ELECTRICAL PENETRATION GRAPH INVESTIGATIONS OF ASIAN CITRUS PSYLLID (*DIAPHORINA CITRI* KUWAYAMA) FEEDING BEHAVIOR: EFFECTS OF INSECTICIDES ON THE POTENTIAL TRANSMISSION OF *CANDIDATUS* LIBERIBACTER ASIATICUS

By

ROSANA HARUMI SERIKAWA

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2011

To my parents and my siblings

ACKNOWLEDGMENTS

I would like to thank the University of Florida, the Entomology and Nematology Department, the Citrus Research and Educational Center, Lynn Steinmetz Scholarship and Fellowship program, and the Citrus Research and Development Foundation for giving me the opportunity and the resources to finish this study. I would also like to thank my major advisor Dr. Michael E. Rogers; the opportunity, words of encouragement, advice and guidance, his support and enthusiasm made my PhD research a lot easier to be accomplished.

I thank all my supervisory committee members, Drs. Lukasz L. Stelinski (co-advisor), Ronald H. Brlansky, Heather J. McAuslane, and Susan E. Webb. Without the help of Drs. Stelinski and Brlansky and all the support necessary on this research, nothing would have being accomplished. I thank Drs. McAuslane and Webb for their support and introducing to me to the EPG technique, which was the whole topic of this dissertation.

I thank Dr. Elaine S. Backus, for all her enthusiasm and research advice; her expertise in the EPG technique helped me to improve my research techniques, my statistical analysis, and research conclusions. Without her I would not have finished my dissertation. I also thank Dr. Timothy A. Ebert, for his friendship and SAS expertise; Dr. Antonios E. Tsagarakis, for his friendship and unlimited psyllid supply; Daniela M. Okuma for being a friend and great assistant in helping me with the experiments and data analysis; Rhonda Schumann, Perciva Mariner, Dalia M. Shawer, Harry E. Anderson, and Guoping Liu for the friendship and great help in this project; Ian Jackson, Wendy Meyer and Angel Hoyte for helping me with the Faraday cages. Additionally, Tania F. Broisler, José Francisco Figueiredo, Jared Ali, Sara Hermann, Sylvia Morais de Souza, Tatiana D. Martinelli, Marina B. Arouca, Luciano Rocha, Fabiano Santinello, Nathalia Vieira, Luiz Henrique Falavigna, Pamela Fávero, André Giorgetti, Bruno Zeuli, and many other friends that passed through my life made my life a lot easier in the USA with their

love and friendship. Finally I would like to thank the most special people in my life: Gabriel Fachini, became my best friend and with all his love, friendship and patience, helped me format and edit my dissertation; my siblings, Marcel A. Serikawa and Simoni S. Serikawa, for their existence, for without them, my life would not have being so fun; and lastly my parents, Tomoko Nakaema Serikawa and Mitsuo Serikawa, with their love, support, and encouragement which made everything possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

ELECTRICAL PENETRATION GRAPH INVESTIGATIONS OF ASIAN CITRUS PSYLLID (*DIAPHORINA CITRI* KUWAYAMA) FEEDING BEHAVIOR: EFFECTS OF INSECTICIDES ON THE POTENTIAL TRANSMISSION OF *CANDIDATUS* LIBERIBACTER ASIATICUS

By

Rosana Harumi Serikawa

August 2011

Chair: Michael E. Rogers
Major: Entomology and Nematology

An Electrical Penetration Graph (EPG) monitor was used to study the feeding behaviors of *Diaphorina citri* Kuwayama, vector of *Candidatus* Liberibacter asiaticus, the presumed causal agent of Huanglongbing (HLB). Effects of insect gender, presence of light, and ability and duration of insecticides to disrupt *D. citri* feeding behaviors responsible for pathogen transmission were examined. Results showed that duration of phloem ingestion was significantly longer for female *D. citri* compared to males. Gender based analysis showed that despite previously being considered a phloem feeder, *D. citri* performs similar amounts of xylem and phloem feeding. Additionally, xylem feeding was more likely to occur during the day and phloem feeding during the night. Application of the soil-applied neonicotinoid insecticide imidacloprid disrupted *D. citri* phloem feeding behaviors suggesting that application of this product to young trees can reduce the likelihood that trees will succumb to HLB. In contrast, the soil-applied carbamate insecticide aldicarb did not disrupt *D. citri* phloem feeding behaviors. In fact, phloem ingestion by *D. citri* was increased on aldicarb-treated plants indicating that use of this product could potentially increase pathogen acquisition rates where aldicarb-treated diseased trees are present. The foliar-applied insecticides chlorpyrifos, fenpropathrin, and imidacloprid

disrupted *D. citri* phloem-feeding behaviors on recently treated plants suggesting that these products will prevent pathogen transmission prior to death of the insect. While application of spinetoram altered *D. citri* feeding behavior, phloem feeding was not disrupted suggesting that pathogen transmission may still occur prior to insect death. In contrast, spirotetramat did not significantly disrupt *D. citri* feeding and thus will not prevent pathogen transmission from occurring. In experiments examining duration of feeding disruption provided by foliar insecticide applications, imidacloprid provided the longest duration of feeding disruption (28 d), followed by fenpropathrin (21 d), and chlorpyrifos (1 d). These results demonstrate that in addition to reducing vector populations in the field, certain soil- and foliar-applied insecticides can prevent pathogen transmission from occurring prior to death of pathogen-carrying *D. citri*. Our findings are of immediate importance for Florida citrus growers who can use this information to improve their current HLB management programs.

CHAPTER 1
INTRODUCTION

Citrus is one of the most widely produced fruits in the world. In 2011, production of 79.1 million metric tons (MMT) of citrus are expected to be produced (USDA 2011). In the USA, 10.9 MMT was produced in the 2009/2010 growing season with citrus production in Florida accounting for 65% of that total; this was a 16% reduction compared to the previous season (USDA 2010a). In Florida, citrus grove acreage totaled 517,100 acres in the 2009/2010 growing season. This was a 13,800 acre reduction from the 2008-09 growing season and almost 33% lower than in 2000 (USDA 2010a). Twelve percent of the citrus losses since 2006 have been attributed to Huanglongbing (HLB) disease, bacterial citrus canker, cold temperatures and commercial development (USDA 2010b). In addition, the increased need for crop inputs to manage citrus diseases and associated arthropod pests has increased production costs making citrus less profitable. Consequently, some growers have become reluctant to replant citrus acreage lost to disease (Pollak and Perez 2008).

One of the main causes for the increased cost of citrus production is the recent introduction of Huanglongbing (HLB) disease. The causal agent of this disease in Florida is believed to be the bacterium *Candidatus* Liberibacter asiaticus. This pathogen was first present in south Florida in 2005 in dooryard trees (Halbert 2005). Since its initial discovery in Florida, HLB has been found in commercial citrus groves in all citrus growing areas of the state making eradication impossible.

In many parts of the world, this disease is referred to as Huanglongbing which translates to "yellow shoot" in Chinese based on one of the primary visual symptoms of the disease. In other parts of the world, it is also known as citrus greening disease, because it causes improper coloration of the fruit (Halbert and Manjunath 2004). Additional symptoms include discolored

and mottled leaves, yellowing of leaf veins, leaf and fruit drop, as well as small deformed fruit which have a bitter taste (Bove 2006). Thus, overall tree health and fruit production can be severely reduced by this disease.

The exact origin of HLB is unknown (Yang 2006); however, it was first described in southern Asia as early as 1919 (da Graca and Korsten 2004, Bove 2006). The causal putative agent of HLB was initially discovered with electron microscopy in 1970 (Bove 2006). The disease is thought to be caused by gram-negative bacterium and there are three known bacterial species thought to be responsible for this disease: *Candidatus* Liberibacter africanus (Laf), *Candidatus* Liberibacter asiaticus (Las) and *Candidatus* Liberibacter americanus (Lam) (Bove 2006). Laf is found in Africa and is vectored by the African citrus psyllid, *Tryoza erytreae* Del Guerico (Hemiptera: Psyllidae). Lam is only found in Brazil and is vectored by Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). Las, the most severe and widely spread, occurs in Florida, Brazil, throughout Asia, the Indian subcontinent and neighboring islands, and the Saudi Arabian peninsula and it is also vectored by *D. citri* (Bove 2006, Polek et al. 2007).

The Asian citrus psyllid is a phloem-feeding insect originating from the Far East and Asia (Mead 1977, Halbert and Manjunath 2004) and was first described on citrus in Taiwan in 1907. It is widely distributed throughout many of the citrus growing regions of the world including Asia, the Middle East, Central and South America, and the Caribbean (Catling 1970, Halbert and Núñez 2004). In the Americas, it was first detected in Brazil in 1942 (Lima 1942) but was not found in Florida until 1998 (Halbert 1998). Since its discovery in Florida, *D. citri* has been recorded from all citrus producing counties within the state. It has now been recorded from Alabama, Arizona, California, Georgia, Hawaii, Louisiana, Mississippi, South Carolina, and

Texas (USDA 2011b). In the absence of the HLB pathogen, *D. citri* was not considered an important pest of citrus in Florida. The direct damage caused by *D. citri* affects mostly new flush and thus pest control measures were only used to keep psyllid populations low on young trees, on which new growth needed to be protected to promote tree development and bring trees into production in as short a time as possible.

Longevity of adult *D. citri* varies from 51-117 days depending on the temperature. Following eclosion from the egg stage, *D. citri* complete five nymphal stages prior to molting to the adult stage. The developmental period from egg to adult is temperature dependent and ranges from 14 -49 d. The optimal development temperature is 25-28 ^{o}C with mean generation time of 28.6 days (Liu and Tsai 2000).

Since the discovery of HLB in Florida, control of HLB has become the primary focus of citrus pest management programs. Currently, the three main components of HLB management strategies in Florida include: 1) the planting of certified disease-free citrus trees, 2) identification and removal of Las-infected trees from groves, and 3) effective psyllid control utilizing broad-spectrum insecticide applications (Brlansky and Rogers 2007). More recently, as an alternative to the removal of Las-infected plants, the use of foliar-nutritional sprays to maintain the health of diseased trees already in production are being used (Spann et al. 2010). Regardless of whether growers choose to remove Las-infected trees or use supplemental nutrient sprays to maintain fruit production on diseased trees, use of insecticide applications continues to be considered necessary. The rationale for insecticide use is to maintain vector populations at low levels so that the rate of disease spread will be reduced. Similar approaches to managing disease spread via controlling vector populations have been practiced in other cropping systems (Perring et al. 1999).

As citrus growers remove diseased trees, either because of HLB or other endemic diseases that render trees unproductive, they must replant new trees in order to maintain long-term economic viability of their grove (Morris and Muraro 2008). The ability to plant new trees and bring them into production where HLB is present is a significant concern for citrus growers. Compared to larger fruit-bearing trees, nonbearing trees produce more new leaf growth (flush) throughout the year, which is required by *D. citri* for oviposition and subsequent nymphal development. Thus, young trees are thought to be more attractive to *D. citri* than mature trees and at greater risk of becoming infected with HLB compared to mature trees which produce fewer flushes per year (Brlansky and Rogers 2007). Because nonbearing trees need to be continually protected due to the frequent production of flush, application of systemic insecticides with extended residual activity is most desirable. Currently, two soil-applied systemic insecticides are commonly used in Florida for *D. citri* control on nonbearing citrus; imidacloprid (Admire® Pro 4.6F, Bayer CropSciences, Research Triangle Park, NC) and thiamethoxam (Platinum 75SG, Syngenta CropProtection, Inc., Greensboro, NC) (Rogers et al. 2011). However, due to pesticide labeling restrictions that limit the use rate on a per acre basis, neither of these products effectively control *D. citri* on large (> 3 m height) bearing trees due to the increased canopy volume which dilutes the effectiveness of the systemic pesticide application. Aldicarb (Temik 15 G, Bayer CropSciences, Research Triangle Park, NC) is the only soil-applied systemic insecticide which has been used with some success for controlling *D. citri* on mature trees (Rogers et al. 2011), however, its usage will be discontinued by the end of 2011 due to food safety concerns (EPA 2010). Consequently, the options for psyllid control on bearing trees are limited to broad-spectrum foliar applied insecticides.

The types of broad-spectrum insecticides used by citrus growers in Florida to control *D. citri* includes several classes with different modes of action including acetylcholinesterase inhibitors such as chlorpyrifos (Losban 4E, DowAgroscience, Indianapolis, IN), the acetylcholine receptor stimulator imidacloprid (Provado 1.6F, Bayer CropSciences, Research Triangle Park, NC), sodium channel modulators including fenpropathrin (Danitol 2.4EC, Valent, Libertyville, IL), and the lipid biosynthesis inhibitor, spirotetremat (Movento 240 SC, Bayer CropSciences, Research Triangle Park, NC). Some of these insecticides require ingestion by the insect or absorption through the cuticle following contact in order to induce toxic effects. In some cases the effects are not always immediate.

Since the HLB putative pathogen is a phloem-limited bacterium, acquisition and inoculation by *D. citri* occurs during the feeding process. Therefore understanding the feeding behaviors of *D. citri* as well as the characteristics of pathogen transmission are important for a better understanding of disease epidemiology and to develop effective vector control strategies.

There are several reports in the literature regarding transmission of Las by the *D. citri*; however, the results are contradictory. Capoor et al. (1974) investigated Las transmission by *D. citri* and reported that the psyllid requires a minimum of 15 min to 24 h of feeding to acquire the pathogen and a minimum of 15 min to 1 h for successful inoculation, implying high transmission efficiency. Conversely, Xu et al. (1988) reported that successful Las acquisition required a feeding access period of 5-7 h. Vuuren and Merwe (1992) and Buitendag and von Broembsen (1993), investigating *T. erytreae*, found that this psyllid only acquires Laf after a 24 h feeding access period. More recently, the rate of inoculation of healthy plants by single adult *D. citri* was found to be only about 4-6% but increased to approximately 88% when groups of 100 individuals were confined on a single plant (Pelz-Stelinski et al. 2010).

The nymphal stage(s) during which *D. citri* are able to acquire the pathogen have not been thoroughly studied. However, fourth and fifth instar *D. citri* have been reported to successfully inoculate healthy plants after a 1-25 d latency period post acquisition (Capoor et al. 1974, Vuuren and Merwe 1992, Roistacher 1991, Xu et al. 1988). In addition, Pelz-Stelinski et al. (2010), found that successful inoculation by adult *D. citri* is much higher if Las is acquired by the nymphal stage compared to acquisition and subsequent inoculation by adult *D. citri*.

The past studies of Las transmission by *D. citri* have measured acquisition and inoculation efficiency as a result of the duration of feeding access periods provided for *D. citri*. Indeed, successful pathogen acquisition and inoculation processes depend on the amount of time that the vector feeds (Power 1991). However, additional experimental variables, such as gender of the vector and environmental factors (light versus dark conditions) can significantly influence the outcome of such studies (Perring et al 1999). Therefore, a better understanding of how these variables affect *D. citri* feeding behavior will be important in the design of future studies on the transmission of Las in order to provide more consistent and repeatable results.

Electrical penetration graph (EPG) monitors have been used for detailed studies of the feeding behaviors of many sap-sucking insects (Powell 1991, Collar et al. 1997a, Tjallingii and Prado 2001, Fereres and Collar 2001). Investigating the transmission of barley yellow dwarf virus by *Rhopalosiphum padi* (L.), Prado and Tjallingii (1994) correlated phloem-associated stylet activities, denoted as waveforms E1 and E2, with salivation and ingestion, respectively. Jiang et al. (2000) linked salivation into phloem (E(pd)1) with inoculation of tomato yellow leaf curl virus by *Bemisia tabaci* (Gennadius). In that study, the inoculation efficiency was positively correlated with the total number and duration of E(pd)1 events. In addition, a 1.8 min period of salivation was sufficient for successful pathogen inoculation (Jiang et al. 2000). Using an EPG

monitor to examine the feeding difference between genders of *Frankliniella occidentalis* (Pergande) (Wetering et al. 1998), showed that while female thrips performed longer and more frequent probes than male thrips, males transmitted the pathogen at a higher rate due to higher mobility as a result of shorter durations of probing and ingestion behaviors performed (Wetering et al. 1998).

EPG monitors have also been used to study the effects of insecticide applications on insect feeding behavior. Joost and Riley (2005) observed that *F. occidentalis* probed more frequently and for longer periods of time on imidacloprid-treated plants than on untreated tomato plants. Their results suggest an increase in inoculation of tomato spotted wilt virus, a persistent and circulative virus, on imidacloprid-treated plants compared with controls. In contrast, when feeding on imidacloprid-treated tomato plants, *F. fusca* exhibited a significant decrease in the number of probes per insect and decreased probing duration when compared to untreated plants (Joost and Riley 2005). Since imidacloprid can work either as an agonist and antagonist, Joost and Riley (2005) suggested that the differences in the feeding behavior of those two species are related to the mode of action of the imidacloprid on the different thrips. In addition, Collar et al. (1997b) did not observe any significant differences in probing behavior of *Myzus persicae* (Sulzer) on imidacloprid-treated versus untreated pepper plants. The first EPG monitoring study of the feeding behavior of a psyllid was performed with the pear psylla, *Psylla pyricola* Foerster, using an AC monitor (Ullman and Mclean, 1988). Salivation (waveform S) and ingestion (waveform I) were observed for both nymphs and adult pear psyllids. Waveforms from the AC monitor available at the time could not distinguish between phloem versus xylem ingestion. However, while histological examination showed that both nymphs and adults ingest from all types of leaf cells, xylem, phloem and bundle sheath cells were found to be the preferred sites for

ingestion. More recently, Bonani et al. (2010) used a Giga-8 DC monitor to identify and histologically correlate (define) *D. citri* feeding waveforms, based in part on their similarity to the well-known aphid waveforms. In addition, Bonani et al. (2010) observed that detectable HLB pathogen acquisition occurred after 1 h of phloem ingestion (waveform E2) by female *D. citri*. However, pathogen inoculation could not be verified but it is likely that it occurs during phloem penetration or salivation (waveforms D and E1, respectively). Bonani (2009) also observed that when *D. citri* fed on young leaves, phloem ingestion (waveform E2) was longer and more frequent compared to feeding bouts on mature leaves. Additionally, 35% more psyllids reached and ingested phloem sap on young leaves compared to mature leaves.

Despite the considerable amount of past studies that have been conducted examining the transmission of Las by *D. citri*, more detailed information is needed regarding *D. citri* feeding behavior in order to develop management strategies that are effective in reducing the spread of HLB. To date, studies investigating the factors affecting *D. citri* feeding behavior have not been conducted. Furthermore, while studies investigating the effects of insecticides on pathogen transmission have been conducted with other insects, it would be presumptuous to make generalizations on how any insecticide will affect a particular insect's feeding behavior without detailed study. As detailed above, insecticides can have variable effects on feeding behaviors of different groups of insects, even within the same taxonomic order. Therefore, the objectives of this dissertation were:

- Determine if gender-based differences in feeding behavior exist for *D. citri* (Chapter 2);
- Examine the effects of dark and light conditions on the feeding behavior *D. citri* (Chapter 2);
- Determine whether the use of soil-applied and foliar-applied insecticides can disrupt *D. citri* feeding behaviors responsible for pathogen transmission (Chapters 3, 4 and 5);

- Determine the duration of *D. citri* feeding disruption that can be expected following the application of foliar-applied insecticides (Chapter 6).

CHAPTER 2
GENDER DIFFERENCE AND EFFECT OF LIGHT AND DARK ON ASIAN CITRUS PSYLLID (*DIAPHORINA CITRI* KUWAYAMA) FEEDING BEHAVIOR

There have been numerous past studies regarding the transmission of Las by *D. citri*, however the collective results reported are inconclusive and contradictory. Capoor et al. (1974) investigated Las transmission by *D. citri* and reported that the psyllid requires a minimum of 15 min to 24 h of feeding to acquire the pathogen and a minimum of 15 min to 1 h for successful inoculation, implying a high efficiency of transmission. In contrast, Xu et al. (1988) reported that feeding access periods of 5-7 h were sufficient for successful inoculation while 1-3 h feeding access periods were not. Additional studies indicated a *D. citri* feeding access period of 24h (Inoue et al. 2009) and 1h of phloem ingestion (Bonani et al. 2010) for successful pathogen acquisition. However, Pelz-Stelinski et al. (2010) recently found that levels of Las are not detectable in adult *D. citri* during the first 7 days after feeding on an infected plant, suggesting a considerable latency period prior to the ability of *D. citri* subsequently inoculate a plant following bacterial pathogen acquisition. Successful inoculation was found to require an average feeding access period of 1 d (Pelz-Stelinski et al. 2010). Nonetheless, recent investigations of Las transmission by *D. citri* have only sought to define bacterial pathogen transmission based on duration of feeding access periods. However, feeding behavior, which is fundamental to transmission of insect-vectored pathogens, can be influenced by variables other than just duration of feeding access period.

The feeding behaviors associated with pathogen transmission can be influenced by variables including host plant recognition and acceptance, pathogen specificity with the vector, vector gender and age (Perring et al. 1999, Weintraub and Beanland 2006). In general, all acquisition and inoculation processes depend on the sequential steps involved in the feeding process. The sequential behaviors associated with insect feeding are plant surface exploration,

labial dabbing, test probing and the subsequent probing of extended duration (Perring et al. 1999). Several stimuli such visual and chemical, influence those feeding behaviors. The knowledge of those stimuli that interfere with insect feeding behaviors can be manipulated to provide control of pathogen transmission (Perring et al. 1999).

One factor that affects the overall success of the pathogen acquisition and inoculation processes is the amount of time that the vector feeds (Power 1991). Studies on factors that might affect this feeding time, such as gender and presence or absence of light, could help to better define the pathogen transmission process.

Female whiteflies, *Bemisia tabaci* Gen., have been shown to be more efficient in the transmission of tomato yellow leaf curl geminivirus than males (Caciagli et al. 1994). In addition, a higher percentage (55%) of female aster leafhoppers, *Macrosteles quadrilineatus* Forbes, were able to transmit phytoplasma yellows to lettuce than males (35%), although the disease spread pattern was significantly more clustered for female than males (Beanland et al. 1999). EPG studies of the feeding behavior of the thrips *Frankliniella occidentalis* (Pergande), showed that female thrips performed longer and more frequent probes than male thrips (Wetering et al. 1998). However, male thrips were more efficient in pathogen transmission due to their frequent moving behaviors associated with the fact that they fed for shorter durations per probe (Wetering et al. 1998).

Studies of host plant selection by *D. citri* have shown that visual cues are also important factor regulating psyllid feeding behavior (Wenninger et al. 2009). More in-depth studies of *D. citri* feeding behavior are still needed to gain a better understanding of the factors affecting feeding. Gender differences and presence of light and dark on the feeding behavior of *D. citri* are not known. Therefore, the objectives of our study were to: 1) determine whether male and female

D. citri differ in their feeding behavior, and 2) examine the effects of the presence of light on *D. citri* feeding activities. These are potentially important variables which could affect the outcome of experiments investigating the transmission of Las by *D. citri*.

Materials and Methods

Plants and Insects

Plants used in experiments consisted of 'sweet orange' (*Citrus sinensis* (L.) Osbeck) seedlings (15-20 cm tall) grown in 120-ml tubes containing Fafard Citrus Mix (Fafard, Agawam, MA). Seedlings were maintained in a pathogen-free greenhouse at 29 ± 3 °C and 60-80% RH. All seedlings were planted and maintained identically to minimize interplant variation. Four weeks prior to initiation of the experiments, plants were pruned to force production of new leaf flushes. Only plants with young leaves (soft leaves, less lignified) were used in the experiments.

Adults *D. citri* (10-15 d) used in experiments were obtained from a greenhouse colony free of *Ca*. Liberibacter asiaticus, reared on 'sour orange' (*Citrus aurantium* L.) and 'sweet orange' at 29 ± 3°C with a photoperiod of 12:12 (L:D) h. Prior to use in EPG recordings, *D. citri* were transferred to a rearing cage (61cm x 61cm x 91cm, Bioquip, Rancho Domingues, CA) containing 'sweet orange' plants for a 48 h acclimation period.

EPG Equipment

The EPG system consisted of a Giga-8 amplifier (Department of Entomology, Wageningen Agricultural University, the Netherlands), analog-to-digital (AD) converter, and custom software for digitizing, recording, and storing EPG recordings. The Giga-8 is an amplifier whose primary circuit is based on a direct current (DC) system (Tjallingii 1978). The monitor contains eight headstage amplifiers (sometimes termed probes) with a fixed input resistance of 10^9 Ω and gain of 50 – 100X. Electrical signals are created by a closed circuit formed by the insect and plant. A headstage amplifier is connected to the insect and the plant electrode is inserted into the soil

surrounding the plant. When the insect is in contact with the plant, the circuit closes, and waveforms are produced in response to the different processes (biological and physical) inherent to the insect and plant association (Walker 2000, Tjallingii 2000). Because the Giga-8 is very sensitive to electrical noise, the headstage amplifier and the plant-insect preparations were housed within a Faraday cage (152 cm x 62 cm x 122 cm). The Giga-8 was connected to an analog-to-digital (A/D) converter (DATAQ ® DI-710UHB, DATAQ instruments, Akron, Ohio) connected via a USB port to a personal computer. All EPG recordings were conducted with 100X gain, a conversion rate of 100 samples per second at 26 ± 2 °C and the substrate voltage set to 15 mV DC.

EPG Recording

After the 48 h acclimation period, psyllids were collected individually using a wet paint brush and immobilized by holding the tips of the wings with a pair of soft forceps (Bioquip Products Inc, Rancho Domingues, CA). A gold wire (18.5 µm in diameter [sold as 0.0010 in], Sigmund Cohn Corp., Mt. Vernon, NY) was cut to 1.5 cm in length and attached to *D. citri* using silver glue (white glue: water: silver powder (8-10µm), 1:1:1 [v:v:w], Inframat Advanced Materials, Manchester, CT). This length of wire was chosen because it allows psyllids to move freely on the leaf surface when selecting a feeding site, but is less likely to come in contact with the plant surface to cause interference in the EPG signal. The gold wire was attached to a psyllid by creating a small loop on one end of the wire and then dipping the loop in silver glue. The glue-coated loop was then held against the dorsal portion of the insect thorax until dry. The other end of the wire was connected using the silver glue to a copper wire (approx. 0.2 mm in diameter, 3 cm in length) which was soldered to a copper nail electrode inserted into the female BNC connector on the headstage amplifier.

After the psyllid was securely attached to the gold wire, the headstage amplifier was connected to the input of the amplifier control box and the psyllid was placed on the abaxial side of a young leaf on a potted citrus seedling. The abaxial side of the leaf was chosen according to the observed preference of psyllid feeding. The leaf was held with the abaxial side up (i.e., inverted) by placing it on top of another leaf from the same plant and securing the two leaves together using a loop made of Scotch® MagicTM tape (3M Center, Saint Paul, MN).

Citrus plants were connected individually to each monitor channel. The plant electrode (copper wire, approx. 2 mm in diameter, 10 cm long) was inserted in the soil at the base of each plant. The soil within each pot was moistened with water prior to the start of each recording to facilitate a closed electrical circuit. Pots were then placed on plastic trays to prevent contact with the Faraday cage. Access time for psyllid feeding on citrus seedlings was 12 h for each recording, in both experiments.

Data were collected, stored, and displayed in real-time using WinDAQ™ Pro software (DATAQ Instruments, Akron, OH). The duration of each waveform event was measured post-acquisition using DATAQ Windaq® Waveform Browser software, version 2.40 (DATAQ Instruments, Akron, OH).

Asian Citrus Psyllid Waveform Terminology

The terminology used for waveforms was based on the conventions of Backus (2000) and the *D. citri* waveform correlations of Bonani et al. (2010). A probe is the amount of time from when the insect's stylets penetrate the leaf until stylets withdrawal from the leaf. A single probe can contain multiple waveform events such as salivation and ingestion (Backus et al. 2007). These behaviors are represented by different waveforms defined by Bonani et al. (2010), summarized below.

Waveform C (Figure 2-1B) was histologically associated with stylet pathway activities and salivary sheath secretion through parenchyma cells. Waveform C is complex in appearance and is comprised of all activities that are occurring during stylet penetration through different leaf tissues. Consequently, it may resemble other types of waveforms. The average frequency of waveform C is 11.5-19.0 Hz (Bonani et al. 2010).

Waveform D (Figure 2-1C) is correlated with salivary sheath termini in phloem tissue (though exactly which of the four phloem cell types is unknown); it seems to represent a transition behavior from pathway to phloem phase. There is no analogous waveform produced by aphids. Consequently, the precise stylet activities by *D. citri* resulting in this waveform are completely unknown. However, they occur after waveform C and end prior to initiation of waveform E1. The average frequency of waveform D is 1.0-3.5 Hz (Bonani et al. 2010).

Waveform E1 (Figure 2-1D) represents the beginning of phloem phase and starts after a potential drop (pd) marking the end of waveform D. Consequently, E1 waveforms only occur after a D waveform. Histological correlation of salivary sheath termini showed E1 to occur in the phloem, however again, no specific cell type could be identified. E1 is hypothesized to correspond to salivation into phloem sieve elements by *D. citri*, based on its similarity with the well-correlated E1 waveforms of aphids. E1 is at a negative voltage level, supporting the suggestion that the stylets tips may be located intracellularly. Therefore, this waveform represents the point in *D. citri* feeding at which inoculation of the circulative, phloem-limited Las bacteria seems most likely to occur, although some inoculation may also occur during waveform D. The average waveform frequency of E1 is 5.0-7.5Hz (Bonani et al. 2010).

Waveform E2 (Figure 2-1E) is also part of phloem phase, and always occurs after an E1 waveform event and, like E1, is on the negative voltage level. E2 definitely corresponds to *D.*

citri phloem ingestion, because Bonani et al. (2010) correlated E2 with salivary sheath termini in phloem tissue, as well as with Las acquisition after performance of E2 for approximately 1 h. The average frequency of E2 waveforms is 3.0-8.0 Hz (Bonani et al. 2010).

Waveform G (Figure 2-2F) is histologically correlated with salivary sheath termini in xylem vessels and has an average frequency of 5.0-7.0 Hz. Again based on an appearance similar to the G waveform of aphids, psyllid waveform G is hypothesized to represent xylem ingestion. G was performed by only 25% of *D. citri* in 160 h of recordings (Bonani et al. 2010). These presumed xylem activities were thought to be related to the insect's need for maintaining water balance.

Experimental Design

Experiment 1. Gender-based differences in *D. citri* feeding behavior.

A total of 15 plants containing young leaves were used for each treatment. Prior to EPG recordings, psyllids from the acclimatization cage were taken and sexed under a microscope and 15 psyllids of each gender were used in this experiment. The feeding behavior of single psyllid was individually recorded per plant and each psyllid EPG recording was considered a unique replicate, thus providing 15 replicates per treatment for analysis.

Experiment 2. Effects of light and dark on *D. citri* feeding behavior.

A total of 15 plants were also used in this experiment for each treatment. For the dark treatment one of the Faraday cage was totally covered with black card boards causing complete darkness. Individual female psyllids were recorded per plant, providing a total of 15 replicates for each treatment. Dark and light treatments were recorded at the same time and unique plants were used for each psyllid recording. Plants and psyllids were acclimated for 1h under their respective treatment prior recordings.

Statistical Analysis

The number of waveform events and duration were analyzed using biologically non-sequential parameters, as described by Backus et al. (2007). Those parameters were grouped by four different levels: cohort, insect, probe and event. At the cohort level, the parameters analyzed were total probing duration (TPD), total number of probes (TNP), total waveform duration (TWD), and total number of waveform events (TNWE). At the insect level, the parameters analyzed were probing duration per insect (PDI), waveform duration per insect (WDI), and number of probes per insect (NPI). At the probe level, the parameters analyzed were waveform duration per probe (WDP), and number of waveform events per probe (NWEP). At the event level, the parameters analyzed were probing (i.e. penetration) duration per event (PDE), total number of probing (penetration) events (TNPE), number of waveform events per insect (NWEI), and waveform duration event per insect (WDEI) (Backus et al. 2007). Another parameter analyzed that was not included in Backus et al. (2007) is the proportion of individuals that produced the specific waveform type (PPW); this parameter was defined in Bonani et al. (2009, 2010). For analysis of probe activities (NPI and PDI) waveforms z and np were combined as one waveform type because z and np are both non-probing behaviors (see first section under Results, below).

Pearson's chi-square test was performed to test the goodness of fit (PROC GLIMMIX, SAS Institute 2001). The waveform duration data were log-transformed before statistical analysis, to improve homogeneity and reduce variability. Data were analyzed by protected ANOVA (PROC GLIMMIX, SAS Institute 2001) with the least significant difference (LSD) test (LSMEANS, SAS Institute 2001) used for pairwise comparisons, to determine whether the waveform parameters analyzed were significantly different between psyllids genders, and light

and dark treatments. Data were also compared in a similar manner among waveforms within treatments. Means were considered significantly different at $\alpha=0.05$.

Results

New Waveform Characterization and Correlations

Waveform np (Figure 2-2A) is a baseline waveform that is highly irregular and spikey. It was visually correlated in every recording with the insect moving on the plant. Further correlation research has been performed and will be described in detail elsewhere (Youn et al., *in press*).

Waveform z (Figure 2-2B) is similar to baseline with low voltage levels, named z by Backus et al. (2007). This waveform represents a non-probing behavior visually correlated herein with times when *D. citri* are motionless, being that they are either resting, dead or have moved off the plant. Thus, unlike most EPG studies, *D. citri* recordings reveal two types of non-probing behavior. Further correlation of these waveforms will be described elsewhere (Youn et al., *in press*).

Experiment 1. Gender-based Differences in *D. citri* Feeding Behavior.

One hundred percent (PPW) of female and male *D. citri* performed pathway/stylet penetration waveforms (waveform C) and non-probing/walking activities (waveform np). One hundred percent of female *D. citri* performed non-walking/non probing activities (waveform z), while only 73.3 % of the male *D. citri* performed the same waveform. Forty percent of both genders penetrated the phloem and salivated (waveforms D and E1, respectively). However, only 20% of the female *D. citri* performed phloem ingestion (waveform E2), although all the males which penetrated into the phloem ingested phloem sap (40%). Approximately 67% of the male *D. citri* ingested xylem fluid (waveform G) while only 27% the female *D. citri* performed the same activity (Table 2-1).

Cohort level. *D. citri* had a total access period of 648,000 s, during which time the 15 female *D. citri* probed a total of 503 times (TNP) and spent 345,611.28 s (TPD) with their stylets inserted in the leaf. For the remaining duration (53.3%) of total access period, female *D. citri* performed non-probing activities such as waveform np and z. In contrast, male *D. citri* spent most of their time (67.8%) performing probing activities (TPD = 439,027.37 s), producing a total of 470 (TNP) probes. The percentages of each waveform duration performed in TPD for both females and males is represented by the total waveform duration (TWD) shown in Figure 2-3.

Insect Level. The general feeding behaviors for male and female *D. citri* did not differ significantly. The number of probes per insect (NPI) ($F = 0.00$; df = 1, 28; $P = 0.97$) and the probe duration per insect (PDI) ($F = 3.28$; df = 1, 28; $P = 0.08$; Table 2-2) were similar. However, when analyzing the waveform duration per insect (WDI), phloem ingestions (waveform E2) were significantly longer for females than males ($F = 9.66$; df = 1, 7; Table 2-1).

Probe level. There was no difference in the number of waveform events per probe (NWEP) between males and females (Table 2-3). Similarly, there was no difference in the waveform duration per probe (WDP) between males and females. However, male *D. citri* produced stylet penetrations (waveform C) which were longer in duration compared to females ($F = 13.13$; df = 2, 971; Table 2-4).

Event level. In contrast to that observed with PDI and NPI, there was a significant difference between males and females in probe duration per event (PDE) (Table 2-1). Male *D. citri* produced longer PDE compared to females. There were significantly more waveform events per insect (NWEI) for females than males. Also females produced more phloem ingestion waveform events (E2) than did males ($F = 7.58$; df = 1, 7; Table 2-5). In addition, there was no

difference in waveform duration per event per insect (WDEI) between males and females (Table 2-6).

