Vector Control and Foliar Nutrition to Maintain Economic Sustainability of Bearing Citrus in Florida Groves Affected by Huanglongbing.

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Stansly et al.: Vector Control and Foliar Nutrition for Management of Huanglongbing

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Abstract

Background: Huanglongbing (HLB) or citrus greening is a bacterial disease vectored by the Asian citrus psyllid (ACP) causing tree decline, and yield loss. Vector control and foliar nutrition are used in Florida to slow the spread of HLB and mitigate debilitating effects of the disease.

A 4 year replicated field study was initiated Feb 2008 in a 5.2-ha commercial block of young 'Valencia' orange trees employing a factorial design to evaluate individual and compound effects vector management and foliar nutriention. Insecticides were sprayed during tree dormancy and when psyllid populations exceeded a nominal threshold. A mixture consisting primarily of micro- and macro-nutrients was applied three times a year corresponding to the principal foliar flushes.

Results: Differences in ACP numbers of from 5 to 13-fold were maintained in insecticide treated and untreated plots. Incidence of HLB estimated by PCR, rose from 30% at the beginning of the study to 95% in only18 months. Highest yields all 4 years were seen from trees receiving both foliar nutrition and vector control. Production for these trees in the 4th was close to pre-HLB regional average for 10 year old 'Valencia' on 'Swingle'. Nevertheless, at current juice prices, the extra revenue generated from the combined insecticide and nutritional treatment did not cover the added treatment costs.

Conclusions: This experiment demonstrated that vector control, especially when combined with enhanced foliar nutrition, could significantly increase yields in a citrus orchard with high incidence of HLB. Economic thresholds for both insecticide and nutrient applications are needed under different market and environmental conditions.

1 INTRODUCTION

Huanglongbing (HLB), also known as citrus greening, is considered to be the most damaging of all citrus diseases[1,2,3]. The causal agent of HLB in Florida is the bacterium *Candidatus* Liberibacter asiaticus (CLas), vectored by the Asian citrus psyllid (ACP) *Diaphorina citri* Kuwayama[1,4]. Trees infected with HLB exhibit chlorotic mottled leaves, nutrient deficient foliage, leaf and fruit loss and in some cases tree death. Fruit may fail to ripen properly with a consequent effect on juice quality, and production is lost due poor fruit set and fruit drop [1,6].

Huanglongbing now occurs in all major citrus growing areas of the world with the exception of the Mediterranean region and Australia[2]. *Diaphorina citri* was first detected in Florida in 1998[7] and quickly spread throughout the state, followed by the first detection of HLB in 2005[8]. Eradication of the disease within the state was never feasible because of widespread distribution prior to detection, the many reservoirs of inoculum and vectors, and a long latency period between infection and symptom expression during which asymptomatic, but infected, trees escape detection [4, 9]. Management recommendations include vector control with insecticides and rogueing of HLB infected trees. Although rigorous practice of these tactics appears to have slowed disease spread in Florida, incidence has increased such that rogueing is no longer an economically viable option for most growers.

Relatively high juice prices beginning in 2009 loosened constraints on production budgets and increased incentives to pursue more aggressive ACP control strategies. Vector control intensified and area wide spray programs of insecticides began, resulting in significant decreases in psyllid populations[10,11,12] The program in southwest Florida focused initially on one and subsequently two applications of broad-spectrum insecticides during late fall and early winter to target a naturally declining psyllid population composed almost exclusively of overwintering adults[13]. Significant suppression was observed for up to 6 months with little impact on populations of key beneficial insects largely absent during this period[12]. This strategy reduced ACP populations during the spring flush and thus the subsequent movement of infected psyllids [14].

Current HLB management programs in Florida parallel similar practices recommended in California against pear decline caused by a phytoplasma and vectored by the pear psylla *Cacopsylla pyricola* [15]. Control of overwintering adults appears to be of fundamental importance for preventing spread of the disease[16], and one or two dormant sprays are recommended to reduce populations to no more than one pear psylla per 100 beat-tray samples by the time trees break dormancy[17]. Furthermore, previous research showed remission of pear decline is more likely if trees remain vigorous by reducing stress caused by inadequate irrigation, nutrient deficiencies, weed competition, and pest damage [18]. Anecdotal reports among Florida citrus growers also indicate that productivity of HLB-infected trees is being maintained by removing stress factors, especially micro-nutrient deficiencies [19]. This study is in part a response to those reports.

Foliar deficiencies of micronutrients are a noted symptom of HLB [20, 21, 22]. A malfunctioning vascular system or changes in membrane permeability can induce systemic or localized nutrient deficiencies [23, 24]. As a result, concentrations of key micronutrients, such as manganese and zinc, may decline in foliar tissue of diseased plants [25]. Koen and Langeneggerpho[26] using an unnamed citrus species infected with *Ca*. L. africanus found that concentrations of potassium were higher in infected plants, while calcium (Ca) and magnesium (Mg) were lower. Aubert [20] found that HLB-infected plants in Réunion contained lower concentrations of calcium (Ca), manganese (Mn), and zinc (Zn).

Foliar applications of micronutrients constitute a strategy being employed by an increasing number of Florida citrus growers to mitigate HLB-induced deficiencies and counter debilitating effects of the disease [19]. These applications often include other materials such as salts of phosphorus acid that are thought to aid assimilation of nutrients and to act against secondary diseases such as root rot caused by *Phytophthora* spp. Salicylic acid applied as a foliar amendment is believed by some to act against the HLB pathogen by activating the systemic acquired resistance (SAR) pathway. These nutrient/SAR programs, coupled with intensive vector control, are purported to lessen disease expression of HLB-infected trees, although corresponding effects on yield have yet to be demonstrated. Indeed, one report concluded that nutrient sprays had no effect on HLB or citrus yield. although their study was limited to 2 years in small plots and conducted in a largely unmanaged orchard[27]. Furthermore, it is not clear to what extent the apparent response observed in commercial orchards is due to vector management, nutrient management, or a combination of both.

We report results from a large scale replicated field study in a functioning commercial citrus orchard. A factorial design was employed to evaluate individual and compound effects of a threshold-based vector management protocol and a popular nutrient/SAR program. Data collected included vector population density, incidence of HLB, fruit quality, and yield. An economic evaluation assesses grower returns under different treatment regimens and fruit price structures.

