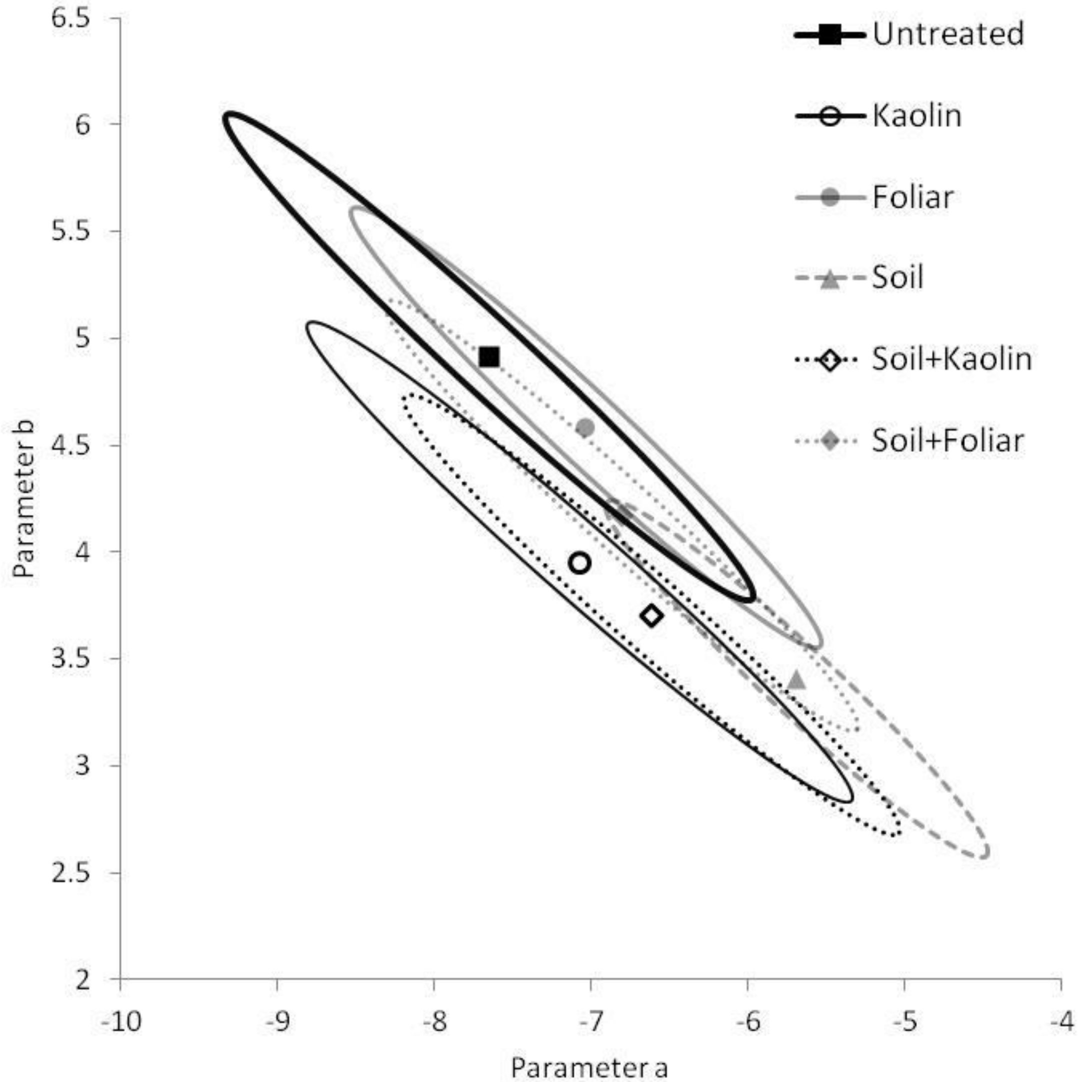

Figure 3-6. Joint confidence regions (α=0.05) of parameters a and b of logistic model for different *D. citri* control programs.