Summary of results. There were slight differences in the feeding behaviors of males and females. When not probing, females remained motionless (z) for longer durations per insect than males, due to longer standing events per insect and the high number of standing still events. In addition, females performed more walking (np) per insect due to longer walking duration per event and a greater number of events per insect.

When stylet-probing, females exhibited shorter pathway activities (C) per insect because each event was shorter. Phloem contact (D) was not significantly different between males and females for any of the parameters analyzed. Female *D. citri* performed longer durations of phloem contact per insect, however phloem contact duration events were slightly shorter in females than males. The frequency of the phloem contacts performed per probe was higher in females than males. For phloem-related behaviors, phloem salivation (E1) did not differ between females than males. However, phloem salivation for females was half as long as in males. In contrast, phloem ingestion (E2) duration per insect was statistically longer for female *D. citri* than males, due to longer phloem ingestion events and the number of times this event was performed per probe. This then lead to a longer duration of phloem ingestion per probe with a higher frequency of phloem ingestion events per insect. In contrast, xylem ingestion (G) was shorter in duration per insect because each event was shorter, performed fewer time, in numerically fewer probes, leading to less time per probe and fewer events per insect.

Overall, females remained motionless and walked more frequently and for longer. Female insects searched less often for vascular tissue, for shorter durations and made fewer probes. Females insects generally took more time to find the phloem but spent less time salivating. Once

feeding on the phloem, females salivated and subsequently ingested for longer durations compared to males. In contrast, females found the xylem and ingested from this tissue for shorter durations than males.

Feeding behavior of female psyllids (within treatment analysis).

Insect level. Analysis of the waveform duration per insect (WDI) indicated high variation among the waveforms ($F = 35.33$; df = 7, 58; $P < 0.0001$). Females performed behaviors related to waveform np, pathway (C) and phloem ingestion (E2) for longer duration compared to other behaviors performed. Waveforms D and E1 were the shortest in duration, as expected (Table 2-1).

Probe level. Analysis of the number of waveforms events per probe (NWEP) ($F = 41.78$; df = 6, 1034; $P < 0.0001$) showed a significantly higher frequency of waveform E1 when compared with other behaviors. The behaviors performed the least number of times were znp, C, and G, which were not significantly different from each other (Table 2-3). For waveform duration per probe (WDP) ($F = 14.61$, df = 6, 1034, $P < 0.0001$), waveform E2 and G were the longest in duration and were not significantly different from one another (Table 2-4). Waveforms C, D, E1 had the shortest durations (Table 2-4).

Event level. There was a significant effect of waveform duration per insect WDEI ($F = 20.50$, df = 7, 58, $P < 0.0001$). Waveforms E2 and G were the longest in duration and did not differ one another (Table 2-6). Waveform D and E1 were the shortest in duration (Table 2-6). There was a significant effect of the number of waveforms events per insect (NWEI) ($F = 17.74$, df = 7, 58, $P < 0.0001$). Waveforms np and C occurred most often and there were no significant difference in the number of timesthe other waveforms were performed (Table 2-5).

Feeding behavior of male psyllids (within treatment analysis).

Insect level. Analysis of the waveform duration per insect (WDI) showed that male *D. citri* had a slightly higher variability among the waveforms than female psyllids ($F = 57.28$, df = 7, 68, $P < 0.0001$). The longest WDI was attributed to waveform C followed by waveform np and shortest durations observed were waveforms D and E1 (Table 2-1).

Probe level. The highest number of waveforms events per probe (NWEP) ($F = 19.43$, df = 6, 978, $P < 0.0001$) was waveform E1, and the waveforms which occurred least often were znp and E2 (Table 2-3). Analysis of waveform duration per probe (WDP) ($F = 12.89$, df = 6, 978, $P < 0.0001$) showed significant differences in duration among the waveforms. Waveforms G and E2 were the longest in duration compared to the other three waveforms (Table 2-4).

Event level. Waveform duration events per insect (WDEI) ($F = 40.77$, df = 7, 68; $P < 0.0001$) were also significantly different between the waveforms produced; waveforms C, E2 and G had the longest durations whereas waveforms D and E1 were shorter in duration (Table 2-6). There were significant differences in the number of waveforms events per insect (NWEI) ($F = 53.21$, df = 7, 68, $P < 0.0001$) with waveforms np and C performed more often than waveforms D, E1, E2 and G (Table 2-5).

Experiment 2. Effects of Light and Dark on *D. citri* Feeding Behavior.

All *D. citri* tested performed both pathway/stylet penetration waveforms (waveform C) and non-probing/walking activities (waveform np) in both light and the dark conditions. Approximately 93% of the *D. citri* performed non-walking/non probing activities (waveform z) in light while 86.7% performed the same waveform in complete darkness. Sixty percent of *D. citri* performed all the phloem activities (phloem penetration, salivation and ingestion, respectively; waveforms D, E1 and E2) in dark conditions, while only 40%, 40%, and 33.3% of the *D. citri* performed phloem penetration, salivation and ingestion, respectively in the presence

of light. Xylem ingestion (waveform G) was performed by 26% of the *D. citri* in light and 53.3% in dark conditions (Table 2-1).

Cohort level. *D. citri* were given a total access period of 648,000 s, during which *D. citri* probed 599 times (TNP) and spent 446,698.11 s (TPD) with their stylets inserted in the leaf in dark conditions. During the remaining time, 68.9% of the total access period, *D. citri* performed non-probing activities (waveforms np and z). In contrast, in light *D. citri* spent less time performing probing activities (TPD = 295,208.26 s) (45.6%), even though the number of probes was higher compared to dark conditions (TNP = 766). The percentage of time spent for each waveform performed in TPD in both light and dark conditions is represented by the total waveform duration (TWD) shown in Figure 2-4.

Insect level. Analysis of the probe duration per insect (PDI) indicated significant differences between feeding behaviors in light and dark conditions ($F = 7.82$; df = 1, 28). In complete darkness, *D. citri* produced probes of longer duration compared to *D. citri* in light conditions (Table 2-2). With respect to waveform duration per insect (WDI), there were significant differences between waveforms z ($F= 4.10$; df=1, 25), np ($F= 4.13$; df=1, 28), E2 ($F= 6.51$; df=1, 12), and G ($F= 4.97$; df=1, 10) (Table 2-1). Waveforms z, np, and G were longer in duration in light conditions while waveforms C and E2 were longer in duration in dark conditions. There was a trend for the mean number of probes per insect (NPI) to be higher in the light than in dark conditions ($F = 2.78$; df = 1, 28; Table 2-2) but this trend was not statistically significant.

Probe level. The presence of light did not affect the number of waveform events per probe (NWEP) (Table 2-3). In contrast, for waveform duration per probe (WDP), waveform C was significantly in dark compared to light conditions ($F = 13.29$, df = 1, 1131; Table 2-4).

Event level. For probe duration per event (PDE), like PDI at the insect level, there were significant differences in between light and dark conditions (F = 18.91; df = 1, 1363; Table 2-2). Even though the number of waveform events per insect (NWEI) were not significantly different between light and dark for any of the waveforms (Table 2-5), the waveform duration event per insect (WDEI) was longer for waveforms z (F= 5.46; df=1, 25) in light whereas waveforms C (F = 5.12; df =1, 28) and E2 (F = 5, 19; df =1, 12) were longer in dark conditions (Table 2-6).

Summary of results. *D. citri* that were recorded in light performed feeding behaviors differently than those recorded in dark. In light conditions, *D. citri* stood still (z) for significantly longer durations per insect, and more often than in light. In light, *D. citri* performed significantly more walking events (np) per insect due to longer walking duration per event and a higher number of events per insect.

In light conditions, pathway activity (C) events per insect were shorter than in dark conditions; however, there were more pathway activities performed in light than dark. Phloem contact (D) was not significantly different for any of the parameters, but in light, phloem contact was twice as long as in dark conditions because of longer durations on the phloem contact event per insect and the higher number of phloem contacts per probe. There was no difference between phloem salivation activities in light and dark conditions. In contrast, phloem ingestion (E2) duration per insect was statistically longer in dark than light conditions because of longer and less frequent events per probe, resulting in longer phloem ingestions. In contrast to phloem ingestion, in light conditions, *D. citri* performed xylem ingestion (G) for significantly longer than in dark with longer duration events per insect and fewer probes probes, resulting in less xylem ingestion events per probe and a slightly higher number of xylem ingestion events per insect.

In conclusion, under light *D. citri* stood still and walked more frequently and for longer times, performing search for vascular tissue less frequently and than under light, despite a similar overall number of probes. Generally to the phloem was penetrated, they ingested for shorter durations than those under dark. In contrast, under light, *D. citri* found and ingested from the xylem more briefly than those under dark conditions.

***D. citri* feeding behavior in light (within treatment analysis).**

Insect level. There was a significant effect of light on waveform duration per insect (WDI) ($F = 79.36$, df = 7, 63, $P < 0.0001$). *D. citri* performed significantly longer durations of waveforms np, and C for significantly longer durations compared to other behaviors; waveforms D and E1 were the shortest in duration (Table 2-1).

Probe level. There was a significant effect of light on the number of waveforms events per probe (NWEP) ($F = 72.62$, df = 6, 1365, $P < 0.0001$). Waveforms E1, followed by waveform D, were performed most often. Conversely, waveforms were znp, C, and G, which were not significantly different from one another, were performed the least number of times (Table 2-3). For waveform duration per probe (WDP) ($F = 37.19$, df = 6, 1365, $P < 0.0001$), waveforms E2 and G were longest in duration whereas waveforms C, and D were the shortest in duration (Table 2-4).

Event level. There was a significant effect of light on waveform duration events per insect (WDEI) ($F = 40.66$, df = 7, 63, $P < 0.0001$). Waveforms E2 and G were the longest in duration and not significantly different from one another (Table 2-6). Waveforms D and E1 were shortest in durations (Table 2-6). In contrast to WDEI, the number of waveforms events per insect (NWEI) showed a lower variation ($F = 31.03$, df = 7, 63, $P < 0.0001$). Waveforms np and C were performed most frequently compared to waveforms D, E1, E2 and G (Table 2-5).

***D. citri* feeding behavior in dark (within treatment analysis).**

Insect level. There was a significant effect of dark conditions on waveform duration per insect (WDI) ($F = 78.42$, df = 7, 76, $P < 0.0001$). Waveform np, C and E2 were characterized by the longest WDI (Table 2-1) whereas waveforms D and E1 were shortest in duration (Table 2-1).

Probe level. The highest number of waveforms events per probe (NWEP) ($F = 48.83$, df = 6, 991, $P < 0.0001$) was waveform E1, followed by waveform D. Waveforms znp, C and G were peformed the least number of times (Table 2-3). There was a significant effect of dark conditions on ($F = 21.81$, df = 6, 991, $P < 0.0001$) waveform duration per probe (WDP) with waveforms G and E2 having the longest durations while waveforms C, D and E1were characterized by the shortest durations (Table 2-4).

Event level. There was a significant effect of dark conditions on waveform duration events per insect (WDEI) ($F = 69.99$, df = 7, 76, $P < 0.0001$). Waveform E2 was performed for the longest duration, followed by waveforms C and G; waveforms D and E1 were shortest in duration (Table 2-6). Unlike WDEI, the number of waveforms events per insect (NWEI) had a lower variance among the waveforms ($F = 31.00$, df = 7, 76, $P < 0.0001$).Waveforms np and C were performed the highest number of times while waveforms D, E1, E2 and G were performed less often (Table 2-5).

Discussion

The objective of the current study was to quantify the effects of gender and light conditions on the feeding behavior of *D. citri*. In general, female psyllids performed non-probing activities (waveform z and np) more often than males. Also, they performed phloem ingestion (E2) more often and for longer durations than males. Male psyllids performed stylet penetration activities (C) and xylem ingestion (G) more often and for longer durations compared to females. The number and duration of probes was not different between males and females. In light conditions,

stylet penetration activities (C) and phloem ingestion (E2) were less frequent and shorter in duration. Phloem ingestion (E2) and xylem ingestion (G) were longer in light than dark for both sexes.

The factors that affect feeding behavior are probably related to both insect physiology (insect age, reproductive stage) and ecology (risk of predation, microenvironment, and temperature). Feeding quantity depends on the nutrient requirements of the insect and potential phagostimulatory effects. Therefore it is common for female insects to feed more in their reproductive period. *D. citri* is sexually mature 2-3 days after eclosion, and capable of oviposition a day after mating (Wenninger and Hall 2007). In our experiments, the insects were 10-15 days old at the time of recording and female and male *D. citri* were in the same cage during acclimation. Therefore, females *D. citri* were likely reproductively mature. This could partially explain why females fed on the phloem more than males. Female *F. occidentalis* (Pergande) also fed on phloem more than males, thus producing more feeding scars than males (Wetering et al. 1998). Male thrips fed occasionally and were more mobile; however, unexpectedly their transmission efficiency was greater than that of females (Wetering et al. 1998). In some cases, gender does not affect feeding behavior of plant feeding insects. For example, honeydew production by *Bucephalogonia xanthophis* (Berg) is not different between males and females (Miranda et al. 2008).

Plants were acclimatized in the dark, so differences in *D. citri* feeding behavior between dark and light could be attributed to nutritional contents of the plant at different times of day. Since *D. citri* is a phloem feeder, sugar and amino content may be the main phagostimulants and could be representing an important factor regulating psyllid feeding activities. For example, *B. xanthophis* fed longer during the light phase probably due to xylem content (Miranda et al.

2008). Even though xylem is poor in nutrients, there are several studies showing that sharpshooters are able to synchronize their feeding behavior according to the fluctuation in the chemical composition of the xylem (Andersen et al. 1989, 1992 and Brodbeck et al.1993). However, Miranda et al. (2008) did not measure the sap content from citrus, and no correlation was made between active sharpshooter feeding and xylem nutritional content. Brodbeck et al (1993) showed that *Homalodisca coagulata* (Say), *Homalodisca insolita* (Walker) and *Cuerna costalis* (F.) feed more on crape myrtle during the photophase than the scotophase. The amino acid content of crape myrtle is 2.5 times higher in photophase compared to scotophase (Brodbeck et al.1993). In contrast, these same species fed more on periwinkle during the scotophase than photophase which corresponded with 1.9 times higher amino acids concentrations during the scotophase than during the photophase.

Generally in herbaceous plants, the products of photosynthesis are accumulated in the leaves during the photophase and they leave the leaves during scotophase. Although, the diurnal pattern of citrus is different, Goldschimitdt and Koch (1996) showed slight daily fluctuations of soluble sugars and starch levels in citrus leaves in which soluble sugars and starch levels were higher during the day and lower during the evening. Since soluble sugars from the leaves are transported through the phloem, sugar concentrations in the phloem could be negatively proportional to that of xylem. Consequently, sugar concentrations in the phloem being higher during the night than during the day.

Mating, oviposition and movement of *D. citri* occurs more frequently during the photophase than the scotophase (Wenninger and Hall 2007). This is likelydue to the use of visual cues for host-plant selection (Wenninger et al. 2009) and mating (Wenninger et al. 2008). This

could explain the greater walking durations observed in the study under light than dark conditions. Xylem ingestion was greater during the light than during the dark.

Overall, these experiments showed that female *D. citri* perform more phloem feeding behaviors compared to males and this phloem feeding behavior is more likely to occur at night. Our results suggest that transmission efficiency is likely influenced both by gender and photophase.

Table 2-1. Mean (± SE) waveform duration per insect (WDI) (s) and the proportion of individuals that produced a waveform type (PPW) for different genders of *Diaphorina citri* feeding and under different light conditions.

Experiment 1: Gender											
Waveform	Female					Male					
	WDI	±	SE	PPW		WDI	±	SE	PPW		p-value
z	5670.19	±	1198.67	15/15	*b*	3925.36	±	1029.94	11/15	*c*	0.5555
np	16294.09	±	2120.02	15/15	*a*	12853.21	±	1890.12	15/15	*b*	0.2945
C	20386.14	±	2119.77	15/15	*a*	25552.90	±	1972.04	15/15	*a*	0.0970
D	166.22	±	60.32	6/15	*c*	103.64	±	30.12	6/15	*d*	0.4344
E1	120.46	±	22.24	6/15	*c*	204.16	±	52.05	6/15	*d*	0.8475
E2	7836.00	±	3070.59	3/15	*ab*	2090.43	±	453.29	6/15	*c*	0.0171
G	3625.52	±	931.00	4/15	*b*	4129.61	±	983.59	10/15	*c*	0.8662
Experiment 2: Light conditions											
	Light					Dark					
Waveform	WDI	±	SE	PPW		WDI	±	SE	PPW		p-value
z	8297.87	±	1721.97	14/15	*b*	4685.02	±	1601.17	13	*b*	0.0538
np	17574.84	±	2475.78	15/15	*a*	11162.15	±	1390.45	15	*a*	0.0517
C	16643.86	±	3038.89	15/15	*a*	20835.76	±	2764.64	15	*a*	0.1679
D	214.70	±	110.62	6/15	*c*	124.56	±	46.31	9	*c*	0.7048
E1	266.74	±	133.26	6/15	*c*	200.08	±	64.58	9	*c*	0.5584
E2	4995.79	±	2747.51	5/15	*b*	12941.43	±	3072.97	9	*a*	0.0254
G	4411.60	±	1105.95	4/15	*b*	1836.89	±	509.39	8	*b*	0.0498

Different letters indicate significant differences (LSD) ($P \leq 0.05$) among waveforms within treatments

Table 2-2. Mean (± SE) probe duration per insect (PDI) (s), number of probes per insect (NPI), and probe duration per event (PDE) (s) for different genders of *Diaphorina citri* feeding and under different light conditions.

Experiment 1: Gender

Female			Male			
PDI	±	SE	PDI	±	SE	p-value
23040.75	±	2332.17	29268.49	±	2192.21	0.0836
NPI	±	SE	NPI	±	SE	p-value
33.8	±	7.03	31.40	±	4.17	0.1066
PDE	±	SE	PDE	±	SE	p-value
602.11	±	74.33	771.57	±	83.52	0.0005

Experiment 2: Light conditions

Light			Dark			
PDI	±	SE	PDI	±	SE	p-value
19680.55	±	3010.29	29779.00	±	1996.34	0.0056
NPI	±	SE	NPI	±	SE	p-value
44.66	±	6.77	31.46	±	5.69	0.1066
PDE	±	SE	PDE	±	SE	p-value
385.38	±	47.80	745.73	±	86.71	<0.0001

Table 2-3. Mean (± SE) number of waveform events per probe (NWEP) and number of probes by waveform (NPw) for different genders of *Diaphorina citri* feeding and under different light conditions.

Experiment 1: Gender											
Waveform	Female					Male					
	NWEP	±	SE	NPw		NWEP	±	SE	NPw		p-value
znp	1.00	±	0.00	507	*c*	1.00	±	0	471	*c*	n/a
C	1.04	±	0.01	503	*c*	1.08	±	0.01	470	*b*	0.0838
D	1.66	±	0.33	9	*b*	1.28	±	0.18	7	*b*	0.3904
E1	2.11	±	0.53	9	*a*	1.85	±	0.34	7	*a*	0.8077
E2	1.50	±	0.50	4	*b*	1.00	±	0	6	*bc*	0.2415
G	1.14	±	0.14	7	*c*	1.23	±	0.13	17	*b*	0.7204
Experiment 2: Light conditions											
Waveform	Light					Dark					
	NWEP	±	SE	NPw		NWEP	±	SE	NPw		p-value
znp	1.00	±	0.00	670	*d*	1.00	±	0	472	*d*	n/a
C	1.05	±	0.01	662	*d*	1.07	±	0.17	471	*d*	0.1981
D	2.00	±	0.42	10	*b*	1.69	±	0.30	13	*b*	0.5410
E1	2.66	±	0.55	9	*a*	2.53	±	0.46	13	*a*	0.8588
E2	1.40	±	0.24	5	*c*	1.25	±	0.13	12	*c*	0.5655
G	1.00	±	0.00	6	*d*	1.12	±	0.12	8	*cd*	0.4082

Different letters indicate significant differences (LSD) ($P \leq 0.05$) among waveforms within treatments

Table 2-4. Mean (± SE) waveform duration per probe (WDP) (s) for different genders of *Diaphorina citri* feeding and under different light conditions.

Experiment 1: Gender									
Waveform	Female				Male				
	WDP	±	SE		WDP	±	SE		p-value
znp	649.68	±	65.51	*b*	501.01	±	46.61	*b*	0.7792
C	608.08	±	84.97	*c*	815.51	±	108.95	*b*	0.0003
D	110.81	±	41.80	*bc*	88.84	±	23.31	*b*	0.8745
E1	80.31	±	15.22	*bc*	174.99	±	47.86	*b*	0.4952
E2	5877	±	2738.56	*a*	2090.43	±	453.29	*a*	0.0772
G	2017.73	±	610.06	*a*	2429.18	±	436.36	*a*	0.8233
Experiment 2: Light conditions									
Waveform	Light				Dark				
	WDP	±	SE		WDP	±	SE		p-value
znp	566.85	±	64.06	*b*	483.69	±	70.22	*b*	0.7956
C	377.12	±	49.97	*c*	663.63	±	84.09	*c*	0.0003
D	128.82	±	30.34	*bc*	86.23	±	21.51	*bc*	0.2490
E1	177.82	±	61.63	*b*	138.51	±	40.98	*bc*	0.3506
E2	4995.79	±	2747.51	*a*	9706.08	±	1912.27	*a*	0.1794
G	2941.07	±	539.34	*a*	1836.89	±	509.39	*a*	0.0971

Different letters indicate significant differences (LSD) ($P \leq 0.05$) among waveforms within treatments

Table 2-5. Mean (± SE) number of waveform events per insect (NWEI) for different genders of *Diaphorina citri* feeding and under different light conditions.

Experiment 1: Gender

Waveform	Female				Male				
	NWEI	±	SE		NWEI	±	SE		p-value
z	6.33	±	1.27	*b*	4.90	±	0.89	*b*	0.5331
np	38.60	±	6.82	*a*	34.40	±	4.12	*a*	0.7065
C	34.93	±	7.08	*a*	33.86	±	4.44	*a*	0.8985
D	2.50	±	0.56	*b*	1.50	±	0.22	*bc*	0.1606
E1	3.16	±	0.83	*b*	2.16	±	0.40	*bc*	0.3927
E2	2.00	±	0.57	*b*	1.00	±	0.00	*c*	0.0283
G	2.00	±	0.40	*b*	2.10	±	0.45	*bc*	0.9680

Experiment 2: Light conditions

	Light				Dark				
Waveform	NWEI	±	SE		NWEI	±	SE		p-value
z	9.57	±	1.68	*b*	9.07	±	1.92	*b*	0.8589
np	51	±	7.29	*a*	37.86	±	5.56	*a*	0.1575
C	46.46	±	6.88	*a*	33.86	±	5.81	*a*	0.1277
D	3.33	±	1.22	*bc*	2.44	±	0.62	*c*	0.5380
E1	4	±	1	*bc*	3.66	±	0.91	*bc*	0.7672
E2	1.4	±	0.24	*c*	1.66	±	0.33	*c*	0.6391
G	1.5	±	0.28	*c*	1.12	±	0.12	*c*	0.1877

Different letters indicate significant differences (LSD) ($P \leq 0.05$) among waveforms within treatments

Table 2-6. Mean (± SE) waveform duration per event per insect (WDEI) (s) for different genders of *Diaphorina citri* feeding and under different light conditions.

Experiment 1: Gender									
Waveform	Female				Male				
	WDEI	±	SE		WDEI	±	SE		p-value
z	964.32	±	219.42	*c*	719.90	±	177.36	*b*	0.5799
np	517.79	±	85.04	*c*	483.87	±	155.48	*b*	0.4161
C	908.95	±	185.76	*bc*	931.60	±	128.17	*a*	0.4457
D	60.51	±	10.46	*d*	66.57	±	14.45	*c*	0.9157
E1	45.96	±	7.22	*d*	107.66	±	41.10	*c*	0.4949
E2	3889.76	±	683.43	*a*	2090.43	±	453.29	*a*	0.0966
G	1789.45	±	350.48	*ab*	2034.97	±	325.73	*a*	0.9461
Experiment 2: Light conditions									
Waveform	Light				Dark				
	WDEI	±	SE		WDEI	±	SE		p-value
z	1404.13	±	523.88	*b*	542.21	±	188.11	*c*	0.0278
np	411.31	±	70.06	*c*	314.53	±	41.77	*c*	0.5911
C	545.92	±	183.3	*c*	928.81	±	175.13	*b*	0.0316
D	51.68	±	11.9	*d*	46.24	±	4.61	*d*	0.9970
E1	59.07	±	13.86	*d*	57.74	±	20.98	*d*	0.5600
E2	4683.1	±	2846.47	*a*	7942.05	±	1439.29	*a*	0.0418
G	2888.07	±	280.9	*a*	1820.95	±	516.66	*b*	0.1734

Different letters indicate significant differences (LSD) ($P \leq 0.05$) among waveforms within treatments

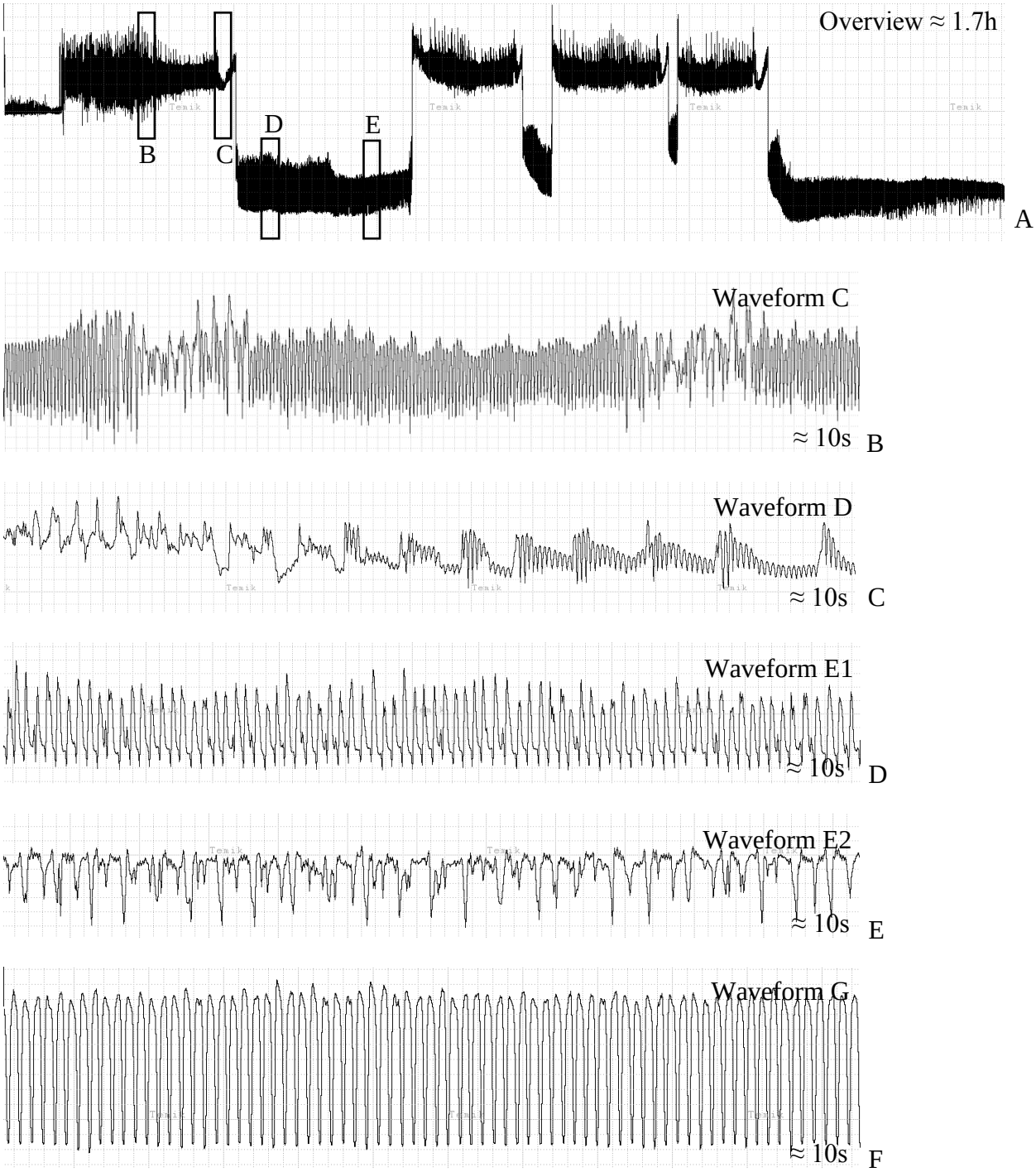

Figure 2-1. Asian citrus psyllid EPG waveforms on sweet orange plants. A) EPG waveform overview. B) Fragment section of the waveform C. C)Fragment section of the waveform D. D) Fragment section of the waveform E1. E) Fragment section of the waveform E2. F) Fragment section of the waveform G. Waveform G is not represented on the waveform overview (A).

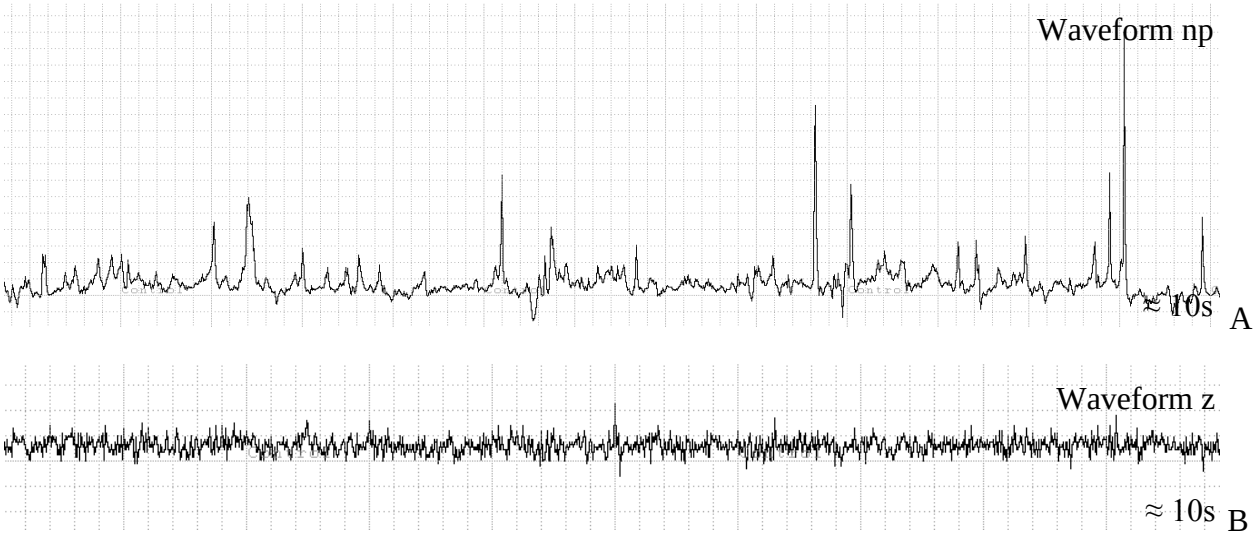

Figure 2-2. Asian citrus psyllid EPG non-probing waveforms A) Waveforms np. B). Waveform z.

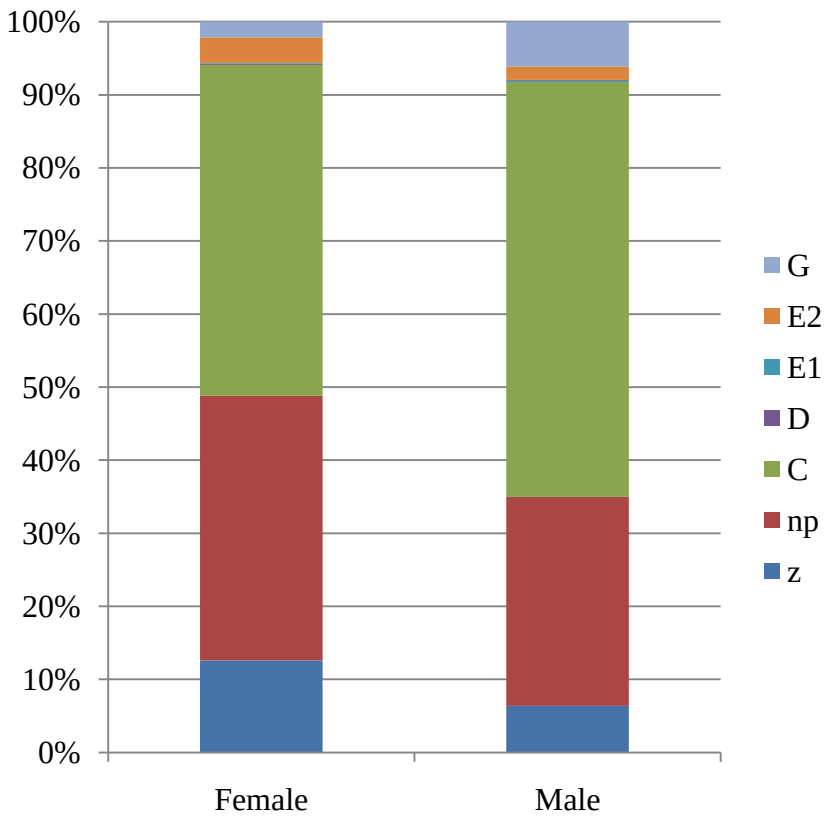

Figure 2-3. Percentage of the total waveform duration (TWD) for female and male *Diaphorina citri* feeding.

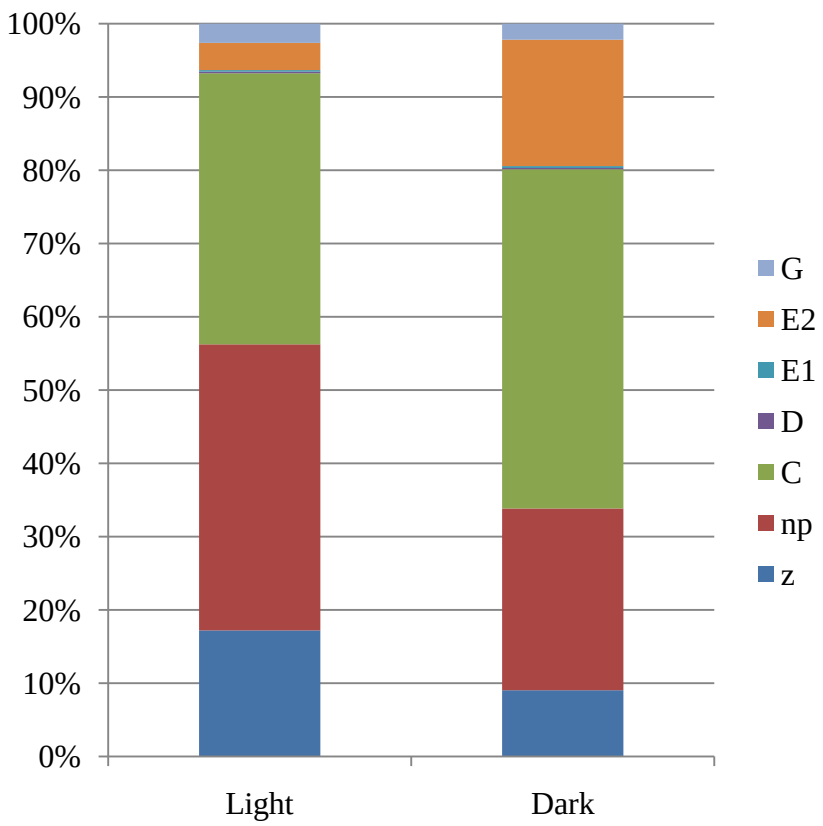

Figure 2-4. Percentage of the total waveform duration (TWD) for *Diaphorina citri* feeding under different light conditions.