2. MATERIALS and METHODS

2.1 Location and Experimental Design The experiment was conducted on a 5.2 ha block of 'Valencia' orange bud-grafted to 'Swingle' citrumelo rootstock and planted June 2001 in Collier Co. Florida (26° 29' N, 81° 21'W). Plant population was 373 trees/ha (151 trees/ac) at 7.3 m between rows and 3.7 m within rows. Standard horticultural practices for Florida citrus were followed [28], including irrigation with micro-sprinklers and weed control by mechanical mowing plus applications of glyphosate once a year or twice in 2011, and of Krovar® (40% bromacil + 40% diuron, Dupont, Wilmingto DE) 5.6 kg/ha (5 lb/ac) during Apr. Ridomil® (mefenoxam, Syngenta Crop Protection, Wilmington DE) was applied in

2010 for protection from root/foot rot caused by *Phytophthora* spp. Methoxyfenozide (Intrepid®, DowAgrosciences, Indianapolis IN) was applied to the entire block on 31 May 2012 at 5.6 kg/ha to control citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillaridae). The following fertilizer applications (NPK or as listed) were made to the soil: Sep08 (13-0-21) 336 kg/ha; Jan09 (12-4-16) 448 kg/ha; May09 (8-0-24) 448 kg/ha; Oct09, Aug10 (K-Mag® = 22% K2O, 11% Mg and 22% S) 224 kg/ha; Oct09, Jan10, Apr10, (UN-32 = 45% NH_4NO_3, 35% urea and 20% water) 186 L/ha, Mar10, May11, Aug11 (0-0-42) 224 kg/ha; Mar10 (9-0-0 liquid) 93 L/ha, May10 Granulite (heat dried biosolids) 1,120 kg/ha; Sep10 (14-0-22) 336 kg/ha, Jan11 (16-4-16) 336 kg/ha; May11, Aug11 (20-0-0+5%Ca liquid) 96 L/ha.

The block was defoliated in 2004 in an attempt to eliminate citrus canker, thus delaying plant growth by approximately one year. Huanglongbing was detected and confirmed in March 2006 by the Florida Department of Agriculture and Consumer Services, Division of Plant Industry (FDACS-DPI). The block was divided Feb 2008 into 16 plots of average area 0.31 ha and containing a mean 108 (range 79-176) trees each.

2.3 Treatments Four treatments were assigned to these plots in a two factor randomized complete block design (Fig. 1). The two factors were insecticide (yes or no) and foliar nutritional (yes or no). Treatments were: (1) nutrition alone, (2) insecticides alone, (4) nutrition + insecticides, and (4) untreated control.

The nutritional regimen (Table 1) was adapted from a program attributed to Mr. Maury Boyd, a citrus grower in southwest Florida [19] and also evaluated by Gottwald et al. [27]. Nutrient applications were initiated March 2008 in designated plots (nutrition-only and insecticide+nutrition treatments) sprayed on the foliage three times a year when major flushes of spring, summer and fall were fully expanded but not yet hardened. Applications were made with an Air-O-Fan airblast sprayer equipped with Albuz® ATR hollow cone nozzles providing an 80° spray pattern with five blue and one green nozzle (2.5 and 3.4 L/min respectively) operating at 10 bars and 5.2 km/hr delivering a total 39 L/min or 982 L/ha (105 gal/ac).

Insecticide treatments to control ACP in plots designated in insecticide alone and insecticide+nutrition began May 2008 using the same equipment and settings. Thereafter, one (Jan 2009) or two (Dec 2009, Feb 2010 and Nov 2010, Jan 2011, Dec 2011 and Feb 2012) dormant sprays of broad-spectrum insecticide were applied in late fall or winter (Table 2). Additional sprays during the growing seasons of 2009 and 2010 were made whenever adult *D. citri* populations in the treated plots surpassed an arbitrary threshold. A threshold of 0.5 adult ACP per "stem tap" sample (explained below) was adopted in 2009 but reduced to 0.2 after the 2010 harvest due to low ACP counts, possibly in response to area wide dormant sprays [10, 11]. Selection of active ingredient was based on recommendations found in the 2010 Florida Citrus Pest Management Guide: Asian Citrus Psyllid and Leafminer ref; http://edis.ifas.ufl.edu/in686.

2.4 Sampling

2.4.1 Asian citrus psyllid adults *D. citri* adults were monitored every 2 weeks from ten randomly selected trees in the middle bed of each plot using the stem-tap sampling method [29,30,31]. For each tree, a white plastic clipboard measuring 28 × 21.6 cm was placed under a randomly chosen branch which was struck three times with a short length of PVC pipe and the number of adult *D. citri* fallen on the board recorded.

2.4.2 Incidence of Huanglongbing Every fifth tree was sampled in every plot for a total of 294 samples taken Nov-08, Apr and Sep-09, Jan, May and Nov-10 and Jan and Apr-11. The most-symptomatic leaves available were chosen for analysis, those exhibiting symptoms of blotchy mottle chlorosis, or in their absence, small up-right leaves with symptoms resembling zinc deficiency. Leaves were bagged and transported on ice immediately to the Southwest

Florida Research and Education Center, University of Florida, Immokalee. Sampling was discontinued after April 2011 when incidence of positive trees had increased to more than 90%.

Visual assessment of the same sample trees to estimate severity of HLB symptoms was conducted on 9 Feb 12 using a scale of 0 to 5 where 0 = no symptoms of HLB, 1= 20% (one sector of tree); 2 = more than 20% but not greater than 50%; 3 = more than 50% but less than 75%; 4 = 75% to 90% and 5 = 100%. Statistical analysis was performed as described below.

2.4.3. Acquisition of pathogen by ACP Colonies of *Diaphorina citri* immatures (1st and 2nd instar nymphs) developing on shoots of treated and untreated trees infested with feral populations of *D. citri* were confined using sleeve cages made from fine mesh organdy that protected nymphs from natural enemies and prevented emerging adults from dispersing. One colony per shoot per tree was caged for a total of two colonies per replicate, eight per treatment. The experiment was repeated June, July, September and December 2009 and February, May, July, September and October 2010. Once adults emerged, (mean 22, range 15657 per cage) all cages were collected and transported on ice in an insulated cooler to the laboratory at SWFREC. Cages were placed in a freezer for 5 minutes to immobilize adults which were then collected using soft camel's hair brush and preserved in 95% EtOH in 2 ml screw cap tubes (Phoenix Research Products, Candler, NC) at -20° C for PCR analysis (see below). Percentage of positive psyllids in each replicate was calculated by dividing the number of positive psyllids by the number processed through PCR. Average of HLB positive psyllids was calculated from four runs in 2009 and five runs in 2010.

2.4.4. PCR Analysis of Plant and Psyllid Samples Total plant DNA was extracted from 100 mg of petiole tissue using the Promega Wizard® 96 DNA Plant isolation kit (Promega, USA). Briefly, tissues were flash frozen under liquid nitrogen prior to pulverization to a fine powder using a Mini-beadbeater (Bio Spec Products Inc., Bartlesville, OK). Samples were then processed as per manufacture's instruction, DNA eluted in 50 µL AE Buffer and stored at -20°C.

Psyllids were processed individually and total DNA was extracted using the Qiagen MagAttract 96 DNA Plant isolation kit (Qiagen, USA) with minor alteration to the procedure. Briefly, psyllids were air dried and transferred individually to a well of a 96-well plate containing 600 µL lysis buffer and silica beads. Psyllids were bead beaten in lysis buffer using a Mini-beadbeater (Bio Spec Products Inc., Bartlesville, OK), centrifuged and lysate supernatant used for DNA extraction. MagAttract Suspension was mixed with molecular grade absolute ethanol in a 1:10 ratio and mixed with lysate. Magnetic beads were washed as per manufacture's instruction; DNA was eluted in 100 µL AE Buffer and stored at -20°C. Each extraction plate of 96-wells included four random wells with "no psyllids" as control, to monitor for the possibility of cross contamination.