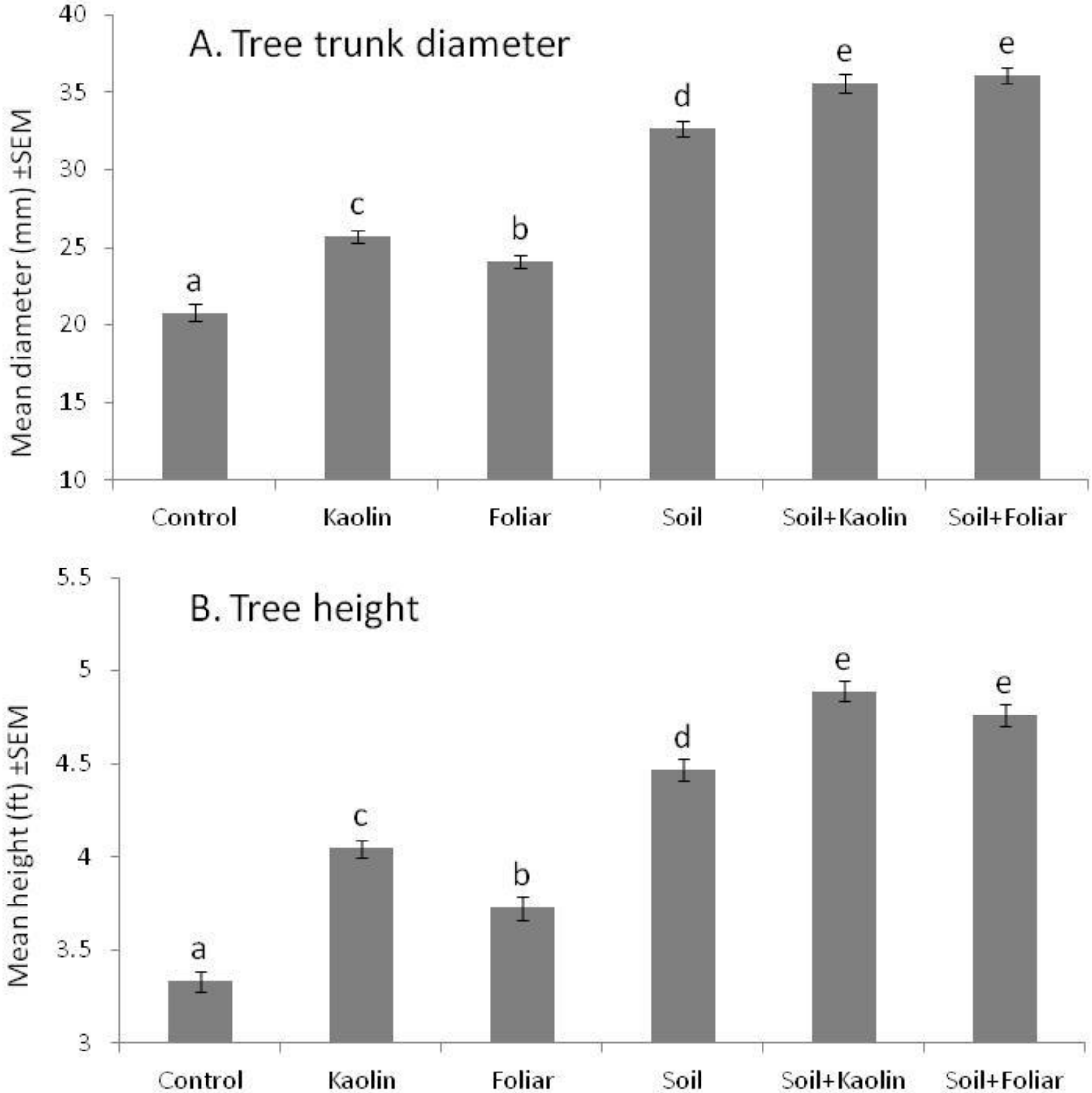

Figure 3-7. Mean (±SEM) diameter and height of experimental trees on different *D. citri* control programs 2 yr after the initial planting. Means labeled with same letter are not significantly different (α=0.05, Fisher's LSD).

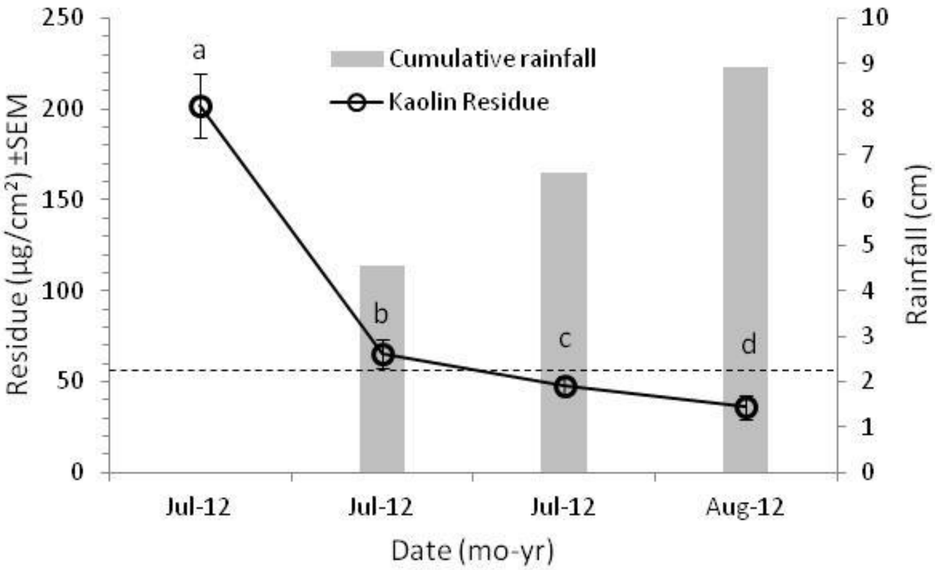

Figure 3-8. Mean (±SEM) mass of dislodgeable kaolin particles on leaf surfaces and cumulative amount of rainfall (based on the data from Florida Automated Weather Network, location: Avalon). Broken line drawn near 56 $\mu g/cm^2$ residue level, above which kaolin showed repellency against *D. citri* in previous laboratory choice study. Means labeled with same letter are not significantly different (α=0.05, Fisher's LSD).

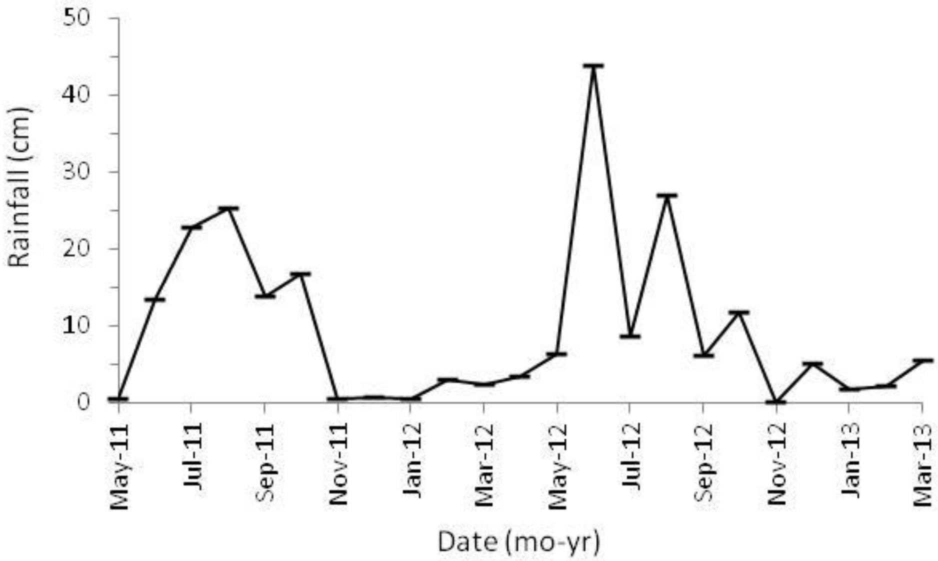

Figure 3-9. Monthly cumulative rainfall obtained from Florida Automated Weather Network (location: Avalon).

CHAPTER 4
IMPACT OF RECURRING INFESTATION AND INOCULATION OF *CANDIDATUS* LIBERIBACTER ASIATICUS BY ASIAN CITRU PSYLLID (HEMIPTERA: LIVIIDAE) ON CITRUS TREE VIGOR

Huanglongbing (HLB), also known as citrus greening disease, is a devastating disease of citrus worldwide causing canopy thinning, premature fruit drop, and twig dieback that result in a yield loss and eventual death of the infected trees (Gottwald 2007). The Asian citrus psyllid, *Diaphorina citri* Kuwayama, transmits Liberibacter species that putatively cause HLB thus considered a serious pest of citrus. When first detected in Florida (Halbert 1998) however, *D. citri* was regarded as a minor pest, because the status of HLB in Florida was unknown, and direct feeding only caused limited leaf damage (e.g., leaf curl and notch) that did not require intensive control measures (Halbert and Manjunath 2004). Such condition changed completely when HLB and its pathogen *Candidatus* Liberibacter asiaticus (*C.* Las) was discovered in Florida (Halbert 2005, Bové 2006), then after *D. citri* became the most important pest of Florida citrus because of its role as a vector. Strategies recommended for HLB management consist mainly of establishing clean nursery production, psyllid vector control using insecticides, and removal of infected trees to reduce inoculum sources (Rogers et al. 2013). In compliance, Florida mandated psyllid-proof enclosure and HLB free certification for budwood sources and nursery productions. Also, regional suppression of *D. citri* populations is being coordinated through Citrus Health Management Areas (CHMA) (Rogers et al. 2013). However, many growers are reluctant to remove the infected yet productive trees because mature trees can still survive for a long time.

