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Assessing Accomplishments since the first Symposium in Grenada (2003)
and Coping with Current Threats to the Region

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AREA-WIDE MANAGEMENT OF ASIAN CITRUS PSYLLID IN FLORIDA

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ABSTRACT: *Diaphorina citri*, the Asian citrus psyllid (ACP) vectors the citrus greening disease also known as huanglongbing (HLB). Vector control is a key strategy for managing this disease, even in infected trees. Oviposition and nymphal development occur in young flush, so flushing patterns drive ACP populations. Adults survive on mature foliage in the absence of flush and are most vulnerable to insecticidal control during long periods of tree dormancy. Significant reduction in the populations has been documented for up to six months following a single application of broad spectrum insecticide during winter in Florida. Beneficial insects such as ladybeetles and lacewings add an important component of pest mortality that should also be conserved. Insecticide sprays during the dormant season impart the additional advantage of minimizing the negative impacts on many beneficial insects that are inactive, cryptic, or not present in citrus groves during these dormant periods. The larger the area sprayed, the more effective is the treatment. Cooperative sprays during winter in southwest Florida covered an excess of 100,000 acres of citrus, more than 70,000 of which were applied by fixed-wing aircraft with excellent results. During the growing season, it is best to base spray decisions on ACP population trends for which an accurate and rapid monitoring program is required. A stem tap procedure, coupled with flush inspection, has provided good results for both research and management purposes. Present efforts are focused on establishing thresholds for ACP control and organizing a web-based platform for managing real time data on psyllid populations for growers and consultants.

KEY WORDS: *Diaphorina citri*, *Candidatus* Liberibacter asiaticus, rouging, foliar application, micronutrients, Serenade®, Saver, dormant season, natural enemies, spring flush

RESUMEN. *Diaphorina citri*, el psílido Asiático de los cítricos (PAC), es el vector de la enfermedad enverdecimiento (“greening”) de los cítricos, también conocida como huanglongbing (HLB). El control del vector es una estrategia clave para el manejo de esta enfermedad, incluso en árboles infectados. Oviposición y desarrollo de las ninfas se produce en brotes jóvenes, y por lo tanto, el patrón de la brotación es un factor determinante a las poblaciones de PAC. En la ausencia de brotes, adultos sobreviven en follaje maduro y son más vulnerables al control químico durante los largos periodos de dormancia (latencia) de los árboles. Se han documentado reducciones significativas de poblaciones de PAC hasta por 6 meses a partir de una sola aplicación de insecticida de amplio espectro durante el invierno en Florida. Los insectos benéficos como mariquitas y las crisopas proporcionan un componente importante a la mortalidad de la plaga y por lo tanto deben ser conservados. Aplicaciones foliares de insecticidas durante la dormancia tiene la ventaja adicional de reducir al mínimo los impactos negativos a los insectos benéficos que están inactivos, crípticos o no atraídos a los cítricos durante dormancia. Cuanto mayor sea el área tratada, más efectivo será el tratamiento. Aplicaciones cooperativas de insecticidas durante el invierno en el suroeste de la Florida han cubierto más de 100.000 hectáreas de cítricos, de los cuales 70.000 fueron aplicados por avioneta con excelentes resultados. Durante la temporada de crecimiento del cultivo, es mejor basar las

decisiones de el uso de insecticidas foliares en fluctuaciones las poblaciones para lo cual se requiere un programa de muestreo preciso y rápido. El muestreo de golpes para adultos junto con la inspección de brotes para ninfas han dado buenos resultados, tanto para fines de investigación como de manejo. Las investigaciones actuales se centran en el establecimiento de los umbrales económicos para el control de PAC como la organización de una plataforma basada en el web para manejo de datos sobre las poblaciones del psílido en tiempo real para agricultores y consultores.

INTRODUCTION

Citrus greening disease, or huanglongbing (HLB), is considered the most debilitating disease of citrus, causing severe loss of foliage and fruit (Figure 1). The causal agent in most of the world, *Candidatus Liberibacter asiaticus*, is vectored by the Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Figure 2). Vector and disease both originated in Asia; were first detected in Florida in 1998 and 2005, respectively; and are now present in much of tropical and subtropical America. Hosts of ACP include all citrus species and many citrus relatives. Halbert and Manjunath (2004) list 56 species in 24 genera. However, at least one of the species listed, *Zanthoxylum fagara* (L.) Sarg, does not support development of ACP (Qureshi, personal communication). Eggs of ACP are laid on "feather flush" and hatch in 2–4 days (Figure 2a). There are five nymphal instars, which are completed in 11–15 days on the same young shoot (Figure 2b). Total development takes 15–47 days, dependent on optimal temperatures of 25–28°C. Females lay as many as 748 eggs in a lifetime at 28°C (Lui and Tsai 2000).