Primers and probes were obtained for *Candidatus* Liberibacter asiaticus (HLBas/HLBr and HLBp (Li et al. 2006). Primers and probes for the plant cytochrome oxidase, COX gene (COXf/COXr and COX-p) were used for an internal control to check the extraction [32]. The internal probe COX-p was labeled with 6-carboxy-4', 5'-dichloro-2', 7'-dimethoxyfluorescein (JOE) reporter dye at the 5'–terminal nucleotide and with BHQ-2 at the 3'–terminal nucleotide. The positive control was DNA from known positive citrus trees located in the SWFREC grove and negative controls were obtained from citrus grown under screen-house conditions at SWFREC and tested annually. The primers and probes for the *wingless* gene (DCF/DCR and DCP [14] were used as an internal control for monitoring the quality of psyllid DNA. A plasmid containing a cloned fragment of the16s rDNA of *Candidatus* L. asiaticus (GenBank Accession No.: EU130556) was generously donated by Dr. M. L. Keremane (USDA-ARS, Riverside, CA) and used to generate positive controls (plasmid plus psyllid DNA). Negative controls consisted of DNA extracted from HLB negative psyllids.

Real-time qPCR was conducted with an ABI 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA) using TaqMan® Fast Universal PCR Master Mix (Applied Biosystems, Foster City, CA) in a 20 μL volume. The standard amplification protocol was initial denaturation at 95°C followed by 40 cycles of reactions (95°C for 3 s, 60°C for 30 s). Data was analyzed using Applied Biosystems 7500 system SDS software version 1.2.

The cycle threshold, or Ct-value, is the minimum number of DNA amplification cycles necessary to detect a signal. The sample (plant or psyllid) was considered negative if the Ct value was greater than 36. If no target DNA was detected after the full 40 cycles, the result was considered "undetermined" Samples with Ct-values less than or equal to 32 were considered positive for HLB and any sample with a Ct-value between greater than 32 and less than 36 were putative positive and resampled[27].

2.4.5. Fruit yield and quality All ripe fruit was harvested from all trees in each plot during the weeks of 26 March 2009, 20 April 2010, 4 April 2011 and 8 March 2012. In 2009, weight of oranges harvested from each plot were estimated based on the number and fraction of 10-box pallet tubs filled, with the assumption that a full tub of oranges weighs 410 kg (10 field boxes at 41 kg/box). In 2010, 2011 and 2012, each tub was weighed using a Gator Deck Scale (Scale Systems, Novi, MI) and the tared weight recorded. One (2010) or two (2012) ½ bushel (17.6 Liter) citrus bags were filled by composite random sample taken from the various tubs that were harvested from each plot. Samples were sent to the University of Florida citrus quality laboratory in Lake Alfred, FL. Juice was de-aerated under vacuum for 2-3 minutes, soluble solids content measured by hydrometer and titratable acidity as citric acid, pH endpoint 8.2. Unfortunately, data were not obtained from the 2011 sample due to insufficient juice caused by freeze damage experienced 18 December 2010.

2.5 Statistical Analysis

Statistical analyses were conducted on both main effects and individual treatments using the General Linear Model Procedure[33]. Main effects were considered if the interaction of the two factors was not significant ($p > 0.05$). Mean separation of individual treatment effects was conducted using *t*-Student test for pair-wise comparisons and Fisher's least significant difference (LSD) test ($\alpha = 0.05$). ACP numbers were analyzed using the cumulative insect × day metric that summarizes insect activity over a given period[34]. This method is analogous to the area under the disease progress curve (AUDPC), also used here calculated per Van der Plank (1963) [35] using disease incidence over time to compare treatment effects. Chi square analysis was used to compare incidence of positive PCR results between particular treatments on individual sample dates. Logistic rate of disease increase ($R_{L)}$ was calculated by linear regression of transformed disease incidence [36] for comparison to published rates of values HLB epidemic rates. Ratings of disease severity were analyzed by ANOVA and significant differences between means were separated by LSD ($P=0.05$) using SAS V9.2 (SAS Systems, Cary, NC). One way ANOVA was used to analyze treatment effects on Ct values less than 40, thus excluding "undetermined results". Proportions of caged psyllids testing positive for HLB were arcsine-transformed and analyzed for both main effects and individual treatments using the General Linear Model Procedure and p-value of 0.05[33]. Statistical analysis of yield was conducted on mean weight of fruit per tree.

2.6 Economic Analysis

A two-step evaluation was conducted using costs of insecticide and nutrient materials, published production enterprise budgets, and the yield data generated by the experiment. The first step was an assessment of whether trees in the untreated control produced a profitable level of fruit. The second step was a marginal analysis that considered only the change in fruit yield by treatment and then compared the value of yield increases (if any) with the added treatment costs for vector control and foliar nutrients. Cost of nutrient/SAR and insecticide materials are listed in Tables 1 and 2, respectively, and summarized in Table 3. These costs were obtained from sale representatives of various fertilizer and chemical supply companies who provided product price information as of June 2011.

3 RESULTS

3.1 Asian citrus psyllid Population levels were consistently less on insecticide-treated trees compared to trees not treated with insecticide over the entire 4 year period (Fig. 2). Numbers per stem tap on trees receiving no insecticide exceeded those on insecticide treated trees by over 13-fold the first year and between 5- to 7-fold in successive years. Despite of these differences, population trends were correlated in insecticide treated and untreated plots ($R = 0.25$, $P < 0.0001$, $N = 768$). The nutrition x insecticide interaction for cumulative × ACP days was not significant for any of the 4 years, permitting main component analyses which showed significant effects of insecticide but not nutrition on ACP numbers each year (Table 4).

3.2 Incidence and severity of huanglongbing

The percentage of trees testing positive for HLB, regardless of treatment in the test block averaged 29.9 ± 1.9% at the first sample date (Nov 2008) and rose to 94.7 ± 1.3% by May 2010 (Fig. 3). Incidence in plots treated with nutrient only was significantly greater than in untreated control plots through Nov 2010 (chi square 4.05 to 12.04, $p = 0.44$ to 0.0005). In contrast incidence in control plots versus plots treated with insecticides or insecticide + nutrients only was significantly different on Nov 2010 and 24 Jan 11 respectively. The logistic rate of disease increase per year, calculated given a first incidence date at 0.001 in January 2006, was $R_L = 2.1$.

Analysis of the AUDPC revealed no significant effect on main components (P = 0.11, F = 3.1, and P = 0.26, F = 1.4 for insecticide and nutrition respectively, df = 1,11). However, the treatment effect was significant (P = 0.27, F = 4.9, df=3,9) with highest AUDPC recorded from trees receiving the nutrition-only compared to all other treatments which were not different from each other.