During the 7 yr period since the first detection of *D. citri* in 1998 until discovery of HLB in 2005, no rigorous control measures were in place for the psyllid vector thus it is generally believed that HLB had already spread throughout the state in citrus growing regions by the time of first discovery (Spann et al. 2011). In fact, when statewide inspections began, *D. citri* and *C.* Las were wide spread that some groves already showed high infection levels. For these groves, removing the infected trees meant losing the majority of yet productive trees or in some cases losing the entire grove, which was not economically feasible. Such circumstances led a commercial citrus producer to keep the infected trees, and try enhancing the nutritional program as an alternative to tree removal (Giles 2011). The symptoms of HLB in infected trees are thought to be associated with nutritional deficiencies that result from restrictions on nutrient uptake, transport, and metabolism induced by Liberibacter infection. Thus, nutrient applications are aimed at recovering these nutritional deficiencies, and prolong tree health and productivity.

Enhanced nutritional program strategy is currently being practiced by many citrus growers in Florida showing positive results of improved tree condition and continued productivity on HLB infected trees. However there exists a major concern for the strategy that the growers may cut back on *D. citri* control efforts. The goal of vector control is to reduce disease spread but if majority of the trees are already infected, growers may feel no need. Cutting back on vector control can facilitate regional increase in *D. citri*, which is counterproductive to established CHMA efforts, and consequently allowing *D. citri* to repeatedly inoculate *C.* Las may hasten the decline of infected trees negating the rehabilitation efforts by nutritional program. Therefore in this study, the impact of recurring *D. citri* infestation and Las inoculation on tree vigor was investigated by comparing the leaf area of two representative branches, tree trunk diameter, height, and canopy volume of Valencia orange trees given different levels of *D. citri* infestation and Las inoculation.

Materials and Methods

Experimental Site and Field Cage

A field trial was conducted at the University of Florida Citrus Research and Education Center (UF CREC) experimental grove (North 40, Lake Alfred, FL). Screen cages (2.74 × 2.74 × 2.74 m) were constructed in the summer of 2010, which consisted of four vertical posts (3.66 m, 4×4 lumbers) that were buried 0.91 m into the ground with concrete base, and four horizontal bars (2.74 m, 2×4 lumbers) that were nailed at the top of each side to secure the posts. The lumber frame was covered with outdoor grade screen material that was psyllid proof. Screen materials were left to drape down to the ground, so the bottom part could be sealed by burying into the soil except that one side was left not buried in order to gain access to inside. A Valencia orange tree, *Citrus sinensis*, was planted in each cage with micro sprinkler irrigation and slow release granular fertilizer.

Insect Material

D. citri were collected in the summer of 2011 from a laboratory colony (UF CREC) that reared psyllids on potted curry leaf trees, *Murraya* k*oenigii*, with no pre-exposure to HLB infected plants. This population was free of *C.* Las, hence referred as clean psyllids and was used to infest the experimental trees without inoculating *C.* Las. For infestation and *C.* Las inoculation, *D. citri* were collected in the summer of 2011 and 2012 mostly from a grove highly infected with HLB (Conserv II, Winter Garden, FL) with small addition from a laboratory colony (UF CREC) that reared psyllids on HLB infected plants. These populations were estimated to contain bacterialiferous psyllids, and were referred as the field collected.

***D. citri* Infestation and Inoculation of *C.* Las**

D. citri were sleeve caged onto the branches of the experimental trees or were freely released inside the field cages for infestation and *C.* Las inoculation. The treatments were applied 1 yr after the initial planting of the experimental trees, and consisted of; 1) untreated control with no *D. citri* infestation, 2) sleeve caging of the field collected *D. citri* one time for infestation and *C. Las* inoculation, 3) sleeve caging of the field collected *D. citri* multiple times for infestation and *C.* Las inoculation, 4) release of clean *D. citri* freely inside the field cage for continuous infestation but not *C.* Las inoculation, and 5) release of the field collected *D. citri* freely inside the field cage for continuous *D. citri* infestation and *C.* Las inoculation. For treatment 1, no *D. citri* were released into the cages and trees were kept psyllid free. For treatments 2 and 3, 100 *D. citri* were sleeve caged for a 2 wk inoculation access period. *D. citri* were sleeve caged only once in case of treatment 2, while it was done four times over the course of 2 yr period in treatment 3. After removing the sleeve cages, some trees were observed with *D. citri* infestation possibly from those that escaped. These trees were sprayed with fenpropathrin (Danitol 2.4 EC) to ensure that no further *D. citri* infestation or Las inoculation could happen. For treatments 4 and 5, 100 *D. citri* were released freely inside each field cage in attempt to establish a continuous psyllid colony. In case of treatment 4, clean *D. citri* were released for continued infestation only, while field collected were released in treatment 5 for continued infestation and *C.* Las inoculation. In 2012, augmentative releases of 100 *D. citri* were done in the field cages where only few psyllids were observed scarcely.

D. citri Recapture and Sub-sampling

D. citri that were sleeve caged or freely released were recaptured or sub-sampled respectively for DNA extraction and PCR detection of *C.* Las to determine if the psyllids in fact contained *C.* Las, hence bacterialiferous. Sleeve cages were enclosed in plastic bags, and then the bags were filled with CO_2 to knock down *D. citri* for recapture. In 2011 (treatments 2 and 3), up to 10 live *D. citri* were recaptured from each sleeve cage; while in 2012 (treatment 3 only), attempts were made to recapture all *D. citri* from sleeve cages whether dead or alive. For treatments 4 and 5 where *D. citri* colonies were established in the field cages, a subsample of 100 psyllids was collected from each cage in 2012 after the augmentative releases. The recaptured *D. citri* were individually processed, while sub-sampled *D. citri* were processed in pooled samples of 10 psyllids. The presence of *C.* Las in *D.* citri was determined by DNA extraction and PCR detection methods as described in Pelz-Stelinski et al. (2010), which utilize 16S rDNA-based TaqMan primer-probe sets designed by Li et al. (2006). Briefly, individual or pooled *D. citri* samples were placed in 1.5 ml microcentrifuge tubes, and homogenized using sterile mortars in a buffer solution (QIAGEN, Valencia, CA), and then lysed overnight at 56°C in a hybridization oven (model 136400, Boekel Scientific, Feasterville, PA). Insect DNA was extracted from the homogenized samples using DNeasy Blood & Tissue kit (QIAGEN) according to manufacturer’s Animal Tissues Spin-Column protocol. The PCR amplification of *C.* Las-specific sequence was conducted using ABI 7500 Real-Time PCR System (Applied Biosystems, Foster city, CA) to determine its presence in extracted insect DNA. In conjunction, amplification reactions also contained primer and probe sets targeting insect wingless (Wg) gene regions for internal control (Thao et al. 2000). Duplicate reactions were conducted per DNA sample, and each PCR run included positive and negative controls.