WEATHER, NEW FLUSHES, AND DISPERSAL

Weather patterns largely determine the pattern of ACP abundance in citrus, primarily through their influence on incidence of new flushes (Qureshi et al. 2009). Adults may live several months feeding on mature citrus leaves, and are thus able to survive long periods in the absence of new flushes needed for reproduction. Those psyllids that survive tree dormancy are able to initiate a new generation on the spring flush, where they enjoy a limitless food supply. With the waning of the spring flush, food and oviposition sites become scarce, so adults disperse in search of fresh sources, spreading disease acquired as nymphs. Therefore, the spring flush serves as a springboard for both the ACP population and the spread of HLB.

KEY STRATEGIES FOR MANAGING HLB

Planting disease-free nursery stock and controlling the psyllid vector are generally agreed-upon key strategies for managing HLB. Roguing symptomatic trees to reduce inoculum has also been recommended, and foliar application of micronutrients to compensate for HLB-induced deficiencies is becoming a widely adapted practice in areas where HLB incidence is high. Here, we focus on psyllid control as a necessary grower practice to mange HLB.

BIOLOGICAL CONTROL OF ACP

Natural enemies of ACP in Florida include predators such as ladybeetles, lacewings, spiders, and an introduced parasitoid *Tamarixia radiata*. The parasitoid is found throughout the state but at generally low incidence (Qureshi et al. 2009). Mass release of *T. radiata* is being investigated as a tactic to increase the impact of the parasitoid and reduce dependence on insecticides (Qureshi and Stansly 2010a). An entomophagous fungus, *Hirsutella citriformis*, may infect many adults during periods of high humidity. This complex of natural enemies is able to inflict upwards of

95% mortality on the ACP population (Qureshi and Stansly 2009). Although biological control alone proved insufficient to prevent the spread of HLB in Florida, it can play an important role in controlling ACP. Therefore, ACP control programs should be designed to conserve or enhance the biological component in an integrated management system (Qureshi and Stansly 2007).

CHEMICAL CONTROL RETARDS SPREAD OF HLB

Insecticidal control appears to be slowing the spread of HLB in Florida. Soil application of the systemic insecticides (i.e., neonicotinoids), imidacloprid, and thiamethoxam to young trees is a key component of insecticidal control necessary to protect young plantings. Aldicarb was widely used on mature trees in Florida with variable success (Qureshi and Stansly 2008), but will be prohibited on citrus in the United States after 2011. As foliar applications, broad-spectrum insecticides directed against adults have proven to be most effective. However, excessive use of broad-spectrum insecticides has also resulted in increased incidence of secondary pests such as leafminers, rust mites, and armored scales due to collateral suppression of their natural enemies. Therefore, these materials are best applied when collateral effects on the beneficial fauna can be minimized.

TIMING OF INSECTICIDAL SPRAYS FOR GREATEST IMPACT ON ACP

When is the best time to spray? We conduct more than 20 field trials during the growing season, and have rarely seen more than four-weeks control from any single application (http://www.imok.ufl.edu/entomology). In contrast, applications of approved organo-phosphates, pyrethroids, or carbamates sprayed during winter when trees are dormant have been shown to cause significant suppression of ACP for five to six months (Figure 3), with little or no effect on ladybeetles, lacewings, or spiders (Figure 4) (Qureshi and Stansly 2010). The dormant spray is critical because it greatly reduces the number of adult ACP entering the spring flush, which is the principal determinant of subsequent vector reproduction and spread of HLB.

AREA-WIDE SPRAYS ENHANCE ACP SUPPRESSION DURING DORMANT SEASON

Citrus growers in southwest Florida and elsewhere have joined together to conduct area-wide sprays to enhance effectiveness during the dormant season (Stansly et al. 2009, 2010a). Two windows for cooperative area-wide sprays have been identified — the first following the fall flush in late November through December, and the second in January until two weeks before the first bud break in February. Any effective insecticide is acceptable. However, organophosphates such as phosmet, dimethoate, or chlorpyrifos, are recommended for the first application, and the pyrethroids, fenpropathrin or zeta-cypermethrin, with their one-day pre-harvest intervals, for the second. The important thing is that effective materials are chosen and that modes of action be rotated. Over 70,000 acres of citrus in the area were sprayed cooperatively by air and an estimated 30,000 by ground during the 2008/09 and 2009/10 seasons (Stansly et al. 2010 a, Table 1). Comparison with an untreated grove showed a 16-fold increase in psyllid populations by May 2009 as compared to treated groves (Figure5).