Average Ct-values decreased from 33.0 ± 0.39 in Nov 2008 to a low of 23.6 ± 25 Jan 2010 indicating rising titer of the target (CLas) DNA. Ct values later rose to 26.8 ± 0.26 in Jan 2011. Lower Ct values in response to nutrition (higher titer) and higher Ct values in response to insecticide (lower titer) were seen in the Sep 2009 and Jan 2010 samples (Table 5A). Lowest Ct values were observed with both treatments that included nutrition on Nov 2010. Only the sample from May 2010 showed no significant treatment effect on Ct-values.

Visual ratings of severity of HLB symptoms towards the end of the test period showed a significant interaction between the two factors of insecticide and nutrition (P=0.01), so only treatment effects are reported. Very significant (F = 11.0, df = 3,289, P < 0001) treatment effects were observed, with highest disease severity ratings seen on trees in control plots at 3.3 ± 0.08, significantly greater than all other treatments indicating more severe expression of symptoms of HLB on untreated trees. Trees receiving insecticide alone received an average disease severity rating of 3.0 ± 0.7, significantly greater than trees receiving nutrition alone or nutrition + insecticide which were not significantly different from each other at 2.7 ± 0.8 and 2.8 ± 0.7, respectively.

3.3 Acquisition of Pathogen by Psyllid Vector Mean incidence of positive psyllids emerging from caged cohorts (10.5 ± 2.9% in 2009 and 9.4 ± 2.5% in 2010) was considerably lower than estimated for trees, with no significant difference between years (F=0.15, P = 0.78, df =1,3). Variation was high, with no infected psyllids in many cohorts while others were 50 to 100% infected. Mean rate of acquisition on trees treated with nutrition-only was

13.4 ± 4.1%, compared to 8.3 ± 2.1 % among remaining treatments. The difference was not significant (F=1.52, P = 0.22, df = 1,3) due perhaps to the high degree of variability.

3.4 Fruit yield and quality Significant treatment effects on yield were observed in all 4 years of the study (F = 4.85; P<0.018 (2009), F = 4.61, P= 0.021 (2010); F = 4.91 P<0.017 (2011), F = 7.63, P< 0.004 (2012), respectively d.f =6,9 for all. Interactions between main effects of insecticide and nutrition were not significant for any year, so effects of each factor were analyzed. Significantly higher yields were observed from trees receiving insecticide application compared to trees not receiving insecticide for the 2010, 2011 and 2012 harvests as well as the combined total of all harvests (Table 6A). Foliar nutrition resulted in significantly increased yields in 2012 but not in 2010, 2011 nor the cumulative yield over the 4 years of the trial.

Looking at treatment effects, insecticides plus nutrients consistently produced the highest yields all 4 years, as well as for the total 4-year production (Table 7B). However, differences with insecticide alone were not significant in 2010 and 2011, or with the untreated control in 2010. Nutrition alone was the poorest treatment in 2010 and 2011, significantly so compared to either treatment with insecticides both years, but not compared to the untreated control.

Yields increased for all treatments in 2012, even the untreated control which improved 2.1-fold from the previous year. Yields from trees treated with nutrition alone improved most, 3.2-fold, with production levels between nutrition + insecticide and insecticide and not significantly different from either. However, combining nutrition with insecticide did result in significant improvement in production over insecticide alone. All three treatments resulted in significantly greater production than the untreated control.

The ratio (brix:acid) in 2010 was less from trees treated with insecticide compared to trees not treated with insecticide (Table 7A). Otherwise all other juice quality effects that year were either not significant (juice per box, brix) or had significant interactions (solids per box, acid). Lower solids per box and higher acid were seen in 2010 with the nutrition-only treatment (Table 7B). No significant effects were seen in 2012 except for brix which was inexplicable higher for the insecticide factor (F=4.9, P = 0.036, df = 3,25,Table 7A) although treatment effects were not significant (F=2.8, P = 0.036, df = 1,9, Table 7B).

3.5 Economic analysis Prior to HLB, production for Valencia oranges on Swingle rootstock in southwest Florida on 7 to 10 year old trees averaged more than 2.5 boxes (102 kg) per tree [37].Yields for all treatments during the first three years of the trial were substantially below these historical averages (Table 6). This trend reversed in 2012 when production under all treatments increased. Yields for the nutrition + insecticide treatment produced over 90 kg/tree, only 7 kg/tree less than the Southwest Florida average for a ten-year old 'Valencia' on 'Swingle' tree prior to HLB.

During the five-years (2001-2005) preceding HLB, grove care costs, production, and delivered-in prices for sweet oranges averaged \$2,100/ha (\$850/ac), 2.83 kg.s./box (6.24 p.s./box), and \$2.49/kg.s. (\$1.13/p.s.) [40, 38]. Assuming harvest and haul costs of \$2.50/box, break-even yields were at least 32 kg per tree. With the advent of HLB, typical grove care costs increased to more than \$3,700 per hectare[40] (\$1,500/ac)[40]. Fruit prices, however, also increased to an average delivered-in price of \$3.81 per kg-solid (\$1.73/p.s.) during the five-years post-HLB (2007-2011) [38]. The combined effects of higher production costs and higher fruit prices increased the break-even production threshold to nearly 38 kg/tree. Production from untreated control plots exceeded this threshold in 3 of the 4 study years (Table 6).

Economic feasibility of the individual treatments was evaluated by comparing the change in revenues under a range of fruit prices with the added costs incurred by each treatment. Costs of the insecticide-only treatment ranged from \$246/ha in 2008/09 to \$689/ha in 2011/12 (Table 3). Only four or five insecticide applications were needed between 2008 and 2010 to maintain ACP populations below the predetermined threshold, requiring an outlay of $246 to $294/ha for material and application costs. Seven applications were made in 2011/2012 with a corresponding increase in cost to $689/ha.

The estimated cost of the nutritional program was $1,588 per hectare (Table 1). The program included two SAR (systematic acquired resistance) products, that if dropped from the nutritional cocktail would reduce costs by $236/ha, or $1,352 of total added costs for the enhanced nutritional program. During the 2011-12 season the costs for the combined insecticide and nutrition treatment were $2,229/ha with the full nutritional program.

Combining results from Tables 6 and 7B indicated that the equivalent in solids harvested in 2012 increased over what was produced from the untreated control by 245, 425, and 531 kg/ha for the insecticide-only, nutrient only, and combined insecticide + nutrient treatments, respectively (Table 8). Fruit prices in this analysis were chosen to encompass a range of market possibilities expected over the next 5 to 10 years. Fruit prices for processed oranges fluctuated between $4.18 and $2.29/kg-solid ($1.90 - $1.04/lb-solid) between 2007 and 2011 [38]. Therefore, the change in revenue was valued at 3 delivered-in (FOB) fruit prices: $3.85, $3.30, and $2.75 per kg-solids ($1.75, $1.50, and $1.25 per lb-solids) and compared against added costs associated with each treatment.