HLB Infection Status

The experimental trees were sampled for DNA extraction and PCR detection to determine the presence of *C.* Las. First sampling was done 1 yr after initial treatments were applied and every 3 mo thereafter. Leaf samples were collected by taking 4 random leaves from each tree. The presence of *C.* Las in sampled leaf tissue was determined by DNA extraction and PCR detection methods as described in Pelz-Stelinski et al. (2010). Briefly, leaf petiole and midvein were separated from collected samples, and cut into small pieces. Then, subsamples of 100 mg were placed in 1.5 ml microcentrifuge tubes containing a 5 mm stainless steel bead, and ground using a bead mill (Tissue-Lyzer II, QIAGEN) under liquid nitrogen for 1 min at 30 Hz/s. Plant DNA was extracted from the ground leaf tissues using DNeasy Plant kit (QIAGEN) according to manufacturer’s Mini protocol. The PCR amplification of *C.* Las-specific sequence was conducted using ABI 7500 Real-Time PCR System (Applied Biosystems, Foster city, CA) to determine its presence in extracted plant DNA. In conjunction, amplification reactions also contained primer and probe sets targeting citrus cytochrome oxidase (Cox) gene regions for internal control (Li et al. 2006). Duplicate reactions were conducted per DNA sample, and each PCR run included positive and negative controls.

Tree Vigor Assessment

Leaf area of two representative branches, trunk diameter, height, and canopy volume of the experimental trees were measured or estimated to assess tree vigor and to compare the impact of different levels of exposure to clean or field collected *D. citri*. Leaf area of two representative branches of each tree was estimated by estimating the total leaf areas of all the shoots attached to the branches. Leaf area of each shoot was estimated using linear regression ($y=1.640x+0.099$; $r^2=0.8508$) produced by Spann and Heerema (2010), which describes the relationship between total shoot leaf area (TSLA, the sum of the areas of all the leaves on each shoot, y) and product (x) of the number of leaves on the shoot (LVS) × the length of the longest leaf on the shoot (LNTH). Tree trunk diameter was measured 5 cm above and below the bud union, and the average was calculated. Tree height was measured using 1.52 m measuring stick or measuring tape if exceeding 1.52 m. Canopy volume was calculated by regarding tree canopy shape as half ellipsoid as described in Morse and Robertson (1987) (Figure 1). Tree height, height to canopy, north-south lateral canopy length (down the row), and east-west lateral canopy length (across the row) were measured, and then the canopy volume was calculated using the formula for one half of the ellipsoid volume (volume = $4/6 \times \pi \times a \times b \times c$).

Statistical Analysis

A tree inside each field screen cage was considered a replicate, and treatments were arranged in a completely randomized design with 10 replicates per treatment initially. However, some of cages from different treatment groups were observed with *D. citri* cadavers infected with unidentified naturally occurring entomopathogenic fungus. Therefore, one cage/replicate was excluded from each treatment, and only 9 replicates were used for statistical analysis. Leaf area of two representative branches, trunk diameter, height, and canopy volume of the experimental trees were compared using ANOVA with Fisher's LSD ($\alpha=0.05$) (PROC ANOVA, SAS Institute 2009).

Results

Bacterialiferous *D. citri*

The recaptured or sub-sampled *D. citri* were processed through DNA extraction and PCR detection of *C.* Las to determine if the psyllids were in fact bacterialiferous. In 2011, a total of 154 live *D. citri* were recaptured from the sleeve cages (treatments 2 and 3), of which 77 (50%) were bacterialiferous. In 2012, a total of 882 *D. citri* (dead and alive) were recaptured from the sleeve cages (treatment 3 only), of which 148 (17%) were bacterialiferous. In case of the sub-sampled *D. citri* that were tested in pools of 10 insects, there were no positive results in treatment 4 where clean psyllids were released, while subsamples from two out of nine field cages showed 50 and 100% positives in treatment 5 where field collected psyllids were released.

HLB Infection and Tree Vigor

The screening of the experimental trees using PCR detection method showed no presence of *C.* Las in every leaf sampled from treatments 1, 2, and 4, while *C.* Las was detected in 22% (2 out of 9 trees) of the leaf samples from both treatments 3 and 5. As for the tree vigor, only treatment 5 showed significant reduction in trunk diameter, height, and canopy volume as compared to the untreated control (Figure 2). In case of the leaf area estimation, all treatments showed significant reductions compared to the untreated control (Figure 2). A general trend of decreased leaf area was observed as *D. citri* infestation and *C.* Las inoculation levels increased.

Discussion

The proportion of bacterialiferous *D. citri* from the field collected populations was a lot lower than expected. Although 50% of live *D. citri* recaptured in 2011 were bacterialiferous, only about 9% of the psyllids that were sleeve caged were recaptured, which does not represent the total population. Almost every *D. citri* that were sleeve caged were recaptured in 2012; however, only 17% of them were bacterialiferous. Consistently, only 4 out of 27 trees that were exposed to field collected *D. citri* were infected. None of the experimental trees that were exposed only once to the field collected *D. citri* were infected. It may be that these trees are infected with very low *C.* Las titer, which cannot be detected using the PCR method. In fact, canopy thinning was the most evident symptom observed on treated trees. Canopy volume estimation depends mostly on the length of branches instead of individual leaves. In contrast, leaf area estimation depends more on the number and sizes of individual leaves. Such aspects seemed to have resulted in leaf area estimation to show more significant reductions compared to other measurements used for assessing tree vigor. However, PCR results could not conclusively support that every tree exposed to the field collected *D. citri* were inoculated with *C.* Las. Thus, a trend of decreased tree vigor might have been the result of *D. citri* infestation alone particularly when there is no difference between treatments 4 and 5.

A recent field trial by Gottwald et al. (2012) was conducted where the majority of the experimental trees were infected with HLB, thus *D. citri* control was discontinued. Although the main focus of that study was not vector control, in its absence, results showed no significant improvement in fruit yield from trees treated with nutritionals compared to untreated control trees. Such results may suggest the importance of vector control even after adapting nutritional programs as an alternative to tree removal. In fact, successful growers who adapted the nutritional programs still emphasize psyllid control as one of the crucial aspect of their HLB management programs (Giles 2011, 2013). In this study, different levels of recurring *D. citri* infestation and *C.* Las inoculation were investigated for their impact on tree vigor. Absence or discontinuing vector control would result in circumstances similar to the treatment 5 of this study where repeated infestation by *D. citri* would occur. The results showed that in such circumstances regardless of *C.* Las inoculation, tree vigor is significantly reduced suggesting that tree decline is being hastened by recurring *D. citri* infestation. Thus, it would be important to continue *D. citri* control efforts even after a grower adapts a nutritional program as an alternative to tree removal.