MONITORING PSYLLID POPULATIONS

During the growing season, it is important to monitor psyllid populations at two-week intervals. Because adults are the target of most sprays and the best time to control them is before a flush, a reliable method of sampling adults is required. The stem-tap method has filled this requirement (Qureshi et al. 2009; Stansly 2009, 2010b; Hall and Hentz 2010). A clear or white plastic clipboard or laminated sheet of paper is held approximately one foot below any branch with a good amount of foliage. The branch is struck sharply three times with the hand or stick. Psyllids falling onto the clipboard are quickly recognized by their wing markings, body shape with pointed abdomen, and characteristic 45-degree stance (Figure 6). Note the number of adult psyllids counted in 10 tap samples from 10 different trees. Other insects of interest that can be observed during tap sampling include pests such as root weevils and leafminer adults, and beneficials such as spiders, ladybeetle adults and larvae, and ant lions. Flush density and percentage shoots infested with psyllids should also be noted. If 10 shoots cannot be found in 20 trees, record the number seen, considering only shoots with new leaves still emerging from the bud. A hand lens is useful to determine whether any stage of psyllid is present (egg, nymph, or adult). Notations should include (1) shoots observed, (2) shoots infested, and (3) number of trees searched to find these shoots. Then the number of infested shoots per tree can be calculated by dividing the number of infested shoots found by the number of trees searched, completing procedures for one stop. Ten stops per block and 10 trees per stop for a total of 100 taps are sufficient to provide a good picture of the psyllid population under most conditions. It is a good idea to make half of these stops around the edges of the block where psyllids often accumulate. Note locations so that hot spots can be identified and treated accordingly. Input the data on a spreadsheet to create an historical record for each block. Forms, data sheets, and more information on monitoring ACP is available at http://www.imok.ufl.edu/entomology/extension/.

FOLIAR NUTRITIONAL PROGRAM AND INSECTICIDAL CONTROL

While it is clear that ACP must be controlled to slow the spread of HLB, is there any value to protecting HLB-infected trees from ACP? Our preliminary data would indicate insecticidal control of ACP on infected trees is beneficial both in terms of fruit quality and yield. An experiment was conducted to evaluate the effects of a popular foliar nutritional program and insecticidal control. A 12-acre block of commercial citrus (‘Valencia’ on ‘Swingle’ citromelo) was divided into 16 plots assigned in a randomized complete block design to four treatments, insecticide alone, nutrition alone, insecticides plus nutrition, and untreated. The nutritional cocktail (Table 2) was applied three times a year, corresponding to spring, summer, and fall flushes. Insecticides were applied during the dormant season and, subsequently, when a threshold was reached of 0.5 (first 2 years) or 0.2 (3rd year) psyllids per tap (Table 3). ACP adults were sampled every two weeks using the stem tap and every fifth tree was tested four times a year for HLB using PCR with 16 S ribosomal DNA primers (Li et al 2006). Ct values of 32 or lower were considered positive. All fruit was harvested and production evaluated in 2009 by counting full and empty tubs and in 2010 by weighing each tub (Figure 7). A 10 sample from each plot was sent to the CREC fruit quality laboratory each year for analysis.

ACP populations were greatest in plots not treated by insecticide, with an overall 12-fold difference maintained the first 30 months (Figure 8). Incidence of HLB started out at around 20% but soon increased to over 80% or more in all plots (Figure 9). Incidence and intensity of HLB was initially greatest in plots treated with nutritionals alone, but soon reached a consistent high in all plots (Figure 10). Brix/acid ratio was least in fruit from untreated trees and greatest from trees receiving both insecticides and nutritionals (Figure 11). Yield in pound solids per tree was least in trees receiving only nutritionals and greatest in trees receiving both nutritionals and insecticides. Main component analysis revealed a significant effect from insecticides (P = 0.041, F = 5.336) but not as yet from nutritionals (P = 0.591. F = 0.307) although a positive trend was evident for this factor as well.

We can conclude from these preliminary results that (1) ACP control is beneficial, even for infected trees, and (2) that insecticidal control has a greater effect on yield than nutritional treatments, but both are necessary for best necessary for optimal production.