CHAPTER 3
EFFECTS OF SOIL-APPLIED IMIDACLOPRID ON ASIAN CITRUS PSYLLID (*DIAPHORINA CITRI* KUWAYAMA) (HEMIPTERA: PSYLLIDAE) FEEDING BEHAVIOR

Imidacloprid is a neonicotinoid insecticide that causes insect mortality by contact and/or ingestion. When applied to the soil surface, imidacloprid is taken up by the plant roots and translocated to citrus leaves as a result of its excellent xylem mobility (Nauen et al. 1999, Sur and Stork 2003). As citrus plants produce new flush, imidacloprid present in the plant xylem moves into the new growth, thus providing an extended period of *D. citri* control. Under typical Florida growing conditions, this systemic activity can last eight weeks or longer (Rogers et al. 2011). In contrast, foliar applications do not provide the same systemic activity, due to the poor phloem mobility of imidacloprid. As a result, imidacloprid does not readily move into flush produced following a foliar application. Thus, the optimal method of imidacloprid application is to nonbearing trees by soil application.

Because *D. citri* ingests primarily from phloem sieve elements and the HLB associated pathogen is a phloem-limited bacterium, pathogen acquisition and inoculation probably occur during stylet activities that occur in the phloem. Acquisition must occur primarily during phloem ingestion; inoculation probably occurs during phloem salivation, because Las is circulative in the vector's body and must re-enter the plant in saliva. Imidacloprid is most mobile in the xylem, from which it then migrates into other plant tissues. It is not known whether acquisition and inoculation of *Candidatus* Liberibacter asiaticus (Las) by *D. citri* could occur before imidacloprid exposure, which would subsequently cause cessation of phloem stylet activities. Therefore, a better understanding of the effects of imidacloprid on stylet penetration behaviors of *D. citri* should help to refine vector control strategies.

Electrical penetration graph (EPG) monitors have been used to study the effects of insecticide applications on insect feeding behavior. Joost and Riley (2005), studying

Frankliniella fusca (Hinds) and *Frankliniella occidentalis* (Pergande), observed that *F. occidentalis* probed plants more frequently and for longer periods of time on imidacloprid-treated plants compared to untreated plants. Their results suggest an increase in the inoculation of the tomato spotted wilt virus (TSWV) on imidacloprid-treated plants, which is a persistent, circulative virus. In contrast, when feeding on imidacloprid-treated tomato plants, *F. fusca* exhibited a significant decrease in the number of probes per insect and probing duration when compared to untreated plants. In addition, Collar et al. (1997b) did not observe any significant differences in probing behavior of *Myzus persicae* (Sulzer) on imidacloprid-treated versus untreated pepper plants.

The first use of an EPG monitor to study the feeding behavior of a psyllid was performed with the pear psylla, *Psylla pyricola* Foerster. Ullman and Mclean (1988) observed salivation (S) and ingestion (I) for both nymphal and adult pear psyllids. Waveforms from the AC monitor used in that study could not distinguish phloem ingestion versus xylem ingestion. However, histological examination showed that both nymphs and adults ingest from all types of leaf cells; xylem, phloem and bundle sheath cells were found to be the preferred sites for ingestion. More recently, Bonani et al. (2010) used a Giga-8 DC monitor to identify and histologically correlate (define) the *D. citri* feeding waveforms, based in part on their similarity to the well-known aphid waveforms.

In the present study, we characterized the feeding behaviors of *D. citri* on citrus plants treated with a soil application of imidacloprid compared with untreated plants. The objective of our study was to determine whether the presence of imidacloprid within a plant disrupts feeding behaviors of *D. citri*, particularly, those hypothesized by Bonani et al. (2010) to be responsible for successful pathogen acquisition and inoculation. The effects of imidacloprid on psyllid

feeding were examined on both young (approximately 2-3 weeks post bud-break in age) and mature leaves. While psyllids prefer to feed on young tender leaves, they can also be found feeding on mature leaves, especially in the winter time when young leaves are not available (Catling 1970). The results of the current study should provide a better understanding of the effect of soil-applied imidacloprid on transmission of Las.

Material and Methods

Plants and Insects

Plants used in the experiments consisted of 'sour orange' (*Citrus aurantium* L.) seedlings (25-30 cm tall) planted in one gallon pots using citrus potting mix (Fafard Citrus Mix, Fafard, Agawam, MA). Seedlings were grown in a pathogen-free greenhouse at 29 ± 3 ^{o}C and 60-80% RH. All seedlings were planted and maintained identically, to minimize interplant variation.

Adult female *D. citri* (10-15 d) used in experiments were obtained from a greenhouse colony free of *Ca.* Liberibacter asiaticus, reared on sour oranges and sweet oranges (*Citrus sinensis* (L.) Osbeck) at 29 ± 3 °C with a photoperiod of 12:12 (L:D) h. Prior to use in EPG recordings, psyllids (10-15 d old) were transferred to a rearing cage (61cm x 61cm x 91cm, Bioquip, Rancho Domingues, CA) containing sour orange plants for a 48 h acclimation period. Of these psyllids held for acclimation, only female *D. citri* were then selected for use in feeding experiments.

EPG Recording and Waveform Analysis

Recordings of *D. citri* feeding for 12 h under constant light conditions on imidacloprid-treated and untreated plants were made using a Giga-8 monitor (Department of Entomology, Wageningen Agricultural University, the Netherlands). Setup of EPG recordings and waveform characterizations were conducted as previously described in Chapter 2.

Experimental Design

Twenty days prior to EPG recordings, citrus plants were treated with imidacloprid (0.750 ml Admire Pro 4.6F) diluted in 250 ml of water applied to the soil surface of each pot. Control plants were treated with tap water only.

Experiment 1. *D. citri* feeding behavior on young leaves.

Four weeks prior to initiation of the experiment a total of 20 plants were pruned to force production of young leaves. From those 20 plants, 10 plants were randomly chosen and treated with imidacloprid 20 days prior to the EPG recording. These plants were treated with imidacloprid at a rate of 0.75ml product (Admire Pro 4.6F) diluted in 250 ml of water applied as a drench to the soil surface of each pot. Control plants were treated with 250 ml of tap water. Only plants that had young leaves were used in this experiment. A total of seven plants containing young leaves were used for each treatment. The feeding behavior of two psyllids was individually recorded on young leaves located on opposite sides of each plant. Because imidacloprid concentration varies leaf-by-leaf, even within the same plant (Mendel et al. 2000), each psyllid EPG recording was considered a unique replicate, thus providing 14 replicates per treatment for analysis.

Experiment 2. *D. citri* feeding behavior on mature leaves.

A total of 10 plants were treated with imidacloprid using the same methods and rates described in the young leaf experiment above. Ten additional plants treated with tap water only served as controls. Two different psyllids were individually recorded feeding on mature leaves on opposite sides of each plant, providing a total of 20 replicates for each treatment.

Statistical Analysis

To compare *D. citri* feeding behavior on imidacloprid-treated and untreated plants, the number of waveform events and their duration were analyzed between treatments using

biologically non-sequential parameters, as described by Backus et al. (2007) and Bonani et al. (2010). These parameters were grouped by cohort, insect, probe and event level. The parameters analyzed at each of these levels were as previously described in Chapter 2.

Pearson's chi-square test was performed to test the goodness of fit (PROC GLIMMIX, SAS Institute 2001). The waveform duration data were log-transformed and the frequencies square root-transformed before statistical analysis, to improve homogeneity and reduce variability. Data were analyzed by protected ANOVA (PROC GLIMMIX, SAS Institute 2001) with the least significant difference (LSD) test (LSMEANS, SAS Institute 2001) used for pairwise comparisons, to determine whether the waveform parameters analyzed were significantly different between the imidacloprid-treated and untreated plants. Data were also compared in a similar manner among waveforms within treatments. Means were considered significantly different at α=0.05.

Results

Experiment 1. Treatment Effects on Feeding, Young Leaves.

One hundred percent (PPW) of *D. citri* performed pathway/stylet penetration waveforms (waveform C) and non-probing/walking activities (waveform np) on both imidacloprid-treated and untreated plants. On the untreated plants, 71.4% (PPW) of *D. citri* penetrated the phloem, salivated and ingested (waveforms D, E1and E2, respectively) and only 35.7% performed non-walking/non probing activities (waveform z). On imidacloprid-treated plants, 42.9% (PPW) of *D. citri* penetrated the phloem (waveform D), 35.7% salivated into the phloem (waveform E1), and only 7.1% ingested phloem sap (waveform E2) (Table 3-1).

Cohort level. *D. citri* had a total access period of 604,800 s, during which psyllids on the imidacloprid-treated plants probed 100 times (TNP) and spent 120,556.32 s (TPD) with their stylets inserted in the leaf. For the majority (80.1%) of the access period, psyllids performed non-

probing activities such as walking, jumping off the leaf (waveform np), or they died from exposure to imidacloprid (waveform z). In contrast, psyllids on untreated plants spent most of their time (63.2%) performing probing activities (TPD = 382,441.72 s) producing 390 (TNP) probes, nearly four times the TNP of *D. citri* on imidacloprid-treated plants. The percentage of each waveform duration performed as part of TPD for both imidacloprid-treated and untreated plants is represented by the total waveform duration (TWD) (Figure 3-1). TWD data clearly show higher percentages of waveform C being produced on untreated plants and higher percentages of waveform z on imidacloprid-treated plants.

Insect level. There was considerable variation among individual insects within the cohort for waveform duration by individual insects (WDi). This variation was observed for both treated and untreated plants, with highest variation on the imidacloprid-treated plants (Figure 3-2). The differences among the waveforms for each individual insect, as demonstrated by WDi between treated and untreated plants, was averaged to give the waveform duration per insect (WDI). Significant differences in WDI were found for non-probing-moving (np) (F = 14.57; df = 1, 26), non-probing-motionless (z) (F = 59.13; df = 1, 17), pathway (C) (F = 18.47; df = 1, 26), phloem salivation (E1) (F = 4.49; df = 1, 14), and xylem ingestion (G) (F = 10.16; df = 1, 9) (Table 3-1). Durations of waveforms np, C, E1 and G were significantly longer on untreated plants, while duration of waveform z was significantly longer on imidacloprid-treated plants (Table 3-1). There also was considerable variation among individual psyllids in terms of the number of probes by insect (NPi) ranging from 3-45 probes on untreated plants, while the NPi on imidacloprid-treated plants ranged from 3-17 probes. Thus, the mean number of probes per insect (NPI) was significantly higher for psyllids on untreated plants compared to imidacloprid-treated plants (F = 19.17; df = 1, 26; Table 3-2). Similar results were also observed for the

duration of those probes (PDI), i.e. psyllids on untreated plants produced probes with significantly longer duration (F = 32.19; df = 1, 26) than psyllids on treated plants (Table 3-2).

Analysis of time to first occurrence of waveform D (T1stD) showed that *D. citri* reached the phloem in an average of 303.9 min, while psyllids on imidacloprid-treated plants took a significantly (F = 4.60; df = 1, 14; P = 0.0414) shorter time, on average only 130.7 min.

Probe level. Analysis of the waveform duration per probe (WDP) revealed a significant difference between treatments for waveforms C (F = 3.97; df = 1, 388) and znp (F = 13.85; df = 1, 406) (Table 3-3). In addition, the number of waveform events per probe (NWEP) was significantly greater on untreated plants for the phloem salivation phase waveform E1 (F = 7.90; df = 1, 36) and significantly less for the znp waveform (F = 26.53, df = 1, 406) (Table 3-4). NWEP for E2 was not significantly different between treatments, although about half the number of events per probe (one vs. two) was performed on treated vs. untreated plants.

Event level. The ACP probing duration per event (PDE), unlike PDI at the insect level, revealed a non-significant difference between treated and untreated plants (Table 3-2). In contrast, significant differences were found in the number of waveform events per insect (NWEI) when imidacloprid-treated and untreated plants were compared. Waveforms np (F = 19.19; df = 1, 26), and C (F = 18.09, df = 1, 26) (Table 3-5) occurred at a significantly higher frequency on untreated plants. NWEI for both E2 and E2 on treated plants were numerically one-fourth the frequency of control plants. The waveform duration per event per insect (WDEI) was significantly longer for waveform G (F = 6.18, df = 1, 9), and C (F = 6.72, df = 1, 26) (Table 3-6) and significantly shorter for waveform z on untreated plants (F = 4.09, df = 1, 17) compared with imidacloprid-treated plants (Table 3-6).

Summary of results. On imidacloprid-treated plants, relative to untreated control plants, psyllids performed their typical feeding behaviors differently, in the following ways. When not probing, individual insects on treated plants were motionless for longer periods of time even though insects performed the same number of standing events. In contrast, treated insects performed less walking on a per insect basis because walking events that occurred were the same length but were performed less often.

During stylet-probing, the duration of pathway activities (waveform C) was shorter for insects on treated plants because each event performed was shorter. Though performed the same number of times per probe, pathway was performed in numerically fewer probes, leading to pathway duration per probe that did not differ between probes. Overall, however, this led to significantly fewer events per insect. Durations of phloem contact (waveform D) were the same for all levels of study, therefore D represents a partly stereotypical behavior. However, there were significantly fewer contacts per probe on treated plants, cancelled out by numerically longer durations per probe. The duration per insect for phloem salivation (waveform E1) was shorter for insects on treated plants, because while each event was the same length, they were performed less often per probe, in numerically fewer probes, leading to shorter duration per probe and significantly fewer events per insect. Phloem ingestion (waveform E2) duration per insect was statistically the same for each treatment, at all levels. However, numerically longer events were performed half as often per probe, in only one-fourth the number of probes, leading to (numerically) twice the mean duration per probe but only one-fourth the number of events per insect. In contrast, xylem ingestion (waveform G) was performed for shorter durations per insect on treated plants because each event was shorter, but performed the same number of times (once

per probe), in numerically fewer probes, leading to less time per probe and significantly fewer events per insect.

Overall, insects on treated plants stood still for longer periods of time and walked less often. Insects on treated plants searched less often for vascular tissue, for shorter periods of time in fewer probes. Insects on imidacloprid-treated plants also required less time to find phloem and salivate into it, during the fewer occasions they found it. However, once there, they salivated and ultimately ingested for the same duration each of those fewer times. In contrast, insects on treated plants found and ingested from xylem more briefly each time, though the same number of times (once) per probe, but in fewer probes.

Experiment 2. Treatment Effects on Feeding, Mature Leaves.

All *D. citri* exposed to untreated mature leaves performed stylet penetration of leaf tissues (waveform C), although only 40% (PPW) of the female *D. citri* reached the phloem and salivated (waveform D and E1), and only 25% ingested phloem sap (waveform E2). In contrast, while 95% of *D. citri* on imidacloprid-treated mature leaves performed stylet penetration, only 25% (PPW) reached the phloem, 20% performed phloem salivation, and only 5% ingested phloem sap (waveform C, D, E1 and E2 respectively) (Table 3-1).

Cohort level. From a total access period of 864,000 s, *D. citri* on the imidacloprid-treated mature leaves probed 99 times (TNP) and spent 42,110.38 s (TPD) with their stylets inserted in the leaf tissues. For the remaining 95.1% of the total access period, psyllids performed non-probing activities (waveforms np and z). In contrast, *D. citri* on untreated plants spent 43.1% of their total access period performing probing activities (TPD = 372,497 s), producing 268 probes (TNP). The percentage of each waveform duration performed in TPD for both imidacloprid-treated and untreated plants are represented by the total waveform duration (TWD) shown in Figure 3-1.

Insect level. Similar to the results from experiments with young leaf tissues, there was also considerable variation in waveform duration among individual insects. This was shown by a higher variation in waveform duration by insect (WDi) on imidacloprid-treated compared to untreated plants (Figure 3-3). Analysis of the mean waveform duration per insect (WDI) showed significantly longer durations for waveforms np (F = 11.84; df = 1, 38), C (F = 36.39; df = 1, 37), D (F= 12.25; df = 1, 11), E1 (F = 4.65; df = 1, 10), and G (F = 6.74; df = 1, 13) (Table 3-1) on untreated plants. The number of probes performed by insect (NPi) also indicated high variation among the insects; *D. citri* on untreated plants performed 2-28 probes while the ones on imidacloprid-treated plants performed 1-15 probes. The significant difference between NPi on treated and untreated plants was apparent in the number of probes per insect (NPI) (F = 14.55; df = 1, 38;Table 3-2), in which untreated plants showed a higher number of probes than the imidacloprid-treated plants. Additionally, PDI was significantly longer for psyllids on untreated plants than on imidacloprid-treated plants (F = 40.9; df = 1, 37; Table 3-2).

Analysis of time to first D (T1stD) indicated that *D. citri* reached the phloem of control plants in an average of 262.15 min and an average of 126.78 min on imidacloprid-treated plants; this difference was not significant.

Probe level. The waveform duration per probe (WDP) was significantly longer for waveform C (F = 19.56, df = 1, 366) on untreated plants but znp was significantly shorter (F = 26.11, df = 1, 396; Table 3-3). In contrast, the number of waveform events per probe (NWEP) was not significantly different between treatments for any waveform analyzed (Table 3-4).

Event level. Psyllid probing duration per event (PDE) indicated significant differences between treated and untreated plants (F = 9.43, df = 1, 616; Table 3-2), with duration per event longer on untreated plants. The number of waveform events per insect (NWEI) was significantly

higher on untreated plants for waveforms np (F = 12.40; df = 1, 38), and C (F = 19.91, df = 1, 37) (Table 3-5). The waveform duration per event per insect (WDEI) was longer for waveforms C (F = 9.24, df = 1, 37), D (F = 5.90, df =1, 11) and G (F = 5.11, df =1, 13) (Table 3-6) for *D. citri* feeding on untreated compared to imidacloprid-treated plants.

Summary of results. Feeding behavior of *D. citri* differed on imidacloprid-treated plants and control plants. When not probing, psyllids on treated plants stood still (z) for longer durations per insect. Even though the durations per insect were not significantly different, their event were two times longer than the event durations on the control plants, making this event even longer, because the frequency of this event per insect was the same between treated and untreated plants. Waveform analysis indicated that psyllids stood still on treated plants half as much as on untreated plants In contrast, on treated plants psyllids performed less walking (waveform np), with shorter duration events per insect and those events were also performed less often.

When stylet-probing, psyllids on treated plants performed shorter pathways activities (waveform C) for a shorter duration per insect because each event was shorter. Furthermore, those pathway activities were characterized by shorter event duration and those events were less frequently performed per insect. In addition, pathway activity was less frequent per probe, and consequently pathway duration per probe was shorter and occurred as fewer events per insect. Durations of phloem contact (D) per insect were significantly shorter on the treated mature leaves than on young leaves because of shorter and less frequent phloem contact per insect. Although, the duration of phloem contact per probe was not significantly different between young and old leaves, there were significantly fewer contacts into the phloem per insect.

As observed with the psyllids on treated young leaves, *D. citri* on the mature leaves also performed phloem salivation (E1) for a shorter duration per insect, with events of the same length as on control plants. Given that these events were the same length, they were performed less often per probe, in numerically fewer probes, leading to fewer probes that were shorter in duration. Phloem ingestion (E2) duration per insect was statistically the same for each treatment, at all levels. Both treatments had similar duration of events. However, there was a noticeable trend for reduced phloem ingestion on treated plants which was 62 fold shorter in duration than that on untreated plants. However only half the number of events per insect occurred on untreated than treated plants. In contrast, xylem ingestion (G) was performed for shorter durations per psyllid on treated plants than on untreated plants because each event was shorter and performed less often per insect. Overall, as observed with young leaves containing imidacloprid, psyllids stood still longer and walked less often on imidacloprid-treated mature leaves. Psyllids searched less often for vascular tissue and for shorter durations of time in fewer probes on untreated than treated plants. Then, as insects in the young treated plants, psyllids on mature treated plants stood still for longer times and walked less often. Insects on treated plants searched less often for vascular tissue, for shorter times in fewer probes. While the presence of imidacloprid in plant tissues reduced the overall duration of phloem ingestion and salivation compared to untreated plants, those psyllids on treated plants that were able to locate and salivate into the phloem did so in a shorter period of time. Psyllids on treated plants found and ingested from xylem less frequently and with fewer probes than on untreated plants.

Discussion

The objective of the current study was to examine the effects of soil-applied systemic applications of imidacloprid on the feeding behavior of *D. citri* to quantify effects of this pesticide on the behaviors that mediate transmission of Las. Overall, the general feeding

behaviors of *D. citri* were disrupted on imidacloprid-treated plants as demonstrated by an increased duration and frequency of standing still, decreased walking and searching for probing sites, and reduced probing behaviors. Similar results were observed for *F. fusca* on imidacloprid-treated pepper, mustard (Groves et al. 2001) and tomato (Joost and Riley 2005).

While all *D. citri* tested on imidacloprid-treated plants died during the course of the 12 h EPG recording due to intoxication, *D. citri* on treated young leaves died sooner on average (4 h) than on old leaves. Consequently, psyllids had an average feeding access period of 4 h before succumbing to the pesticide treatment. In this access period, a considerable percentage of the *D. citri* were able to reach the phloem (waveforms D, E1, and E2).

Because the associated agent, *Candidatus* Liberibacter asiaticus, is a phloem-limited bacterium (Bove 2006), it is likely that bacterial acquisition and inoculation require phloem ingestion and salivation, respectively. Bonani et al. (2010), examining *D. citri* feeding on HLB-affected citrus plants, observed that detectable Las acquisition occurred after 1 h of the *D. citri* ingestion waveform (E2). In our study, *D. citri* feeding on young leaves of plants treated with imidacloprid had an average ingestion period of 1 h (0.02 h for mature leaves). However, only 14% of psyllids exposed to young treated leaves produced waveform durations longer than 1 h, and from the total of six phloem contact events only two were longer than 1 h. In contrast, psyllids on mature control plants had a total of 42 phloem contacts, in which two waveforms were also longer than 1 h. Consequently, a few psyllids might have a slightly higher chance of acquiring Las during longer, although rare, phloem ingestion (E2) events on an infected tree. On the other hand, the probability of inoculation is probably reduced because both numbers and durations of phloem salivation (E1) events were reduced on treated plants. Although not significantly different due to small sample size, we suggest that these changes in phloem

salivation and ingestion could be biologically important for Las transmission on imidacloprid-treated plants. Thus, if those insects were feeding on a Las positive plant, they would have a reduced, but probably biologically relevant, chance of acquiring Las. However, they would not be able to transmit, due to the pathogen latency period in the psyllid. Studies suggest a latency period of 24 h to 25 d (Xu et al. 1988, Roistacher 1991), thus as our results show, psyllids would be dead before they were able to transmit. In addition, *D. citri* are poor vectors of Las when they acquire the bacteria as adults Pelz-Stelinski et al. (2010).

Compared to feeding on mature leaves, *D. citri* on young citrus leaves performed numerically more probes which were also longer in duration on both imidacloprid-treated and untreated plants. This result is probably related to leaf tenderness, which may influence the ease of successful stylet penetration. Bonani (2009) showed that during a *D. citri* access period of 5 h, 50% of the psyllids on young leaves reached the phloem and ingested while only 15% of the psyllids reached the phloem on the mature leaves reached the phloem. It was postulated that this was due to higher lignifications of the cell walls of mature leaves (Bonani 2009). The ability of some psyllids to perform extensive bouts of phloem-associated behaviors on the young leaves of imidacloprid-treated plants may be due to uneven distribution of imidacloprid within a leaf. Mendel et al. (2000) found uneven concentrations of imidacloprid throughout the parenchyma and low concentrations close to the vascular bundles; however, younger shoots had higher imidacloprid concentrations. Similar results were also found by El-Hamady et al. (2008) when examining the distribution of radioactive imidacloprid in cotton. In addition, El-Hamady et al. (2008) found higher concentrations of imidacloprid nearest to the edges of young leaves with concentrations at much lower levels near the leaf veins. Because psyllids often feed closer to leaf veins, those insights could explain the ability of some *D. citri* to successfully ingest from phloem

on imidacloprid-treated plants. Likewise, the high variability seen in waveform duration by individual insects could be a result of the location of the phloem sieve elements chosen by those individual psyllids, which was not accounted for in this study.

Implications for managing the spread of HLB. The current results demonstrate that the benefits of soil-applied imidacloprid applications extend beyond reducing psyllid populations. Prior to causing mortality, imidacloprid application can potentially disrupt psyllid feeding behaviors such that successful pathogen acquisition and inoculation are both less likely to occur. Our findings also support other studies that have shown imidacloprid-treated plants to be repellent to *D. citri*. During the course of our EPG recordings, we observed that following a short test probe, *D. citri* often jumped off imidacloprid-treated plants, whereas psyllids on untreated plants rarely left the plant. This was indicated by the high percentage of time spent by *D. citri* in non-probing behaviors for both young and mature leaves of imidacloprid-treated plants. Similarly, Liu and Trumble (2004), examining the feeding behavior of *B. cockerelli* on tomato, found a significantly higher jumping frequency and a longer off-of-plant duration for imidacloprid-treated compared to untreated tomato plants. Boina et al. (2009) also reported sublethal effects of imidacloprid resulting in repellence of *D. citri* when encountering citrus with low levels of imidacloprid present. Further studies are needed to determine the duration of this feeding disruption provided by soil applications of imidacloprid to help guide growers in developing effective psyllid control programs for protection of young trees from HLB disease.

Table 3-1. Mean (± SE) waveform duration per insect (WDI) (s) and the proportion of individuals that produced a waveform type (PPW) for *Diaphorina citri* feeding on young and mature leaf tissues of imidacloprid-treated and untreated plants.

Experiment 1: Young Leaves

Waveform	Untreated control				Imidacloprid				
	WDI	±	SE	PPW	WDI	±	SE	PPW	p-value
z	6333.82	±	982.71	5/14	32043.71	±	2781.96	14/14	<0.0001
np	15375.28	±	2807.67	14/14	6371.35	±	1101.15	14/14	0.0008
C	22205.09	±	2154.62	14/14	5529.63	±	1505.27	14/14	0.0002
D	747.57	±	180.88	10/14	544.34	±	226.05	6/14	0.2337
E1	1378.41	±	272.65	10/14	308.01	±	77.70	6/14	0.0525
E2	3269.93	±	930.40	10/14	3601.42	±	2061.69	5/14	0.2295
G	1764.50	±	290.79	10/14	94.08	±	N/A	1/14	0.0110

Experiment 2: Mature leaves

Waveform	Untretead control				Imidacloprid				
	WDI	±	SE	PPW	WDI	±	SE	PPW	p-value
z	13915.58	±	3769.54	6/20	32390.61	±	2815.86	20/20	0.2800
np	22217.05	±	2822.68	20/20	10627.13	±	2710.06	20/20	0.0014
C	13287.12	±	2143.33	20/20	2182.41	±	600.03	19/20	< 0.0001
D	669.77	±	284.14	8/20	64.59	±	21.03	5/20	0.0050
E1	250.65	±	99.78	8/20	46.51	±	8.05	4/20	0.0565
E2	7598.13	±	3520.96	5/20	76.18	±	N/A	1/20	0.0722
G	4381.29	±	1219.61	14/20	59.54	±	N/A	1/20	0.0222

Table 3-2. Mean (± SE) probe duration per insect (PDI) (s), number of probes per insect (NPI), and probe duration per event (PDE) (s) for *Diaphorina citri* feeding on young and mature leaf tissues of imidacloprid-treated and untreated plants.

Experiment 1: Young Leaves

Untreated control			Imidacloprid			
PDI	±	SE	PDI	±	SE	p-value
27317.27	±	2555.20	7187.86	±	2083.68	0.0003
NPI	±	SE	NPI	±	SE	p-value
21.14	±	2.76	8.00	±	1.19	0.0002
PDE	±	SE	PDE	±	SE	p-value
498.09	±	42.04	488.49	±	73.26	0.6301

Experiment 2: Mature leaves

Untreated control			Imidacloprid			
PDI	±	SE	PDI	±	SE	p-value
18270.98	±	2376.14	2216.34	±	608.17	<0.0001
NPI	±	SE	NPI	±	SE	p-value
14.05	±	1.96	5.80	±	0.91	0.0005
PDE	±	SE	PDE	±	SE	p-value
750.88	±	74.07	345.17	±	44.54	0.0022

Table 3-3. Mean (± SE) waveform duration per probe (WDP) (s) for *Diaphorina citri* feeding on young and mature leaf tissues of imidacloprid-treated and untreated plants.

Experiment 1: Young Leaves							
Waveform	Untreated control			Imidacloprid			
	WDP	±	SE	WDP	±	SE	p-value
znp	834.20	±	118.26	4726.59	±	955.99	0.0002
C	1075.96	±	119.07	973.41	±	194.26	0.0469
D	219.95	±	21.94	272.17	±	96.22	0.2577
E1	492.29	±	106.76	184.81	±	30.92	0.1791
E2	1634.96	±	530.41	3001.19	±	1520.88	0.6543
G	1260.35	±	212.84	94.08	±	N/A	0.0610

Experiment 2: Mature leaves							
Waveform	Untreated control			Imidacloprid			
	WDP	±	SE	WDP	±	SE	p-value
znp	1871.75	±	264.61	7038.63	±	1107.05	<0.0001
C	987.84	±	125.49	418.75	±	67.57	<0.0001
D	336.58	±	140.68	53.82	±	19.99	0.1538
E1	123.63	±	41.45	46.51	±	8.05	0.8918
E2	4748.83	±	1803.21	76.18	±	N/A	0.2707
G	2667.44	±	807.48	59.54	±	N/A	0.1977

Table 3-4. Mean (± SE) number of waveform event per probe (NWEP) and number of probes by waveform (NPw) for *Diaphorina citri* feeding on young and mature leaf tissues of imidacloprid-treated and untreated plants.

Experiment 1: Young Leaves

Waveform	Untreated control				Imidacloprid				
	NWEP	±	SE	NPw	NWEP	±	SE	NPw	p-value
znp	1.04	±	0.01	296	1.44	±	0.12	112	<0.0001
C	1.48	±	0.09	290	1.36	±	0.13	100	0.5417
D	4.44	±	0.42	29	3.25	±	0.81	12	0.1164
E1	5.50	±	0.61	28	2.40	±	0.54	10	0.0080
E2	2.10	±	0.33	20	1.00	±	0.00	5	0.0860
G	1.00	±	0.00	14	1.00	±	N/A	1	N/A

Experiment 2: Mature leaves

Waveform	Untreated control				Imidacloprid				
	NWEP	±	SE	NPw	NWEP	±	SE	NPw	p-value
znp	1.00	±	0	282	1.00	±	0	116	N/A
C	1.26	±	0.07	268	1.07	±	0.03	99	0.1028
D	3.38	±	0.87	16	1.33	±	0.21	6	0.1740
E1	3.75	±	0.99	16	1.50	±	0.29	4	0.2809
E2	1.63	±	0.32	8	1.00	±	N/A	1	0.5406
G	1.35	±	0.15	23	1.00	±	N/A	1	0.6382

Table 3-5. Mean (± SE) number of waveform events per insect (NWEI) for *Diaphorina citri* feeding on young and mature leaf tissues of imidacloprid-treated and untreated plants.

Experiment 1: Young Leaves

Waveform	Untretaed control			Imidacloprid			
	NWEI	±	SE	NWEI	±	SE	p-value
z	1.40	±	0.25	3.36	±	0.77	0.1588
np	21.43	±	2.75	8.21	±	1.25	0.0002
C	30.36	±	4.52	9.71	±	1.76	0.0002
D	13.30	±	3.35	6.50	±	2.25	0.1703
E1	15.40	±	4.49	4.00	±	1.03	0.0787
E2	4.20	±	1.60	1.00	±	N/A	0.2158
G	1.40	±	0.22	1.00	±	N/A	0.5987

Experiment 2: Mature leaves

Waveform	Untreated control			Imidacloprid			
	NWEI	±	SE	NWEI	±	SE	p-value
z	4.50	±	1.50	3.65	±	0.52	0.5018
np	14.65	±	2.11	6.50	±	0.99	0.0011
C	16.9	±	2.29	5.57	±	0.99	< 0.0001
D	6.75	±	2.63	1.60	±	0.24	0.1568
E1	7.5	±	2.96	1.50	±	0.29	0.1923
E2	2.60	±	0.81	1.00	±	N/A	0.4664
G	2.214	±	0.43	1.00	±	N/A	0.4833

Table 3-6. Mean (± SE) waveform duration per event per insect (WDEI) (s) for *Diaphorina citri* feeding on young and mature leaf tissues of imidacloprid-treated and untreated plants.

Experiment 1: Young Leaves

Waveform	Untreated control			Imidacloprid			
	WDEI	±	SE	WDEI	±	SE	p-value
Z	6333.82	±	982.71	32043.71	±	2781.36	0.0591
Np	1596.30	±	878.21	803.10	±	115.45	0.7411
C	985.46	±	188.93	486.88	±	87.46	0.0154
D	56.93	±	4.55	70.01	±	25.60	0.7770
E1	129.67	±	38.58	78.01	±	8.45	0.8140
E2	1145.72	±	255.27	2585.39	±	1396.73	0.8712
G	1438.90	±	250.49	94.08	±	N/A	0.0346

Experiment 2: Mature leaves

Waveform	Untreated control			Imidacloprid			
	WDEI	±	SE	WDEI	±	SE	p-value
z	7358.75	±	4491.82	14084.04	±	2953.50	0.2973
np	2345.87	±	470.63	1909.68	±	558.24	0.3545
C	838.11	±	127.95	419.64	±	67.18	0.0043
D	110.63	±	22.85	41.15	±	9.85	0.0335
E1	37.19	±	7.81	31.56	±	1.95	0.9808
E2	3330.71	±	1192.10	76.18	±	N/A	0.1099
G	2383.16	±	670.29	59.54	±	N/A	0.0410

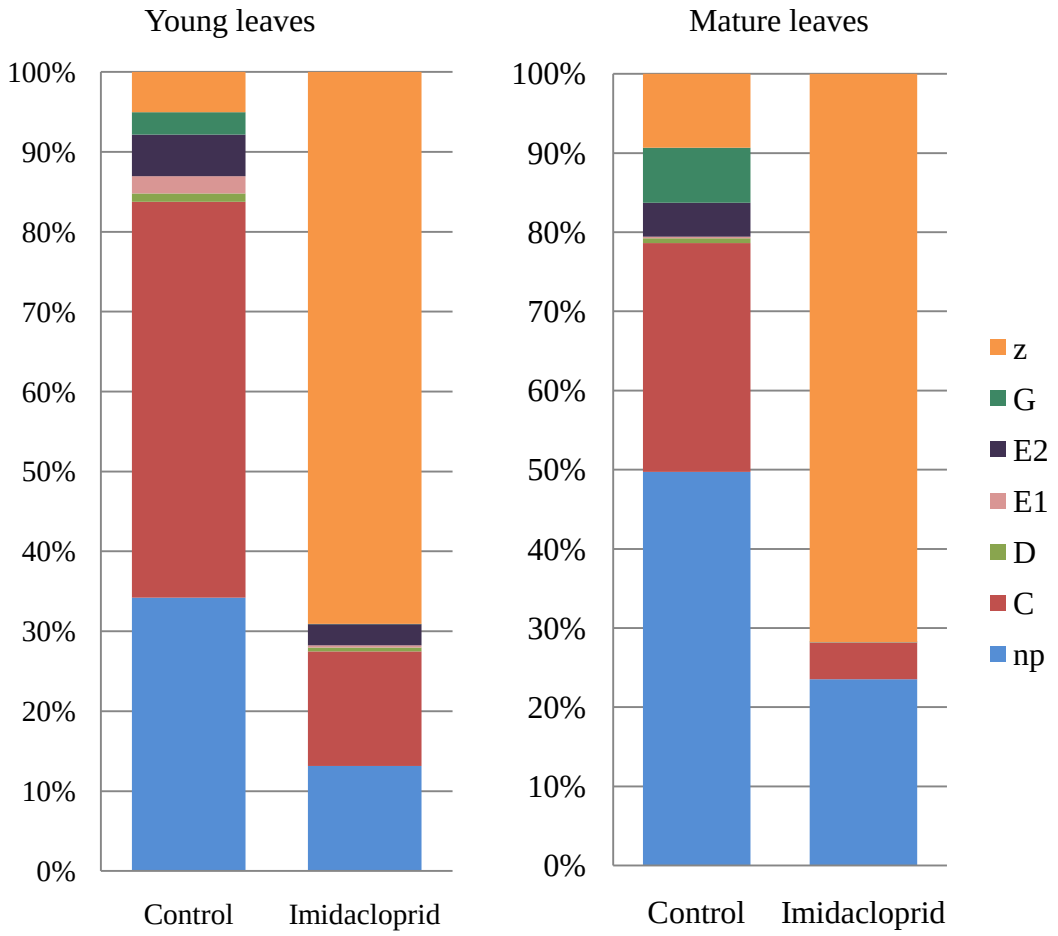

Figure 3-1. Percentage of the total waveform duration (TWD) for *Diaphorina citri* feeding on young and mature leaf tissues of imidacloprid-treated and untreated plants.