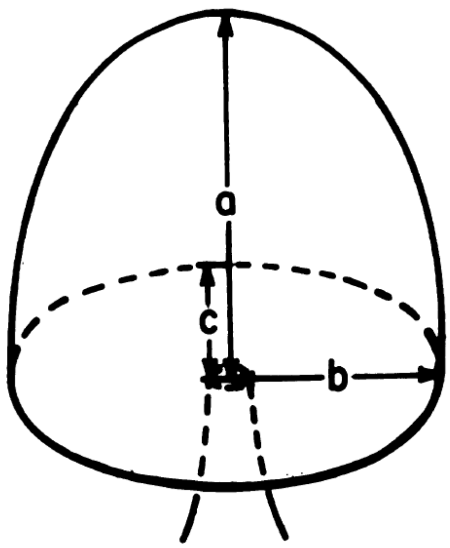

Figure 4-1. Schematic drawing of tree canopy from Morse and Robertson (1987), which approximates the canopy shape as a half ellipsoid. a = canopy height, b = skirt radius down the row, c = skirt radius across the row.

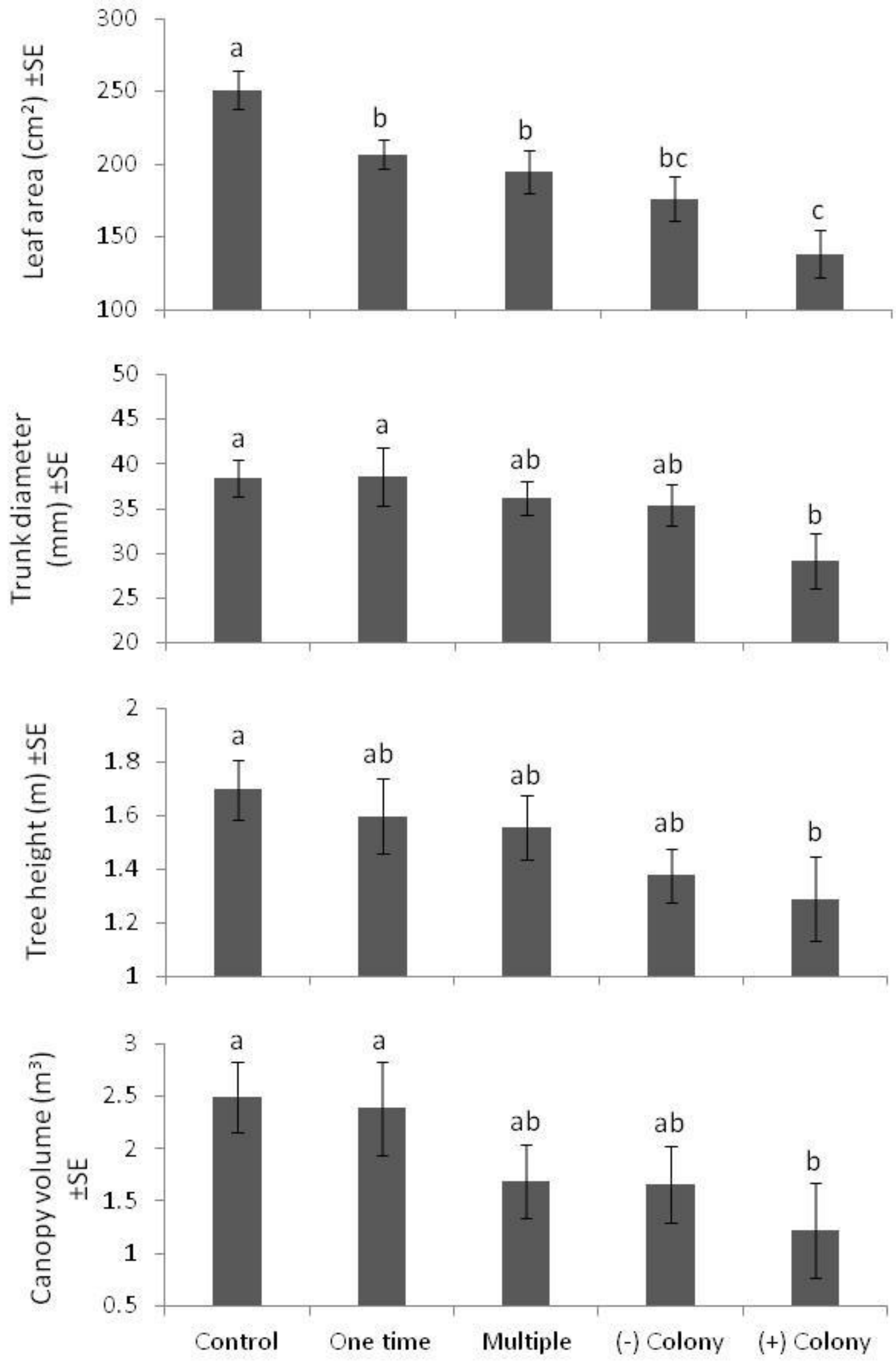

Figure 4-2. Mean (±SE) leaf area of two representative branches, trunk diameter, height, and canopy volume of the experimental trees with respect to different treatments. Measurements were taken on January 2013, about 2.5 yr after initial planting.

CHAPTER 5
CONCLUSION

Almost 9 yr after the initial discovery of citrus greening disease (Huanglongbing, HLB) in Florida, the Florida citrus industry is experiencing a severe damage and rapidly losing the bearing acreage. For 2013-14 growing season, the USDA forecasts the lowest production of Florida citrus since the freeze-affected 1989-1990 season (USDA-NASS 2013b). In order to maintain citrus production, new plantings must be brought into production to replace the infected trees that are quickly becoming unproductive. Coca-Cola Co. recently announced that it will invest $2 billion to help growers plant 25,000 acres of orange trees. The biggest challenge is whether the growers will be able to protect these new plantings from HLB infection. Although there are studies investigating potential ways to cure the HLB, none of them are available right now and the growers are left with what is currently available to protect the new plantings.