CONCLUSIONS

In conclusion, young trees need to be protected from psyllids with systemic insecticides, and the dormant season is the best time to make foliar applications of broad spectrum insecticides for control of adult ACP. The most effective way to apply these 'dormant sprays' is to spray all the citrus in a particular area within a relatively narrow window of time. ACP populations should be monitored all year but especially during the growing season, and significant population increases should receive additional sprays of selective products such as spinetoram or spriotetramat. The former is effective against adults and nymphs and the latter against immature stages only but is systemic. Frequent applications of horticultural spray oil are another option. Psyllid control is the most effective measure a grower can take to manage HLB, even on infected trees.

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Table 1. Number of aerial sprays, number of groves, and total acreage sprayed during two seasons in SW Florida (Stansly et al. 2010a).

2008-2009

# Sprays	# Groves	Total acreage
1	36	49,228
2	7	22,688
Grand Total	43	71,916

2009-2010

# Sprays	# Groves	Total acreage
1	35	56,618
2	10	7,081
3	3	9,481
Grand Total	48	73,180

Table 2. Composition of the nutritional blend used during this trial. In 2008 nutritional applications were conducted on Mar 19, Jun 3, and Oct 16. During 2009 on Mar 23, Jun 17, and Aug 31, and in 2010 on Mar 25 and Jul 31 (Arevalo et al. unpublished).

Product	Quantity per acre
Serenade® Max WP (fungicide/bactericide)	2.25 lb
Saver (Salicylic acid)	1 qt
3-18-20 with K-Phite	8 gal
13-0-44 fertilizer	8.5 lb
Techmangan (MG Sulfate)	8.5 lb
Zinc Sulfate	2.8 lb
Sodium Molybdate	0.85 oz
Di-Oxy Solv Organic	2 qt
Epsom Salts	8.5 lb
435 oil	5 gal

Table 3. Date, product, active ingredient (a.i), and rate of insecticide applications made in designated treated plots from 2008 to 2010. All applications were conducted when the scouting results indicated ACP populations above 0.5 adult ACP per "stem-tap" sample (Arevalo et al, unpublished).

App. Code (Fig.1)	**Date**	**Product**	**a.i**	**Rate**	**Company**
1	May 2, 2008	Danitol 4EC	fenpropathrin	16 fl oz/ac.	Valent USA Corp.
2	Aug 7,2008	Delegate WG	spinetoram	4 oz/ac.	Dow Agrosciences
3	Nov ,2008	Delegate WG	spinetoram	4 oz/ac.	Dow Agrosciences
4 (Dormant spray)	Jan 14, 2009	Mustang	zeta-cypermethrin	4.3 fl oz./ac.	FMC.
5	May 20,09	Movento	spirotetramat	10 fl oz./ac.	Bayer CropSciences
6	Sep 29, 2009	Lorsban 4E	chlorpyrifos	3 pt/ac	Dow Agrosciences
7 (Dormant spray)	Dec 23, 2009	Dimethoate 4EC	dimethoate	1 pt/fl ac.	Helena Chemical
8 (Dormant spray)	Feb, 16, 2010	Danitol 4EC	fenpropathrin	12 ft. oz/ac.	Valent USA Corp.
9	May 31, 2010	Delegate WG	spinetoram	5 fl. oz/ac.	Dow Agrosciences
10*	May 31, 2010	Intrepid 2F	methoxyfeno zide	8 fl.oz/a c	Dow Agrosciences
11	July 30, 2010	Lorsban 4E	chlorpyrifos	3 pt/ac	Dow Agrosciences

* Made to all plots for citrus leafminer control.

Figure 1. HLB-affected young 'Valencia' tree showing symptoms of die back and fruit drop (Photo: P. Stansly).

Figure 2. (A) adult, (B) eggs, and (C) 1st instar nymphs, and (D) 4th and 5th instar nymphs of *Diaphorina citri* (Photos: M. Rogers).