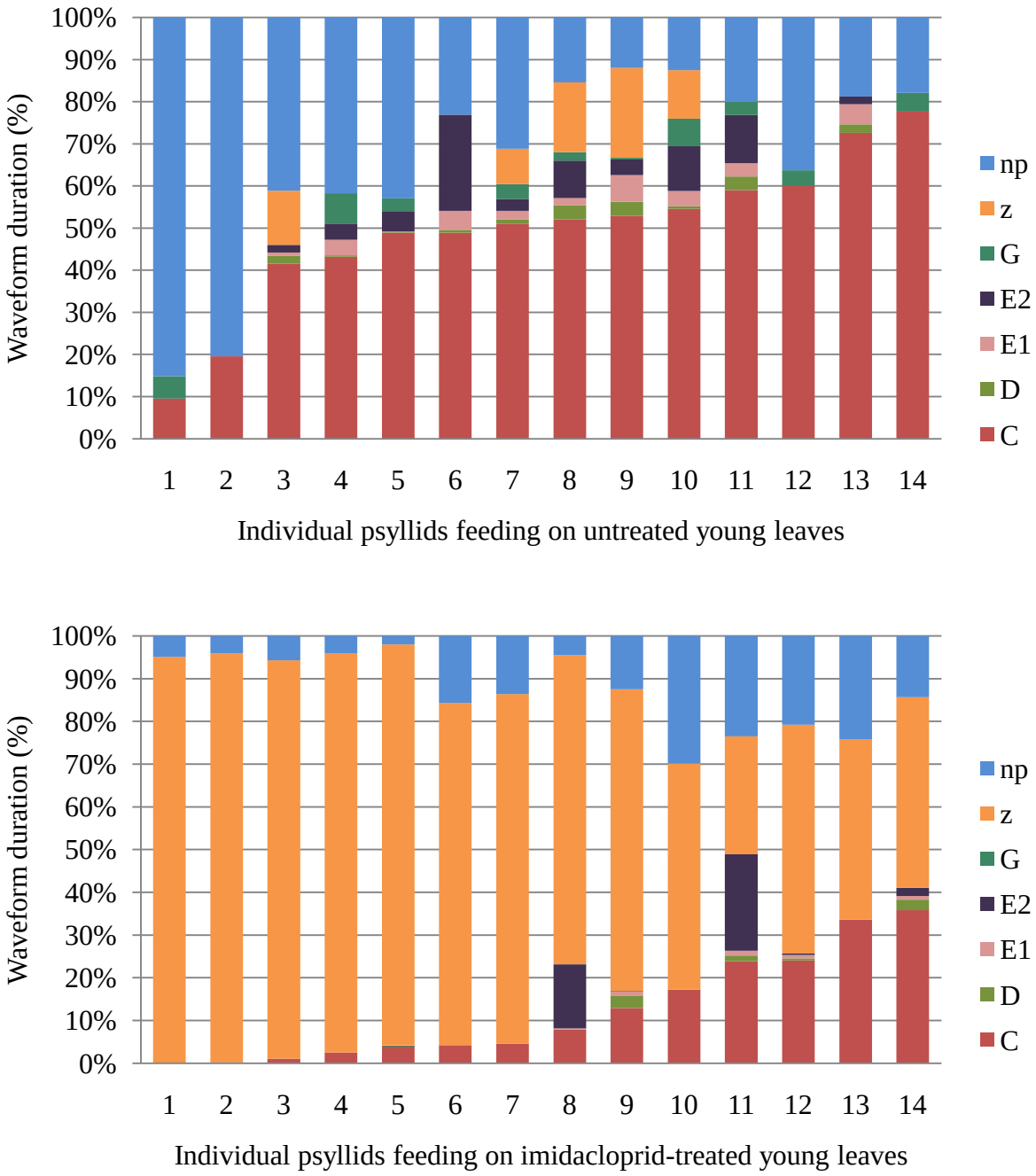

Figure 3-2. Percentage of the waveform duration by insect (WDi) for *Diaphorina citri* feeding on young leaf tissues of imidacloprid-treated and untreated plants.

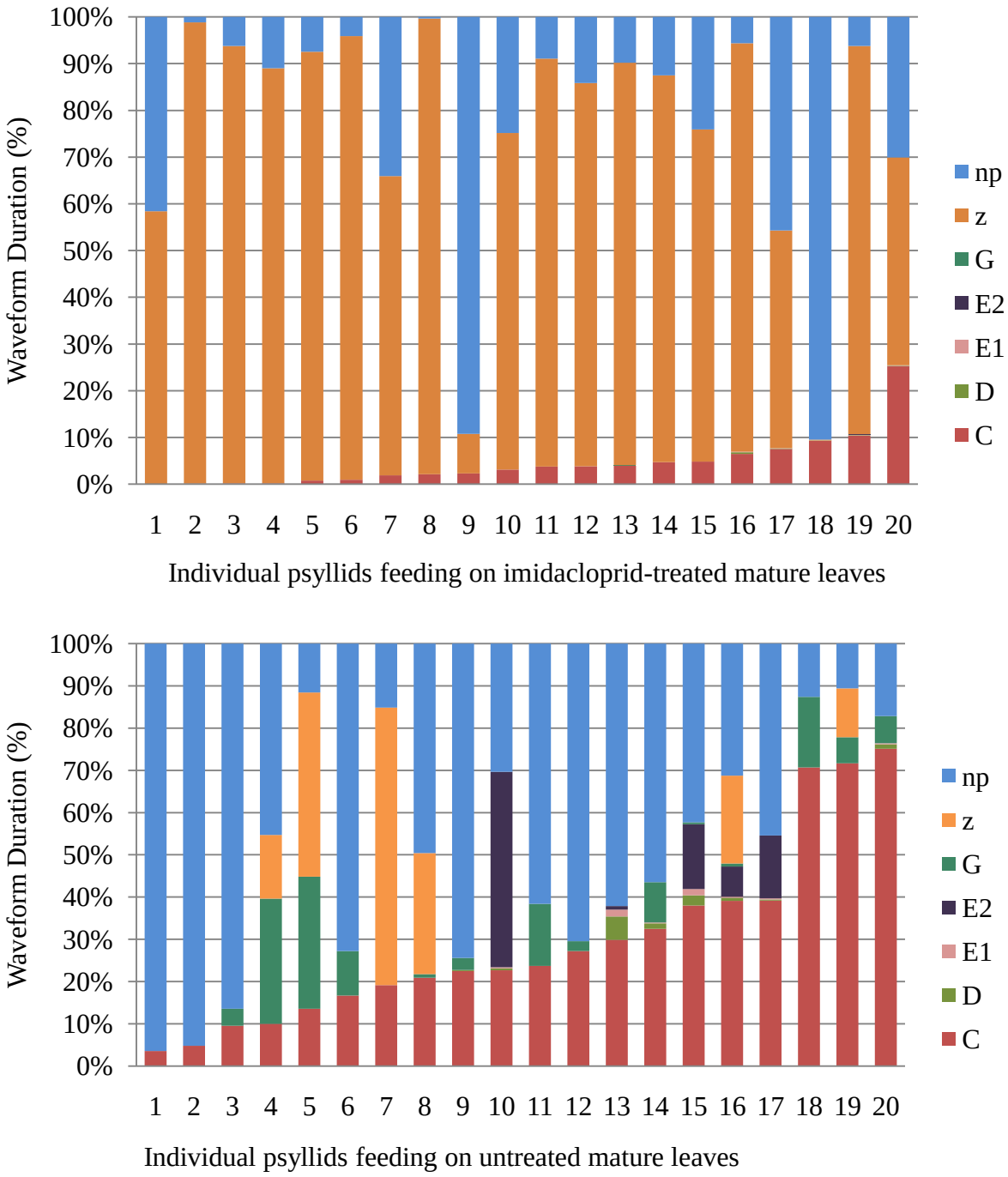

Figure 3-3. Percentage of the waveforms duration by insect (WDi) for *Diaphorina citri* feeding on mature leaf tissues of imidacloprid-treated and untreated plants.

CHAPTER 4
EFFECTS OF ALDICARB ON ASIAN CITRUS PSYLLID (HEMIPTERA: PSYLLIDAE) FEEDING BEHAVIOR AND THEIR POTENTIAL IMPACTS ON TRANSMISSION

Soil-applied systemic insecticides are commonly used in Florida citrus production for control of plant-feeding pests. Due to their long residual period of activity within treated plants, these systemic insecticides typically provide control of target pests for longer durations of time compared to foliar insecticide sprays. Currently, three soil-applied systemic insecticides are registered for use in Florida citrus: aldicarb (Temik ® 15G, Bayer CropSciences, Research Triangle Park, N.C.), imidacloprid (Admire® Pro 4.6F, Bayer CropSciences, Research Triangle Park, N.C.) and thiamethoxam (Platinum 75SG, Syngenta CropProtection, Inc., Greensboro, N.C.) (Rogers et al, 2011). Of these products, aldicarb is the only systemic product for which the product label permits its use at high enough quantities of active ingredient to provide control of target pests on large (> 9 ft height) bearing citrus trees. For this reason, aldicarb has been commonly used in Florida citrus production since the 1970's for control of insect and mite pests on both young and mature citrus trees.

Aldicarb is a systemic carbamate insecticide that is applied as a granular formulation incorporated into the soil surface surrounding the plant. The maximum use rate of aldicarb in Florida citrus is 36.99 kg/ha. Soon after being applied, aldicarb granules dissolve in the presence of soil moisture and the product is then readily absorbed by the plant root system for translocation throughout the plant (Ware 1994). Because of concerns of groundwater contamination, past use of aldicarb has been restricted to the Florida dry season (November 15th through April 30th). More recently however, the worldwide registration of aldicarb is being cancelled with use in Florida citrus no longer permitted after December 31, 2011.

Since the discovery of HLB in Florida in 2005, aldicarb has been viewed as an important tool for controlling *D. citri* and managing the spread of HLB (Qureshi and Stansly 2008).

However, the true value of use of this product over the past six years for controlling the spread of HLB is not well understood. Thus, the objective of the current investigation was to evaluate the effects of aldicarb (Temik 15G®) applications on *D. citri* feeding behavior and determine the potential impacts on Las transmission.

Materials and Methods

Plants and Insects

Plants used in the experiments consisted of 'sweet orange' (*Citrus sinensis* (L.) Osbeck) seedlings (15-20 cm tall) planted in 120ml tubes containing mix Fafard Citrus potting Mix (Fafard, Agawam, MA), grown in a pathogen-free greenhouse at 29 ± 3 °C and 60-80% RH. All seedlings were planted and maintained identically to minimize interplant variation. Female psyllids (10-15d post-emergence) were obtained from a greenhouse colony (free of Las) reared on citrus at 29 ± 3 °C and 12:12 (L:D) h photoperiod.

EPG Recording and Waveform Analysis

Recordings of *D. citri* feeding for 12 h under constant light conditions on aldicarb-treated and untreated plants were made using a Giga-8 monitor (Department of Entomology, Wageningen Agricultural University, the Netherlands). Setup of EPG recordings and waveform characterizations were conducted as previously described in Chapter 2.

Effects of Aldicarb on *D. citri* Feeding Behavior

Twenty days prior to EPG recordings, a subset of plants was treated with 0.0046g of aldicarb (Temik 15G) (field rate) applied to the soil surface of each pot. After insecticide application, the pots were covered with a layer (1cm) of potting soil to avoid insecticide spill during watering.

A total of 15 plants were treated with aldicarb, and 15 additional plants were used as controls. One young leaf was randomly selected from each plant on which a single psyllid was placed and feeding behavior then recorded.

Statistical Analysis

To compare *D. citri* feeding behavior on aldicarb-treated and untreated plants, the number of waveform events and their duration were analyzed between treatments using biologically non-sequential parameters, as described by Backus et al. (2007) and Bonani et al. (2010). These parameters were grouped by cohort, insect, probe and event level. The parameters analyzed at each of these levels were as previously described in Chapter 2.

Pearson's chi-square test was performed to test the goodness of fit (PROC GLIMMIX, SAS Institute 2001). The waveform duration data were log-transformed and the frequencies square root-transformed before statistical analysis, to improve homogeneity and reduce variability. Data were analyzed by protected ANOVA (PROC GLIMMIX, SAS Institute 2001) with the least significant difference (LSD) test (LSMEANS, SAS Institute 2001) used for pairwise comparisons, to determine whether the waveform parameters analyzed were significantly different between the aldicarb-treated and untreated plants. Means were considered significantly different at $\alpha=0.05$.

Confirmation of Aldicarb in Treated Plants

After recordings of *D. citri* feeding behavior were completed, leaf samples were collected for analysis to confirm the presence of aldicarb in the plant tissue. Since the plants used in the recordings were small and a minimum of 5 g of leaf tissue was needed for proper analysis, leaves from 5 plants of each treatment was combined to obtain a sufficient quantity of leaf material for analysis. Leaf samples were sent to the Waters Agricultural Laboratory (Camilla, GA) for analysis of aldicarb content using HPLC/UV chromatography with detection at 205nm.

Results

One hundred percent (PPW) of *D. citri* performed pathway/stylet penetration waveforms (Waveform C) and non-probing/walking activities (Waveform np) on both aldicarb-treated and untreated plants. On the untreated plants, 71.4% (PPW) of *D. citri* penetrated the phloem and salivated (Waveforms D and E1, respectively) and 64.3% ingested phloem (Waveform E2). Approximately seventy-nine percent (78.6%) of the psyllids performed xylem activities (Waveform G) on untreated plants and only 7.1% spend time with non-walking/non probing activities (Waveform z). On aldicarb-treated plants, 78.6% (PPW) of *D. citri* penetrated the phloem (Waveform D) and salivated into it (Waveform E1), 57.1% ingested phloem sap (Waveform E2), 85.7% ingested xylem sap (Waveform G) and 64.3% performed non-walking/non-probing activities (Table 4-1).

Cohort level. *D. citri* had a total access period of 604,800 s, during which psyllids on untreated plants performed 217 (TNP) probes and spent 405,759.3 s (TPD) with their mouth parts inserted into the leaf. Psyllids on aldicarb-treated plants probed 195 times (TNP) in which the total duration of the probes (TPD) was 337,361.09 s. The percentage of each waveform duration performed in TPD are represented by the total waveform duration (TWD) shown in Figure 4-1. There was only a small difference between *D. citri* feeding on treated and untreated plants. Those differences were investigated in more detail at the insect level.

Insect level. The probe duration per insect (PDI) on untreated plants averaged 28,982.81 s, while on aldicarb-treated plants it averaged 24,097.22 s (Table 4-2). The number of probes per insects (NPI) was also observed, and no significant difference was found between *D. citri* feeding on aldicarb-treated and untreated plants ($F = 0.16$; df = 1, 26) (Table 4-2).

ANOVA indicated no significant difference between treatments (F = 0.3469; df = 1, 26). The waveform duration performed per psyllid (WDI) was also not significantly different (Table 4-1).

Analysis of time to first D (T1stD) showed that *D. citri* on untreated plants required on average 238.70 min to reached the phloem, while psyllids on aldicarb-treated plants took an average 252.23 min, this difference was not significantly different (F = 0.03; df = 1, 19; p = 0.8721).

Probe level. Similar to that observed at the insect level, the number of waveform events per probe (NWEP) did not differ significantly between aldicarb-treated and untreated plants for any of the waveforms (Table 4-3). In contrast, waveform duration per probe (WDP), differed significantly for waveforms znp (F = 11.49; df = 1, 421; Table 4-4), but not for any of the other waveforms.

Event level. *D. citri* probing duration per event (PDE), unlike PDI and NPI at the insect level, revealed a significant difference between treated and untreated plants (F = 7.76; df = 1,1076; Table 4-2). In addition, waveform duration per event (WDE) was significant different for waveforms np (F = 7.42; df = 1,435), E1 (F = 4.92; df = 1,204), and E2 (F = 4.61; df = 1, 47), in which non-probing activities were longer on control plants than aldicarb-treated plants (Table 4-7). Also, phloem activities were longer on aldicarb-treated plants than untreated plants (Table 4-7). However, the number of waveforms events performed per insect (NWEI) was not significantly different between *D. citri* on aldicarb-treated and untreated plants (Table 4-5). In addition, waveform duration per event per insect (WDEI), as NWEI, were not significantly different between treatments (Table 4-6).

Confirmation of Aldicarb in Treated Plants

Because there was no apparent difference in the parameters analyzed for *D. citri* feeding on aldicarb-treated compared to untreated plants, analysis of aldicarb content within the leaves was conducted to confirm the presence of aldicarb in treated plants. The mean (±SE) content of aldicarb per 5 g of plant tissue for aldicarb-treated plants was 0.94 ± 0.62 ppm, while aldicarb was not detected in untreated plants.

Summary of results. Psyllids on aldicarb-treated plants performed their typical feeding behaviors in a manner similar to psyllids on untreated control plants. The differences between the two treatments were very small, with no significant difference for most parameters analyzed. However, those small differences indicate some toxic effect of aldicarb on the psyllids tested. For example, when not probing, psyllids on treated plants stood still (z) two times longer per insect than those on untreated plants. However, their duration per event per insect was shorter due to a higher number of standing events that was performed 2x more frequently. In addition, insects on aldicarb-treated plants performed less walking (np) per insect because events durations were smaller but they were performed almost in the same frequency as on untreated plants. However, when looking into the general non-probing (walking + standing still) (znp) behavior, there was significantly longer non-probing behavior per probe on aldicarb treated plants than untreated plants since this behavior represents the combination of the standing still and walking behavior.

When stylet-probing, insects on treated plants made slightly shorter pathway activities (C) per insect. This difference is due to a smaller number of events per insect and the same number of stylet-probing per probe. Durations of phloem contact (D) on aldicarb-treated plants were half the duration of that for the control plants. This was probably due to the short duration of phloem contacts performed per insect (10x smaller than control). Even though those parameters were

shorter, there was no significant difference between phloem contact duration per probe or per event on treated and untreated plants. Salivation into the phloem (E1) was slightly shorter in duration per insect on aldicarb-treated compared to untreated plants; this was due to the small difference in the duration of events per insect. However, phloem salivation was longer per event on treated than untreated plants, indicating that even though phloem salivation events were performed less often, they were longer on aldicarb-treated plants. Phloem ingestion (E2) duration per insect was smaller, because each event was shorter in duration per insect. In addition, the number of phloem ingestion events per probe was smaller per insect, in a numerically fewer phloem ingestions. Even though phloem salivation durations were not significant different per probe, the phloem ingestions were slightly higher on aldicarb-treated plants, which lead to significantly longer phloem ingestions per event. While phloem salivation events were less frequently performed per insect on aldicarb-treated plants than untreated plants, those events were longer on the treated plants than on untreated plants. In contrast, xylem ingestion (G) was performed for longer durations per insect on aldicarb-treated plants than untreated, due to slightly longer events per insect. Overall, except for longer standing still events and walking less frequently on aldicarb-treated compared to untreated plants, there was no other differences in the parameters analyzed.

Discussion

The objective of the current study was to examine the effects of soil-applied systemic applications of aldicarb on the feeding behavior of *D. citri* to determine the potential effects of this insecticide on the behaviors that mediate transmission of Las. The overall analysis of *D. citri* feeding behavior indicated no effect of this insecticide on psyllid probing behavior. However, differences were found when examining the event level, where waveform duration per event (WDE) was higher during the phloem activities of salivation and ingestion on aldicarb-treated

plants than controls. Those results showed that *D. citri* ingested phloem sap and salivated into phloem for longer durations per event on aldicarb treated plants when compared to psyllids on untreated plants.

Ragab (1981) suggested that this insecticide might have a direct effect on mineral metabolism of cotton plants, mainly the ones that are involved in nitrogen and phosphorous metabolism. Balayannis (1983) showed that aldicarb applications increased the leaf content of water-soluble sugars in tobacco and the concentration of iron, manganese and zinc in the leaves and roots. Also, aldicarb decreased leaf nitrate reductase activity and the concentrations of nicotine and crude protein. Plant nutrition has a direct effect on insect behavior. For example, studies on *Psylla pyricola* Foerster showed a higher production of honeydew when feeding on pear leaves with very low nitrogen content, indicating compensatory feeding effect due to the low nutrition of the leaves (Pfeiffer and Burts 1984). Consequently, aldicarb may have caused increased phloem ingestion by *D. citri* feeding on aldicarb-treated plants. Previous studies have shown an increase of brix, yield, and peel color in citrus fruit sampled from trees treated with aldicarb along with an increase in calcium and potassium content in the citrus leaves (Wheaton et al. 1985). The nitrogen content was not measured however and increases in nitrogen content might also affect *D. citri* feeding behavior (Tsagkarakis and Rogers, unpublished).

After 12 h of feeding on aldicarb treated plants, none of the *D. citri* was found dead. For this reason citrus plants were sent for residual analysis to confirm the presence of the insecticide in tested plants. Residual analysis confirmed existence of aldicarb within the plants. The actual residual concentration necessary to cause psyllid toxicity is unknown. However studies looking into the efficiency of aldicarb treatments for the control of *Tryoza erytreae* (South African citrus psyllid) showed poor efficiency in egg and nymphal control even when applying 227 g of

aldicarb per tree with trees averaging 23.2 m^2 canopy (Catling, 1969). However, nymphs were controlled using 907.2 g per tree (de Villiers, 1969 cited by Catling 1969). Such dosages have not been permitted in Florida due to concerns of groundwater contamination. In contrast, Qureshi and Stansly (2008) showed that aldicarb successfully reduced *D. citri* populations, when applied 2-3 months prior to spring flushes at recommended rates. However, when they caged adult psyllids for 25 d on the aldicarb-treated plants, mortality was below 50% following 25 days (Qureshi and Stansly, 2008).

During the 12 h access period, *D. citri* probed for 6.7h on aldicarb treated plants, during which time they were able to reach the phloem and salivate (Wavefom D and E1) for an average of 0.26 h, and ingest (Waveform E2) for more than 3 h. Those results differ from the investigations examining soil-applied imidacloprid (Chapter 3). *D. citri* feeding behavior on imidacloprid-treated citrus resulted in mortality within a 6 h access period. Also, during this access period phloem ingestion averaged 1.0 h for psyllids on young leaves and it did not occur on mature citrus leaves (Chapter 3).

Implication for managing the spread of HLB. The current results indicate that aldicarb has negligible effects on the feeding behaviors of *D. citri*. However, there was a slight indication that aldicarb treatment may enhance Las transmission given the longer salivation and phloem ingestion events on aldicarb-treated plants. Bonani et al. (2010), observed Las acquisition 1 h after initiation of the ingestion waveform (Waveform E2) by *D. citri*. The efficiency of Las acquisition by adult *D. citri* is low (Pelz-Stelinski et al. 2010) and there is a latency period anywhere from 24 h to 25 d (Xu et al. 1988, Roistacher 1991). However, since aldicarb applications can result in less than 50% of adults being controlled under field conditions

(Qureshi and Stansly 2008), this further supports our findings that *D. citri* can likely inoculate aldicarb-treated plants with Las.

The current study underscores the importance of EPG studies for vector-transmission dynamics in order to improve management of insect vectored plant diseases. Although aldicarb usage will be banned in citrus groves as of December 31, 2011, investigations such as this one demonstrate that unlike imidacloprid, not all soil-applied systemic insecticides can disrupt transmission of Las by *D. citri*.

Table 4-1. Mean (± SE) waveform duration per insect (WDI) (s) and the proportion of individuals that produced a waveform type (PPW) for *Diaphorina citri* feeding on aldicarb treated and untreated citrus plants.

Waveform	Untreated control				Aldicarb				
	WDI	±	SE	PPW	WDI	±	SE	PPW	p-value
z	5750.40	±	N/A	1/15	10273.44	±	2507.34	9/15	0.8771
np	16040.72	±	2991.02	14/15	14201.18	±	2412.40	14/15	0.9049
C	15519.65	±	2786.05	14/15	14407.25	±	2083.64	14/15	0.6542
D	998.22	±	526.03	10/15	442.15	±	132.11	11/15	0.3747
E1	622.69	±	145.02	10/15	500.85	±	103.65	11/15	0.4605
E2	15595.10	±	5568.77	9/15	11321.59	±	4204.89	8/15	0.5627
G	2901.74	±	411.41	11/15	3228.84	±	368.71	12/15	0.4627

Table 4-2. Mean (± SE) probe duration per insect (PDI) (s), mean number of probes per insect (NPI), and probe duration per event (PDE) (s) for *Diaphorina citri* feeding on aldicarb treated and untreated citrus plants.

Untreated control			Aldicarb			
PDI	±	SE	PDI	±	SE	p-value
28982.81	±	3297.99	24097.22	±	3473.27	0.3469
NPI	±	SE	NPI	±	SE	p-value
15.86	±	3.12	14.36	±	2.00	0.9804
PDE	±	SE	PDE	±	SE	p-value
698.38	±	119.62	677.43	±	98.11	0.0054

Table 4-3. Mean (± SE) number of waveforms event per probe (NWEP) and number of probes by waveform (NPw) for *Diaphorina citri* feeding on aldicarb-treated and untreated plants.

Waveform	Untreated control				Aldicarb				
	NWEP	±	SE	NPw	NWEP	±	SE	NPw	p-value
znp	1.01	±	0.01	222	1.01	±	0.01	201	N/A
C	1.47	±	0.11	217	1.48	±	0.10	195	0.7792
D	3.63	±	0.67	24	3.33	±	0.44	24	0.9902
E1	4.92	±	0.85	24	3.71	±	0.46	24	0.3488
E2	1.89	±	0.31	19	1.27	±	0.19	11	0.1613
G	1.00	±	0.00	20	1.13	±	0.07	23	0.0984

Table 4-4. Mean (± SE) waveform duration per probe (WDP) (s) for *Diaphorina citri* feeding on aldicarb-treated and untreated plants.

Waveform	Untreated control			Aldicarb			
	WDP	±	SE	WDP	±	SE	p-value
znp	1037.48	±	174.12	1469.20	±	221.95	0.0008
C	994.74	±	135.09	1013.69	±	144.13	0.0864
D	413.94	±	225.64	202.65	±	29.56	0.8264
E1	260.11	±	45.00	229.55	±	37.70	0.5609
E2	7388.84	±	3063.89	8233.89	±	3384.07	0.3469
G	1666.76	±	208.30	1684.61	±	219.85	0.7665

Table 4-5. Mean (± SE) number of waveforms events per insect (NWEI) for *Diaphorina citri* feeding on aldicarb treated and untreated citrus plants.

Waveform	Untreated control			Aldicarb			
	NWEI	±	SE	NWEI	±	SE	p-value
z	1.00	±	N/A	2.33	±	0.53	0.3787
np	15.93	±	3.13	15.29	±	2.10	0.8495
C	22.93	±	4.72	20.71	±	3.33	0.9784
D	8.80	±	3.42	7.27	±	2.07	0.8328
E1	11.80	±	4.96	8.00	±	2.08	0.6359
E2	3.89	±	1.81	1.75	±	0.49	0.2967
G	1.81	±	0.26	2.17	±	0.40	0.5224

Table 4-6. Mean (± SE) waveform duration event per insect (WDEI) (s) for *Diaphorina citri* feeding on aldicarb-treated and untreated plants.

Waveform	Untreated control			Aldicarb			
	WDEI	±	SE	WDEI	±	SE	p-value
z	5750.40	±	N/A	5474.08	±	1642.25	0.6771
np	1664.81	±	509.49	1066.00	±	201.14	0.6760
C	847.72	±	145.90	784.42	±	110.45	0.9596
D	602.18	±	549.32	57.36	±	7.85	0.2978
E1	120.36	±	35.88	98.40	±	30.54	0.6406
E2	8916.69	±	4030.00	8527.49	±	3764.27	0.9152
G	1738.52	±	174.83	1776.21	±	257.55	0.9834

Table 4-7. Mean (± SE) waveform duration per event (WDE) (s) for *Diaphorina citri* feeding on aldicarb-treated and untreated plants.

Waveform	Untreated control			Aldicarb			
	WDE	±	SE	WDE	±	SE	p-value
z	5750.40	±	N/A	4402.90	±	1229.04	0.4568
np	1007.04	±	169.29	929.04	±	96.40	0.0067
C	676.87	±	78.92	695.02	±	69.31	0.2039
D	113.43	±	62.49	60.80	±	3.73	0.7364
E1	52.77	±	6.74	61.90	±	9.72	0.0277
E2	4010.17	±	1752.20	6469.48	±	2787.80	0.0370
G	1595.96	±	218.89	1440.43	±	182.44	0.8607

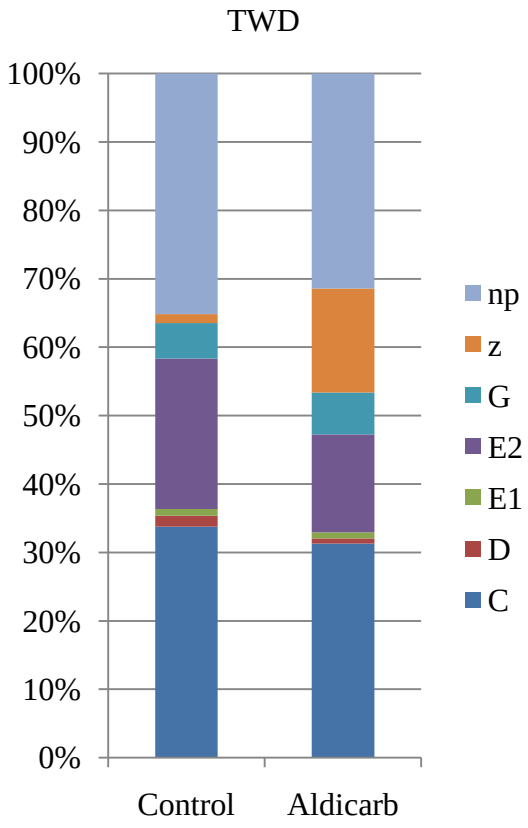

Figure 4-1. Percentage of the total waveform duration (TWD) for *Diaphorina citri* feeding on aldicarb-treated and untreated citrus plants.

CHAPTER 5
EFFECTS OF FIVE DIFFERENT FOLIAR-APPLIED INSECTICIDES ON ASIAN CITRUS PSYLLID (*DIAPHORINA CITRI*) FEEDING BEHAVIOR AND THEIR POSSIBLE IMPLICATION FOR LAS TRANSMISSION

One of the components of HLB management programs used by Florida citrus growers is the use of broad-spectrum foliar insecticide applications to reduce psyllid populations. The rationale for this approach is that by decreasing psyllid populations, the number of psyllids inoculating healthy citrus plants will also decrease. Consequently the number of insecticide sprays in citrus has greatly increased since 2005. Insecticides commonly used by growers in Florida to control psyllids belong to several classes with different modes of action. These include acetylcholinesterase inhibitors such as chlorpyrifos, the acetylcholine receptor stimulator imidacloprid, sodium channel modulators including fenpropathrin, and the lipid biosynthesis inhibitor spirotetremat. Some of these insecticide products require ingestion by the insect or absorption onto the cuticle following contact in order to induce toxic effects, which are not always immediate.

Because *D. citri* feeds primarily from phloem sieve elements and the HLB associated agent is a phloem-limited bacterium, bacterial acquisition and inoculation probably occur during stylet activities within the phloem. Pathogen acquisition likely occurs during phloem ingestion while inoculation probably occurs during phloem salivation. Las is circulative within the vector's body and must re-enter the plant in saliva. Given that some insecticides increase vector activities and thus enhance pathogen spread (Lowery and Boiteau 1988, Roberts et al. 1993), a better understanding of the effects of insecticides on the stylet penetration behaviors of *D. citri* should help refine vector control strategies.

Electrical penetration graph (EPG) monitors have been used to study the effects of insecticide applications on insect feeding behavior. Topically applied and injected pymetrozine

inhibited stylet insertion of *Aphis fabae* Scopoli, *Macrosiphum euphorbia* (Thomas), *Myzus persicae* (Sulzer) and *Aphis gossypii* Glover on broad beans, potato plants, Chinese cabbage, and cucumber respectively. High doses of pymetrozine caused irreversibly feeding disruption while low doses only affected aphid feeding temporary. Pymetrozine showed no toxic effect on aphids, in contrast, it showed to cause aphids death due to starvation (Harrewij 1997). In addition, EPG studies on *Frankliniella fusca* (Hinds) and *F.occidentalis* on tomato plants showed that *F. occidentalis* probed more frequently and for longer periods of time on imidacloprid-treated plants than on untreated plants suggesting an increase in the inoculation of the tomato spotted wilt virus (TSWV) on imidacloprid-treated plants. In contrast, *F. fusca* when feeding on imidacloprid-treated tomato plants exhibited a significant decrease in the number of probes per insect and probing duration when compared to untreated plants (Joost and Riley 2005).

In the present study, we characterized the feeding behaviors of *D. citri* on citrus plants treated with five different foliar-applied insecticides: chlorpyrifos (Lorsban 4E™, DowAgroSciences, Indianapolis, IN), fenpropathrin (Danitol 2.4 EC™, Valent U.S.A. Corporation, Walnut Creek, CA), imidacloprid (Provado 1.6F™, Bayer CropScience, Research Triangle Park, NC), spinetoram (Delegate WG™, DowAgroSciences, Indianapolis, IN), and spirotetramat (Movento 240SC™, Bayer CropScience, Research Triangle Park, NC) and compared them with untreated-plants. The objective was to determine whether the presence of these insecticides on a plant is able to disrupt feeding behaviors of *D. citri*, especially those hypothesized to be responsible for successful pathogen acquisition and inoculation (Bonani et al., 2010).

Materials and Methods

Plants and Insects

Plants used in the experiments consisted of 'sweet orange' (*Citrus sinensis* (L.) Osbeck) seedlings (20-25 cm tall) planted in 120ml tubes with Fafard Citrus potting Mix (Fafard, Agawam, MA). Seedlings were grown in a pathogen-free greenhouse at 29 ± 3 °C and 60-80% RH. All seedlings were planted and maintained identically to minimize interplant variation.

Adult female *D. citri* (10-15 d) used in experiments obtained from a greenhouse colony free of *Ca.* Liberibacter asiaticus, reared on citrus at 29 ± 3 °C with a photoperiod of 12:12 (L:D) h. Prior to use in EPG recordings, female and male psyllids were transferred to a rearing cage (61cm x 61cm x 91cm, Bioquip, Rancho Domingues, CA) containing 'sweet orange' plants for a 48 h acclimation period. Of these psyllids held for acclimation, only female *D. citri* were selected for use in feeding experiments.

EPG Recording and Waveform Analysis

Recordings of *D. citri* feeding for 6 h under constant light conditions on insecticide-treated and untreated plants were made using a Giga-8 monitor (Department of Entomology, Wageningen Agricultural University, the Netherlands). Setup of EPG recordings and waveform characterizations were conducted as previously described in Chapter 3.

Effects of Foliar Insecticides on *D. citri* Feeding Behavior

Examination of the effect of foliar-applied insecticides on *D. citri* feeding behavior were conducted as five separate experiments in which each experiment evaluated one of the following insecticides: 1) chlorpyrifos (Lorsban 4E), 2) fenpropathrin (Danitol 2.4EC), 3) imidacloprid (Provado 1.6F), 4) spinetoram (Delegate WG), and 5) spirotetramat (Movento 240SC) (Table 5-1).

Twenty four hours prior to EPG recordings, the insecticides were applied to 'sweet orange' plants using a 500 ml plastic spray bottle until insecticide runoff from the leaves was achieved. All insecticides were applied at concentrations which correspond to the recommended field rate listed in the 2011 Florida Citrus Pest Management Guide (Rogers et al. 2011).

Twenty 'sweet orange' seedlings were used for each treatment, for which a single psyllid was recorded on feeding on each plant for 12 h. At the end of each recordingthose insects that had wiring problems resulting in poor waveform recording quality were discarded from the analysis.