Frequent use of the insecticides to control the Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Liviidae), which transmits the presumed causal agent of HLB, *Candidatus* Liberibacter asiaticus (*C.* Las), is the main if not the only strategy currently available for protecting young non-bearing citrus trees. However, protecting young non-bearing trees is very difficult given a number of circumstances. *D. citri* exclusively reproduce on newly developing shoots (flushes), hence are known to be attracted to the citrus trees that are flushing. In case of mature trees, there are only about 2-3 major flushing periods in a growing season. However, young trees continuously flush multiple times throughout the year, hence more attractive of *D. citri* compared to the mature trees. Consequently, young trees are considered vulnerable throughout the year thus in need of a continuous insecticide coverage especially when even a single *D. citri* is capable of transmitting *C.*

Las. Therefore, it is necessary to adjust from the passive approach of pest management where insecticides were applied after the monitored pest population exceeds the threshold level to more proactive approach where insecticide coverage is maintained throughout the growing season. This transition to more frequent use of insecticides and maintaining a longer duration of coverage may bring adverse impacts in a long run, but the devastating effects of HLB necessitates and justifies such intensive measures.

The most effective insecticides are broad spectrum nerve poisons in organophosphate, pyrethroid, neonicotinoid and spinosyn class of chemistries. The neonicotinoids in particular are the most useful tool because they can be applied in soil for a plant uptake of the active ingredient, and then persist systemically within the plant for a longer duration of time compared to foliar applied insecticides. Such characteristics also reduce exposure to beneficial insects that are non-phytophagous in general. There are a number of different products available for use in citrus so application regimes can be developed to achieve a season-long residual activity. However, all products share a single mode of action thus increase the potential for a resistance development. The results from a field trial that evaluated a season-long application regime of neonicotinoid soil drenches (Chapter 3) showed how rapidly in a matter of months *D. citri* could develop resistance. Exposure to a single mode of action existing throughout the season and a higher chance of exposure to a sublethal level during plant uptake and fade out periods are some of the important aspects to consider when using soil applied systemic neonicotinoids. Additional application of insecticides with different modes of action must be implemented in order to prevent resistance development and maintain the efficacy of the neonicotinoids, which are the backbone of current young tree protection.

Foliar applied insecticides provide a quick suppression of *D. citri* population, but residual activity is generally short in a matter of weeks. It might actually be shorter than what the field data suggests. Typical field efficacy trials monitor pest population for a certain period after an insecticide spray and suggest the duration of efficacy based on how long it takes for a subsequent pest population to infest the treated plots. Such method however does not take into account the repopulation period. Phytophagous insects generally need a time to search for the host, and examine the acceptance of the host in order for an infestation to occur. This period of searching and tasting is not accounted for in field efficacy trials. More accurate investigation of the residue profiles using chemical analysis techniques may be necessary especially in Florida where dynamic weather conditions involving thunderstorms and heavy rainfalls greatly influence the longevity of insecticide residues. Currently, up to 12 sprays are being applied by the growers for *D. citri* control. Most of these would be conventional broad spectrum nerve poisons, continued frequent use of which entail adverse impacts thus alternative strategies are recommended to reduce growers' reliance on these insecticides.

Kaolin particle film is one of the alternatives that showed great potential as shown in chapter 2. Application can be done using traditional handgun or air-blast sprayer, although nozzles should be inspected more often for clogging. Interference on the behaviors associated with *D. citri* feeding in particular, would be useful to reduce *C.* Las transmission as demonstrated in the field trial (chapter 3) Hall et al. (2007) reported that the cost of kaolin application is less expensive than typical conventional insecticide applications and is comparable to that of oil sprays. Kaolin may also persist longer during dry season compared to other short lived organophosphate foliar applied insecticides. More importantly, kaolin residue level can be estimated visually. The activity of kaolin particle film is through a white physical coating on leaf surfaces that prevent *D. citri* settling. Although residue analysis technique was developed to estimate the baseline residue level, growers should be able to assess quite easily whether the plant canopy is coated in white colored kaolin particles or not. Inexpensive option that can provide similar level of *D. citri* suppression as conventional foliar sprays along with feeding deterrent activity would be the most beneficial aspects of kaolin particle film to the growers.

In conclusion, soil applied systemic neonicotinoids in combination with frequent applications of kaolin particle film to maintain physical coating on leaf surfaces along with occasional applications of broad spectrum foliar sprays as resistance management for neonicotinoids would collectively be the best protection based on the tools that are currently available.

LIST OF REFERENCES

Arevalo H.A., J.A. Qureshi, and P.A. Stansly. 2011. Sampling for Asian citrus spyllid (ACP) in Florida citrus groves. Entomology and Nematology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. ENY-857 (IN867). (http://edis.ifas.ufl.edu/in867).

Backus, E. A., A. R. Cline, M. R. Ellerseick, and M. S. Serrano. 2007. *Lygus hesperus* (Hemiptera: Miridae) feeding on cotton: New methods and parameters for analysis of nonsequential electrical penetration graph data. Ann. Entomol. Soc. Am. 100: 296-310.

Bassanezi, R.B., L.H. Montesino, N. Gimenes-Fernandes, P.T. Yamamoto, T.R. Gottwald, L Amorim, and A. Bergamin Filho. 2013. Efficacy of area-wide inoculum reduction and vector control on temporal progress of huanglongbing in young sweet orange plantings. Plant Disease 97:789-796.

Belasque, J., R.B. Bassanezi, P.T. Yamamoto, A.J. Ayres, A. Tachibana, A.R. Violante, A. Tank Jr., F. Di Giorgi, F.E.A. Tersi, G.M. Menezes, J. Dragone, R.H. Jank Jr., and J.M. Bove. 2010. Lessons from Huanglongbing management in Sao Paulo State, Brazil. J. Plant Pathol. 92(2):285-302.

Bonani, J.P., A. Fereres, E. Garzo, M.P. Miranda, B. Appezzato-Da-Gloria, and J.R.S. Lopes. 2010. Characterization of electrical penetration graphs of the Asian citrus psyllid, *Diaphorian citri*, in sweet orange seedlings. Entomol. Exp. Appl. 134: 35-49.

Bové, J.M. 2006. Huanglongbing: a destructive, newly emerging, century-old disease of citrus. J. Plant Pathol. 88: 7-37.

Brlansky, R.H., M.M. Dewdney, M.E. Rogers. 2013. Huanglongbing (citrus greening). pp. 105-106. *In* Rogers, M.E., M.M. Dewdney, and T.M. Spann (eds.) 2013 Florida citrus pest management guide. University of Florida, Gainesville, FL.

Childers C.C., and D.S. Achor. 1991. Feeding and oviposition injury to flowers and developing floral buds of 'Navel' orange by *Frankliniella bispinosa* (Thysanoptera: Thripidae) in Florida. Ann. Entomol. Soc. Am. 84(3): 272-282.

Davies, F.S., and L.K. Jackson. 2009. Citrus Growing in Florida 5th ed. University Press of Florida, Gainesville, FL.

Dewdney, M.M., and J.H. Graham. 2013. Citrus canker. pp. 101-104. *In* Rogers, M.E., M.M. Dewdney, and T.M. Spann (eds.) 2013 Florida citrus pest management guide. University of Florida, Gainesville, FL.