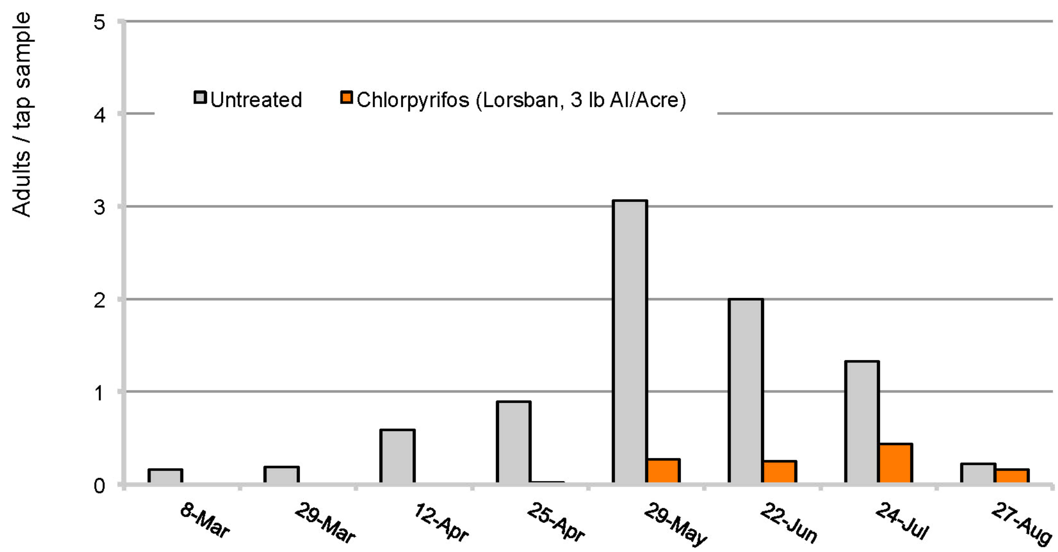

Figure 3. Adult psyllids per tap sample in trees sprayed or not with chlorpyrifos on January 15, 2007 (Qureshi and Stansly 2010).

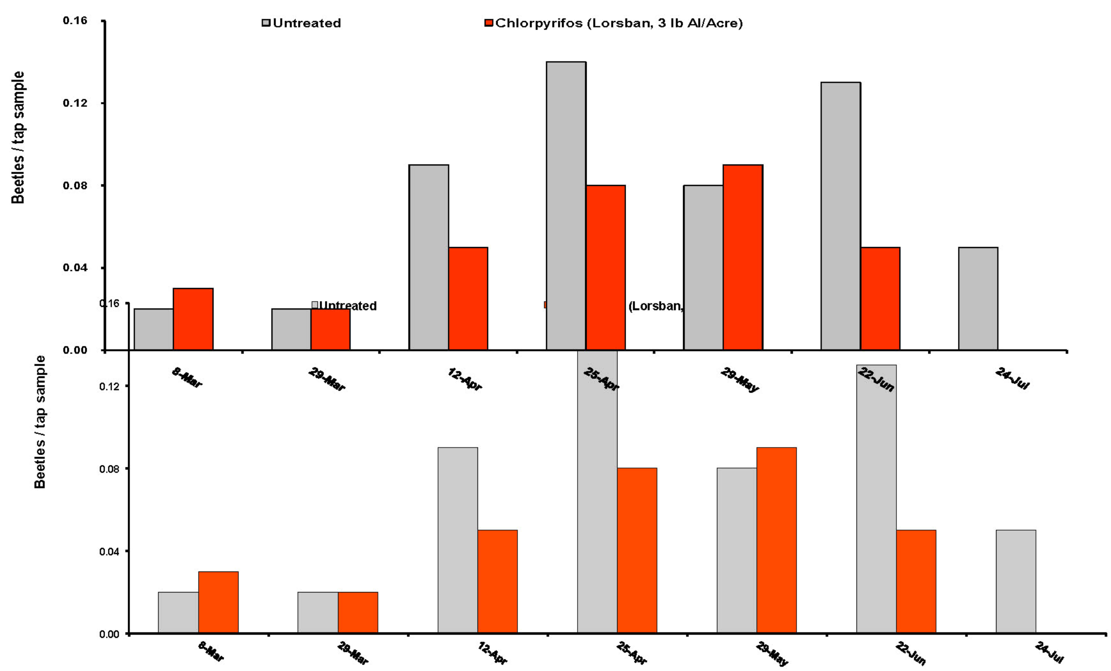

Figure 4. Ladybeetles per tap in trees sprayed or not with chlorpyrifos on January 15, 2007 (Qureshi and Stansly 2010).