Statistical Analysis

Feeding behavior of *D. citri* on plants treated with one of the five selected insecticides was compared to untreated plants as previously described in Chapter 2. However, parameters were only analyzed at the cohort and insect level for each insecticide vs. untreated comparison. Pearson's chi-square test was performed to test the goodness of fit (PROC GLIMMIX, SAS Institute 2001) and waveform duration parameter data were log transformed before statistical analysis to improve homogeneity of variances. Data were analyzed by ANOVA (PROC GLIMMIX, SAS Institute 2001) with the least significant difference (LSD) test (LSMEANS, SAS Institute 2001) used to determine if the waveform parameters analyzed were significantly different between the insecticide-treated and untreated plants. Means were considered significantly different at α=0.05.

Results

Chlorpyrifos

One hundred percent (PPW) of *D. citri* performed pathway/stylet penetration waveforms (Waveform C) and non-probing/walking activities (Waveform np) on both chlorpyrifos-treated and untreated plants. On the untreated plants, 64.3% (PPW) of *D. citri* penetrated the phloem,

51.2% salivated and 42.9% ingested (Waveforms D, E1 and E2, respectively), 64.3% ingested xylem sap (Waveform G), and 78.6% performed non-walking/non probing activities (Waveform z). On chlorpyrifos-treated plants, none of the insects penetrated into the phloem (Waveform D), and consequently none of the insects salivated or ingested phloem sap (Waveforms E1and E2), 14.3% ingested xylem sap (Waveform G), and 100% performed non-walking/non probing activities (Waveform z) (Table 5-1).

At the cohort level, *D. citri* had a total access period of 604,800s, during which psyllids on the chlorpyrifos-treated plants probed 221 times (TNP) and spent 44,406.195s (TPD) with their stylets inserted into the leaf. Formajority (92.7%) of the access period *D. citri* performed non-probing activities such as walking, jumping off the leaf, or they died from chlorpyrifos exposure. In contrast, *D. citri* on untreated plants spent most of their time (65.2%) performing probing activities (TPD = 394,031.59s) producing 643 (TNP) probes.

The differences observed at the cohort level can also be seen when analyzed at the insect level. The number of probes per insect (NPI) was significantly higher for *D. citri* on untreated plants compared to chlorpyrifos-treated plants ($F = 8.66$; df = 1, 26; $P = 0.0067$; Table 5-2). Similar results were also observed for the duration of those probes (PDI). Psyllids on untreated plants produced significantly longer probes ($F = 48.02$; df = 1, 26; $P < 0.0001$) than on treated plants (Table 5-3). For waveform duration per insect (WDI), there were significant differences found between treatments for waveforms C ($F = 39.04$; df = 1, 26; $P < 0.0001$), np ($F = 17.5$; df = 1, 26; $P = 0.0003$), and z ($F = 24.66$; df = 1, 23; $P < 0.0001$); waveforms np and C were significantly longer in duration on untreated than plants treated whereas waveform z was significantly longer on chlorpyrifos-treated plants (Table 5-1). Statistical analyses on D, E1, and E2 are not available because *D. citri* did not perform those behaviors prior to death on

chlorpyrifos-treated plants (Table 5-1). Similar results were also obtained with waveform duration per event per insect (WDEI); the duration of waveform C (F = 12.56; df = 1, 26; P = 0.0015) was longer on untreated plants whereas the duration of waveform z was longer on chlorpyrifos-treated plants (F = 35.46; df = 1, 23; P = <0.0001). Analysis of the number of waveforms events per insect (NWEI), indicated a significantly higher occurrence of the waveforms np (F = 7.96; df = 1, 26; P = 0.0090) and C (F = 10.06; df = 1, 26; P = 0.0039) on untreated compared to chlorpyrifos-treated plants.

Fenpropathrin

All *D. citri* performed pathway/stylet penetration waveforms (Waveform C) and non-probing/walking activities (Waveform np) on both fenpropathrin-treated and untreated plants. On the untreated plants, 38.5% (PPW) of *D. citri* penetrated and salivated into the phloem (Waveform D and E1), 23.1% ingested phloem sap (Waveform E2), 61.5% ingested xylem sap (Waveform E2), and 76.9% performed non-walking/non probing activities (Waveform z). On fenpropathrin-treated plants, none of the psyllids penetrated into the phloem and consequently none salivated or ingested phloem sap (Waveform D, E1 and E2, respectively). Also, none of the *D. citri* performed Waveform G, and 100% performed non-walking/non probing activities on fenpropathrin-treated plants (Waveform z) (Table 5-4).

At the cohort level, *D. citri* had a total access period of 280,800s, during which psyllids on the fenpropathrin-treated plants probed 25 times (TNP) and spent 864.74s (TPD) with their stylets inserted into the leaf. During the majority (99.7%) of the access period, psyllids performed non-probing activities such as walking, jumping off the leaf, or they died from fenpropathrin exposure. In contrast, psyllids on untreated plants spent most of their time (63.2%) performing probing activities (TPD = 177,392 s) producing 125 (TNP) probes.

At the insect level, the NPI was significantly higher for *D. citri* on untreated plants compared to fenpropathrin-treated plants ($F = 27.4$; df = 1, 26; $P < 0.0001$; Table 5-2). Similar results were also shown for the duration of those probes (PDI); psyllids on untreated plants produced longer probes ($F = 224.07$; df = 1, 20; $P < 0.0001$) than psyllids on treated plants (Table 5-3). For the waveform duration per insect (WDI), significant differences between treatments were found for waveforms C ($F = 154.98$; df = 1, 20; $P < 0.0001$), np ($F = 55.66$; df = 1, 26; $P < 0.0001$), and z ($F = 36.35$; df = 1, 23 $P < 0.0001$). Waveform np and C were significantly longer in duration on untreated compared to treated plants whereas waveform z was significantly longer in duration on fenpropathrin-treated plants (Table 5-4). *D. citri* did not perform waveforms D, E1, E2 and G, since they were intoxicated prior to the performance of those behaviors. Analysis on the waveform duration events per insect (WDEI), indicated a significantly longer duration of waveform C ($F = 60.11$; df = 1, 20; $P < 0.0001$) on untreated plants compared to fenpropathrin-treated plants. In contrast, waveform z was significantly longer ($F = 32.82$; df =1, 23; $P < 0.0001$) on fenpropathrin-treated compared to untreated plants (Table 5-4). In addition, ANOVA showed a significantly higher frequency of waveform events per insect (NWEI) for waveforms np ($F = 28.33$; df = 1, 26; $P = < 0.0001$) and C ($F = 24.27$; df =1, 20; $P < 0.0001$) on fenpropathrin-treated plants compared to untreated plants (Table 4).

Imidacloprid

One hundred percent of the *D. citri* on untreated plants performed pathway/stylet penetration waveforms (Waveform C), non-probing/walking activities (Waveform np), 20% (PPW) penetrated and salivated into the phloem (Waveforms D and E1, respectively), 6.7% ingested phloem sap (Waveform E2), 26.7% ingested xylem sap, and 66.67% performed non-walking/non probing activities (Waveform z). On imidacloprid-treated plants 100% of the *D. citri* performed Waveform np, 86.7% performed Waveform C, and none of the psyllids

penetrated into the phloem, salivated, ingested phloem sap, and ingested xylem sap (Waveform D, E1, E2, G respectively), and 100% performed non-walking/non probing activities (Waveform z) on imidacloprid-treated plants (Table 5-5).

At the cohort level, *D. citri* had a total access period of 280,800 s, during which psyllids on the imidacloprid-treated plants probed 76 times (TNP) and the majority of their time (96.5%) was spent in non-probing activities. *D. citri* spent 9954.22s (TPD) with their mouth parts inserted into the leaf tissues. In contrast, psyllids on untreated plants spent most of their time (64%) performing probing activities (TPD = 179,828.88 s), producing 253 (TNP) probes.

The differences observed at the cohort level can also be seen when the insects were analyzed at the insect level. The number of probes per insect (NPI) was significantly higher for *D. citri* on untreated plants compared to imidacloprid-treated plants (F = 19.37; df = 1, 28; P = 0.0001; Table 5-2). Similar results were also observed for the duration of those probes (PDI). Psyllids on untreated plants produced probes that were significantly longer in duration (F = 43.99; df = 1, 26; $P < 0.0001$) than psyllids on treated plants (Table 5-3). For waveform duration per insect (WDI), significant differences were observed for waveforms C (F = 40.34; df = 1, 26; $P < 0.0001$), np (F = 12; df = 1, 26; P = 0.0016), and z (F = 32.86; df = 1, 23; $P < 0.0001$). Waveforms np and C were significantly longer in duration on untreated compared with treated plants and waveform z was significantly longer on imidacloprid treated than untreated plants (Table 5-5). Waveforms D, E1, E2, and G could not be statistically compared due to the low number of psyllids that performed those waveforms on imidacloprid-treated plants. For WDEI, waveforms C (F = 22.87; df = 1, 26; $P < 0.0001$) and z (F = 28.37; df =1, 23; $P < 0.0001$) were significantly different; waveform C was longer in duration on untreated-plants whereas waveform z was longer on imidacloprid-treated plants. The number of waveforms events per

insect occurred at a significantly higher frequency for waveforms np (F = 17.53; df = 1, 28; P = 0.0003) and C (F = 18.50; df =1, 26; P = 0.0002) for *D. citri* on untreated plants compared to imidacloprid-treated plants.

Spinetoram

One hundred percent (PPW) of *D. citri* performed pathway/stylet penetration waveforms (Waveform C) and non-probing/walking activities (Waveform np) on both spinetoram-treated and untreated plants. On the untreated plants, 33.3% (PPW) of *D. citri* penetrated the phloem, salivated, and ingested (Waveforms D, E1and E2, respectively), 66.7% ingested xylem sap (Waveform G), and 93.3% performed non-walking/non probing activities (Waveform z). On spinetoram-treated plants, 13% of the psyllids penetrated into the phloem, salivated and ingested phloem sap (Waveform D, E1, and E2, respectively), 6.7% ingested xylem sap (Waveform G), and all (100%) *D. citri* performed non-walking/non probing activities (Waveform z) (Table 5-6).

At the cohort level, *D. citri* had a total access period of 604,800s, during which psyllids on the spinetoram treated plants probed 301 times (TNP) and spent 65,167.41s (TPD) with their stylets inserted into the leaf. For the majority (89.2%) of the access period, *D. citri* performed non-probing activities such as walking, jumping off the leaf, or they died from spinetoram exposure. In contrast, *D. citri* on untreated plants spent most of their time (59.1%) performing probing activities (TPD = 357,680.49s) producing 455 (TNP) probes.

At the insect level, significant difference were found for probe duration per insect (PDI), (F= 43.99; df = 1, 28; P < 0.0001; Table 5-3). However, the number of probes per insect (NPI) did not differ between spinetoram-treated and untreated-plants (F = 1.78; df = 1, 28; P = 0.1933; Table 5-2). For waveform duration per insect (WDI), significant differences were found for waveform C (F = 39.67; df = 1, 28; P < 0.0001), np (F = 6.92; df = 1, 28; P = 0.0137), G (F = 14.80; df = 1, 9; P = 0.0039), and z (F= 34.84; df = 1, 27; P < 0.0001); waveforms C, np and G

were significantly longer in duration on untreated plants and waveform z was significantly longer in duration on spinetoram-treated plants (Table 5-6). Waveform duration event per insect (WDEI), showed that waveform C (F = 27.24; df = 1, 28; P < 0.0001) and G (F = 33.04; df = 1, 9; P = 0.0003) were significantly longer on untreated plants than spinetoram-treated plants. WDEI for waveform z (F = 31.99; df = 1, 9; P < 0.0001) was significantly longer in duration on sprinetoram-treated compared to untreated plants. The number of waveforms events per insect (NWEI), was not significantly different between spinoteran-treated and untreated plants.

Spirotetramat

One hundred percent (PPW) of *D. citri* performed pathway/stylet penetration waveforms (Waveform C) and non-probing/walking activities (Waveform np) on both spirotetramat-treated and untreated plants. On the untreated plants, 33.3% (PPW) of *D. citri* penetrated the phloem and salivated (Waveforms D, and E1, respectively) and 26.7% ingested phloem (Waveform E2). Sixty percent of the psyllids performed xylem activities (Waveform G) on untreated plants and 86.7% spend their time performing non-walking/non probing activities (Waveform z). On spirotetramat treated plants, 40% (PPW) of *D. citri* penetrated the phloem (Waveform D), salivated (Waveform E1), and ingested phloem sap (Waveform E2). Also on spirotetramat-treated plants, 66.7% ingested xylem sap and 73.3% performed non-walking/non-probing activities (Waveform z) (Table 5-7).

At the cohort level *D. citri* had a total access period of 604,800s, during which psyllids on untreated plants performed 588 (TNP) probes and spent 401,039s (TPD) with their mouth parts inserted into the leaf. On spirotetramat-treated plants, psyllids probed 531 times (TNP) and the total duration of probes (TPD) was 391,820s. At the insect level, analyses of the number of probes per insect (NPI) and the probe duration per insect (PDI) indicated no significant difference between spirotetramat-treated and untreated plants. Also, the waveform duration

(WDI) was not significantly different for waveform C (F = 0.36; df = 1, 28): P = 0.5555), waveform D (F = 0.44; df = 1, 9); P =0.5248), waveform E1 (F = 1.00; df = 1, 9); P = 0.3433), waveform E2) (F = 0.11; df = 1, 8; P = 0.7524), waveform G (F= 1.55; df = 1, 17; P = 0.2300), waveform z (F = 2.79; df = 1, 22; P = 0.1088), waveform np (F = 0.44; df = 1, 28; P = 0.5138) between treated and untreated plants (Table 5-7). Similarly, no significant differences were found for the waveform duration event per insect (WDEI) and number of waveforms events per insect (NWEI), with one exception where WDEI for waveform G (F = 5.74; df = 1, 22; P = 0.0255), was significantly shorter in duration on untreated plants than spirotetramat-treated plants (Table 5-7).

Summary of Results. Figure 5-1 shows the stereotypical feeding behavior of *D. citri* on plants treated with one of the five insecticides examined in this study. *D. citri* on spirotetramat-treated plants performed normal feeding when compared to control plants. During the 12 h recording, psyllids on spirotetramat-treated plants did not differ for any of the parameters analyzed and performed phloem penetration, salivation and ingestion normally.

Chlorpyrifos, fenpropathrin and imidacloprid reduced feeding and increased non-probing activities (Figure 5-1). These insecticides caused a quick knockdown effect and induced psyllid mortality prior to phloem contact.

The general feeding behavior of psyllids on spinetoram-treated plants was reduced, however, psyllids were still able to reach the phloem, salivate and ingest phloem sap prior to intoxication. However, pathway/stylet activities were abnormal on spinetoram-treated plants and fewer numbers of *D. citri* reached the phloem on this treatment compared with the control (Figure 5-2).

Discussion

The goal of this study was to understand what effects foliar insecticide applications have on the transmission of Las by *D. citri*. The results showed that there are differences between insecticides with different modes of action in terms of the ability to disrupt the feeding behaviors associated with pathogen transmission. Chlorpyrifos, fenpropathrin and imidacloprid all reduced *D. citri* phloem feeding behaviors whereas spinetoram and spirotetramat did not prevent phloem feeding behaviors. Chlorpyrifos, fenpropathrin, imidacloprid and spinetoram mainly act as a contact insecticides and act directly on the insect nervous system (acetylcholinesterase inhibitors, sodium channel modulators, acetylcholine receptor stimulator, and disruption of nicotinic/gamma amino butyric acid (GABA)-gated chloride channels, respectively). Spirotetramat has limited contact activity and is mainly effective following digestion (Nauen et al. 2008). Spirotetramat is a tetramic acid that results in lipid biosynthesis inhibition, acting mainly on the insects' juvenile stages and at adult reproduction (Nauen et al. 2008).

Our results indicated that *D. citri* exposed to chlorpyrifos-treated plants required on average 4.02h for 100% mortality and none of the psyllids were able to reach the phloem. Chlorpyrifos (785 g AI/ha) caused 90% mortality of *Cacopsylla melanoneura* (Förster), vector of the apple proliferation disease pathogen, when overwintering adults are exposed for 1 day, while 100% mortality occurs 3 days after treatment (Baldessari et al. 2010). Additionally, field experiments in citrus showed that chlorpyrifos treatments were very effective in the control of the *D. citri*, when applied during the Florida winter months (Qureshi and Stansly, 2010).

Fenpropathrin-treated plants caused a quick knockdown of *D. citri*. Psyllids took in average 0.62 h to die with a total probing time of 0.24 h. This short probing duration was also observed for susceptible *Myzus persicae* (Sulzer); aphids exposed to etofenprox-treated plants, had a total probing time of 0.27 h (Jo et al. 2009). In addition, the sharpshooter *Homalodisca*

coagulata (Say), vector of oleander leaf scorch (*Xylella fastidiosa*), was killed within an average of 4 hours. Quantification of honeydew secretions, indicated a significant reduction of probing and settling on fenpropatrin-treated than untreated plants. Transmission of the pathogen for these sharpshooters was reduced by 50% (Bethke et al. 2001).

D. citri exposed to foliar-applied imidacloprid-treated plants were killed within an average of 1.41 h, and their feeding activities were extremely reduced when compared to the untreated plants. In addition, none of the psyllids were able to reach the phloem. Similar results were obtained with *Frankliniella fusca* (Hinds) on tomato plants treated with soil-applied imidacloprid (Joost and Riley 2005). The number and duration of probes were reduced when compared with untreated plants. However results obtained with *Frankliniella occidentalis* (Pergrande) were different compared with the current study (Joost and Riley 2005). This thrips exhibited longer and more frequent probes when in contact with imidacloprid-treated tomato plants than controls. Psyllids feeding behavior on foliar-applied imidacloprid was also different than those obtained with soil-applied imidacloprid (Chapter 3). Foliar-applied imidacloprid caused faster knockdown, than soil-applied imidacloprid (Chapter 3). Foliar applied imidacloprid completely prevent behaviors associated with Las transmission, while similar probing was observed with the systemic soil application (Chapter 3). This is likely because psyllids must initiate feeding to obtain the active ingredient in the case of the soil-applied formulation. It took longer (7.3 h) for mortality to occur on spinetoram-treated plants compared to the other insecticide evaluated (except spirotetramat). In additional to the long feeding access period, a small percentage of the *D. citri* were able to reach the phloem and perform phloem penetration, salivation and ingestion (Waveform D, E1, and E2, respectively) on spinetoram-treated plants suggesting that Las transmission could occur despite presence of recently applied residues. In addition to those

measurements, it was observed stylet penetrations (C) were not normal on spinetoram-treated plants as compared to the controls. Waveform C did not have a pattern and instead showing long frequencies followed by shorter ones, the waveforms appeared chaotic. Consequently, spinetoram-treated plants had some anti-feeding effects on psyllids.

In contrast, feeding behavior of *D. citri* was not affected on spirotetramat-treated plants with similar amounts of phloem salivation and ingestion occurring on treated and untreated plants. These results were similar to our findings for *D. citri* feeding on aldicarb-treated plants (Chapter 4).

Table 5-1. Mean (± SE) waveform duration per insect (WDI) (s), waveform duration event per insect (s), and number of waveform events per insect (NWEI) for *Diaphorina citri* feeding on chlorpyrifos-treated and untreated citrus plants.

Waveform	Treatments	Parameters											
		WDI	±	SE		PPW	WDEI	±	SE		NWEI	±	SE	
np	Untreated control	9860.62	±	1175.79	*	14/14	315.12	±	46.45	ns	10.23	±	1.41	*
	Chlorpyrifos	4556.51	±	648.48		14/14	265.24	±	37.27		2.73	±	0.43	
C	Untreated control	20298.24	±	2637.48	*	14/14	707.24	±	123.93	*	11.92	±	1.49	*
	Chlorpyrifos	2971.49	±	864.15		14/14	403.22	±	243.07		2.78	±	0.55	
D	Untreated control	222.97	±	63.63	n/a	9/14	58.27	±	2.86	n/a	4.20	±	0.80	n/a
	Chlorpyrifos		±			0/14		±				±		
E_1	Untreated control	393.68	±	126.93	n/a	8/14	69.72	±	16.17	n/a	4.80	±	0.86	n/a
	Chlorpyrifos		±			0/14		±				±		
E_2	Untreated control	12850.59	±	3367.56	n/a	6/14	4769.72	±	1376.24	n/a	1.75	±	0.48	n/a
	Chlorpyrifos		±			0/14		±				±		
G	Untreated control	3066.55	±	647.42	ns	9/14	2160.55	±	390.73	ns	1.50	±	0.27	n/a
	Chlorpyrifos	1402.70	±	122.27		2/14	1402.70	±	122.27			±		
z	Untreated control	8411.21	±	2485.60	*	11/14	1127.13	±	277.65	*	2.00	±	0.47	ns
	Chlorpyrifos	37014.68	±	1273.64		14/14	9016.63	±	1532.42		1.60	±	0.34	

*Significant difference from the untreated control $P \leq 0.05$

ns Non significant difference from the untreated control $P > 0.05$

Table 5-2. Mean (± SE) number of probes per insect for *Diaphorina citri* on chlorpyrifos, fenpropathrin, imidacloprid, spinetoram, treated and untreated citrus plants.

Treatment	NPI	±	SE	p-value
Chlorpyrifos	16.50	±	2.43	0.0067
Untreated control	35.64	±	6.66	
Fenpropathrin	2.67	±	0.49	<0.0001
Untreated control	10.00	±	1.40	
Imidacloprid	6.07	±	1.59	0.0001
Untreated control	2.42	±	1.59	
Spinetoram	21.00	±	2.77	0.1933
Untreated control	30.67	±	6.70	
Spirotetramat	27.13	±	4.63	0.2377
Untreated control	34.20	±	4.41	

Table 5-3. Mean (± SE) probe duration per insect (PDI) (s) for *Diaphorina citri* feeding on chlorpyrifos, fenpropathrin, imidacloprid, spinetoram, treated and untreated control plants.

Treatment	PDI	±	SE	p-value
Chlorpyrifos	3171.87	±	861.02	<0.0001
Untreated control	28145.11	±	2610.06	
Fenpropathrin	96.08	±	38.92	<0.0001
Untreated control	13645.56	±	1652.54	
Imidacloprid	765.71	±	242.88	<0.0001
Untreated control	11988.59	±	1690.97	
Spinetoram	4344.49	±	1182.35	<0.0001
Untreated control	23845.37	±	2415.16	
Spirotetramat	26121.39	±	3121.59	0.6751
Untreated control	26735.96	±	2637.55	

Table 5-4. Mean (± SE) waveform duration per insect (WDI) (s), waveform duration event per insect (s), and number of waveform events per insect (NWEI) for *Diaphorina citri* on feeding fenpropathrin-treated and untreated citrus plants.

Waveform	Treatments	Parameters											
		WDI	±	SE		PPW	WDEI	±	SE		NWEI	±	SE	
np	Untreated control	6050.48	±	992.77	*	13/13	677.84	±	92.97	ns	10.23	±	1.41	*
	Fenpropathrin	986.51	±	139.38		15/15	526.67	±	109.33		2.73	±	0.43	
C	Untreated control	9178.22	±	1318.21	*	13/13	840.77	±	105.01	*	11.92	±	1.49	*
	Fenpropathrin	96.08	±	38.92		9/15	49.43	±	20.91		2.78	±	0.55	
D	Untreated control	300.13	±	61.88	n/a	5/13	75.38	±	13.29	n/a	4.20	±	0.80	n/a
	Fenpropathrin		±			0/15		±				±		
E_1	Untreated control	287.44	±	62.50	n/a	5/13	59.88	±	7.91	n/a	4.80	±	0.86	n/a
	Fenpropathrin		±			0/15		±				±		
E_2	Untreated control	6327.93	±	2464.02	n/a	3/13	4045.22	±	1964.84	n/a	1.75	±	0.48	n/a
	Fenpropathrin		±			0/15		±				±		
G	Untreated control	3728.24	±	698.45	n/a	8/13	2837.43	±	733.54	n/a	1.50	±	0.27	n/a
	Fenpropathrin		±			0/15		±				±		
z	Untreated control	3012.28	±	1086.52	*	10/13	2553.64	±	1136.81	*	2.00	±	0.47	ns
	Fenpropathrin	19401.05	±	208.44		15/15	16518.67	±	1544.85		1.60	±	0.34	

*Significant difference from the untreated control $P \leq 0.05$

ns Non significant difference from the untreated control $P > 0.05$

Table 5-5. Mean (± SE) waveform duration per insect (WDI) (s), waveform duration event per insect (s), and number of waveform events per insect (NWEI) for *Diaphorina citri* feeding on imidacloprid-treated and untreated citrus plants.

Waveform	Treatments	Parameters											
		WDI	±	SE		PPW	WDEI	±	SE		NWEI	±	SE	
np	Untreated control	11377.44	±	1883.16	*	15/15	627.38	±	109.35	ns	18.87	±	2.57	*
	Imidacloprid	2815.08	±	492.39		15/15	528.69	±	119.75		7.33	±	7.33	
C	Untreated control	9432.35	±	1485.20	*	15/15	638.56	±	94.03	*	17.93	±	2.55	*
	Imidacloprid	765.71	±	242.88		13/15	150.07	±	56.33		5.85	±	1.74	
D	Untreated control	112.39	±	12.67	n/a	3/15	93.59	±	22.50	n/a	1.33	±	0.33	n/a
	Imidacloprid		±			0/15		±				±		
E_1	Untreated control	95.52	±	35.94	n/a	3/15	85.23	±	41.78	n/a	1.33	±	0.33	n/a
	Imidacloprid		±			0/15		±				±		
E_2	Untreated control	22761.76	±		n/a	1/15	22761.76	±		n/a	1.00	±		n/a
	Imidacloprid		±			0/15		±				±		
G	Untreated control	3730.18	±	1134.17	n/a	4/15	3078.55	±	1277.87	n/a	1.50	±	0.50	n/a
	Imidacloprid		±			0/15		±				±		
z	Untreated control	4311.73	±	1210.06	*	10/15	1612.30	±	465.41	*	3.60	±	1.08	ns
	Imidacloprid	21521.43	±	481.05		15/15	9743.81	±	1892.93		3.27	±	0.44	

*Significant difference from the untreated control $P \leq 0.05$
ns Non significant difference from the untreated control $P > 0.05$

Table 5-6. Mean (± SE) waveform duration per insect (WDI) (s), waveform duration event per insect (s), and number of waveform events per insect (NWEI) for *Diaphorina citri* feeding on spinetoram-treated and untreated citrus plants.

Waveform	Treatments	Parameters											
		WDI	±	SE		PPW	WDEI	±	SE		NWEI	±	SE	
np	Untreated control	16890.22	±	2072.11	*	15	617.14	±	91.11	ns	33.47	±	6.67	ns
	Spinetoram	9398.50	±	824.47		15	468.81	±	69.87		23.67	±	2.60	
C	Untreated control	17953.47	±	2046.91	*	15	749.75	±	100.58	*	31.93	±	6.56	ns
	Spinetoram	3625.26	±	797.77		15	182.43	±	33.60		20.40	±	2.76	
D	Untreated control	224.21	±	59.85	ns	5	84.71	±	16.76	ns	3.00	±	0.94	ns
	Spinetoram	109.84	±	68.89		2	65.16	±	24.21		1.50	±	0.50	
E_1	Untreated control	306.25	±	95.43	ns	5	94.39	±	18.13	ns	3.40	±	0.93	ns
	Spinetoram	321.36	±	162.80		2	100.16	±	20.88		3.00	±	1.00	
E_2	Untreated control	13687.99	±	5499.34	ns	5	10913.17	±	4293.62	ns	1.20	±	0.20	ns
	Spinetoram	4896.88	±	4661.68		2	2507.24	±	2272.04		1.50	±	0.50	
G	Untreated control	1728.63	±	296.40	*	10	1288.72	±	137.69	*	1.30	±	0.15	ns
	Spinetoram	161.12	±			1	161.12	±			1.00	±		
z	Untreated control	4566.52	±	1246.12	*	14	1171.13	±	345.35	*	4.36	±	1.05	ns
	Spinetoram	31254.27	±	1621.34		15	7118.70	±	1084.35		6.00	±	1.01	

*Significant difference from the untreated control $P \leq 0.05$

ns Non significant difference from the untreated control $P > 0.05$

Table 5-7. Mean (± SE) waveform duration per insect (WDI) (s), proportion of insects performing a specific waveform (PPW), waveform duration event per insect (s) (WDEI), and number of waveform events per insect (NWEI) for *Diaphorina citri* feeding on spirotetramat-treated and untreated citrus plants.

Waveform	Treatments	Parameters											
		WDI	±	SE		PPW	WDEI	±	SE		NWEI	±	SE	
np	Untreated control	14784.33	±	2006.10	ns	15/15	464.42	±	84.64	ns	37.47	±	5.13	ns
	Spirotetramat	12906.29	±	1867.91		15/15	567.14	±	132.17		29.47	±	4.68	
C	Untreated control	20687.67	±	2847.53	ns	15/15	912.06	±	376.43	ns	35.87	±	4.68	ns
	Spirotetramat	19038.67	±	2627.90		15/15	818.28	±	152.28		27.73	±	4.66	
D	Untreated control	237.73	±	71.68	ns	5/15	93.23	±	11.36	ns	2.40	±	0.51	ns
	Spirotetramat	283.71	±	57.43		6/15	83.48	±	7.63		3.33	±	0.61	
E_1	Untreated control	275.54	±	89.11	ns	5/15	99.44	±	20.57	ns	2.60	±	0.75	ns
	Spirotetramat	387.84	±	77.11		6/15	89.65	±	17.26		4.50	±	0.85	
E_2	Untreated control	12041.82	±	5630.75	ns	4/15	8638.85	±	2973.73	ns	1.25	±	0.25	ns
	Spirotetramat	11611.99	±	5371.13		6/15	9613.43	±	5108.85		1.67	±	0.33	
G	Untreated control	4441.93	±	952.70	ns	9/15	2921.30	±	934.90	*	1.78	±	0.22	ns
	Spirotetramat	3249.88	±	690.09		10/15	2512.75	±	542.87		1.40	±	0.31	
z	Untreated control	4015.24	±	1545.90	ns	13/15	883.68	±	349.42	ns	6.69	±	3.42	ns
	Spirotetramat	8144.86	±	1775.13		11/15	2149.25	±	411.73		4.09	±	0.89	

*Significant difference from the untreated control $P \leq 0.05$

ns Non significant difference from the untreated control $P > 0.05$

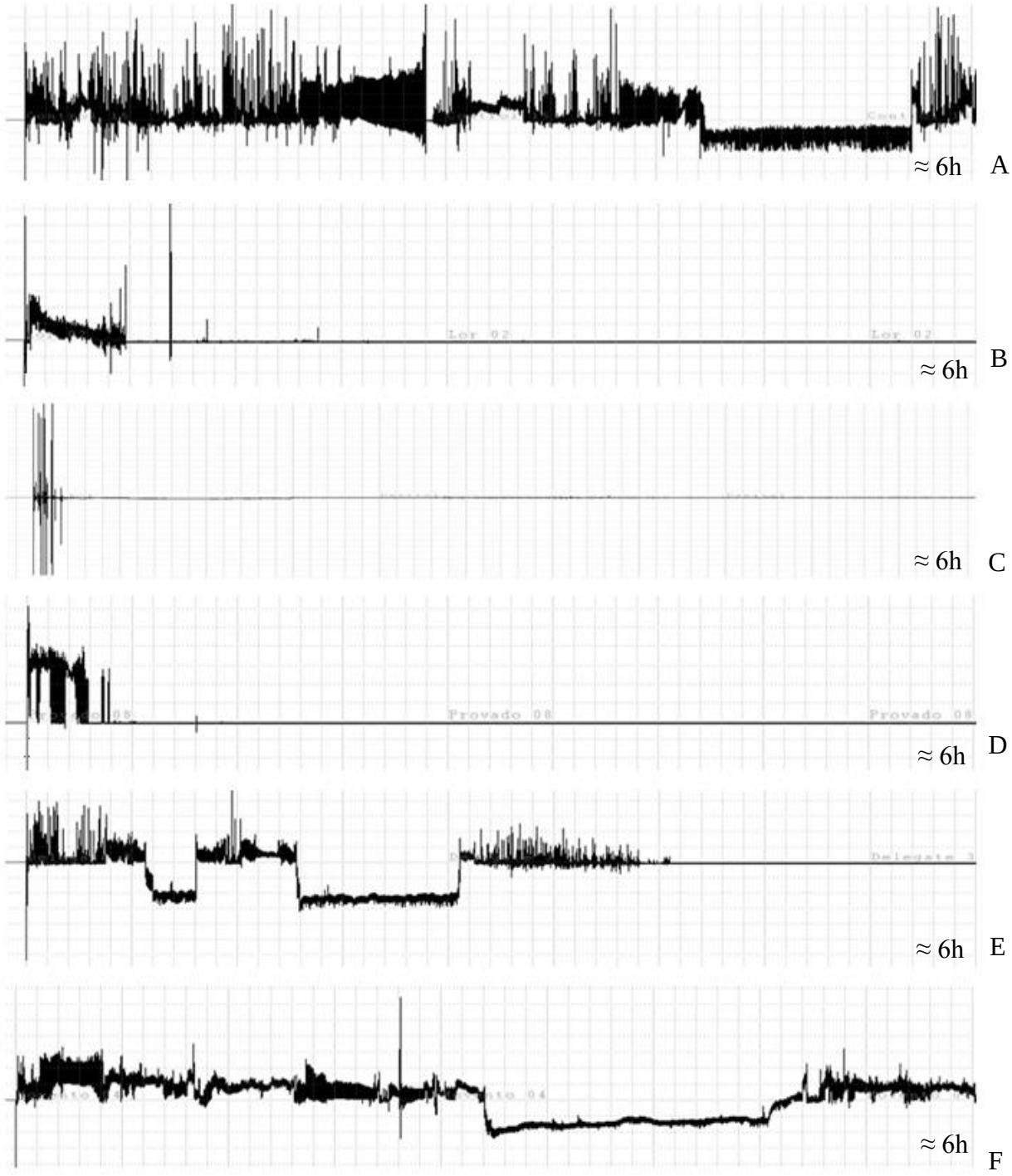

Figure 5-1. Representative Asian citrus psyllid EPG waveforms on sweet orange plants treated with: A) Untreated control; B) chlorpyrifos; C) fenpropathrin; D) imidacloprid; E) spinetoram; F) spirotetramat.