(FAOSTAT) The Statistics Division of the Food and Agriculture Organization of the United Nations. 2011. Browse Data. (http://faostat3.fao.org/faostat-gateway/go/to/browse/Q/*/E)

(FDACS-DPI) Florida Department of Agriculture and Consumer Services – Division of Plant Industry. 2011. Sections (TRS) positive for Huanglongbing (HLB, Citrus Greening) in Florida. FDACS-DPI. Tallahassee, FL. (http://freshfromflorida.ssd.thinkcreativeinternal.net/content/download/24035/486944/StatewidePositiveHLBSections.pdf)

(FDACS-DPI) Florida Department of Agriculture and Consumer Services-Division of Plant Industry. 2012. Comprehensive report on citrus canker in Florida. (http://www.freshfromflorida.com/content/download/24084/487233/cankerflorida.pdf)

Gatineau, F., F. Bonnot, T.H. Yen, D.H. Tuan, N.D. Tuyen, and N.T. Truc. 2010. Effects of imidacloprid and fenobucarb on the dynamics of the psyllid *Diaphorian citri* Kuwayama and on the incidence of *Candidatus* Liberibacter asiaticus. Fruits 65:209-220.

Giles, F. 2011. Daring to be different. Florida Grower 104(6): 8-10.

Giles, F. 2013. HLB: what we know today. (http://www.growingproduce.com/citrus/hlb-what-we-know-today/)

Glenn, D.M., and G. Puterka. 2005. Particle films: a new technology for agriculture, pp. 1-45. *In* J. Janick (ed.), Horticultural reviews, vol. 31. Wiley, Hoboken, NJ.

Glenn, D.M., G. Puterka, T. Vanderzwet, R.E. Byers, and C. Feldhake. 1999. Hydrophobic particle films: A new paradigm for suppression of arthropod pests and plant diseases. J. Econ. Entomol. 92(4): 759-771.

Gottwald, T.R. 2010. Current epidemiological understanding of citrus huanglongbing. Annu. Rev. Phytopathol. 48:119-139.

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

Gottwald, T.R., J.V. da Graa, and R. B. Bassanezi. 2007. Citrus Huanglongbing: the pathogen and its impact. Plant Health Prog. (http://www.apsnet.org/publications/apsnetfeatures/Pages/HuanglongbingImpact.aspx).

Grafton-Cardwell, E.E., L.L. Stelinski, and P.A. Stansly. 2013. Biology and management of Asian citrus psyllid, vector of the Huanglongbing pathogens. Annu. Rev. Entomol. 58:413-432.

Halbert, S.E. 1998. Entomology Section. pp. 6-7. *In* N.C. Coile (ed.), Tri-ology 37(3): 6-7.

Halbert, S.E. 2005. The discovery of huanglongbing in Florida. p. 50. *In* T.R. Gottwald, W.N.Dixon, J.H. Graham, and P. Berger (eds.), Proceedings, Second International Citrus Canker and Huanglongbing Research Workshop. 7-11 November 2005, Orlando, FL.

Halbert, S.E. and K.L. Manjunath. 2004. Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease in citrus: A literatures review and assessment of risk in Florida. Fla. Entomol. 87(3):935-941.

Hall, D.G., and L.G. Albrigo. 2007. Estimating the relative abundance of flush shoots in citrus with implications on monitoring insect associated with flush. HortScience 42(2):364-368.

Hall, D.G., and R. Nguyen. 2010. Toxicity of pesticides to *Tamarixia radiata*, a parasitoid of the Asian citrus psyllid. BioControl 55(5): 601-611.

Hall, D.G., M.G. Hent, and R.C. Adair, Jr. 2008. Population ecology and phenology of *Diaphorina citri* (Hemiptera: Psyllidae) in two Florida citrus groves. Environ. Entomol. 37(4):914-924.

Hall, D.G., S.L. Lapointe, and E.J. Wenninger. 2007. Effects of a particle film on biology and behavior of Diaphorina citri (Hemiptera: Psyllidae) and its infestation in citrus. J. Econ. Entomol. 100(3): 847-854.

Hall, D.G., T.R. Gottwald, E. Stover, and A.C. Beattie. 2013. Evaluation of management programs for protecting young citrus plantings from Huanglongbing. HortScience 48(3):330-337.

Hoy, M.A., and R. Nguyen. 2000. Classical biological control of Asian citrus psylla. Citrus Industry 81(12): 48-50.

Hoy, M.A., R. Nguyen, and A. Jeyaprakash. 1999. Classical biological control of the Asian citrus psylla-release of *Tamarixia radiata.* Citrus Industry 80(9): 20-22.

Inoue, H., H, Ohnishi, T. Ito, K. Tomimura, S. Miyata, T. Iwanami, and W. Ashihara. 2009. Enhanced proliferation and efficient transmission of *Candidatus* Liberibacter asiaticus by adult *Diaphorina citri* after acquisition feeding in the nymphal stage. Ann. Appl. Biol. 155:29-36.

Jifon, J.L., and J.P. Syvertsen. 2003. Kaolin particle film applications can increase photosynthesis and water use efficiency of ‘Ruby Red’ grapefruit leaves. J. Amer. Soc. Hort. Sci. 128(1): 107-112.

Lapointe, S.L., C.L. McKenzie, and D.G. Hall. 2006. Reduced oviposition by *Diaprepes abbreviatus* (Coleoptera: Curculionidae) and growth enhancement of citrus by surround particle film. J. Econ. Entomol 99(1): 109-116.

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

Liu, Y.H., and J.H. Tsai. 2000. Effects of temperature on biology and life table parameters of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae). Ann. Appl. Biol. 137(3):201-206.

Madden, L.V., G. Hughes, and F. van den Bosch. 2007. Temporal analysis I: quantifying and comparing epidemics. pp. 92-108. *In* L.V. Madden, G. Hughes, and F. van den Bosch (eds.), The study of plant disease epidemics. American Phytopathological Society Press, St. Paul, MN.

McClean, A.P.D., and R.E. Schwarz. 1970. Greening of blotchy-mottle disease in citrus. *Phytophylactica* 2:177-194.

McKenzie, C.L., S.L. Lapointe, W.B. Hunter, and G.J. Puterka. 2002. Efficacy of Surround for control of Asian citrus psyllid on citrus, 2000. Arthropod Manage. Tests 27: D8.

Mead, F.W. 1977. The Asiatic citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae), pp. 1-44. Entomology Circular 180. Florida Department of Agriculture and Consumer Services, Division of Plan Industry, Gainesville, FL.

Miller, W.P. and D.M. Miller. 1987. A micro-pipette method for soil mechanical analysis. Commun. in Soil Sci. Plant Anal. 18(1): 1-15.