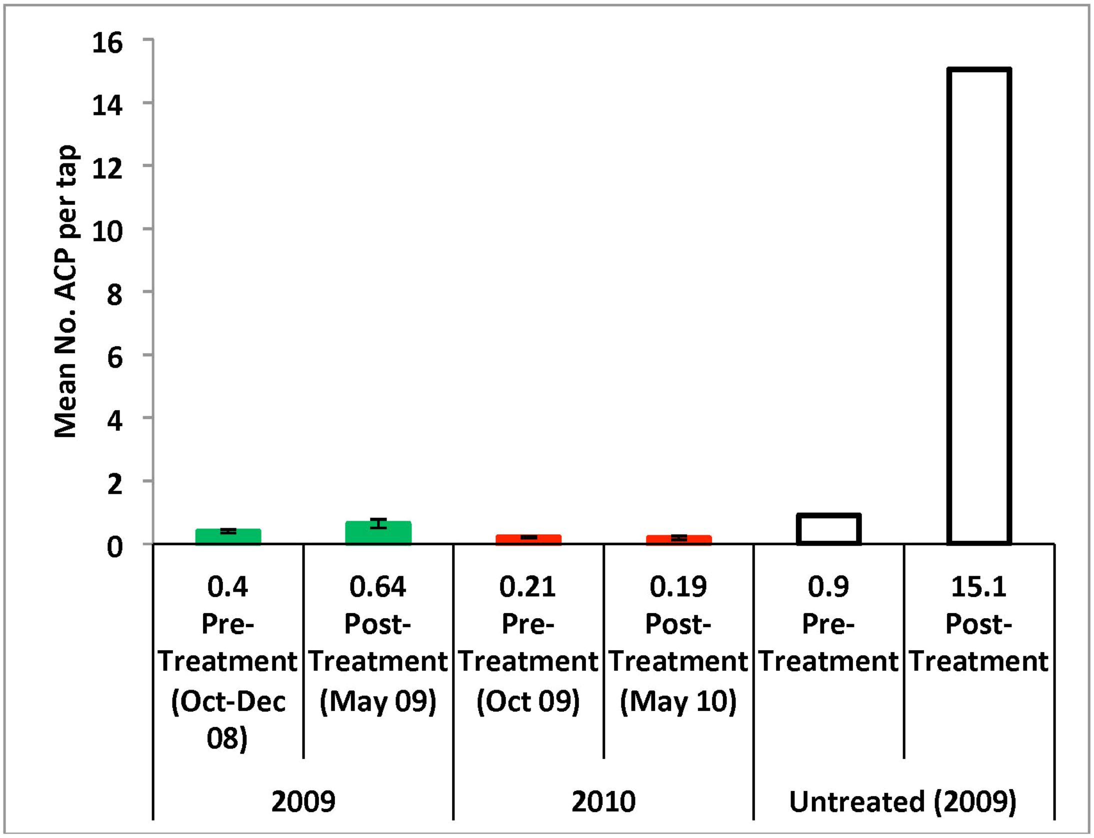

Figure 5. Overall populations of adult ACP during 2009 and 2010 in 240 and 248 treated blocks of citrus, respectively, and during 2009 in two untreated blocks in southwest Florida (Stansly et al. 2010 a).

Figure 6. The stem-tap method is a rapid and accurate means of monitoring adult ACP populations that was used by state and grower personnel to assess effectiveness of dormant sprays (Photos: H Arevalo).

Figure 7. Oranges were hand picked into 10-box tubs by supervised crews. Tared weight of oranges in each tub was recorded in the field using a Gator Deck Scale 500 ± 1 lb (Photo: P. Stansly).