Production gains in 2011-12 from the insecticide-only treatment nearly offset the added costs of $689/ha at the lowest fruit price of $2.75/kg.s. Fruit prices would have to be at least $2.81/kg.s ($1.27/p.s.) before the value of added production would fully pay for the added insecticide costs. The enhanced foliar nutritional (EFN) without insecticides was profitable in 2012 only under the highest fruit price ($3.85/kg.s.). If the SAR products did not contribute to greater production, then the cost of EFN would decrease by $236/ha and would have been profitable at a fruit price of $3.30/kg-s. The insecticide+nutritional treatment produced the highest gain in production, but also the highest cost. Even at the highest fruit price ($3.85/kg.s.), the amount of increased production from the insecticide+nutritional treatment did not add sufficient revenue to completely offset the cost of the treatments. A delivered-in fruit price of more than $4.07/kg.s. ($1.85/p,s.) would have been necessary to cover all the costs of the combined insecticide and nutritional treatment. If the SAR products were removed (less $236/ha), the break-even price would fall to $3.75/kg.s. ($1.70/p.s.).

4 DISCUSSION

4.1 Psyllid populations and HLB incidence Only four insecticide applications per year were necessary to significantly reduce adult psyllid numbers as indicated by stem tap samples from 2008 through the 2011 harvest. Insecticidal treatments were increased to 7 the next year, including a second dormant spray application in February 2012. Even though insecticides greatly reduced psyllid numbers, population trends correlated between insecticide treated and untreated plots, indicating that the main drivers of population change were the same for all, presumably weather and tree flushing patterns. Furthermore, we saw psyllid numbers remain distinctly different over months in adjacent plots no larger than 0.3 ha, indicating limited movement of adults from treated to untreated areas. These results seem to contradict the general notion that ACP adults are constantly on the move[41, 42]. Rather, it would appear that movement requires some stimulus, such as overcrowding or insufficient food; conditions that might occur more often in abandoned than managed citrus groves.

HLB moved rapidly throughout the block, likely following flights of ACP with the termination of spring and summer flushing (Fig. 3). Applications of insecticides were apparently too late and/or insufficient to detectably slow progress of the disease, even though numbers of ACP were reduced significantly by the sprays. A lack of significant effect on HLB incidence may also have been due to high incidence of latent infection at the beginning of the trial that could have remained undetectable for 1-2.5 years[4]. Some movement among plots is also likely.

January 2006 was used as a starting point for the epidemic to calculate the R_L (logistic rate), given that HLB was detected in the block in March 2006. The estimated R_L of 2.10 fell within the range of 1.37 to 2.37 presented by Gottwald[4] for eight plantings in Florida. This result supports his statement that epidemics of HLB are rapid, although not his conclusion that it would be 'rare' for a planting with high incidence not to be removed because of non-productiveness[4].

In contrast to insecticides, we observed higher incidence of HLB and lower Ct values in trees treated with nutrients alone (Fig. 3, Table 5). Higher initial incidence and lower Ct values, sustained through Jan 2010 may have been due to chance location of these plots on the periphery of the block (Fig 1). The existence of pronounced edge effects in distribution of HLB infected trees is well documented and supported by inverse power function (IPF) analysis[4]. Edge effects may form adjacent to canals, ponds, pastures or woods and would be most pronounced at corners where two edges meet.

We saw no nutrient effect on psyllid numbers (Table 4) so the effect cannot be attributed to attraction by ACP to increased growth of new foliage. Improved tree health of nutrient-treated trees might provide a more favorable environment for the Clas bacteria to replicate and reach detectable levels. However, we did not observe low Ct values for the combined nutrient + insecticide treatment until Nov. 2010. (Table 5B). In apparent contradiction to PCR results, we observed significantly reduced severity of HLB symptoms in nutrient-treated trees compared to control trees or trees receiving only insecticides. These observations agree with our results on yield and support declarations of growers, consultants, and other researchers that foliar nutrients attenuate HLB symptoms, although clearly not from any inhibitory effect on bacterial titer.

4.2 Yield effects and economic considerations. Significant yield effects were seen from vector control each year after 2009 and for the combined 4 harvests of the trial (Table 6A). In contrast, a significant effect of foliar nutrition was seen only in 2012 when yields doubled from the previous 3 years. Poor yields in 2010 and 2011 were attributed, at least in part, to adverse growing conditions - an untimely application of glyphosate 3 weeks before harvest in 2010 and a freeze in December 2010 which affected the 2011 harvest. Fortunately, two freeze events during the winter of 2012 caused little apparent damage, and production that year better reflected the true potential of the block.

The combined nutrient + insecticide treatment consistently resulted in the highest level of fruit production every year and over all four years, although differences with insecticide alone treatment were not significant in 2010 and 2011 (Table 6B). Poor yield response those years from trees treated with nutrients alone may have been due to the trend for higher incidence of HLB in those plots as discussed above. However, production rebounded in nutrient only-treated trees in 2012, coming close to the pre-greening regional average [37], and indicating a degree of compensation for the effects of HLB.

Gottwald et al. [27] reported no yield response from 'Valencia' orange trees grafted to 'Swingle' citrumelo with a similar mixture of nutrients and SARs tested on small (4-tree) plots replicated 3 times in an abandoned Florida orchard. No data were provided on psyllid populations and their study ran for only 2 years. Without the insecticide component, our results would have agreed with theirs for the first 3 years, during which we saw no yield response from nutrients alone. The combined nutrients + insecticide treatment, however, always provided the highest numerical yields among the four treatments, and nutrients alone rebounded the 4th year with significantly better yields than the untreated control. These results suggest that longer term studies are necessary to adequately evaluate effects of such treatments on HLB infected trees, and that vector control is an indispensable component for management of the disease.

The combined treatment of insecticides + foliar nutrition consistently produced the greatest yield gains relative to the untreated control in this experiment, but also was the most expensive and might not be profitable in its present form over the long term economic conditions facing the Florida process citrus industry. The objective of this experiment, however, was to evaluate the consequential effects one set of vector control and nutrient protocols, not necessarily their profitability. Fine tuning the various components of insecticide and nutritional programs could substantially reduce costs and increase the likelihood that citrus growers could manage HLB infected trees profitably in Florida.

This research is the first study to show that productivity of HLB infected citrus groves can be enhanced by vector control and applications of foliar micro- and macro-nutrients. Further research is necessary to determine the specific components in both the insecticide and micro-nutrient programs that will achieve the greatest yield gains at the least cost, and to evaluate these under a variety of environmental and horticultural conditions. Our study demonstrates that, although it is may be possible to live with HLB, the cost of maintaining production once trees are infected is considerably greater than in an HLB free environment. Vector control and rogueing of symptomatic trees to protect from HLB are also expensive practices. Most of the world’s juice production comes from areas where HLB is now endemic, so it follows that prices must increase if production is to remain profitable. The process citrus industry will be challenged to maintain consumer demand for juice on the one hand and reduce production costs on the other if profitability is to be sustained in an HLB world.