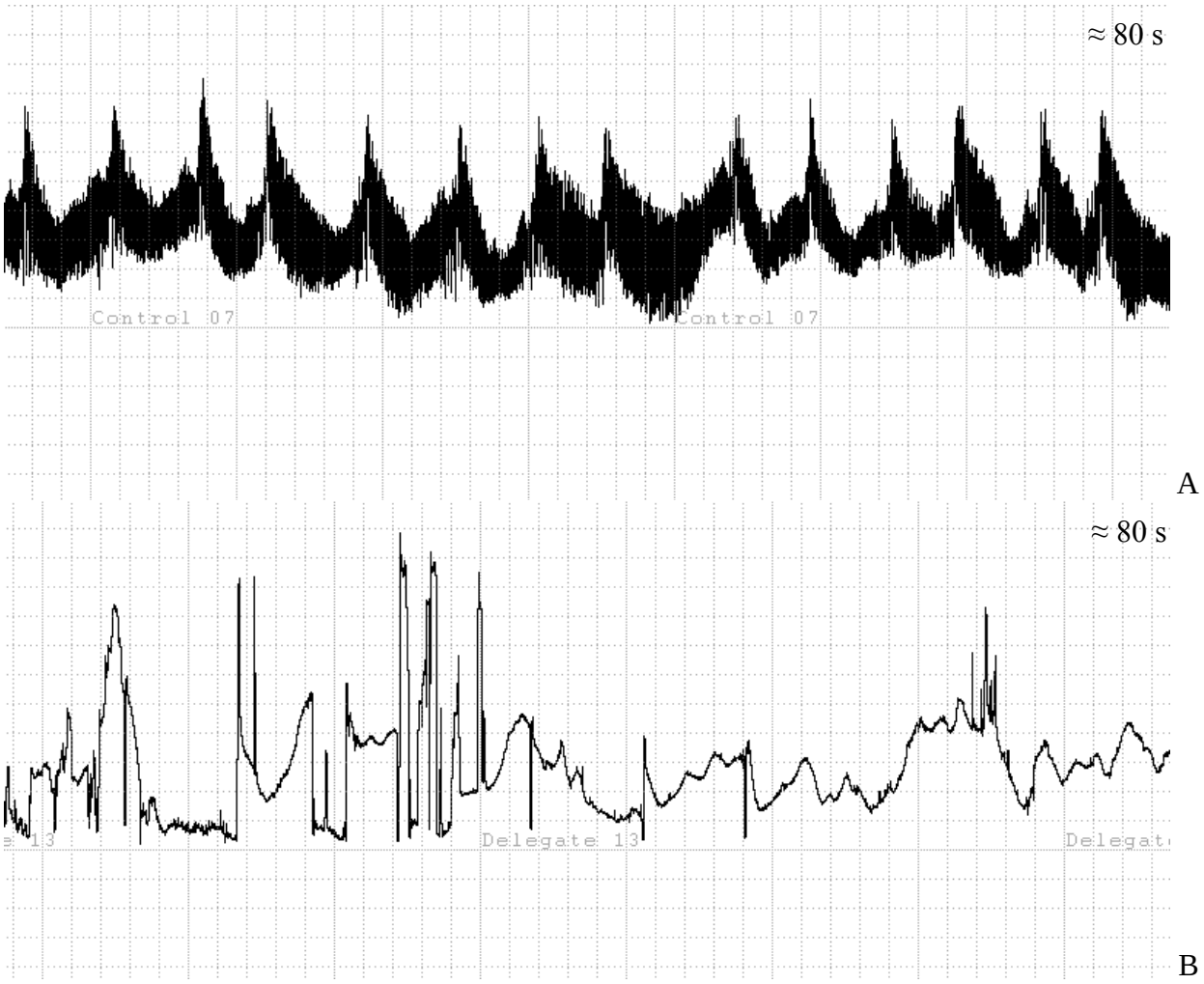

Figure 5-2. Asian citrus psyllid EPG waveforms C on. A) Untreated control; B) spinetoram-treated plants (difficulties in stylet penetration).

CHAPTER 6
RESIDUAL ACTIVITY OF FIVE DIFFERENT FOLIAR-APPLIED INSECTICIDES ON ASIAN CITRUS PSYLLID (*DIAPHORINA CITRI*) FEEDING BEHAVIOR AND THEIR POSSIBLE IMPLICATIONS FOR LAS TRANSMISSION

Several investigations of insecticide efficacy have been conducted against *D.citri*. In field experiments, various insecticides and rates were analyzed against *D. citri* (Childers and Rogers 2005). Chlorpyrifos (Lorsban 4E), fenpropathrin (Danitol 2.4 EC), and imidacloprid (Provado 1.6F) provided good adult knockdown control up to 5 DAT, and depending on the application date, the residual effect of fenpropathrin and imidacloprid were up to 15 DAT (Childers and Rogers 2005). Similar results were obtained by Qureshi et al. (2009) when looking into foliar applied imidacloprid. In addition, Qureshi et al. (2009) found significantly fewer adults psyllids 7 DAT on plants treated with spirotetramat (Movento 240SC), but nymphs were reduced even after 24 DAT. Although residual activity studies are based on nymphal and adult mortality, they do not determine effects on psyllid feeding behavior. Since, Las pathogen is a phloem restricted bacterium, transmission probably occur during feeding activities, which take place in the phloem. Acquisition likely occurs during phloem ingestion and inoculation probably takes place during phloem salivation. Consequently, insecticides that disrupt psyllid feeding behavior prior to causing mortality are the most likely to prevent spread of disease.

Information concerning the effects of the residual activity on the *D. citri* feeding behavior is very important in the improvement of psyllid control and the subsequent success of HLB management programs. In Chapter 5, *D. citri* feeding behavior was examined when exposed to the fresh residues of five different insecticides. In this study, the duration of feeding disruption provided by those same five insecticides is examined in detail. More specifically, the objective of this study was to determine residual activity 1, 7, 14, 21 and 28 DAT of chlorpyrifos (Lorsban

4E), fenpropathrin (Danitol 2.4EC), imidacloprid (Provado 1.6F), spinetoram (Delegate WG), and spirotetramat (Movento 240SC) in terms of disrupting *D. citri* feeding behavior.

Materials and Methods

Plants and Insects

Plants used in the experiments consisted of 'sour orange' (*Citrus aurantium* L.) seedlings (25-30 cm tall) planted in one gallon pots using citrus potting mix (Fafard Citrus Mix, Fafard, Agawam, MA). Seedlings were grown in a pathogen-free greenhouse at 29 ± 3 °C and 60-80% RH. All seedlings were planted and maintained identically, to minimize interplant variation.

Adults *D. citri* (10-20 d) used in experiments were obtained from a greenhouse colony free of *Ca.* Liberibacter asiaticus, reared on sour oranges and sweet oranges (*Citrus Sinensis* (L.) Osbeck) at 29 ± 3°C with a 12:12 (L:D) h photoperiod. Prior to use in EPG recordings, psyllids (10-20 d old) were transferred to a rearing cage (61cm x 61cm x 91cm, Bioquip, Rancho Domingues, CA) containing sour orange plants for a 48 h acclimation period. Of the psyllids held for acclimation, both female and male *D. citri* were then selected for use in feeding experiments.

EPG Recording and Waveform Analysis

Recordings of *D. citri* feeding for 6 h under constant light conditions on insecticide-treated and untreated plants were made using a Giga-8 monitor (Department of Entomology, Wageningen Agricultural University, the Netherlands). Setup of EPG recordings and waveform characterizations were conducted as previously described in Chapter 3.

Effects of Residual Foliar Insecticides on Psyllid Feeding Behavior

The experiments investigating the residual effect of several foliar-applied insecticides were divided into five different experiments. Each experiment was conducted 1, 7, 14, 21, and 28 DAT. Fifty plants per treatment were treated individually using one of the following insecticides:

1) chlorpyrifos (Lorsban 4E), 2) fenpropathrin (Danitol 2.4EC), 3) imidacloprid (Provado 1.6F), 4) spinetoram (Delegate WG), and 5) spirotetramat (Movento 240SC).

Twenty four hours prior to the first EPG recordings, insecticides were applied to 'sour orange' plants, using a 500ml plastic bottle spray to wet the entire plant canopy until insecticide runoff from the leaves. Each insecticide was applied at the recommended field rate as described in Chapter 5. Between recording dates (eg. 7, 14, 21 or 28 DAT) plants were weathered outdoors inside of a screen cage (180 cm x 360 cm x 180 cm, Bioquip, Rancho Domingues, CA). Temperature and relative humidity, inside and outside of the screen cage were measured throughout the experiment using a HOBO® data logger (Onset Computer Corporation, Bourne, MA).

Ten 'sour orange' seedlings were used for each treatment, and two psyllids were recorded per plant on separate leaves at the same time. At the end of each recording, any insects that had wiring problems resulting in poor quality EPG recordings were discarded from the analysis.

Residual Analysis

Following EPG recordings, leaves from control, chlopyrifos and imidacloprid-treated plants were sampled to obtain 5g of leaf material per treatment. Plant samples were then sent to the Waters Agricultural Laboratory (Camilla, GA) for residual analysis. Residual activities were analyzed by HPLC/UV chromatography.

Statistical Analysis

For each recording date, *D. citri* feeding behaviors were compared between the treatments. Analyses of feeding parameters were conducted at the cohort and insect levels as previously described in Chapter 2.

Pearson's chi-square test was performed to test the goodness of fit (PROC GLIMMIX, SAS Institute 2001). Data were analyzed by ANOVA (PROC GLIMMIX, SAS Institute 2001)

with the least significant difference (LSD) test (LSMEANS, SAS Institute 2001) used to determine if the waveform parameters analyzed were significantly different between the treatments. Means were considered significantly different at α=0.05.

Results

Experiment 1. One Day After Treatment

All *D. citri* tested performed non-probing/walking activities (waveform np) on both treated and untreated plants. In addition, 100% (PPW) of the psyllids performed non-walking/non-probing activities (waveform z) on fenpropathrin and imidacloprid-treated plants, while 93.3%, 86.7% , 66.7 % and 60% (PPW) of the insects performed the same behavior on spinetoram, chlorpyrifos, spirotetramat-treated and untreated plants, respectively.

One-hundred percent (PPW) of the *D. citri* performed stylet penetration (waveform C) on control, chlorpyrifos, imidacloprid, spinetoram and spirotetramat-treated plants; the same behavior was observed on 80 % (PPW) of *D. citri* on fenpropathrin-treated plants. However, none of the psyllids on chlopyrifos, fenpropathrin and imidacloprid-treated plants reached the phloem and salivated. However, 20% (PPW) of the insects penetrated and salivated in the phloem (D and E1, respectively) on untreated plants while 13.3% (PPW) and 6.7% of the *D. citri* tested performed those same waveforms on spirotetramat and spinetoram-treated plants. In addition, only psyllids on spirotetramat-treated plants performed phloem ingestions (waveform E2). Xylem ingestion (waveform G) was performed on chlorpyrifos, spinetoram, spirotetramat-treated and untreated plants by 13.3%, 26.7%, 60 % and 46.7% of *D. citri*, respectively.

Cohort level. *D. citri* had a total access period of 324,000 s during which psyllids on the untreated plants (control) probed 933 times (TNP) and spent 107,532.96 s (TPD) with stylets inserted into the citrus leaves. However, psyllid probing on spirotetramat-treated plants was decreased to 636 times (TNP) and psyllids spent more time probing (TPD=189,584.91 s). In

addition, psyllids on spinetoramspinetoram-treated plants probed 601 times (TNP) and spent 90,956.07 s (TPD) probing. In contrast, probe frequencies and durations were reduced on fenpropathrin, chlorpyrifos and imidacloprid-treated plants. Psyllids on fenpropathrin-treated plants performed 318 probes (TNP) and spent 10,358.05 s (TPD) with stylets inserted into the leaf tissue. On chlorpyrifos-treated plants, the total number of probes was 311 and the total probe duration 44,019.06 s. On imidacloprid-treated plants, they probed 178 times (TNP) and for a duration of 10,383.91 s (TPD).

Insect level. The differences found at cohort level, were also found to be significantly different for the number of probes per insect (NPI). Psyllids on control, spinetoram and spirotetramat-treated plants performed significantly more probes than *D. citri* feeding on chlorpyrifos, fenpropathrin and imidacloprid-treated plants ($F = 9.32$; df = 5, 83; $P < 0.0001$; Table 6-1). In addition, the duration of probes per insect (PDI), was significantly longer in duration on spirotetramat-treated plants, than on untreated and spinetoram-treated plants ($F= 9.17$; df = 5, 81; $P < 0.0001$). The shortest durations occurred on imidacloprid and fenpropathrin-treated plants (Table 6-2). Significant differences were also found in the waveform duration per insect (WDI). Psyllids on control, chlorpyrifos, spinetoram and spirotetramat-treated plants non-probed/walked (np) for longer periods of time ($F = 8.68$; df= 5, 84; $P < 0.0001$) than psyllids on fenpropathrin and imidacloprid-treated plants. If the psyllids were not probing and walking, they were performing non-probing/standing still behaviors, which were significantly longer on the chlorpyrifos, fenpropathrin and imidacloprid-treated plants ($F = 19.26$; df = 5, 70; $P < 0.0001$) than on control and spirotetramat-treated plants. Thus, pathway/stylet penetration (waveform C) ($F = 18.19$; df = 5, 81; $P < 0.0001$) was longer in duration on the control plants, spinetoram and spirotetramat-treated plants but shorter on fenpropathrin and imidacloprid-treated plants. In

addition, xylem ingestion (waveform G) (F = 0.61; df = 3, 18; P = 0.61; Table 6-3) was not different among the treatments.

Experiment 2. Seven Days After Treatment

One hundred percent (PPW) of *D. citri* tested performed non-probing/walking activities (waveform np) on chlorpyrifos, fenpropathrin, spinetoramspinetoram, spirotetramat-treated, and untreated plants, while 92.9 % of the psyllids performed the same behavior on imidacloprid-treated. When psyllids were not walking or probing, they were non-probing/standing still, consequently one hundred percent (PPW) of *D. citri* performed non-walking/non probing activities (z) on spinetoram-treated plants, while 92.9% (PPW) performed the same behavior on imidacloprid and fenpropathrin-treated plants. In addition, 78.6%, 50% and 35.7% (PPW) of the psyllids non-probed/stood still on chlorpyrifos, spirotetramat-treated, and untreated plants respectively.

One hundred percent (PPW) of *D. citri* performed pathway/stylet penetration (C) on chlorpyrifos, spinetoram, spirotetramat-treated and untreated plants, while 92.9% and 78.6% (PPW) of the psyllids performed the same behavior on fenpropathrin, and imidacloprid-treated plants. However, only 7.1% (PPW) of the psyllids on chlorpyrifos, spirotetramat, and on control plants were able to penetrate and salivate into the phloem (D and E1, respectively). In addition, only 7.1% (PPW) of *D. citri* performed phloem ingestion (E2) on control plants. Xylem ingestion (G) was performed by 50 % (PPW) of *D. citri* exposed to chlorpyrifos, spirotetramat-treated and untreated plants, while 35.7% (PPW) of *D. citri* performed this same behavior on spinetoram-treated plants.

Cohort level. *D. citri* had a total access period of 302,000 s on control plants during which psyllids inserted and withdrew their stylets 582 times (TNP), which lasted for 152,652.98 s (TPD). In addition, psyllids on the chlopyrifos and spirotetramat-treated plants had a total probe

duration (TPD) of 117,595.37 s and 117,090.00 s respectively, with a total number of probes (TNP) performed at 593 and 313 times, respectively. Psyllids exposed to spinetoram-treated plants, probed 359 times (TNP) and with a total duration of 69,471.09 s (TPD). Psyllids on imidacloprid-treated plants probed 135 times (TNP) and spent 9,331.80 s (TPD) probing. On fenpropathrin-treated plants, psyllids probed 117 times (TNP) and spent 7,125.33 s (TPD) with their stylets inserted into the leaf tissue.

Insect level. Psyllids on spirotetramat-treated and untreated plants exhibited significantly higher NPI, than those on fenpropathrin and imidacloprid-treated plants ($F = 8.38$; df = 5, 77; $P < 0.0001$; Table 6-4). In addition, the duration of those probes per insect (PDI) was significantly longer for psyllids on untreated plants than on spirotetramat-treated plants ($F = 18.28$; df = 5, 74; $P < 0.0001$) and was the shortest on fenpropathrin and imidacloprid-treated plants (Table 6-4). Significant differences were also found for the parameter WDI. Non-probing/standing still (z) behavior was longer in duration for psyllids on imidacloprid and fenpropathrin-treated plants ($F = 6.63$; df =5, 57; $P < 0.0001$) with shorter duration found for psyllids on control, chlorpyrifos, spinetoram and spirotetramat-treated plants. Non-probing/walking behaviors (np) were significantly longer in duration on chlorpyrifos, spinetoram, spirotetramat and untreated plants compared to fenpropathrin and imidacloprid-treated plants ($F = 10.50$; df = 5, 77; $P < 0.0001$).

Stylet penetration (waveform C) ($F =17.33$; df = 5, 74; $P < 0.0001$), was longer on control plants than spirotetramat and chlorpyrifos-treated plants. Stylet penetrations were shortest on fenfopathrin and imidacloprid-treated plants. Xylem ingestion (G), was not significantly different among the treatments tested ($F = 0.34$; df = 3, 22; $P = 0.7990$; Table 6-4).

Experiment 3. Fourteen Days After Treatment

One hundred percent (PPW) of *D. citri* performed non-probing/walking activities (np) on control, chlorpyrifos, fenpropathrin and spirotetramat-treated plants. In addition, the same

behaviors were performed by 93.3% (PPW) of psyllids exposed to imidacloprid and spinetoram-treated plants. If the insects were not walking or probing, they were performing non-probing/standing still behaviors (z). This behavior was observed for all (PPW) of the *D. citri* on control, chlorpyrifos and fenpropathrin-treated plants; on 93.3 % (PPW) of the psyllids on imidacloprid and spinetoram-treated plants; and on 80 % (PPW) of the insects on spirotetramat-treated plants.

All of the (PPW) of *D. citri* performed pathway/stylet penetration (C) on control, chlorpyrifos and fenpropathrin-treated plants and the same behavior was performed by 93.3 % (PPW) of the psyllids on imidacloprid, spinetoram and spirotetramat-treated plants. For psyllids probing the phloem, only 13.3 % (PPW) of those exposed to chlorpyrifos, spirotetramat-treated and untreated plants were penetrated and salivated into the phloem (D and E1, respectively). However, only 13.3 % and 6.7% (PPW) of *D. citri* on control and spinetoram-treated plants were ingested phloem sap (E2).

Xylem ingestion (G) was performed by 53.3 % (PPW) of *D. citri* on spirotetramat-treated plants, 40 % (PPW) on control plants, 33.3 % (PPW), chlorpyrifos-treated plants and 20 % (PPW) on spinetoram-treated plants. In addition, none of the psyllids performed xylem ingestions on imidacloprid and fenpropathrin-treated plants.

Cohort level. *D. citri* had a total access period of 324,000 s during which psyllids on the control plants psyllids probed 965 times (TNP) and spent 111,842.24 s (TPD) with their probing activities. Psyllids on spinetoram-treated plants probed 844 times (TNP) and spent 89,068.19 s (TPD) with their mouth parts inserted into the leaves. In addition, insects on chlorpyrifos-treated plants psyllids probed 694 times (TNP) with a total duration of 104,961.59 s (TPD). On the spirotetramat-treated plants, psyllids probed 577 times (TNP) with a total duration of 155,952.30

s (TPD). However, total frequency and duration of probing was greatly reduced on fenpropathrin and imidacloprid-treated plants. Psyllids on fenpropathrin-treated plants probed 196 times (TNP) and spent 26,223.16 s (TPD) feeding. On imidacloprid-treated plants, psyllids probed 177 times (TNP) and spent 9,199.91 s (TPD) performing this behavior.

Insect level. The differences found between TPD and TNP, were also observed with the number of probes per insect (NPI) and probe duration per insect (PDI). NPI was significantly higher (F = 5.74 df = 5, 82; P = 0.0001; Table 6-5) for *D. citri* on control, chlorpyrifos, spinetoram and spirotetramat-treated plants, whereas it was shortest on fenpropathrin and imidacloprid-treated plants. PDI was also longest on control, chlorpyrifos, spinetoram and spirotetramat-treated plants (F = 3.81; df = 5, 81; $P < 0.0001$) and shortest on fenpropathrin and imidacloprid-treated plants (Table 6-5). In addition, insects stood still (z) for the longest durations (F = 7.65; df = 5, 79; $P < 0.0001$) on the fenpropathrin and imidacloprid-treated plants and the shortest duration on control, chlorpyrifos, spinetoram and spirotetramat-treated plants. The opposite results were observed for the non-probing/walking behavior (np) (F = 9.58; df = 5, 82; $P < 0.0001$). These behaviors were performed for the longest duration on control, chlorpyrifos, spinetoram and spirotetramat-treated plants and shortest duration on fenpropathrin and imidacloprid-treated plants. For WDI, there were significant differences between treatments for pathway/stylet penetration (waveforms C) (F = 11.67; df = 5, 81; $P < 0.0001$). The longest duration of waveform C occurred for control, chlorpyrifos, spinetoram and spirotetramat-treated plants with shorter durations on fenpropathrin and imidacloprid-treated plants. However, there was a high percentage of psyllids performing stylet penetration. Waveform D did not occur in all treatments. For psyllids which performed waveform D, there was no significant difference between treatments (F = 0.42; df = 1, 2; P = 0.58) which was also the case for *D. citri*

performing phloem salivation (E1) (F = 0.00; df = 1, 2; P = 0.99) and phloem ingestions (E2) (F = 0.87; df = 1, 1; P = 0.5214; Table 6-5).

For WDI, xylem ingestion (G) was significantly different between treatments (F = 5, 27; df = 3, 18; P = 0.0087). The longest duration occurred on the control plants and the shortest durations were on chlorpyrifos, spinetoram and spirotetramat-treated plants.

Experiment 4. Twenty-one Days After Treatment

All psyllids (PPW) on treatments tested performed non-probing/walking activities (np). When they were not probing or walking, they were standing still. Consequently, 100%, 93.3%, 73.3 % and 53.3 % (PPW) of the psyllids on imidacloprid, chlorpyrifos, spinetoram-treated and untreated plants, respectively, performed non-probing/non-walking (z) activities. In addition, 80 % (PPW) of *D. citri* performed waveform z on fenpropathrin and spirotetramat-treated plants.

All psyllids on all of the treatments performed pathway/stylet penetration (C). However, only 26.7 % and 20% of *D. citri* penetrated and salivated in the phloem (D and E1, respectively) on control and chlorpyrifos-treated plants, respectively. Thus, only 6.7 % (PPW) of *D. citri* performed the same behavior on fenpropathrin and spirotetramat-treated plants. Of the psyllids which reached the phloem, only 20 % (PPW) performed phloem ingestion (E2) on control and chlorpyrifos-treated plants. Also, 6 % (PPW) performed the same behavior on fenpropathrin-treated plants. In addition to phloem ingestion, a large percentage of the psyllids performed xylem ingestion (G). Specifically, 60%, 46.7 %, 40 %, 33.3 % and 6.7 % (PPW) of the tested psyllids performed waveform G on control, spirotetramat, chlorpyrifos, spinetoram and fenpropathrin-treated plants respectively.

Cohort level. *D. citri* had a total access period of 324,000 s during which psyllids on the control plants psyllids probed 493 times (TNP) and spent 153,742.06 s (TPD) feeding behavior. Psyllids on the spirotetramat-treated plants probed 402 times (TNP) and had a total probing

duration of 103,795.11 s (TPD). Similarly, psyllids on chlorpyrifos-treated plants probed 301 times (TNP) and spent 110,890.77 s (TPD) with their stylets inserted into the leaves. However, psyllids on spinetoram-treated plants probed 708 times (TNP), and for (TPD= 82,140.57 s). In addition, psyllids on fenpropathrin and imidacloprid-treated plants performed a total number of probes (TNP) of 339 and 209 respectively, with total probing duration (TPD) of 39,600.71 s and 19,495.92 s, respectively.

Insect level. The differences found in the cohort level were also analyzed at the insect level. The number of probes per insect (NPI) was higher for psyllids on control and spinetoram-treated plants than on chlorpyrifos, fenpropathrin, spirotetramat and imidacloprid-treated plants (F = 3.87; df = 5, 84; P = 0.0033; Table 6-6); *D. citri* on imidacloprid-treated plants had the lowest NPI. In addition, for the probe duration per insect (PDI), there was a significantly longer duration (F = 8.12; df = 5, 84; $P < 0.0001$) for psyllids on control, chlorpyrifos, spinetoram and spirotetramat-treated plants compared to fenpropathrin and imidacloprid-treated plants (Table 6-6). Significant differences were also found in the waveform duration per insect (WDI). Non-probing/non walking activities (z) were longer on imidacloprid-treated plants (F = 3.05; df = 5, 66; P = 0.015) than control, chlorpyrifos, spinetoram and spirotetramat-treated plants. The non-probing/walking waveforms (np) (F = 2.40; df = 5, 84; P = 0.044) were significantly longer in duration on control, chlorpyrifos, fenpropathrin, spinetoram and spirotetramat-treated plants compared to imidacloprid-treated plants (Table 6-6). Thus, pathway/stylet penetration (C) was longest on chlorpyrifos, spinetoram and spirotetramat-treated plants (F = 6.31; df = 5, 84; $P <$ 0.0001,) than on fenpropathrin and imidacloprid-treated plants. There was no difference among the treatments for phloem penetration (D) (F = 2.07; df = 3, 5; P = 0.2200), phloem salivation

(E1) (F = 0.88; df = 3, 5; P = 0.5090), phloem ingestion (E2) (F = 0.1; df = 2, 4; P = 0.8500) and xylem ingestion (G) (F = 0.55; df = 4, 23; P = 0.703; Table 6-6).

Experiment 5. Twenty-eight Days After Treatment

All (PPW) of the *D. citri* performed non-probing/walking activities (np) on control, chlorpyrifos, fenpropathrin, imidacloprid and spinetoram-treated plants. In addition, 92.7 % (PPW) of *D. citri* performed the same behavior on spirotetramat-treated plants. While psyllids were not walking and probing, they were performing non-probing/standing still (z) events. Consequently 92.7 %, 85.7 %, and 57.1% (PPW) of *D. citri* performed waveform z on imidacloprid, spinetoram, and chlorpyrifos-treated plants, respectively. Also 64.3 % (PPW) of *D. citri* performed standing still events on fenpropathrin and spirotetramat-treated plants. For WDI, all *D. citri* performed pathway/stylet penetration (C) on control, fenpropathrin, and spinetoram-treated plants. Also, 92.9 % of psyllids (PPW) on chlorpyrifos-treated plants and 85.7 % (PPW) on imidacloprid, and spirotetramat-treated plants performed stylet penetration. Only a few *D. citri* reached and penetrated the phloem (waveform D) on control, chlorpyrifos and imidacloprid-treated plants (21.4 %), and 7.1 % (PPW) on spirotetramat-treated plants. Of those, only 21.4 % (PPW) of *D. citri* salivated into the phloem (E1) on chlorpyrifos and imidacloprid-treated plants; 14.3 % on control plants and 7.1 % (PPW) performed the same behavior on spirotetramat-treated plants. However, only 14.3 % (PPW) of *D. citri* were able to perform phloem ingestion (E2) on chlorpyrifos and imidacloprid-treated plants, while 7.1 % performed the same behavior (PPW) on control plants and spirotetramat-treated plants. In addition, a high percentage of psyllids were able to ingest xylem. Specifically 71.4 %, 64.3 %, 50 %, 42.7 %, 21.4 %, and 7.1 % of *D. citri* ingested xylem (G) on chlorpyrifos, control, spirotetramat, spinetoram, fenpropathrin, and imidacloprid-treated plants.

Cohort level. There was a considerable amount of variation at the cohort level data 28 DAT. *D. citri* had a total access period of 302,000 s. Psyllids on control, spirotetramat, fenpropathrin, spinetoram, chlorpyrifos and imidacloprid-treated plants performed a TNP of 371, 323, 317, 313, 263, and 141, respectively. Those probes lasted (TPD) 109,293.61 s; 143,469.34 s; 80,848.41 s; 109,116.97 s; 119,905.33 s and 53,097.00 s, respectively.

Insect level. There were significant differences in the number of probes per insect (NPI) (F = 2.57; df = 5, 77; P = 0.0334; Table 6-7) between treatments. Total probe frequency was higher for psyllids on control, fenpropathrin, spinetoram and spirotetramat-treated plants compared to chlorpyrifos and imidacloprid-treated plants. PDI was significantly longer on spirotetramat-treated plants (F = 4.08; df = 5, 73; P = 0.0025) than on imidacloprid-treated plants (Table 6-7). In addition, for waveform duration per insect WDI, non-probing-moving (np) (F = 3.97; df = 5, 77; P = 0.0030) was longer on control, chlorpyrifos, fenpropathrin, imidacloprid and spirotetramat-treated plants than spinetoram-treated plants. Also, non-probing and standing still (z) (F = 3.82; df = 5, 54; P = 0.0049) was longer on imidacloprid-treated plants than on control, chlorpyrifos, spinetoram and spirotetramat-treated plants. Pathway/stylet penetration waveforms (C) (F = 3.98; df = 5, 73; P = 0.0030) was longer on spirotetramat-treated plants than on imidacloprid-treated plants. However, phloem penetration (D) (F = 4.33; df = 3, 6; P = 0.0601), phloem salivation (E1) (F = 0.63; df = 3, 5; P = 0.6273), phloem ingestion (E2) (F = 1, 32; df = 3, 3; P = 0.4587) and xylem ingestion (G) (F = 1.00; df = 5, 30; P = 0.4328) were not significantly different between treatments (Table 6-7).

Summary of results. Overall, 1 DAT, the duration of the probing activities was reduced on fenpropathrin and imidacloprid treated plants compared with untreated plants. Feeding activities ceased fastest on imidacloprid and fenpropathrin-treated plants as a result of decreased

probe, a reduction in the number and duration of stylet penetrations (C), phloem activities (waveforms D, E1 and E2), xylem ingestion (G), and non-probing/walking (np). Consequently, there was an increase in the non-probing/standing still activities (z) by *D. citri* on fenpropathrin and imidacloprid treated plants. Chlopyrifos, spinetoram and spirotetramat did not affect probing activities compared to untreated plants. However, psyllids on chlorpyrifos-treated plants probed half as long as on untreated plants. Psyllids on spinetoram and spirotetramat-treated plants probed for the same duration or even longer than psyllids on untreated plants. In addition, psyllids on spinetoram and spirotetramat-treated plants penetrated the phloem and salivated normally; however, psyllids were able ingest phloem only on spirotetramat-treated plants.

At 7 DAT, psyllids on chlorpyrifos-treated plants doubled the time spent probing. In contrast, all the other insecticides had slightly smaller number and duration of probes, but only fenpropathrin, imidacloprid and spinetoram treated-plants were significantly different than the control. Fenpropathrin, imidacloprid and spinetoram showed smaller duration in stylet penetration behaviors (C), and no performance of phloem activities (D, E1 and E2). Consequently, insects had longer durations on the non-probing/standing still (z) events, although, they had a smaller duration of non-probing/walking (np) when compared to chlorpyrifos, control and spirotetramat-treated plants.

At 14 DAT, feeding was disrupted on fenpropathrin and imidacloprid-treated plants. Psyllids on fenpropathrin and imidacloprid-treated plants had their stylet penetration (C), phloem activities (waveform D, E1, and E2), and xylem ingestion (G) reduced when compared to chlorpyrifos, control, spinetoram, spirotetramat-treated plants. Consequently they also had their non-probing/standing (z) duration increased. Since those insecticides were still causing insect mortality, the non-probing/walking (np) events were also reduced. Although *D. citri* on untreated

plants did not successfully reach the phloem, psyllids on chlorpyrifos and spinetoram-treated plants were successful in performing phloem penetration (D), salivation (E1), and ingestion (E2) behaviors. Compared to the other insecticides, *D. citri* feeding on spirotetramat-treated plants did not show any significant difference in feeding behaviors when compared to untreated plants.

There were still significant reductions 21 DAT in probe duration for *D. citri* feeding on fenpropathrin and imidacloprid treated plants. However, the frequency of probes started to homogenize and fenpropathrin-treated plants showed not to be significantly different from control, chlorpyrifos, and spirotetramat-treated plants. In addition, waveform duration per insect were still different for the duration of the stylet penetrations (C) on both fenpropathrin and imidacloprid treated plants. Non-probing/walking events were significantly smaller for imidacloprid-treated plants and the non-probing/standing still events were significantly higher. Phloem activities (D, E1 and E2), were performed by psyllids on fenpropathrin-treated plants and were not significantly difference from the control, chlorpyrifos, and spirotetramat-treated plants. In addition, there was no significant difference in xylem feeding behavior between any of the treatments. At 28 DAT, the imidacloprid treatment was the only case where *D. citri* were still performing probes of shorter duration compared to control plants. This was a direct result of shorter durations of the stylet penetrations (C) and xylem ingestion (G) yet longer non-probing/standing still (z) events. Although, a small percentage of those psyllids were able to find the phloem, salivate and ingest into the phloem. However, the number of *D. citri* that were able to perform these phloem behaviors was too small to allow statistical comparison. There was no significant difference in *D. citri* feeding behavior on chlorpyrifos, fenpropathrin, spinetoram, and spirotetramat-treated plants when compared to control plants.

The average of the probe duration per insect (PDI), average of number of probes per insect (NPI) and total waveform duration (TWD) through the whole experiment are shown on figures 6-1, 6-2 and 6-3, respectively. TWD of waveforms np, C, G, D, E1 and E2 shown to be negatively proportional to the residual concentration of chlorpyrifos and imidacloprid (figure 6-3), while NPI, and TWD of waveform z, were positively proportional.

Residual Analyses and Temperature Recording

The average residual activity of chlorpyrifos and imidacloprid in leaf tissues are represented in figure 6-4. Temperature and relative humidity variations were measured through the insecticide residual break down; they were also recorded through the experiment and are represented in the figure 6-4. Temperatures did not differ inside and outside of the cage, but relative humidity did differ. Humidity inside of the cage was significantly higher than outside of the cage ($F = 28.57$; df = 1, 1486; $P < 0.0001$).

Discussion

The current study investigated the effect of residual activity of five different insecticides on *D. citri* feeding behavior. The data indicated that feeding by *D. citri* was reduced on fenpropathrin and imidacloprid-treated plants for up to 21 and 28 days, respectively. Chlorpyrifos affected psyllid phloem contact for only 1 DAT. *D. citri* on spinetoram-treated plants were able to reach the phloem 1 DAT; however, they did perform probes of shorter duration 1 and 7 DAT. Treatment of plants with spirotetramat did not affect psyllid feeding. In fact, *D. citri* probed for longer durations on spirotetramat-treated plants compared to untreated plants.

Breakdown of insecticides depends on, environmental factors (temperature, relative humidity, and wind speed), and plant factors (species and plant growth) (Edwards, 1975).

Temperature was not affected by our cages; however, relative humidity was higher inside of cages.

In order to trace insecticide break down, chlorpyrifos and imidacloprid leaf samples were analyzed. Ideally, all the insecticides should have been analyzed, but not all the residual activity analyses were available. Feeding behaviors indirectly indicated breakdown of chlorpyrifos, fenpropathrin, imidacloprid and spinetoram-treated plants. Psyllids on those treated plants showed a negative correlation on probing, stylet penetration, phloem penetration, salivation and ingestion, and xylem ingestion when compared to the chlorpyrifos and imidacloprid residual concentrations. Thus we are able to see a positive relationship of the non-walking/standing still event when comparing to chlorpyrifos and imidacloprid residual concentrations.

Chlorpyrifos, fenpropathrin, and imidacloprid are mainly contact insecticides and act directly on the insect nervous system (acetylcholinesterase inhibitors, sodium channel modulators, and acetylcholine receptor stimulators, respectively). These insecticides can cause over stimulation of the nervous system quickly killing the insects. Consequently, chlorpyrifos, fenpropathrin, and imidacloprid cause a rapid effect on the insect nervous system which could explain the immediate reduction in *D. citri* feeding behaviors in this study.

Spinetoram is also a contact insecticide. However, this insecticide acts via disruption of the nicotinic/GABA-gated chloride channels triggering either an up-regulation or a down-regulation of neurotransmitters, which is slower acting but still results in insect death (Casida and Quistad 2004) and could explain the fact that *D. citri* were still able to perform phloem feeding behaviors prior to the occurrence of mortality, even 1 DAT.

Spirotetramat has limited contact activity and is mainly effective after insect digestion and prevents formation of lipids required for reproduction or ecdysis (Nauen et al. 2008), which

would explain the lack of noticeable effects on psyllid feeding behavior which may take a much longer period of time for subleathal effects on feeding behavior to occur.

In addition to different modes of action, insecticides formulation characteristics could have some affect on the feeding behavior of *D. citri.* Insecticide breakdown also depends on the insecticide characteristics, such as active ingredient stability, volatility, and formulation (Edwards 1975). Therefore, it is possible that the five different insecticides tested could have degraded at different rates affecting psyllid behavior differentially. Chlorpyrifos (Lorsban 4E) and fenpropathrin (Danitol 2.4 EC) formulations were applied as emulsifiable concentrates. Imidacloprid (Provado 1.6F) as a flowable, spinetoram (Delegate WG) as a wettable granulate and spirotetramat (Movento 240SC) is a suspension concentrate. Emulsifiable concentrated insecticides applied to plants have their active ingredients immediately available for insect control. However, those types of insecticides are not as persistent as granulates and they are easily washed away (Edwards 1975, Montemurro et al. 2002). Flowables and suspension concentrates are a liquid suspension. After application, they form small crystals on the treated surfaced, and since those crystals are insoluble, they have a better residual activity. Wettable granulates, are insecticides that are not immediately active and the active ingredient releases within 1 to 30 h after applications which depends on light exposure (Montermurro et al. 2002). These facts could explain the longer residual activity of products such as imidacloprid compared to chlorpiryfos and fenpropathrin and perhaps why spinetoram (a wettable granule) was more effective 7 DAT compared to 1 DAT.