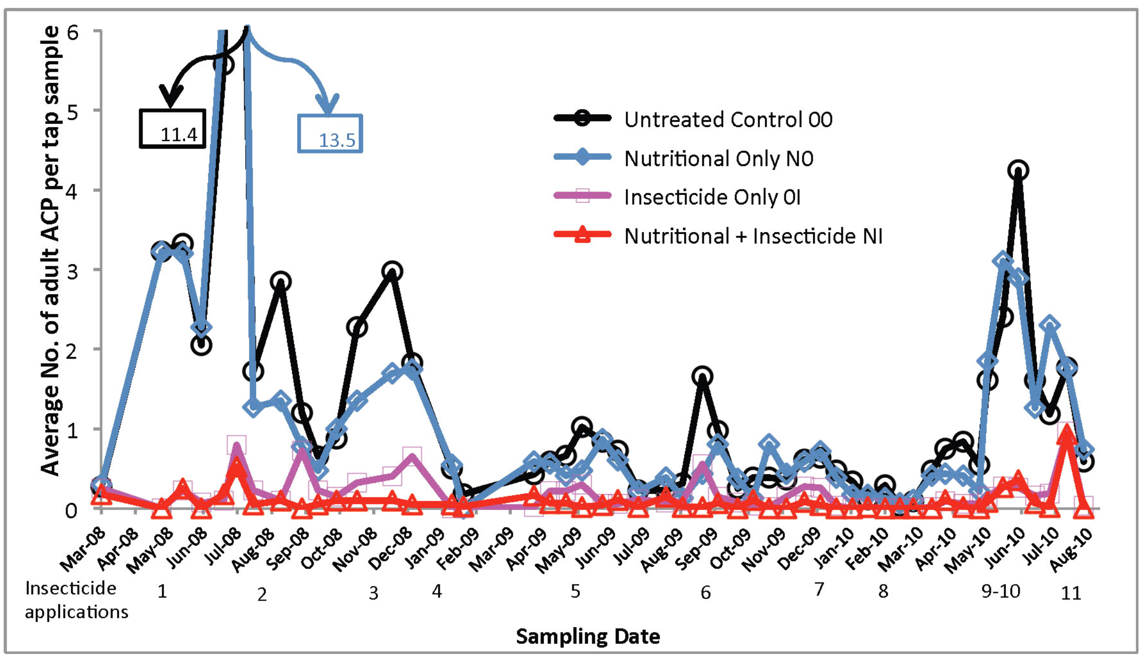

Figure 8. Effect of insecticide and/or nutritional applications on density adult ACP as assessed by tap samples from March 2008 to August 2010 (numbers at bottom labeled insecticide applications correspond to applications indicated in Table 3).

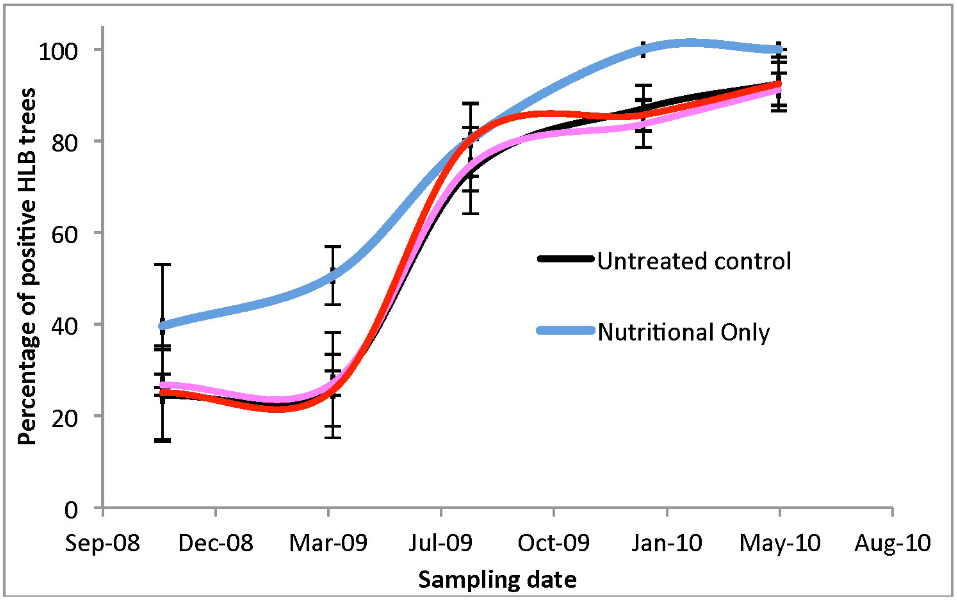

Figure 9. Percentage of positive samples for HLB on each treatment. Means ± SEM followed by the same letter or without a letter are not statistically significant within the same date using Least Square Means Comparison (α = 0.05) (Arevalo et al., unpublished).