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6.0 References

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7.0 Figure legends

Figure 1. Plot plan, 5.2 ha, 1,728 trees 'Valencia' orange on 'Swingle' citrumelo planted Collier Co. FL, in 2001. Block was divided Feb 2008 into 16 plots 0.31 ha and containing a mean 108 (range 79-176) trees each sorted in a RDBD with 4 replications and 4 treatments: Pink: insecticides only; Blue: nutrition-only; Red: insecticides + nutrition; White: untreated, no insecticides or nutrition.

Figure 2. Mean number of ACP adults per tap sample taken at 2 week intervals.

Figure 3. Mean incidence (%) ± standard error of HLB positive trees by treatment as indicated by PCR analysis of every 5th tree in the entire block on 8 sample dates from Nov 2008 through April 2011.

Tables

Table 1. Composition of the nutrition + systemic acquired resistance (SAR) inducer blend used during this trial.

Product	Quantity Unit/ac[1]- appl	Cost $/unit[2]	Function	Company
Serenade Max WP (*Bascillus subtilis*)	2.25 lb	$11.75	SAR inducer	AgraQuest, Inc.
SAver (Potassium salicylate)	1 qt	$5.50	SAR inducer	Plant Food Systems
3-18-20 with K-Phite	8 gal	$12.00	Macronutrients	Plant Food Systems
13-0-44 fertilizer	8.5 lb	$0.72	Macronutrients	Diamond R
Techmangam (Mg Sulfate)	8.5 lb	$0.75	Micronutrients	Diamond R
Zinc Sulfate	2.8 lb	$0.90	Micronutrients	Diamond R
Sodium Molybdate	0.85 oz	$1.50	Micronutrients	Diamond R
Epsom Salts	8.5 lb	$0.30	Micronutrients	Diamond R
435 oil	5 gal	$5.50	Adjuvant	PetroCanada
Number of applications:				3x/year
Nutrient material costs:				$1,056/ha
SAR material costs:				$236/ha
Total cost, material + application of the full EFN				$1,588/ha

Notes:

[1] Products purchased in English units.

[2] Cost of materials from a June 2011 survey of fertilizer and agricultural chemical suppliers in US dollars.

Table 2. Date, product, active ingredient (a.i.), rate of insecticide applications, unit cost of material in US dollars sprayed in designated treated plots from 2008 to 2010. All applications were conducted when scouting results indicated *D. citri* populations above 0.5 adult *D. citri* per "stem-tap" sample in 2008 or 0.2 subsequently.

Season	**Date**	**Product**	**a.i**	**Rate Unit/ac**	**Cost $/unit**	**Company**
2008-09						
Growing	2008 May 2	Danitol 4EC	fenpropathrin	16 oz/ac.	$1.01	Valent USA Corp.
Growing	2008 Aug 7	Delegate WG	spinetoram	4 oz/ac.	$6.50	Dow Agrosciences
Growing	2008 Nov	Delegate WG	spinetoram	4 oz/ac.	$6.50	Dow Agrosciences
Dormant	2009 Jan 14	Mustang	zeta-cypermethrin	4.3 oz/ac.	$1.50	FMC.
2009-10						
Growing	2009 May 20	Movento	spirotetramat	10 oz/ac.	$6.28	Bayer CropSciences
Growing	2009 Sep 29	Lorsban 4E	chlorpyrifos	3 pt/ac	$4.75	Dow Agrosciences
Dormant	2009 Dec 23	Dimethoate 4EC	dimethoate	1 pt/ ac.	$5.00	Helena Chemical
Dormant	2010 Feb 16	Danitol 4EC	fenpropathrin	12 oz/ac.	$1.01	Valent USA Corp.
2010-11						
Growing	2010 May 31	Delegate WG	spinetoram	5 oz/ac.	$6.50	Dow Agrosciences
Growing	2010 July 30	Lorsban 4E	chlorpyrifos	3 pt/ac	$4.75	Dow Agrosciences
Dormant	2010 Nov 23	Imidan 70W	phosmet	1 lb/ac	$8.30	Gowan Co.
Dormant	2011 Jan	Danitol	fenpropathrin	8 oz/ac.	$1.01	Valent USA

	20	4EC				Corp.
Growing	2011 Mar 15	Danitol 4EC	fenpropathrin	12 oz/ac.	$1.01	Valent USA Corp.?
2011-12						
Growing	2011 Apr 28	Dibrom 8E	nayled	16 oz/ac	$0.83	AMVAC Chem. Corp.
Growing	2011 May 12	Delegate WG	spinetoram	5 oz/ac	$6.50	Dow Agrosciences
Growing	2011 June 7	Movento MPC	spirotetramat	16 oz/ac	$6.28	Bayer CropSciences
Growing	2011 July 19	Agri-flex	abamectin+ thiamethoxam	5 oz/ac	$3.40	Syngenta Crop Protection
Growing	2011 Sept 12	Dimethoate 4E	dimethoate	1 pt/ac	$0.38	BASF Corp.
Dormant	2011 Dec 7	Imidan 70 W	phosmet	0.75 lb/ac	$8.30	Gowan Co.
Dormant	2012 Feb 2	Danitol 4EC	fenpropathrin	12 oz/ac	$1.01	Valent USA Corp.

Table 3. Summary of annual number of spray applications, material cost, and total cost of insecticidal treatments.

	Spray Season[1]			
	2008-09	**2009-10**	**2010-11**	**2011-12**
Ground sprays (number)	3	3	3	5
Aerial sprays (number)[2]	1	1	2	2
Application costs (US dollars ha-yr)[3]	$62	$62	$74	$84
Material costs (US dollars /ha-yr)[4]	$184	$232	$184	$605
Total cost (US dollars /ha-yr)	$246	$294	$258	$689

Notes

[1] Spray season defined as one production cycle from end of harvest (April) through beginning of harvest the next year (March). First sprays of the trial applied in May 2008.

[2] Normally an aerial spray although a ground application was actually used because of small plot size.

[3] Application cost of ground sprays with PropTec and aerial sprays assumed to be $16.06 and $12.36 per hectare respectively.

[4] Material costs based on quantity and cost information presented in Table 2.

Table 4. Mean ± SEM cumulative insect × days for the interval between harvests. A. Main component analysis. B. Treatment effects.

A	Year Prior to Harvest			
Factor	2008-2009	2009-2010	2010-2011	2011- 2012
Insecticide	57 ± 16 b	29 ± 8 b	60 ± 16 b	34± 8 b
No Insecticide	753 ± 112 a	171 ± 33 a	312 ± 40 a	229 ± 62 a
Nutrition	378 ± 196 a	83 ± 41 a	184 ± 78 a	108 ± 46 a
No Nutrition	432 ± 208 a	117 ± 47 a	189 ± 70 a	156 ± 83 a

B	Year Prior to Harvest			
Treatment	×2008-2009	2009-2010	2010-2011	2011-2012
Insecticide+nutrition	32 ± 1 b	20 ± 9 b	50 ± 15 b	39 ± 8 b
Insecticide	83 ± 12 b	40 ± 4 b	71 ± 17 b	29 ± 8 b
Nutrition	725 ± 97 a	148 ± 34 a	318 ± 43 a	176 ± 41a
Control	782 ± 139 a	196 ± 32 a	307 ± 44 a	282 ± 74 a

*Means followed by the same letter in the same column within factors (A) or among treatments (B) are not statistically different (LSD, α = 0.05).