Results from this study demonstrate that while some insecticides may cause relatively rapid mortality of adult psyllids, there is considerable variability that exists among these products in terms of the duration of feeding disruption provided. While some insecticides provided

feeding disruption lasting 3-4 weeks (fenpropathrin and imidacloprid), protection provided by other products was much shorter. Overall, the results of this study can be used to help guide citrus growers in product selection and also determine when additional applications will be necessary.

Table 6-1. Mean (± SE) number of probe per insect (NPI) for *Diaphorina citri* feeding on chlorpyrifos, fenpropathrin, imidacloprid, spinetoram, and spirotetramat-treated and untreated citrus plants up to 28 d after treatment (DAT).

Treatments	DAT[a]																	
	1				7				14				21				28			
	NPI	±	SEM		NPI	±	SEM		NPI	±	SEM		NPI	±	SEM		NPI	±	SEM	
Untreated control	62.60	±	10.58	*a*	41.85	±	8.98	*ab*	64.93	±	14.53	*a*	33.33	±	6.17	*ab*	26.85	±	3.88	*a*
Chlorpyrifos	21.53	±	3.22	*b*	22.78	±	3.82	*c*	46.73	±	8.68	*a*	20.73	±	2.51	*bc*	19.21	±	4.64	*ab*
Fenpropathrin	23.50	±	8.26	*b*	9.35	±	1.70	*d*	14.00	±	2.52	*b*	23.46	±	5.70	*bc*	23.50	±	4.62	*a*
Imidacloprid	12.86	±	1.89	*b*	11.30	±	2.42	*d*	13.50	±	2.07	*b*	14.86	±	2.76	*c*	11.00	±	3.04	*ab*
Spinetoram	41.06	±	5.97	*a*	26.64	±	5.96	*bc*	61.07	±	16.11	*a*	48.06	±	9.53	*a*	23.07	±	4.86	*a*
Spirotetramat	42.66	±	4.99	*a*	43.14	±	7.30	*a*	38.93	±	8.45	*a*	27.33	±	4.06	*bc*	25.23	±	4.58	*a*

[a] Means with different lower case letters are not significantly different (LSMEANS, $P < 0.05$)

Table 6-2. Mean (± SE) probe duration per insect (PDI) (s) for *Diaphorina citri* feeding on chlorpyrifos, fenpropathrin, imidacloprid, spinetoram, and spirotetramat-treated and untreated citrus plants up to 28 d after treatment (DAT).

	DAT[a]															
	1				7				14				21			
Treatment	PDI	±	SE		PDI	±	SE		PDI	±	SE		PDI	±	SE	
Untreated control	119.78	±	22.43	*ba*	181.73	±	24.55	*a*	124.27	±	19.14	*a*	170.82	±	28.21	*a*
Chlorpyrifos	48.91	±	12.76	*b*	139.41	±	26.87	*ba*	116.62	±	23.04	*a*	123.21	±	24.72	*a*
Fenpropathrin	14.39	±	5.75	*c*	9.14	±	2.42	*c*	29.14	±	19.15	*b*	44.00	±	20.82	*b*
Imidacloprid	11.54	±	3.82	*c*	14.14	±	3.37	*c*	10.95	±	3.01	*b*	21.66	±	6.41	*b*
Spinetoran	101.06	±	15.28	*ba*	82.70	±	17.21	*b*	106.03	±	28.39	*a*	91.27	±	19.65	*a*
Spirotetramat	210.65	±	25.30	*a*	139.99	±	21.72	*ba*	182.09	±	28.54	*a*	115.33	±	22.64	*a*

[a] Means with different lower case letters are not significantly different (LSMEANS, $P < 0.05$)

Table 6-2. Continued

	DAT			
	28			
Treatment	PDI	±	SE	
Untreated control	130.13	±	25.88	*ba*
Chlorpyrifos	153.72	±	24.41	*ba*
Fenpropathrin	96.25	±	28.71	*b*
Imidacloprid	73.75	±	26.97	*c*
Spinetoran	129.90	±	26.37	*ba*
Spirotetramat	199.26	±	26.06	*a*

[a] Means with different lower case letters are not significantly different (LSMEANS, $P < 0.05$)

Table 6-3. Mean (± SE) waveform duration per insect (WDI) (s) for *Diaphorina citri* feeding on chlorpyrifos, fenpropathrin, imidacloprid, spinetoram, and spirotetramat-treated and untreated plants 1 d after treatment (DAT).

1st Day Waveforms	z				np				C				G			
Treatment	WDI	±	SE		WDI	±	SE		WDI	±	SE		WDI	±	SE	
Untreated control	45.82	±	19.07	*c*	166.02	±	1768.48	*a*	89.43	±	15.22	*ab*	59.79	±	10.39	*a*
Chlorpyrifos	208.70	±	24.03	*ab*	109.91	±	1327.96	*a*	36.34	±	7.13	*b*	94.28	±	79.05	*a*
Fenpropathrin	296.40	±	22.12	*ab*	35.41	±	495.54	*b*	14.39	±	5.76	*c*	n/a	±	n/a	*n/a*
Imidacloprid	305.89	±	9.68	*a*	41.05	±	322.58	*b*	11.50	±	3.80	*c*	n/a	±	n/a	*n/a*
Spinetoram	163.33	±	24.58	*b*	93.13	±	826.14	*a*	92.14	±	13.66	*a*	33.03	±	6.10	*a*
Spirotetramat	76.41	±	38.00	*c*	96.80	±	968.17	*a*	165.11	±	21.28	*a*	57.23	±	10.50	*a*

[1] Means with different lower case letters are not significantly different (LSMEANS, $P < 0.05$)

Table 6-3. Continued

Waveforms	D				E1				E2			
Treatment	WDI	±	SE		WDI	±	SE		WDI	±	SE	
Untreated control	2.77	±	2.30	*a*	7.77	±	7.44	*a*	n/a	±	n/a	*n/a*
Chlorpyrifos	n/a	±	n/a	*n/a*	n/a	±	n/a	*n/a*	n/a	±	n/a	*n/a*
Fenpropathrin	n/a	±	n/a	*n/a*	n/a	±	n/a	*n/a*	n/a	±	n/a	*n/a*
Imidacloprid	n/a	±	n/a	*n/a*	n/a	±	n/a	*n/a*	n/a	±	n/a	*n/a*
Spinetoram	0.95	±	n/a	*a*	0.64	±	n/a	*a*	n/a	±	n/a	*n/a*
Spirotetramat	1.47	±	0.90	*a*	0.68	±	5.50	*a*	81.80	±	11.55	*n/a*

[1] Means with different lower case letters are not significantly different (LSMEANS, $P < 0.05$)

Table 6-4. Mean (± SE) waveform duration per insect (WDI) (s) for *Diaphorina citri* feeding on chlorpyrifos, fenpropathrin, imidacloprid, spinetoram, and spirotetramat-treated and untreated plants 7 d after treatment (DAT).

1st Week															
Waveforms	z				np				C				G			
Treatment	WDI	±	SE		WDI	±	SE		WDI	±	SE		WDI	±	SE	
Untreated control	31.96	±	15.27	*d*	151.05	±	22.02	*a*	153.19	±	21.16	*a*	47.29	±	16.34	*a*
Chlorpyrifos	94.07	±	34.77	*cd*	150.69	±	23.69	*a*	106.44	±	21.48	*ab*	65.63	±	11.96	*a*
Fenpropathrin	286.24	±	26.33	*ab*	86.49	±	32.22	*b*	9.14	±	2.42	*c*	n/a	±	n/a	*n/a*
Imidacloprid	303.83	±	9.08	*a*	45.38	±	7.08	*b*	14.14	±	3.37	*c*	n/a	±	n/a	*n/a*
Spinetoram	124.49	±	22.79	*bc*	157.12	±	18.79	*a*	60.31	±	11.24	*b*	62.69	±	23.14	*a*
Spirotetramat	97.91	±	33.66	*cd*	175.16	±	16.75	*a*	107.09	±	20.23	*ab*	65.15	±	20.00	*a*

[1] Means with different lower case letters are not significantly different (LSMEANS, $P < 0.05$)

Table 6-4. Continued

Waveforms	D				E1				E2			
Treatment	WDI	±	SE		WDI	±	SE		WDI	±	SE	
Untreated control	11.7725	±	n/a	*n/a*	18.70	±	n/a	*n/a*	45.95	±	n/a	*n/a*
Chlorpyrifos	0.4	±	n/a	*n/a*	1.56	±	n/a	*n/a*	n/a	±	n/a	*n/a*
Fenpropathrin	n/a	±	n/a	*n/a*	n/a	±	n/a	*n/a*	n/a	±	n/a	*n/a*
Imidacloprid	n/a	±	n/a	*n/a*	n/a	±	n/a	*n/a*	n/a	±	n/a	*n/a*
Spinetoram	n/a	±	n/a	*n/a*	n/a	±	n/a	*n/a*	n/a	±	n/a	*n/a*
Spirotetramat	2.58	±	n/a	*n/a*	2	±	n/a	*n/a*	n/a	±	n/a	*n/a*

[1] Means with different lower case letters are not significantly different (LSMEANS, $P < 0.05$)

Table 6-5. Mean (± SE) waveform duration per insect (WDI) (s) for *Diaphorina citri* feeding on chlorpyrifos, fenpropathrin, imidacloprid, spinetoram, and spirotetramat-treated and untreated plants 14 d after treatment (DAT).

2nd Week

Waveforms	z				np				C				G			
Treatment	WDI	±	SE		WDI	±	SE		WDI	±	SE		WDI	±	SE	
Untreated control	75.90	±	21.88	*b*	152.25	±	17.58	*a*	76.26	±	13.34	*a*	119.96	±	20.03	*a*
Chlorpyrifos	97.89	±	28.72	*b*	142.81	±	17.79	*a*	92.98	±	17.37	*a*	56.13	±	13.36	*b*
Fenpropathrin	288.77	±	23.73	*a*	41.62	±	10.62	*b*	29.14	±	19.15	*b*	n/a	±	n/a	*n/a*
Imidacloprid	301.63	±	10.83	*a*	44.05	±	5.77	*b*	10.95	±	3.01	*b*	n/a	±	n/a	*n/a*
Spinetoram	98.59	±	26.93	*b*	152.39	±	24.97	*a*	77.75	±	19.24	*a*	31.97	±	4.28	*b*
Spirotetramat	79.02	±	35.25	*b*	126.62	±	20.50	*a*	145.30	±	27.74	*a*	63.84	±	9.63	*b*

* Means with different lower case letters are not significantly different (LSMEANS, $P < 0.05$)

Table 6-5. Continued

Waveforms	D				E1				E2			
Treatment	WDI	±	SE		WDI	±	SE		WDI	±	SE	
Untreated control	n/a	±	n/a	*n/a*	n/a	±	n/a	*n/a*	n/a	±	n/a	*n/a*
Chlorpyrifos	1.50	±	1.05	*a*	2.44	±	0.05	*a*	32.73	±	30.49	*a*
Fenpropathrin	n/a	±	n/a	*n/a*	n/a	±	n/a	*n/a*	n/a	±	n/a	*n/a*
Imidacloprid	n/a	±	n/a	*n/a*	n/a	±	n/a	*n/a*	n/a	±	n/a	*n/a*
Spinetoram	0.61	±	0.00	*a*	4.31	±	3.58	*a*	178.13	±	n/a	*a*
Spirotetramat	n/a	±	n/a	*n/a*	n/a	±	n/a	*n/a*	n/a	±	n/a	*n/a*

*Means with different lower case letters are not significantly different (LSMEANS, $P < 0.05$)

Table 6-6. Mean (± SE) waveform duration per insect (WDI) (s) for *Diaphorina citri* feeding on chlorpyrifos, fenpropathrin, imidacloprid, spinetoram, and spirotetramat-treated and untreated plants 21 d after treatment (DAT).

3rd Week

Waveforms	z				np				C				G			
Treatment	WDI	±	SE		WDI	±	SE		WDI	±	SE		WDI	±	SE	
Untreated control	74.40	±	33.48	*b*	152.85	±	24.20	*ab*	105.80	±	23.32	*a*	58.89	±	8.61	*a*
Chlorpyrifos	72.16	±	24.73	*b*	172.79	±	23.14	*a*	81.76	±	17.36	*a*	54.13	±	11.79	*a*
Fenpropathrin	151.83	±	30.78	*ab*	197.89	±	27.30	*a*	30.48	±	10.21	*b*	26.56	±	n/a	*a*
Imidacloprid	223.08	±	22.58	*a*	115.23	±	22.82	*b*	21.65	±	6.41	*b*	n/a	±	n/a	*n/a*
Spinetoram	100.90	±	27.85	*b*	198.10	±	20.56	*a*	73.15	±	15.82	*a*	54.31	±	31.06	*a*
Spirotetramat	101.52	±	28.55	*b*	166.82	±	21.92	*a*	89.45	±	18.98	*a*	55.18	±	13.96	*a*

*Means with different lower case letters are not significantly different (LSMEANS, $P < 0.05$)

Table 6-6. Continued

Waveforms	D				E1				E2			
Treatment	WDI	±	SE		WDI	±	SE		WDI	±	SE	
Untreated control	1.92	±	0.85	*a*	4.26	±	2.45	*a*	140.11	±	67.21	*a*
Chlorpyrifos	4.06	±	0.81	*a*	5.53	±	1.70	*a*	89.40	±	29.21	*a*
Fenpropathrin	1.68	±	n/a	*a*	2.42	±	n/a	*a*	172.15	±	n/a	*a*
Imidacloprid	n/a	±	n/a	*n/a*	n/a	±	n/a	*n/a*	n/a	±	n/a	*n/a*
Spinetoram	n/a	±	n/a	*n/a*	n/a	±	n/a	*n/a*	n/a	±	n/a	*n/a*
Spirotetramat	0.95	±	n/a	*a*	0.86	±	n/a	*a*	n/a	±	n/a	*n/a*

*Means with different lower case letters are not significantly different (LSMEANS, $P < 0.05$)

Table 6-7. Mean (± SE) waveform duration per insect (WDI) (s) for *Diaphorina citri* feeding on chlorpyrifos, fenpropathrin, imidacloprid, spinetoram, and spirotetramat-treated and untreated plants 28 d after treatment (DAT).

4th Week																
Waveforms	z				np				C				G			
Treatment	WDI	±	SE		WDI	±	SE		WDI	±	SE		WDI	±	SE	
Untreated control	76.99	±	29.76	*b*	186.01	±	20.64	*a*	95.43	±	21.00	*ab*	43.03	±	5.87	*ab*
Chlorpyrifos	82.47	±	28.48	*b*	154.25	±	17.71	*a*	81.50	±	14.90	*ab*	72.50	±	23.55	*ab*
Fenpropathrin	110.25	±	45.07	*b*	200.34	±	36.55	*a*	81.61	±	21.07	*b*	68.31	±	34.75	*a*
Imidacloprid	243.31	±	29.22	*a*	78.32	±	17.48	*a*	54.26	±	19.94	*c*	9.72	±	n/a	*b*
Spinetoram	106.87	±	35.37	*b*	176.71	±	22.95	*b*	103.44	±	22.78	*ab*	61.75	±	17.25	*ab*
Spirotetramat	53.89	±	35.65	*b*	146.20	±	22.32	*a*	161.23	±	23.85	*a*	59.48	±	11.21	*a*

[a] Means with different lower case letters are not significantly different (LSMEANS, $P < 0.05$)

Table 6-7. Continued

Waveforms	D				E1				E2		
Treatment	WDI	±	SE		WDI	±	SE		WDI	±	SE
Untreated control	1.68	±	0.61	*a*	1.06	±	0.66	*a*	84.91	±	n/a
Chlorpyrifos	3.19	±	1.20	*a*	3.75	±	1.83	*a*	96.54	±	6.55
Fenpropathrin	n/a	±	n/a	*n/a*	n/a	±	n/a	*n/a*	n/a	±	n/a
Imidacloprid	1.81	±	0.06	*a*	2.20	±	0.51	*a*	105.94	±	44.98
Spinetoram	n/a	±	n/a	*n/a*	n/a	±	n/a	*n/a*	n/a	±	n/a
Spirotetramat	0.19	±	n/a	*b*	305.70	±	n/a	*a*	2088.00	±	n/a

[a] Means with different lower case letters are not significantly different (LSMEANS, $P < 0.05$)

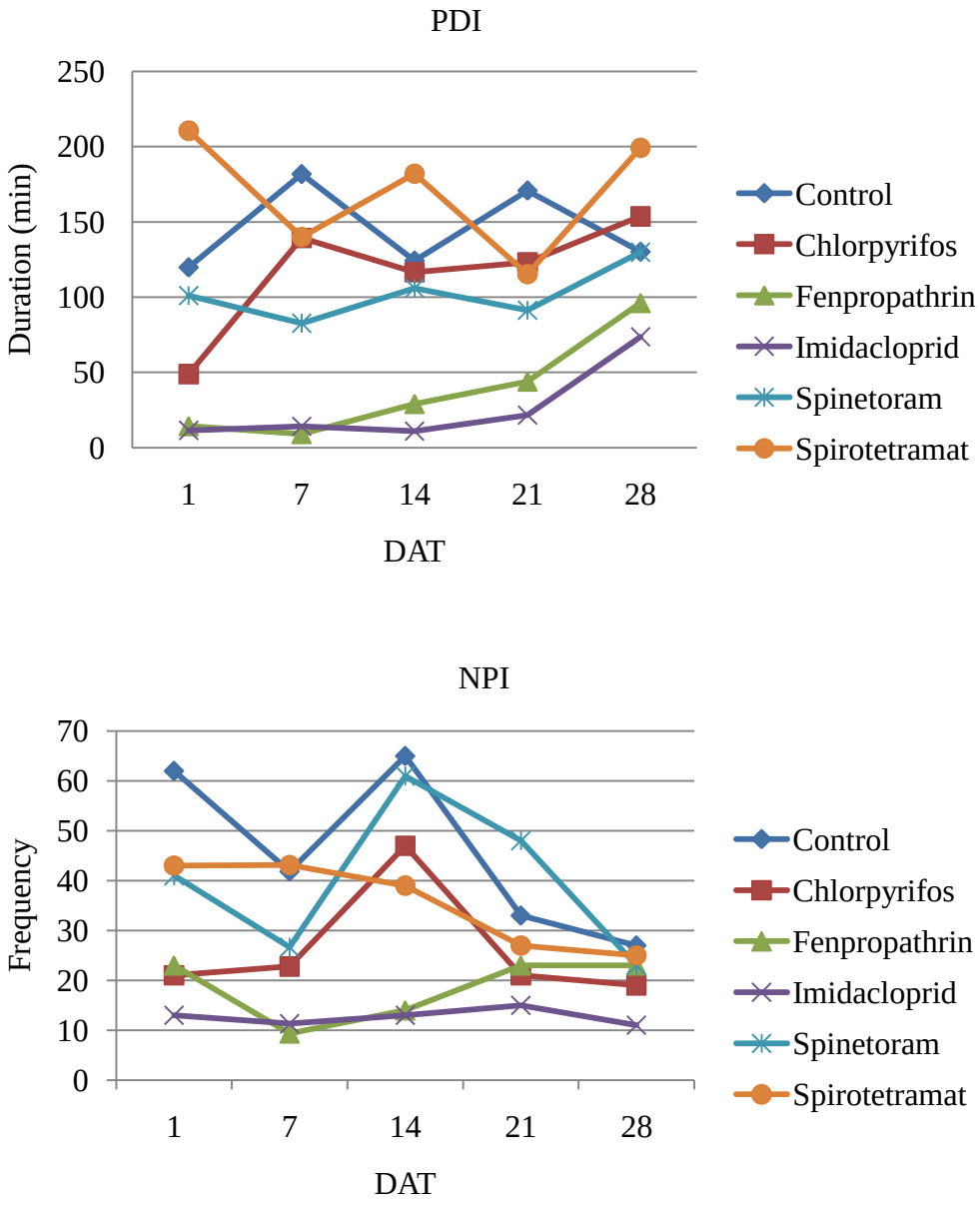

Figure 6-1. Mean probe duration per insect (PDI) and the mean number of probes per insect (NPI) for *Diaphorina citri* feeding on chlorpyrifos, fenpropathrin, imidacloprid, spinetoram, and spirotetramat-treated and untreated citrus plants through 28 d after treatment (DAT).

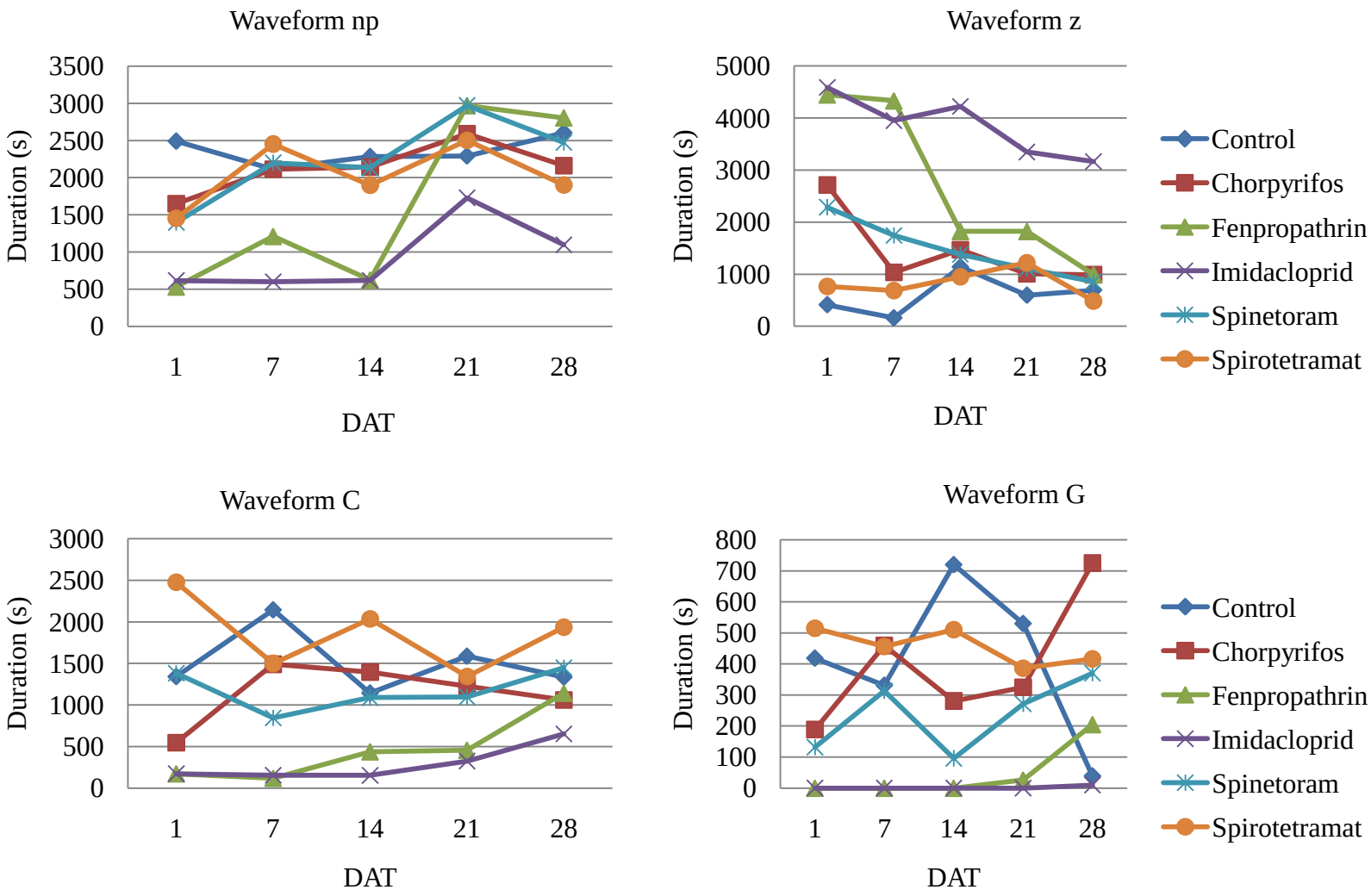

Figure 6-2. Total waveform duration (TWD) for *Diaphorina citri* feeding on chlorpyrifos, fenpropathrin, imidacloprid, spinetoram, and spirotetramat-treated and untreated citrus plants through 28 d after treatment (DAT).

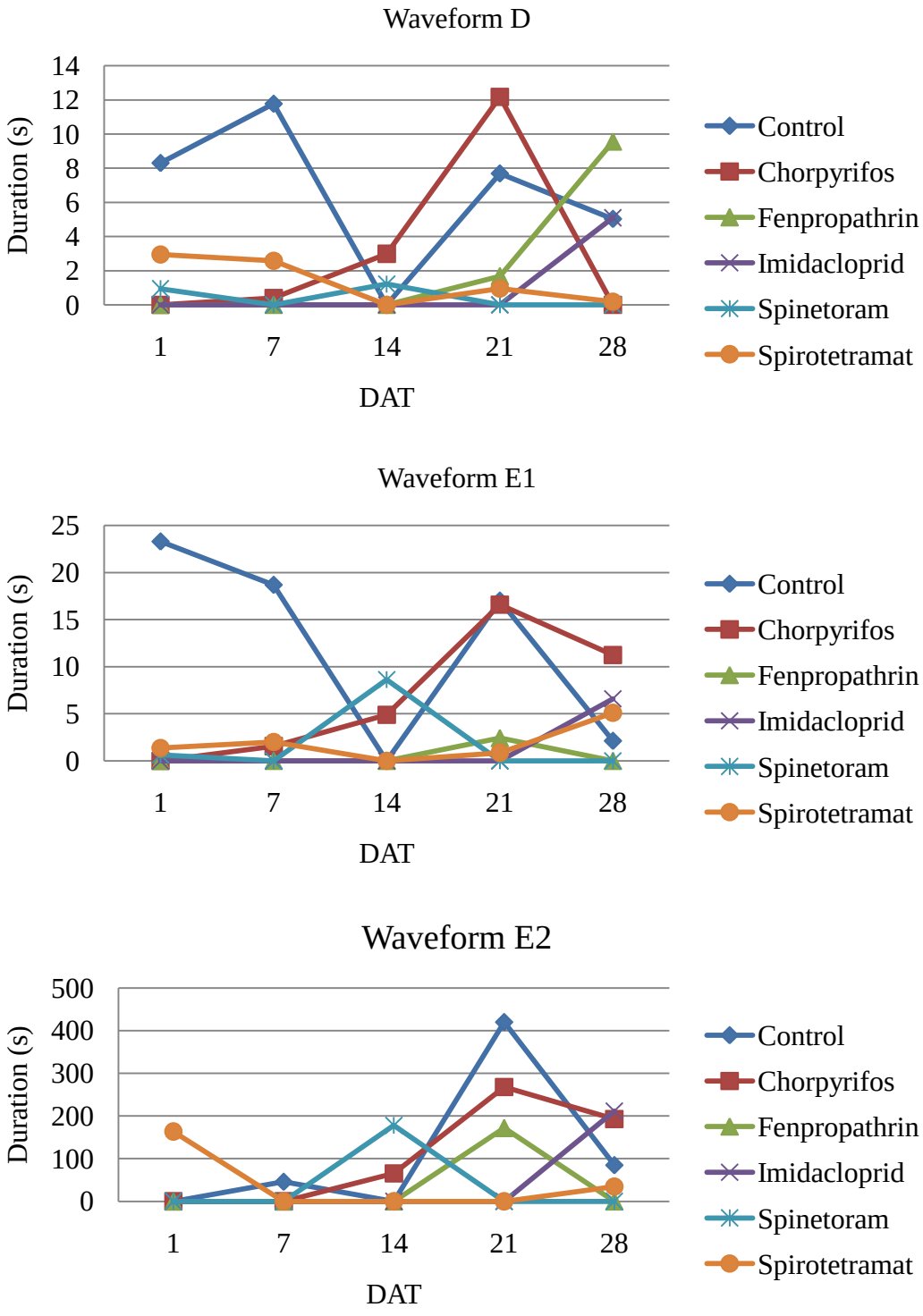

Figure 6-3. Total waveform duration (TWD) for *Diaphorina citri* feeding on chlorpyrifos, fenpropathrin, imidacloprid, spinetoram, and spirotetramat-treated and untreated citrus plants through 28 d after treatment (DAT).

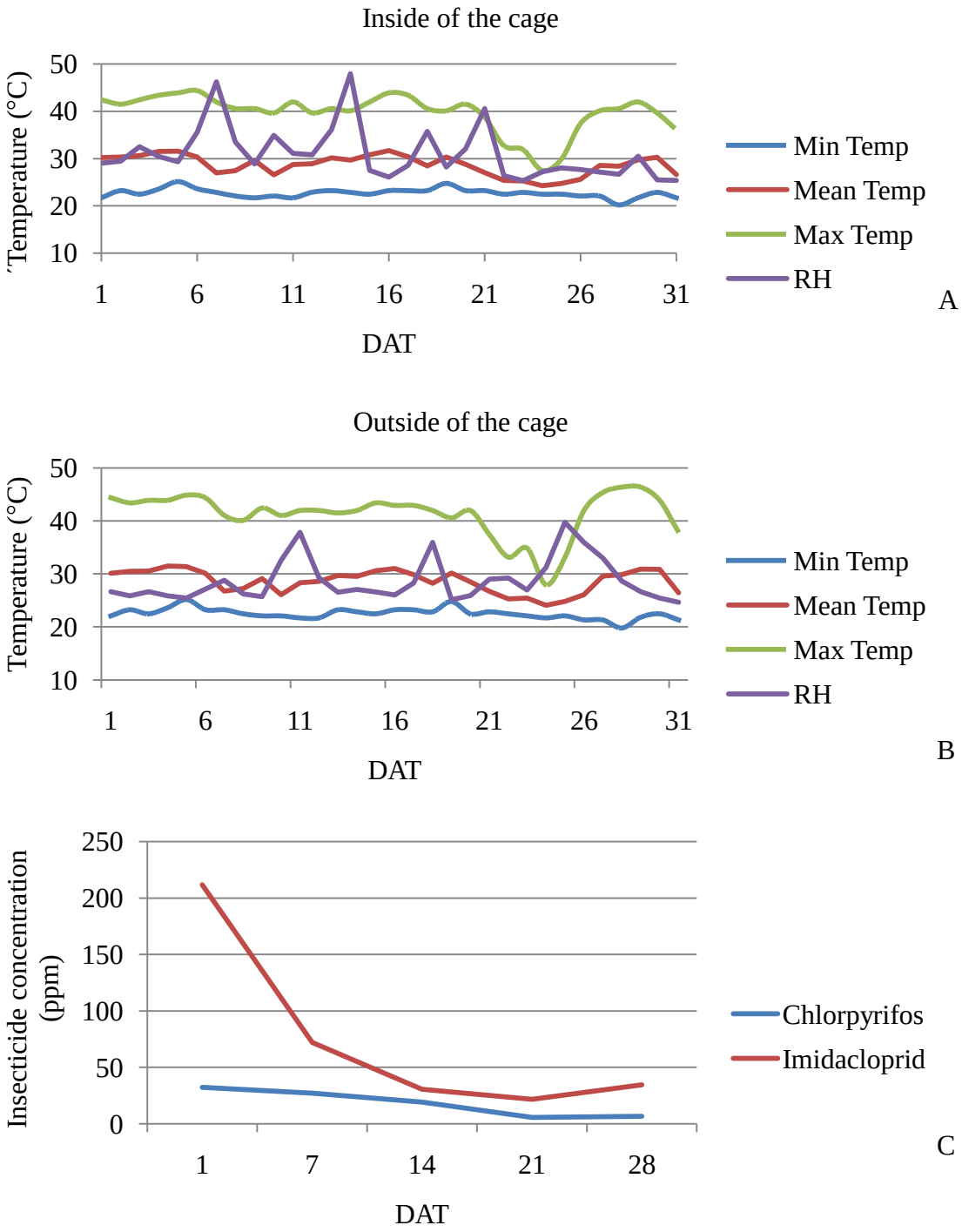

Figure 6-4. Temperature, relative humidity and insecticide concentrations through 28 d after treatment. A) Temperature (max, average and min) and relative humidity (RH) inside of the cage. B) Temperature (max, average and min) and relative humidity (RH) outside of the cage. C) Insecticide residues

CHAPTER 7
SUMMARY

Since ‘Huanglongbing’ (HLB) was first found in Florida, the estimated per acre pest management costs for mature citrus groves have increased nearly 41% (Morris and Muraro 2008). These additional costs are due to scouting for HLB infected trees and an increased use of pesticides for controlling the disease vector, the Asian citrus psyllid (*Diaphorina citri* Kuwayama). The HLB associated pathogen (*Candidatus* Liberibacter asiaticus) is a phloem-limited bacterium and is transmitted during the feeding activities of *D. citri*. Consequently, studies designed to gain a better understanding of psyllid feeding behavior are justified. In the research reported here an electrical penetration graph (EPG) monitor was used to record psyllid feeding activities in order to determine the effects of gender, presence of light and insecticides on *D. citri* feeding behaviors.

EPG was first developed by McLean (1964) and later modified by Tjallingii and Prado (2001) to allow quantification of the probing and feeding behaviors of certain sap-sucking insects, thus making it possible to unravel the mechanisms underlying pathogen acquisition and transmission (McLean 1964 and Tjallingii and Prado 2001). The first EPG studies with *D. citri* were conducted by Bonani et al. (2010). In those studies, the basic Asian citrus psyllid feeding behaviors were characterized. Bonani et al. (2010) also was able to correlate phloem ingestion (Waveform E2) by *D. citri* with acquisition of *Candidatus* Liberibacter asiaticus. However, bacterial inoculation could not be correlated with phloem penetration or salivation (waveforms D and E,1 respectively) but there was a strong indication that this might be happening during either of these feeding behaviors.

In Chapter 2 of the current research, the effects of gender and photoperiod on *D. citri* feeding behavior were investigated. When comparing the feeding behaviors of *D. citri* between

genders, males reached the phloem (waveform D) 20% more often than females. However, when females were successful in penetrating the phloem, the duration of phloem ingestion (waveform E2) was significantly longer for females when compared to males.

One possible explanation for female *D. citri* performing more phloem ingestion than males is the physiological needs of females that are related to reproduction. In other insects, it is common for females to feed more frequently during their reproductive period (Nation 2002). *D. citri* is sexually mature 2-3 days after emergence, therefore, it is likely that most (if not all) female psyllids used in this study were reproductively viable. Whether longer durations of phloem feeding behaviors will result in increased pathogen acquisition and/or inoculation remain uncertain. In studies done with *Frankliniella occidentalis,* male thrips were responsible for higher rates of pathogen transmission despite the fact that female thrips fed for longer durations of time (Wetering et al. 1998). Consequently, conclusions regarding the transmission efficiency of male versus female *D. citri* could not be made since the definitive studies of transmission using psyllids containing bacteria were not conducted.

The effect of light presence on *D. citri* feeding behavior was also presented in Chapter 2. Results showed that non-probing activities (waveform z and np), phloem penetration and salivation (waveform D and E1, respectively), and xylem ingestion (waveform G) were generally longer in duration during the light. Conversely, stylet penetration (waveform C) and phloem ingestion (waveform E2) were longer in duration during the dark. Within treatment analysis indicated some effects of light on xylem ingestion (waveform G) and phloem ingestion (waveform E2) with phloem ingestion waveforms being longer in duration during the dark. However, there was no significant difference in the presence of light. Since *D. citri* is considered to be a phloem feeder, sugar and amino acid content are likely the main phagostimulants

regulating psyllid feeding activities. Differences found in *D. citri* feeding between light and dark periods could be related to changes in nutrient availability in the plant. Studies with *Bucephalogonia xanthophis* (Miranda et al. 2008), and other sharpshooters (Andersen et al. 1989, 1992 and Brodbeck et al.1993) have shown that those insects are able to synchronize their feeding behavior according to the fluctuation in xylem fluid chemistry. Goldschimitdt and Koch (1996) showed slight daily fluctuations of soluble sugars and starch levels in citrus leaves, such that soluble sugars and starch levels were higher during the day and lower during the evening. Since soluble sugars from the leaves are transported through the phloem, sugar concentrations in the phloem are negatively inverted, and thus are higher during the night and lower during the day.