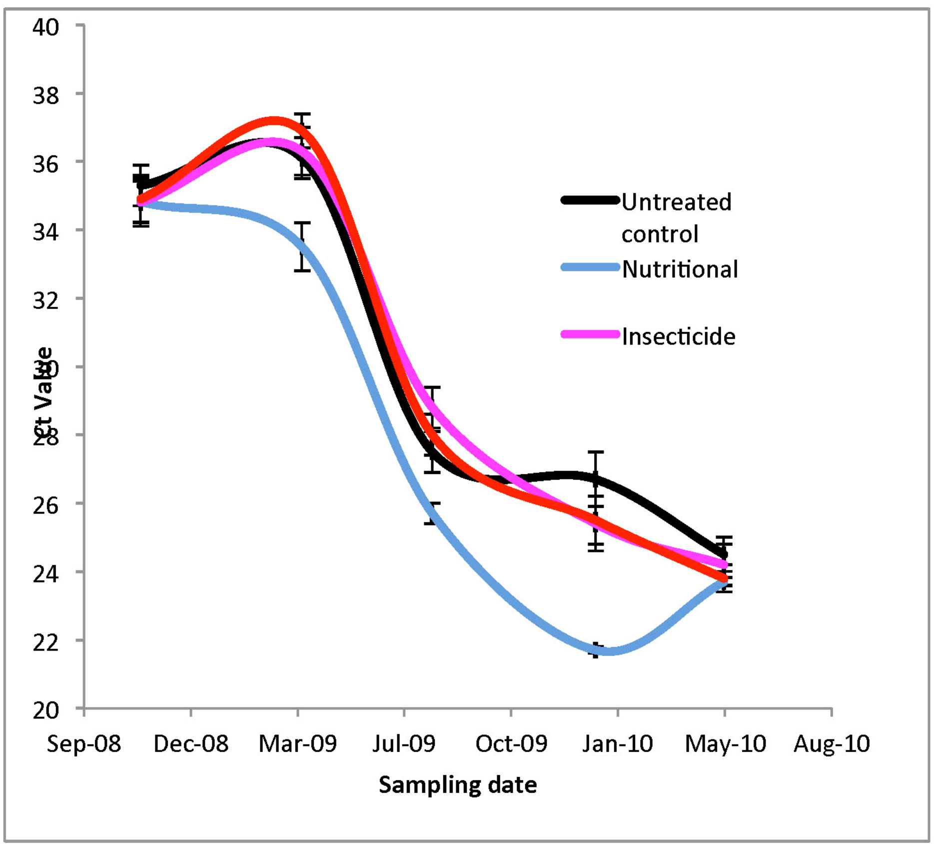

Figure 10. Intensity of HLB as expressed by cycle threshold (Ct) values ± standard error of the mean over two years. Ct value is the number of annealing/disassociation cycles required for sufficient amplification of target DNA to be detected by real time PCR. The lower the number, the higher is the bacterial titer in the original sample. Means ± SEM followed by the same letter or without a letter are not statistically significant using Least Square Means Comparison (α = 0.05) (Arevalo et al. 2010).

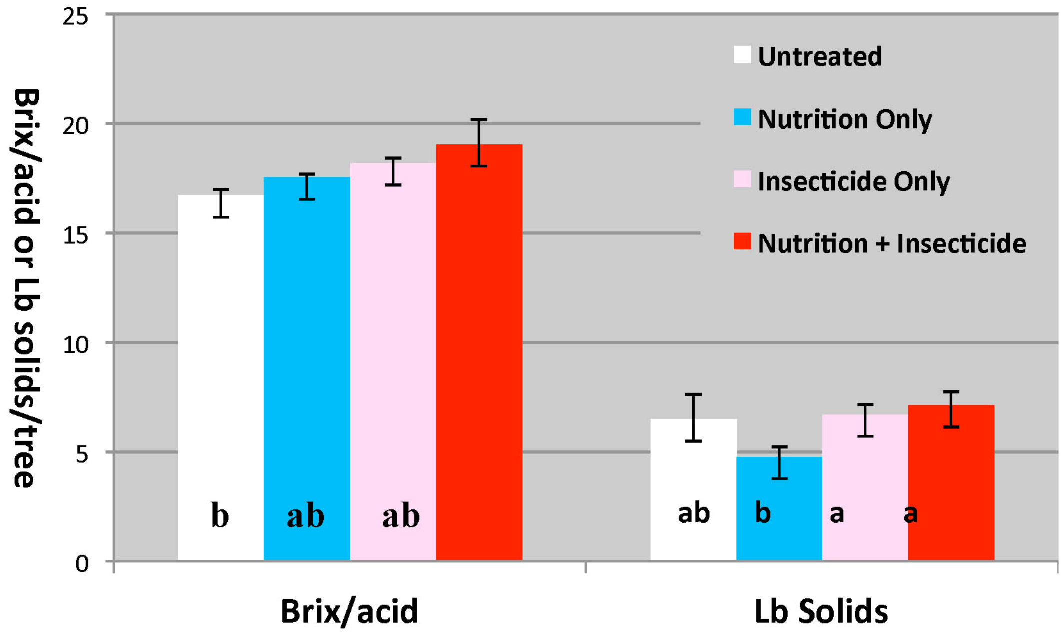

Figure 11. Brix/acid ratio and yield (lb solids/tree) of fruit from 'Valencia' orange on 'Swingle' citromelo trees in a commercial citrus block treated or not 3 to 4 times a year for 2 years with an insecticide rotation which maintained an overall ratio of approximately 12:1 ACP adults in treated: untreated plots. Block is 12 acres divided into 16 plots randomly assigned for insecticide treatment or untreated check. Analysis of variance showed that differences between treatments were statistically significant ($P < 0.05$) (Arevalo et al., unpublished).