ANOVAS (A), 2008-2009: F = 15.05; d.f =6, 9; P<0.001 (model), P=0.97 (interaction), P=0.495 (Nutrition), P<0.001(Insecticide). 2009 – 2010: F = 5.16; d.f =6, 9; P= 0.015 (model); P=0.616 (interaction); P=0.231(Nutrition); P<0.001(Insecticide). 2010 – 2011: F = 20.81; d.f =6,9; P<0.001 (model); P=0.519 (interaction). P=0.822 (Nutrition);

P<0.001(Insecticide); 2011-2012: F = 5.44; d.f.=6,9; P<0.012 (model), P=0.180 (Interaction), P=0.001 (Nutrition), P=0.258 (Insecticide). ANOVAS (B), 2008-2009: F = 15.05; df =6, 9; P<0.001 (model), P=0.97 (interaction), P=0.495 (Insecticide), P<0.001(Nutrition), 2009 – 2010: F = 5.16; d.f =6, 9; P= 0.015(model); P=0.616 (interaction); P=0.231 (Insecticide); P<0.001 (Nutrition), 2010 – 2011: F = 20.81; d.f =6,9; P<0.001 (model); P=0.519(nteraction). P=0.822 (Insecticide); P<0.001(Nutrition); 2011-2012: F = 5.44; d.f.=6,9; P<0.012 (model), P=0.180 (interaction), P=0.001 (Nutrition), P=0.258 (Insecticide)

Table 5. Mean ± SEM Ct values for PCR analysis of leaf tissue from experimental plots. A. Main effects; B., Treatment effects

A. Main Effects

Factor	13-Nov-08	10-Apr-09	2-Sep-09	11-Jan-10	10-May-10	1-Nov-10	24-Jan-11	26-Apr-11
Insecticide	33.6 ± 0.5 a	33.4 ± 0.7	28.1 ± 0.4 a	24.4 ± 0.5 a	23.9 ± 0.3 a	24.7 ± 0.4a	26.9 ± 0.4 a	25.4 ± 0.3 a
No Insecticide	32.4 ± 0.6 a	30.9 ± 0.6	26.5 ± 0.3 b	22.9 ± 0.2 b	23.8 ± 0.2 a	25.3 ± 0.4	26.8 ± 0.3 a	25.4 ± 0.3 a
Nutrition	32.6 ± 0.5 a	31.2 ± 0.6	26.6 ± 0.3 b	22.8 ± 0.3 b	23.8 ± 0.2 a	23.4 ± 0.2	26.3 ± 0.4 a	24.7 ± 0.2 b
No Nutrition	33.5 ± 0.6 a	33.0 ± 0.8	28.1 ± 0.4 a	24.6 ± 0.5 a	23.9 ± 0.3 a	27.0 ± 0.5	26.8 ± 0.4 a	26.3 ± 0.4 a

B. Treatment Effects

Treatment	13-Nov-08	10-Apr-09	2-Sep-09	11-Jan-10	10-May-10	1-Nov-10	24-Jan-11	26-Apr-11
Insecticide-only	33.1 ± 0.9 a	32.7 ± 1.2 a	28.8 ± 0.8 a	24.9 ± 0.8 a	24.0 ± 0.5 a	26.2 ± 0.7 b	26.9 ± 0.6 a	26.7 ± 0.6 a
Insect + Nutrition	33.9 ± 0.7 a	33.9 ± 0.9 a	27.5 ± 0.5 a	24.0 ± 0.5 a	23.9 ± 0.4 a	23.6 ± 0.3 c	26.9 ± 0.5 a	24.4± 0.3 c
Nutrition-only	30.6 ± 0.7 b	29.2 ± 0.8 b	25.7 ± 0.1 b	21.8 ± 0.1 b	23.8 ± 0.1 a	23.2 ± 0.3 c	26.9 ± 0.4 a	24.9 ± 0.4 bc
Untreated	33.9 ± 0.8 a	33.1 ± 1.0 a	27.5 ± 0.5 a	24.3 ± 0.5 a	23.9 ± 0.4 a	27.7 ± 0.6 a	26.6 ± 0.5 a	25.9 ± 0.5 ab

*Means followed by the same letter within factors (A) or within columns (B) are not statistically different (LSD, $P < 0.05$). No letter after a mean in (A) indicates a significant interaction term.

ANOVAS, 6A Nov 2008: F = 5.46; d.f =6, 209; P<0.001 (model), P=0.318 (Insecticide), P<0.298 (Nutrition). Apr 2009: F = 4.95; d.f =6,138; P<0.001 (model), P=0.066(Insecticide), P=0.330 (Nutrition). Sept 2009: F = 6.40; d.f.=6,284; P<0.000(model), P<0.001(Insecticide), P<0.005 (Nutrition). Jan 2010: F = 6.92; d.f.=6,270; P<0.000 (model), P=0.016(Insecticide), P<0.001 (Nutrition). May 2010: F = 0.17; d.f.=6,286; P=0.984 (model), P=0.726(Insecticide), P=0.877 (Nutrition). Nov 2010: F = 10.71; d.f.=6,289; P<0.000(model), P=0.246(Insecticide), P<0.001 (Nutrition). January 2011: F = 0.84; d.f.=6,283; P=0.541(model), P=0.764(Insecticide), P=0.859 (Nutrition). April 2011: F=4.15; d.f.=6,288; P<0.001(model), P=00.675(Insecticide), P<0.001 (Nutrition).

ANOVAS,6B Nov 2008: F = 5.46; d.f =6, 209; P<0.001 (model), P=0.322(Insecticide), P=0.0.993(Insecticide+Nutrition), P<0.015 (Nutrition). Apr 2009: F = 4.07; d.f =6,155; P<0.001 (model); P=0.349 (Insecticide). P=0.435(Insecticide+Nutrition); P=0.004(Nutrition); Aug 2009: F = 6.82; d.f.=6,297; P<0.000(model), P=0.033 (Insecticide), P=0.584 (Insecticide+Nutrition), P=0.0.005 (Nutrition); Jan 2010: F = 6.92; d.f.=6,270; P<0.000 (model), P=0.601 (Insecticide), P<0.000 (Insecticide+Nutrition), P=0.000 (Nutrition); May 2010: F = 0.17; d.f.=6,286; P=0.984 (model), P=0.807(Insecticide), P=0.887 (Insecticide+Nutrition), P=0.9160.258 (Nutrition); Nov 2010: F = 10.71; d.f.=6,289; P<0.000(model), P=0.029 (Insecticide), P=0.000 (Insecticide+Nutrition), P=0.000 (Nutrition); January 2011: F = 0.84; d.f.=6,283; P=0.541(model),P=0.718 (Insecticide), P=0.730 (Insecticide+Nutrition), P=0.764 (Nutrition); April 2011: F=4.15; d.f.=6,288; P<0.001(model), P=0.187 (Insecticide), P=0.017 (Insecticide+Nutrition), P=0.105 (Nutrition).