Reduction in feeding behaviors during light could also be a result of increases in other behaviors that must occur during the photophase thus leaving less time for feeding behaviors to occur. For example, *D. citri* perform higher mating, oviposition and dispersal behaviors during the photophase (Wenninger and Hall 2007). These biological attributes of *D. citri* could explain the longer durations and increased frequency of walking and standing still during the light and longer phloem ingestion during the dark.

Chapters 3-6 examine the effects of soil-applied systemic and broad-spectrum foliar-applied insecticides on the *D. citri* feeding behavior. Since some insecticides have been shown to increase pathogen transmission rates in some pathosystems (Joost and Riley 2005), it is therefore important to know what effects insecticide applications will have on *D. citri* and subsequent potential transmission of Las. More specifically, can insecticide applications be used to disrupt psyllid feeding behaviors associated with pathogen acquisition and inoculation prior to

insecticide-induced mortality? Also, how long will such effects last, and are there any unintended consequences such as an increased potential for bacterial transmission?

The effect of the soil-applied imidacloprid on *D. citri* feeding behavior is presented in Chapter 3. Overall, the general feeding behavior of *D. citri* was disrupted on imidacloprid-treated plants as demonstrated by an increased duration and number of times in which the insect stood still or attempted to jump off plants, a decrease in walking and searching behaviors, a reduction in the number of probes, and a reduction on mean durations per event at the probe and insect level. While all *D. citri* tested on imidacloprid-treated plants died during the course of the 12 h EPG recording due to imidacloprid exposure, *D. citri* feeding on young leaves of imidacloprid-treated plants took on average 4 h (6 h for mature leaves) to die. Consequently, psyllids had an average feeding access period of 4 h before succumbing to the pesticide treatment. During this feeding access period, some of the psyllids tested were able to perform phloem related behaviors (waveforms D, E1, and E2). In addition, compared to feeding on mature leaves, *D. citri* on young citrus leaves performed a numerically higher number of probes which were also longer in duration on both imidacloprid-treated and untreated plants. This result is probably related to leaf tenderness, which may influence the ease of successful stylet penetration. Results from this study demonstrate that the benefits of soil-applied imidacloprid applications extend beyond reducing the overall psyllid population. Prior to causing mortality, imidacloprid application can potentially disrupt the psyllid feeding process such that successful pathogen acquisition and inoculation are both less likely to occur. It was also found that, on rare occasions, the probability of acquisition of Las could actually increase as a result of slight increases in phloem ingestion behaviors (waveform E2). However, this may not matter epidemiologically due to the pathogen latency

period in the insect coupled with the fact that psyllids would be dead before being capable of successfully inoculating a healthy tree with the pathogen.

In Chapter 4, the effects of another systemic insecticide, aldicarb (Temik 15 G), on psyllid feeding behavior was investigated. Aldicarb is a soil-applied systemic carbamate insecticide that has been widely used in Florida commercial citrus production for psyllid control. However, one noticeable difference in terms of control provided compared to soil-applied imidacloprid is that the mortality of adult psyllids resulting from exposure to aldicarb is not as rapid. The overall results of this study showed no significant reduction in adult *D. citri* feeding behavior between aldicarb-treated and untreated plants when data were analyzed at both the insect and probe levels. Analysis of walking (np), stylet penetration (C), phloem penetration (D) and phloem salivation (E1) behaviors were slightly reduced and incidence of insects standing still (z), phloem ingestion (E2) and xylem ingestion (G) were slightly increased on aldicarb-treated plants. However, significant differences were found at the event level, in which waveform duration per event (WDE) was higher for the phloem-related activities of salivation and ingestion on aldicarb-treated plants when compared to controls. Thus, *D. citri* on aldicarb-treated plants ingested from and salivated into phloem for longer durations per event when compared to psyllids on untreated plants indicating a potentially negative effect of aldicarb application. In other words, these results suggest that aldicarb applications could lead to increased pathogen transmission rates. While aldicarb does provide long-term reduction in psyllid populations through control of developing nymphal populations, these results point to the need for additional use of foliar insecticide applications targeting adult psyllids to offset these negative effects.

The effect of broad-spectrum foliar-applied insecticides and their residual activity on the feeding behavior of *D. citri* was studied in Chapters 5 and 6, respectively. In Chapter 5,

differences in feeding disruption provided by foliar-applied insecticide with differing modes of action was found. Overall, *D. citri* on chlorpyrifos, fenpropathrin, and imidacloprid-treated plants had a reduction in the probe duration, number of probes, walking activities, stylet penetration, phloem activities and xylem ingestion, while psyllids on spinetoram-treated plants only had reductions in the probe duration, walking activities, stylet penetration, and xylem ingestion behaviors. However, psyllids on spinetoram-treated plants were still able to reach the phloem. In contrast, there was no effect of spirotetramat application on any *D. citri* feeding behaviors when compared with untreated plants.

For each of the insecticides evaluated, the more important differences noted were in the level of feeding disruption provided as follows: *D. citri* exposed to chlorpyrifos-treated plants took an average 4.02 h to achieve 100% mortality with probing behaviors performed an average of 0.88 h per insect. Psyllids feeding on fenpropathrin-treated plants took an average of 0.62 h to die with an average probing duration of only 0.03 h per insect. Similar results were obtained for psyllids exposed to foliar-applied imidacloprid-treated plants, in which average time to mortality was 1.41 h and the average probing duration was reduced to 0.21 h per insect. Psyllids on spinoteram-treated plants probed an average of 1.20 h per insect and required 7.31 h to achieve 100% mortality. For spirotetramat-treated plants, there was no resulting psyllid mortality during the 12 h of recording and there was also no discernable effects on probing duration which averaged 7.26 h per insect.

The differences found in psyllid feeding behavior on plants treated with these various insecticides is likely due to the manner in which these different modes of action affect the insect to cause mortality. Chlorpyrifos, fenpropathrin, imidacloprid and spinetoram act primarily as a contact insecticides inhibiting the insect's nervous system (acetylcholinesterase inhibitors,

sodium channel modulators, acetylcholine receptor stimulator, and disruption of nicotinic/gamma amino butyric acid (GABA)-gated chloride channels, respectively). Spirotetramat, on the other hand, has limited contact activity and is mainly effective following ingestion by the insect (Nauen et al. 2008). Spirotetramat is a tetramic acid that acts as a lipid biosynthesis inhibitor affecting primarily the insects' juvenile stages and adult reproduction (Nauen et al. 2008).

The results from this study demonstrate that certain foliar-applied insecticides may have important benefits in terms of reducing the spread of HLB that extended beyond just reducing the overall population levels of the insect vector. Prior to causing mortality, chlorpyrifos, fenpropathrin, imidacloprid and spinetoram application can potentially disrupt the psyllid feeding process such that successful pathogen acquisition and inoculation are both less likely to occur. It was also found that psyllids on spinetoram-treated plants reach the phloem of the citrus plants prior to mortality, albeit, stylet penetrations were abnormal indicating that some feeding disruption might still be occurring. Spirotetramat was the only treatment that did not affect psyllid feeding behaviors. Even though this insecticide has been shown to reduce psyllid populations under field conditions via effects on immature stages, infected adult psyllids that land on a healthy plant to feed may still successfully inoculate that plant with the pathogen. Thus, similar to aldicarb as discussed above, citrus growers using spirotetramat should consider combining this insecticide with another that will provide the desired level of adult psyllid control.

In Chapter 6, the residual effects, or the duration of feeding disruption provided by the insecticides evaluated in Chapter 5, were examined. For chlorpyrifos, at 1DAT, while there was no significant difference in probing duration, psyllids on chlorpyrifos performed half of the

probing durations when compared to untreated plants. Phloem related feeding behaviors were prevented up to 7 DAT on chlorpyrifos treated plants when chlorpyrifos residues on the leaf surface averaged 27.13 ppm. For both fenpropathrin and imidacloprid-treated plants, there was an immediate reduction 1 DAT in *D. citri* probe duration, number of probes, non-probing/walking activities, stylet penetration, phloem activities and xylem ingestion when compared to untreated plants. Over time, the duration of those behaviors increased as insecticide residue levels decreased. Fenpropathrin residues provided significant disruption of phloem related feeding behaviors by *D. citri* up to 21 DAT. Disruption of phloem feeding behaviors for psyllids on imidacloprid-treated plants lasted up to 28 DAT. At this time imidacloprid residue levels were 34.66 ppm, which was 83% lower than the concentration found at 1DAT. Psyllids on spinetoram-treated plants performed probes which were shorter in duration, but disruption of phloem feeding behaviors was not prevented, even at 1 DAT. The decrease in probing duration for psyllids on spinetoram-treated plants was only significant 7 DAT. Psyllids on spirotetramat-treated plants performed normal phloem activities throughout the entire experiment. The results from this study demonstrate that while some insecticides may cause relatively rapid mortality of adult psyllids, there is considerable variability that exists among these products in terms of the duration of feeding disruption provided. While some insecticides provided feeding disruption lasting 3-4 weeks (fenpropathrin and imidacloprid), protection provided by other products was much shorter. Overall, the results of this study can be used to help guide citrus growers in product selection and also determine when additional applications will be necessary.

In conclusion, utilizing an electrical penetration graph monitor to study psyllid feeding behavior, we have a better understanding of how certain aspects of psyllid biology (gender) and environment (photoperiod) influence *D. citri* feeding behavior. These two factors have been

shown to be important variables which could affect the outcome of experiments investigating the transmission of *Candidatus* Liberibacter asiaticus by *D. citri*. Future studies by researchers dealing with pathogen transmission should be aware of and account for these factors which could confound the results of planned experiments. Unaccounted for, these factors may have played a role in reported inconsistencies in past studies of psyllid transmission of the HLB associated bacterium. With regards to managing the spread of HLB, this work sheds new light on the benefits provided by insecticides commonly used for psyllid control. We have shown that such benefits go beyond just reducing overall psyllid populations, but certain products may actually prevent bacterial transmission (acquisition and or inoculation) for an extended period of time via disruption of psyllid phloem feeding behaviors. Additionally, it was shown that certain insecticides that have been commonly relied upon as stand-alone psyllid control products may not have provided as much reduction in pathogen spread as desired or perhaps in some cases, enhanced pathogen spread by increasing certain psyllid feeding behaviors. This new information should be useful in helping citrus growers refine their current HLB/psyllid management programs based on a better understanding of how these insecticides affect psyllid feeding behavior.

LIST OF REFERENCES

Almeida, R. P. P. and E. A. Backus. 2004. Stylet penetration behaviors of *Graphocephala atropunctata* (Signoret) (Hemiptera, Cicadellidae): EPG waveform characterization and quantification. Ann. of the Entomol. Soc. of Am. 97: 838-851.

Andersen, P.C., B. V. Brodbeck, and R. F. Mizell III. 1989. Metabolism of amino acids, organic acids and sugars extracted from the xylem fluid of four host plants by *Homalodisca coagulata*. Entomol. Exp. Appl. 50: 149-159.

Andersen, P. C., R. F. Mizell III, B. V. Brodbeck, T. G. Beckman, and G. Krewer. 2008. Abundance and consumption rate of glassy-winged sharpshooter (Hemiptera: Cicadellidae) on peaches and plums. J. Entomol. Sci. 43:394-407.

Aubert, B. 1987. *Trioza-Erytreae* (Del-Guercio) and *Diaphorina-Citri* Kuwayama (Homoptera, Psylloidea), the 2 Vectors of Citrus Greening Disease - Biological Aspects and Possible Control Strategies. Fruits 42: 149-162.

Backus, E. A. 1988. Sensory systems and behaviours which mediate hemipteran plant-feeding: a taxonomic overview. J. Insect Physiol. 34: 151-165.

Backus, E. A. 2000. Our own jabberwocky: clarifying the terminology of certain piercing-sucking behaviors of homopterans, pp. 1-13. *In* G. P. Walker and E. A. Backus [eds.], Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior. Thomas Say Publications in Entomology, Entomological Society of America, Lanham, MD.

Backus, E. A., J. Habibi, F. Yan, M. Ellersieck. 2005. Stylet penetration by adult *Homalodisca coagulata* on grape: electrical penetration graph waveform characterization, tissue correlation, and possible implications for transmission of *Xylella fastidiosa*. Ann. Entomol. Soc. Am. 98(6): 787-813.

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.

Backus, E. A., W. J. Holmes, F. Schreiber, B.J. Reardon, and G. P. Walker. 2009. Sharpshooter X wave: correlation of an Electrical Penetration Graph Waveform with Xylem Penetration Supports a Hypothesized Mechanism for *Xylella fastidiosa* Inoculation. Ann. Entomol. Soc. Am.102: 847-867.

Balayannis P.G. 1983. Effect of soil applications of aldicarb on the growth, yield, and chemical composition of tobacco plants. *J. Agric. Food Chem. 31:*1351-1355.

Baldessari, M., F. Trona, G. Angeli, and C. Ioriatti. 2010. Effectiveness of five insecticides for the control of adults and young stages of *Cacopsylla melanoneura* (Förster) (Hemiptera: Psyllidae) in a semi-field trial. Pest. Manag. Sci. 66: 308-312.

Beanland, L., C.W. Hoy, S.A. Miller, and L.R. Nault. 2000. Influence of aster yellows phytoplasma on the fitness of aster leafhopper (Homoptera: Cicadellidae) Ann. Entomol. Soc. Am. 93: 271-276.

Bethke J.A., M. J. Blua, and R. A. Redak. 2001. Effect of selected insecticides on *Homalodisca coagulata* (Homoptera: Cicadellidae) and transmission of oleander leaf scorch in a greenhouse study. J. Econ. Entomol. 2001. 94:1031-1036.

Boina, D. R., E. O. Onagbola, M. Salyani, and L. L. Stelinski. 2009. Antifeedant and sublethal effects of imidacloprid on Asian citrus psyllid. Pest Manag. Sci. 65 :870-877.

Bonani, J. P. 2009. Caracterização do aparelho bucal e comportamento alimentar de *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) em *Citrus sinensis* (L.) Osbeck. PhD dissertation. Escola Superior de Agricultura Luiz de Queiroz (ESALQ), Piracicaba-SP, Brazil.

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, *Diaphorina citri*, in sweet orange seedlings. Entomol. Exp. Appl. 134: 35-49.

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

Brlansky, R.H. and Rogers, M.E. 2007. Citrus huanglongbing: understanding the vector-pathogen interaction for disease management. APSnet. http://www.apsnet.org/publications/apsnetfeatures/Pages/Huanglongbing.aspx

Brodbeck, B.V., R.F., Mizell III, and P.C.Andersen, 1993. Physiological and Behavioral Adaptations of Three Species of Leafhoppers in Response to the Dilute Nutrient Content of Xylem Fluid. J. of Insect Physiol. 39: 73-81.

Brück, E., A. Elbert, R. Fischer, S. Krueger, J. Kühnhold, A. M. Klueken, R. Nauen, J.F. Niebes, U. Reckmann, H.J. Schnorbach, R. Steffens, and X. van Waetermeulen. 2009. Movento, an innovative ambimobile insecticide for sucking insect pest control in agriculture: Biological profile and filed performance. Crop Prot. 28:838-844.

Buitendag, C. H., and L. A. Von Broembsen. 1993. Living with citrus greening in South Africa, pp. 269- 273 *In* P. Moreno, J. V. da Graça, and L. W. Timmer [eds.], Proc. 12th Conference of the International Organization of Citrus Virologists. University of California, Riverside.

Capoor, S. P., D. G. Rao, and S.M. Viswanath. 1974. Greening disease of citrus in the Deccan Trap Country and its relationship with the vector, *Diaphorina citri* Kuwayama." Proceedings of the Sixth Conference of the International Organization of Citrus Virologists. 43-49.

Catling, H. D. 1969. The control of citrus psylla, *Trioza erytreae* del Guercio (Homoptera:Psyllidae). S. Afr. Citrus J. 426:9-16.

Catling, H. D. 1970. Distribution of the psyllid vectors of citrus greening disease, with notes on the biology and bionomics of *Diaphorina citri*. F.A.O. Plant Protection Bulletin. 18: 8-15.

Caciagli, P., D. Bosco, and L. Al-Bitar. 1995. Relationships of the sardinian isolate of tomato yellow leaf curl geminivirus with its whitefly vector *Bemisia tabaci* Gen. Eur. J. Plant Pathol.101: 163-170.

Casida, J.E., and G.B. Quistad. 2004. Why insecticides are more toxic to insects than people: the unique toxicology of insects. J. Pestic. Sci. 29: 81-86.

Childers, C.C, and M. E. Rodgers. 2005. Chemical control and management approaches of the asian citrus psyllid, *Diaphorina citri* Kuwayama (homoptera: psyllidae) in Florida citrus. Proc. Fla. State Hort. Soc. 118:49-53.

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

Chung, K. and X. C. Fan. 1990. Successful integrated management of huanlungbin disease in several farms of guangodng and Fujian, by combining early eradication with target insecticide sprayings. pp. 145-148. *In* Proceedings, 4th International Asia Pacific Conference on Citrus Rehabilitation, Chian Mai, Thailand.

Collar, J.L., C. Avillar, and A. Fereres. 1997a. New correlations between aphid stylet paths and nonpersisten virus transmission. Environ. Entomol. 26: 537-544.

Collar, J.L., C. Avillar, M. Duque, and A. Fereres. 1997b. Behavioral response and virus vector ability of *Myzus persicae* (Homoptera: Aphididae) probing on pepper plants treated with aphicides. J. of Econ. Entomol. 6: 1628-1634.

Da Graca, J., and L. Korsten. 2004. Citrus huanglongbing: review, present status and future strategies. Dis. Fruits veg. 1: 229-245.

Edwards, C.A. 1975. Factors that affect the persistence of pesticides in plants and soils. Pure Appl. Chem. 42:39-56.

El-Hamady, S. E., R. Kubiak, and A.S. Derbalah. 2008. Fate of imidacloprid in soil and plant after application to cotton seeds. Chemosphere 71: 2173-2179.

EPA. 2010. Pesticides registration: agreement to terminate all uses of aldicarb. http://www.epa.gov/oppsrrd1/REDs/factsheets/aldicarb_fs.html

Fereres, A. and J. L. Collar. 2001. Analysis of noncirculative transmission by electrical penetration graphs. pp. 87-109. In: F. K. Harris, O. P. Smith and J. E. Duffus, Virus-insect-plant interactions, Academic press, San Diego, CA.

Goldschmidt E.E. & Koch E.K. 1996. Citrus. pp. 797–823. In E. Zamski & A. A.Scaffer. Photoassimilate Distribution in Plants and Crops. Marcel Dekker, New York, NY.

Gottwald, T. R., da Graça, J. V., and R. B. Bassanezi. 2007. Citrus Huanglongbing: The pathogen and its impact. Online. Plant Health Progress doi:10.1094/PHP-2007-0906-01-RV. http://www.apsnet.org/publications/apsnetfeatures/Pages/HuanglongbingImpact.aspx

Grafton-Cardwell1, E.E., Godfrey, K.E., Rogers, M.E., Childers, C.C. and Stansly, P.A., 2005. Asian citrus psyllid. http://ccpp.ucr.edu/news/PsyllidbrochureAug05.pdf

Groves, R.L., C.E. Sorenson, J.F., Walgenbach, and G.G. Kennedy. 2001. Effects of imidacloprid on transmission of tomato spotted wilt tospovirus to pepper, tomato, and tobacco by *Frankliniella fusca* Hinds (Thysanoptera: Thripidae). Crop Prot. 20:439-445.

Halbert, S. E. 1998. Entomology Section. Tri-ology. 37: 6-7.

Halbert, S.E. 2005. Pest alert: citrus greening/Huanglongbing. Florida Department of Agriculture and Consumer Services. Division of Plant Industry. (http://www.doacs.state.fl.us/pi/chrp/greening/citrusgreeninalert.htlm)

Halbert, S. E. and K. L. Manjunath. 2004. Asian citrus psyllids (Sternorrhyncha : Psyllidae) and greening disease of citrus: A literature review and assessment of risk in Florida. Fla. Entomol. 87: 330-353

Harrewijn, P. and H. Kayser. 1997. Pymetrozine, a fast-acting and selective inhibitor of aphid feeding in-situ studies with electronic monitoring of feeding behavior. Pestic. Sci. 49:130-140.

Hunter, W. A., and D. E. Ullman, 1989. Analysis of mouthpart movement during feeding of *Frankliniella occidentalis* (Pergande) and *F. schultzei* Trybom (Thysanoptera: Thripidae). Int. J. of Insect Morphol. 18: 161–171.

Inoue, H., and J. Hirao. 1978. Transmission efficiency of rice waika virus by the green rice leafhoppers, *Nephotettix spp*. (Hemiptera: Cicadellidae). Appl. Entomol Zool 13:264-2733.

Jiang, Y. X., C. de Blas, L. Barrios, and A. Fereres. 2000. Correlation between whitefly (Homoptera: Aleyrodidae) feeding behavior and transmission of tomato yellow leaf curl virus. Ann. Entomol. Soc. Am. 93:573-579.

Jo, C. W., C. R. Park, K. S. Yoon, M. A. Kang, H. R. Kwon, H.B. Seok, E. J. Kang, M. J. Seo, Y. Y. Yu, and Y. N. Youn. 2009. Feeding behavior of etofenprox resistant green peach aphid (*Myzus persicae*) against thiamethoxam and fenpropathrin. Korean J. Appl. Entomol. 48: 493-501.

Joost, P.H. and D. G. Riley. 2005. Imidacloprid effects on probing and settling behavior of *Frankliniella fusca* and *Frankliniella occidentalis* (Thysanoptera: Thripidae) in tomato. Hortic. Entomol.. 98:1622-1629.

Laszlo, P. 2007. Citrus: A History, University of Chicago Press, Chicago, IL.

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

Lima. A. M. C. da. 1942. Insetos do Brasil: Homópteros. Rio de Janeiro: Escola Nacional de Agronomia, v.3, p.1-327.

Liu, D. G. and J. T. Trumble. 2004. Tomato psyllid behavioral responses to tomato plant lines and interactions of plant lines with insecticides. J. Econ. Entomol. 97: 1078-1085.

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. 137:201-206.

Lowery, D.T. and G. Boiteau. 1988. Effects of five insecticides on the probing, walking, and settling behavior of the green peach aphid and the buckthorn aphid (Homoptera: Aphididae) on potato. J. Econ. Entomol. 181:208-214.

Mahmoud, M. F., G. A. EI-Kady, M.A.M. Osman, and I.M. Bahgat. 2009. Efficiency of spinetoram as a biopesticide to onion thrips (*Thrips tabaci* Lindeman) and green peach aphid (*Myzus persicae* Sulzer) under laboratory and field contidions. J. Biopest. 2:223-227.

McLean, D. L. and M. G. Kinsey. 1964. A technique for electronically recording aphid feeding and salivation. Nature. 202: 2

Mead, F. W. 1977. The Asiatic citrus psyllid, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae). Entomology Circular, Division of Plant Industry, Florida Department of Agriculture and Consumer Services. 180: 4.

Mendel, R. M., U. Reckmann, and F. Führ. 2000. Xylem transport of the pesticide imidacloprid in citrus. Acta Hort. (ISHS) 531:129-134
http://www.actahort.org/books/531/531_18.htm

Miranda, M.P., D.N.Viola, R.N. Marques, J.P. Bonani, and J.R.S. Lopes. 2008. Feeding sites and food intake of *Bucephalogonia xanthophis* (BERG) (Hemiptera: Cicadellidae), a sharpshooter vector of *Xylella fastidioas*, on citrus plants. Rev. Bras. Frutic. 30: 913-918.

Montemurro, N., F. Grieco, G.Lacertosa, and A. Visconti. 2002. Chlorpyrifos decline curves and residue levels from different commercial formulations applied to oranges. J. Agicul and Food Chem. 50: 5975-5980.

Montesino, L.H., J.H.C. Coelho,M.R. Felippe, and P.T. Yamamoto. 2006. Xylem sap ingestion form healthy "pera" and "Valencia" sweet Orange (*Citrus sinensis* (L.) Osbeck) and infected ones by *Xylella fastidiosa*, *Oncometopia facialis* and *Dilobopterus costalimai* (Hemiptera: Cicadellidae). Rev. Bras. Frutic. 28: 199-204.

Morris, A. and R. Muraro. 2008. Economic Evaluation of Citrus Greening Management and Control Strategies. Extension Digital Information Source (EDIS) FE 712, Food and Resource Economics Department, University of Florida, June, 2008. http://edis.ifas.ufl.edu/FE712.

Nation, J.L., 2002. Insect Physiology and Biochemistry. CRC Press, Boca Raton.

Nauen, R., U. Reckmann, S. Armborst, H.P. Stupp, and A. Elbert. 1999. Whitefly-active metabolites of imidacloprid: biological efficacy and translocation in cotton plants. Pestic. Sci. 55:265-271.

Nauen, R. U., Reckmann, J. Thomzik, and W. Thielert. 2008. Biological profile of spirotetramat (Movento) a new two-way systemic (ambimobile) insecticide against sucking pest species. Pflanzenschutz-Nachrichten Bayer 61: 403–436.

Olson, E. R., G.P. Dively, and J. O. Nelson. 2004. Bioassay determination of the distribution of imidacloprid in potato plants: implications to resistance development. J. Econ. Entomol. 97:614-620.

Pelz-Stelinski, K. S., R. H. Brlansky, T. E. Ebert and M. E. Rogers. 2010. Transmission parametersfor *Candidatus* Liberibacter asiaticus by Asian citrus psyllid (Hemiptera: Psyllidae). J. Econ. Entomol. 103:1531-1541.

Pfeiffer, D. G., and E. C. Burts. 1984. Effect of tree fertilization on protein and free amino acid content and feeding rate of pear psylla (Homoptera: Psyllidae). Environ. Entomol. 6:1487-1490.

Perring, T. M., N. M. Gruenhagen, and C. A. Farrar. 1999. Management of plant viral diseases through chemical control of insect vectors. Annu. Rev. Entomol. 44:457-481.

Polek, M., G. Vidalakis, and K. Godfrey. 2007. Citrus bacterial canker disease and huanglongbing (Citrus greening).Publication 8218. University of California Agriculture and Natural Resources. Oakland, California (http://citrusent.uckac.edu/8218CitrusCankerandHuanglongbing.pdf)

Pollak S.L., and Perez, A. 2008. A report from the economic research service: Fruit and tree nut outlook. USDA. (http://www.ers.usda.gov/publications/fts/2008/03MAR/FTS331.pdf). Accessed on October 16th, 2008.

Powell, G. 1991. Cell membrane punctures during epidermal penetrations by aphids: consequences for the transmission of two potyviruses. Ann. Appl. Biol. 119:313-321.

Power, A. G. 1991. Virus spread and vector dynamics in genetically diverse plant populations. Ecology 72:232-241.

Prado, E. and W. F. Tjallingii. 1994. Aphid activities during sieve element punctures. Entomol. Exp. Appl. 72: 157:165.

Purcell, A. H. 1982. Insect vector relationships with prokaryotic plant pathogens. Annu. Rev. Phytopathol. 20:397-417.

Qureshi, J. A. and P. A. Stansly. 2008. Rate, placement and timing of aldicarb applications to control Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), in oranges. Pest Manag. Sci. 64: 1159-1169.

Qureshi, J.A., M. E. Rodgers, D. G. Hall, and P. A. Stansly. 2009. Incidence of Invasive *Diaphorina citri* (Hemiptera: Psyllidae) and Its Introduced Parasitoid *Tamarixia* J. Econ. Entomol. 102:247-256.

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

Ragab, S.M. 1981. Cotton growth and nutrient uptake following Temik (aldicarb) application. J. Agr. Sci. 97: 731-737.

Reese, J. C., W. F. Tjallingii, M. van Helden, and E. Prado. 2000. Waveform comparisons among AC and DC eletronic monitoring systems for aphid (Homopetra: Aphididae) feeding behavior. 70-101. In G. P. Walker and E. A. Backus. Principles and aplications of electronic monitoring and other techniques in the study of homopteran feeding behavior. Thomas Say Pubs. in Entomology. Entomological society of America.

Roberts, J. M. F., C. J. Hodgson, L. E. N. Jackai, G. Thottappilly, and S.R. Singh. 1993. Interactions between two synthetic pyrethroids and the spread of two non-persistent viruses in cowpea. Ann. Appl. Biol. 122:57-67.

Rogers, M. E., P. A. Stansly, and L. L. Stelinski. 2010. 2010 Florida citrus pest management guide: Asian citrus psyllid and citrus leafminer. ENY-734. IFAS extension.

Rogers, M. E., P. A. Stansly, and L. L. Stelinski. 2011. 2011 Florida citrus pest management guide: Asian citrus psyllid and citrus leafminer. ENY-734. IFAS extension.

Roistacher, C. N. 1991. Techniques for biological detection of specific citrus graft transmissible diseases. pp. 35-45. Greening. Food Agric. Organ., Rome.

SAS Institute. 2001. PROC user's manual, version 6th ed. SAS Institute, Cary, NC.

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

Serrano, M. S., E. A. Backus, and C. Cardona. 2000. Comparison of AC electronic monitoring and held data for estimating tolerance to *Empoasca kraemeri* (Homoptera: Cicadellidae) in common bean genotypes. J. Econ. Entomol. 93: 1796-1809.

Spann, T.M., R.A. Atwood, M.M., Dewdney, R.C. Ebel, R. Ehsani, G. England, S. Futch, T. Gaver, T. Hurner, C. Oswalt, M.E. Rogers, F. M. Roka, M. A. Ritenour and M. Zekri. 2010. IFAS guidance for huanglongbing. Citrus Industry. http://www.crec.ifas.ufl.edu/academics/faculty/rogers/PDF/Spann%20et%20al%202010.pdf

Steer, B. T. 1973. Diurnal variations in photosynthetic products and nitrogen metabolism in expanding leaves. Plant Physiol. 51: 744-748.

Sur. R., and A. Stork. 2003. Uptake, translocation and metabolism of imidacloprid in plants. B. Insectol. 56(1): 35-40.

Tiwari, S., K. Pelz-Stelinski, and L. Stelinski. 2011. Effect of *Candidatus* Liberibacter asiaticusi nfection on susceptibility of Asian citrus psyllid, Diaphorina citri, to selected insecticides. Pest. Manag. Sci. 67: 94–99

Tjallingii, W.F.1978. Electronic recording of penetration behaviour by aphids. Entomol. Exp. Appl. 24: 721–730.

Tjallingii, W. F. 2000. Comparison of AC and DC systems for electronic monitoring of stylet penetration activities by homopterans, pp. 41-69. *In* G. P. Walker and E. A. Backus [eds.], Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior. Entomological Society of America, Lanham, MD.

Tjallingii, W. F. and E. Prado. 2001. Analysis of circulative transmission by electrical penetration graphs. Pp. 69-85. Virus-insect-plant-interactions. F. K. Harris, O. P. Smith and J. E. Duffus. San Diego, Academic press.

UF/IFAS. 2008. Eradication. Impact of eradication. http://www.crec.ifas.ufl.edu/extension/canker/eradication.htm

Ullman, D. E. and D. L. Mclean. 1988. The probing behavior of the summer-form pear psylla. Entomol. Exp. Appl.47: 115-125.

USDA. 2010a. Citrus Fruits: 2010 summary. http://usda.mannlib.cornell.edu/usda/current/CitrFrui/CitrFrui-09-23-2010.pdf

USDA. 2010b. Florida Citrus statistics 2009-2010. http://www.nass.usda.gov/Statistics_by_State/Florida/Publications/Citrus/fcs/2009-10/fcs0910.pdf

USDA. 2011a. Citrus: World Markets and Trade. Citrus: 2010/11 Forecast. http://usda.mannlib.cornell.edu/usda/current/citruswm/citruswm-01-27-2011.pdf

USDA. 2011b. 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

Van Den Berg, M.A., Dippenaar-Schoeman, A.S., Deacon, V.E., and S.H., Anderson, 1992. Interactions between the citrus psylla, *Trioza erytreae* (Hemiptera: Triozidae), and spiders in an unsprayed citrus orchard in the Transvaal Lowveld. Entomophaga 37: 599–608.

Van Rensburg, G. D. J., and J.H. Giliomee. 1990. Comparison of females and males of *Cicadulina anestae* and *C. mbila* (Homoptera: Cicadellidae) as vectors of maize streak virus. Phytophylactica. 22: 241-243.

Van Vuuren, S. P. v. and M. J. v. d. Merwe. 1992. Efficacy of citrus psylla, *Trioza erytreae*, as vector of citrus greening disease. Phytophylactica 24: 285-288.

Walker, G. P. 2000. A beginner's guide to electronic monitoring of homopteran feeding behavior, pp. 14-20. In G. P. Walker and E. A. Backus [eds.], Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior. Thomas Say Publications in Entomology, Entomological Society of America, Lanham, MD.

Ware, G.W. 1994. Chapter 4: Insecticides. pp. 41-74. In G.W.Ware. The Pesticide Book, Fourth Edition, Thomson Publications, Fresno, CA.

Weintraub, P. G., and L. Beanland. 2006. Insect vectors of phytoplasmas. Annu. Rev.of Entomol. 51: 91-111.

Wenninger, E. J. and D. G. Hall. 2007. Daily timing of mating and age at reproductive maturity in *Diaphorina citri* (Hemiptera: Psyllidae). Fla. Entomol. 90: 715-722.

Wenninger, E. J., L. L. Stelinski, and D. G. Hall. 2008. Behavioral evidence for a female-produced sex attractant in *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). Entomol. Exp. Appl. 128: 450–459.

Wenninger, E.J., L.L. Stelinski, and D.G. Hall. 2009. Roles of olfactory cues, visual cues, and mating status in orientation of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) to four different host plants. Environ. Entomol.38: 225-234.

Wetering, F. van, Hulshof, J., Posthuma, K., Harrewijn, P., Goldbach, R., and D. Peters, 1998. Distinct feeding behavior between sexes of *Frankliniella occidentalis* results in higher scar production and lower tospovirus transmission by females. Entomol. Exp. Appl. 88: 9–15.

Wheaton, T. A., C. C. Childers, L. W. Timmer, L. W. Duncan, an S. Nikdel. 1985. Effects of aldicarb on yield, fruit quality, and tree condition of Florida citrus. Proc. Fla. State Hort. Soc. 98:6-10.

Xu, C. F., Y. Xia, K.B. Li, and C. Ke. 1988. Further study of the transmission of citrus Huanglungbin by a psyllid, *Diaphorina citri* Kuwayama. pp. 243-248. *In* Proceedings, 10th Conference of the International Organization of Citrus Virologists. IOCV. Riverside, CA.

Yang, Y., M. Huang, G. Andrew, G.A.C. Beattie, Y, Xia, G. Ouyang, and J. Xiong. 2006. Distribution, biology, ecology and control of the psyllid *Diaphorina citri* Kuwayama, a major pest of citrus: a status report for China. Int J. Pest Manage. 52(4): 343-352.

Youn Y., E.A. Backus, R. H. Serikawa, and L.L. Stelinski. in press. Correlation of an electrical penetration graph waveform with walking by asian citrus psyllid, *Diaphorina citri* kuwayama. Fla Entomol.

BIOGRAPHICAL SKETCH

Rosana Harumi Serikawa was born in São Carlos, SP – Brazil. Rosana was educated at Colégio São Carlos for elementary and middle school and Anglo for high school. Since she was always helping her mother in the chemistry lab, she got involved with several science projects in middle and high school. After a national general exam, she was accepted to the Universidade Estadual Paulista – Faculdade de Ciências Agrárias e Veterinárias – Jaboticabal, where she majored in agronomic engineering as an undergraduate. Her first trip to the United States was in 2003 when she was invited to do an internship at University of Nebraska – Lincoln, which she continued on to complete her M.Sc. in *Entomology* under the advisement of John E. Foster, studying population genetics of sugar beet root aphids. She moved to Florida to pursue a Ph.D. under Michael E. Rogers, where her research was based on the feeding behavior of the Asian citrus psyllid. Upon the completion of her Ph.D. at the University of Florida, Rosana plans to accept an opportunity to work as Research Entomologist at DuPont –Brazil.