Morris, A., and R. Muraro. 2008. Economic evaluation of citrus greening management and control strategies. Extension Digital Information Source, FE 712, Food and Resource Economics Department, University of Florida. (http://www.crec.ifas.ufl.edu/extension/greening/PDF/EconomicEvaluation.pdf).

Morris, R.A., C. Erick, and M. Estes. 2009. Survey to estimate the rate of greening and canker infection in Florida citrus groves. Citrus Industry 90(10):16-18.

Morse, J.G., and C.A. Robertson. 1987. Calculating canopy area of citrus trees and surface area of fruits. Fla. Entomol. 70(1):168-171.

(NRC) National Research Council 2010. Strategic planning for the Florida citrus industry: addressing citrus greening. National Academies Press, Washington, D.C.

Pelz-Stelinski, K.S., R.H. Brlansky, T.A. Ebert, and M.E. Rogers. 2010. Transmission parameters for *Candidatus* Liberibacter asiaticus by Asian Citrus Psyllid (Hemiptera: Psyllidae). J. Econ. Entomol.103 (5):1531-1541.

Peng, L., J.T. Trumble, J.E. Munyaneza, and T. Liu. 2011. Repellency of a kaolin particle to potato psyllid, *Bactericera cockerelli* (Hemiptera: Psyllidae), on tomato under laboratory and field conditions. Pest Manag. Sci. 67(7): 815-824.

Puterka, G.J., D.M. Glenn, and R.C. Pluta. 2005. Action of particle films on the biology and behavior of pear psylla (Homoptera: Psyllidae). J. Econ. Entomol 98(6): 2079-2088.

Rogers, M.E. 2012. Protection of young trees from the Asian citrus psyllid and HLB. Citrus Industry 93:10-15.

Rogers, M.E., P.A. Stansly, and L.L. Stelinski. 2013. Asian citrus psyllid and citrus leafminer. pp. 39-44. *In* Rogers, M.E., M.M. Dewdney, and T.M. Spann (eds.) 2013 Florida citrus pest management guide. University of Florida, Gainesville, FL.

Saour, G. 2005. Efficacy of kaolin particle film and selected synthetic insecticides against pistachio psyllid Agonoscena targionii (Homoptera: Psyllidae) infestation. Crop Protection. 24: 711-717.

SAS Institue 2009. SAS/STAT® 9.2 User's Guide, Second Edition. Cary, NC: SAS Institute Inc.

Serikawa, R.H., E.A. Backus, and M.E. Rogers. 2012. Effects of soil-applied imidacloprid on Asian citrus psyllid (Hemiptera: Psyllidae) feeding behavior. J. Econ. Entomol. 105(5): 1492-1502.

Spann, T.M., A.W. Schumann, R. Rouse, and R. Ebel. 2011. Foliar nutrition for HLB. Citrus Industry 92(6):6-10.

Spann, T.M., and R.J. Heerema. 2010. A simple method for non-destructive estimation of total shoot leaf area in tree fruit crops. Scientia Horticulturae. 125:528-533.

Spreen, T.H. 2012. That shrinking feeling. Florida Grower 105:22.

Teixeira, D.C., C. Saillard, S. Eveillard, J.L. Danet, P.I. da Costa, A.J. Ayres, and J. Bové. 2005. '*Candidatus* Liberibacter americanus', associated with citrus huanglongbing (greening disease) in Sao Paulo State, Brazil. Int. J. Syst. Evol. Microbiol. 55(5):1857-1862.

Thao, M.L., N.A. Moran, P. Abbot, E.B. Brennan, D.H. Burckhardt, and P. Baumann. 2000. Cospeciation of psyllids and their primary prokaryotic endosymbionts. Appl. Environ. Microbiol. 66: 2898-2905.

Tiwari, S., N. Killiny, and L.L. Stelinski. 2013. Dynamic insecticide susceptibility changes in Florida populations of *Diaphorina citri* (Hemiptera: Psyllidae). J. Econ. Entomol 106(1): 393-399.

Tiwari, S., R.S. Mann, M.E. Rogers, and L.L. Stelinski. 2011. Insecticide resistance in field populations of Asian citrus psyllid in Florida. Pest Manag. Sci. 67(10): 1258-1268.

(UNCTAD) United Nations Conference on Trade and Development. 2011. Description and technical characteristics. (http://www.unctad.info/en/Infocomm/Agricultural_Products/Citrus-fruit/Characteristics/Description-and-technical-cahracteristics/)

(USDA) U.S. Department of Agriculture. 2012. National quarantine citrus greening and Asian citrus psyllid. (http://www.aphis.usda.gov/plant_health/plant_pest_info/citrus_greening/downloads/pdf_files/nationalquarantinemap.pdf)

(USDA-NASS) U.S. Department of Agriculture – National Agricultural Statistical Service. 2013a. Florida Citrus Statistics 2011-2012. (http://www.nass.usda.gov/Statistics_by_State/Florida/Publications/Citrus/fcs/2011-12/fcs1112.pdf)

(USDA-NASS) U.S. Department of Agriculture – National Agricultural Statistical Service. 2013b. November forecast maturity test results and fruit size. (http://www.nass.usda.gov/Statistics_by_State/Florida/Publications/Citrus/cit/2013-14/cit1113.pdf)

von Luxburg, U., and V.H. Franz. 2009. A geometric approach to confidence sets for raios: Fieller's theorem, generalizations and bootstrap. Statistica Sinica 19:1095-1117.

Wise, J., and M. Whalon. 2009. A systems approach to IPM integration, ecological assessment and resistance management in tree fruit orchards. pp. 325-345. *In* I. Ishaaya, A. Horowitz (eds.), Biorational control of arthropod pests: application and resistance management. Springer Science+Business Media B.V., Dordrecht, Netherlands.

Wise, J.C., K. Kim, E.J. Hoffmann, C. Vandervoort, A. Gokce, and M.E. Whalon. 2007. Novel life stage targets against plum curculio, *Conotrachelus nenuphar* (Herbst), in apple integrated pest management. Pest Manag. Sci. 63:737-742.

Xu, C.F., Y.H. Xia, K.B. Li, and C. Ke. 1988. Further study of the transmission of citrus huanglongbing by a psyllid, *Diaphorina citri* Kuwayama. pp. 243-248. *In* L.W. Timmer, S.M. Garnsey and L. Navarro (eds.), Proceedings of the 10th Conference of International Organization of Citrus Virologists, 17-21 November 1986, Valencia. University of California, Riverside, CA.

BIOGRAPHICAL SKETCH

Ki Duk Kim was born in Seoul, South Korea. Ki came to the United States when he was 13 years old. He received B.S. and M.S. degrees at Michigan State University, and then came to the University of Florida for Ph.D. degree in entomology and nematology under Dr. Michael E. Rogers. His research experiences mostly involved integrated pest management in tree fruit systems.