Table 6. Yield of oranges in kg per tree for each of 4 harvests and the sum of all 4 harvests.

A.

B. Treatment Effects

	2009	2010	2011	2012	4 years combined
Insecticide+nutrition	54.1 ± 6.4 a	46.2 ± 4.6 a	46.4 ± 3.1 a	90.6 ± 1.8 a	237.3 ± 12.3 a
Nutrition-only	40.4 ± 8.1 b	28.0 ± 4.0 b	25.5 ± 2.9 c	82.7 ± 3.4 ab	176.9 ± 17.0 b
Insecticide-only	38.9 ± 7.8 b	42.7 ± 3.1 a	41.6 ± 5.9 ab	77.8 ± 0.5 b	201.4 ± 16.4 b
Untreated	40.4 ± 2.9 b	37.1 ± 7.2 ab	32.1 ± 6.3 bc	66.7 ± 2.44 c	176.7 ± 15.4 b
Effective tree age[1]	7	8	9	10	
Average SWFla Production (kg/tree)[2]	108	106	115	97	

Means within factors (A) or among treatments (B) followed by the same letter are not significantly different (LSD, $P > 0.05$).

ANOVAS (A), Kg per Tree Harvest 2009: F = 4.85; d.f =6, 9; P<0.018(model), P=0.103 (interaction); P=0.1080 (Nutrition); P<0184(Insecticide). 2010: F = 4.61; d.f =6, 9; P= 0.021(model); P=0.116(interaction);, P=0.455(Nutrition); P<0.010(Insecticide); 2011: F = 4.91; d.f =6,9; P<0.017 (model);, P=0.177(interaction). , P=0.773 (Nutrition); P=0.003(Insecticide). 2012: F = 7.63; d.f.=6,9; P< 0.004 (model); P=0.540 (Interaction); P=0.000 (Nutrition); P=0.005 (Insecticide). ANOVAS (B) Kg per Tree Harvest 2009: F = 4.85; d.f =6, 9; P<0.018(model), P=0.049 (Insecticide+Nutrition); P=0.983 (Nutrition); P=0.797 (Insecticide). 2010: F = 4.61; d.f =6, 9; P= 0.021(model); P=0.114 (Insecticide+Nutrition); P< 0.109(Nutrition); P=0.311 (Insecticide). 2011: F = 4.91; d.f =6,9; P<0.017 (model);, P=0.028 (Insecticide+Nutrition); P<0.245(Nutrition); P=0.107(Insecticide). 2012: F = 7.63; d.f.=6,9; P< 0.004 (model); P=0.000 (Insecticide+Nutrition); P<0.058(Nutrition); P=0.013 (Insecticide).

[1] Study block planted in June 2001. The block was defoliated in 2004 in an attempt to eliminate citrus canker. Thus effective age of the study block when the trial was initiated was estimated to be 6 years.

[2] Average fruit production (kg/tree) in southwest Florida by tree age for ‘Valencia’ on Swingle planted at 381 trees per hectare reported in Roka, Rouse, and Muraro, 2000.

Table 7. Juice quality of randomly chosen fruit sampled from harvest bins 2010 and 2012. A. Main Effects. B. Treatment Effects

A. Main Effects

Year	Factor/ Treatment	Juice (Kg/Box)	Solids (Kg/box)	Acid (% w/w)	Brix (TSS)	Ratio
2010	Insecticide	22.31 ± 0.35 a	2.40 ± 0.04	0.58 ± 0.02	10.71 ± 0.16 a	18.62 ± 0.6 a
	No Insecticide	22.91 ± 0.33 a	2.32 ± 0.08	0.62 ± 0.02	10.58 ± 0.27 a	17.12 ± 0.22 b
	Nutrition	22.10 ± 0.34 a	2.29 ± 0.06	0.57 ± 0.02	10.38 ± 0.20 a	18.29 ± 0.60 a
	No Nutrition	23.12 ± 0.21 a	2.43 ± 0.06	0.63 ± 0.02	10.98 ± 0.19 a	17.45 ± 0.33 a
2012	Insecticide	24.43 ± 0.35 a	2.64 ± 0.06 a	0.64 ± 0.02 b	10.81 ± 0.15 b	17.08 ± 0.37 a
	No Insecticide	24.47 ± 0.17 a	2.74 ± 0.05 a	0.69 ± 0.02 a	11.18 ± 0.16 a	16.34 ± 0.35 a
	Nutrition	24.25 ± 0.33 a	2.70 ± 0.06 a	0.66 ± 0.02 a	11.13 ± 0.16 a	16.92 ± 0.43 a
	No Nutrition	24.65 ± 0.19 a	2.68 ± 0.04 a	0.66 ± 0.02 a	10.87 ± 0.15 a	16.50 ± 0.29 a

B. Treatment Effects

Year	Treatment	Juice (Kg/Box)	Solids (Kg/box)	Acid (%)	Brix (TSS)	Ratio
2010	Insecticide+nutrition	22.7 ± 0.60 a	2.44 ± 0.02 a	0.57 ± 0.03 b	10.8± 0.23 ab	19.5 ± 1.13 a
	Nutrition	21.5 ± 0.06 a	2.15 ± 0.04 b	0.57 ± 0.01 b	10.0 ± 0.18 b	17.5 ± 0.16 ab
	Insecticide	21.9 ± 0.26 a	2.36 ± 0.07 a	0.59 ± 0.01 b	10.8 ± 0.24 ab	18.2 ± 0.2 ab
	Untreated	22.3 ± 0.31 a	2.50 ± 0.08 a	0.67 ± 0.03 a	11.2 ± 0.29 a	16.7 ± 0.28 b
2012	Insecticide+nutrition	24.2 ± 0.67 a	2.63 ± 0.10 a	0.6 ± 0.00 a	10.85 ± 0.21 a	17.4 ± 0.7 a
	Nutrition	24.3 ± 0.16 a	2.78 ± 0.51 a	0.7 ± 0.00 a	11.40 ± 0.20 b	16.4 ± 0.47 a
	Insecticide	24.7 ± 0.24 a	2.66 ± 0.05 a	0.6 ± 0.00 a	10.77 ± 0.22 a	16.8 ± 0.22 a
	Untreated	24.6 ± 0.30 a	2.70 ± 0.08 a	0.7 ± 0.00 a	10.96 ± 0.22 ab	16.3 ± 0.55 a

*Means followed by the same letter within factors (A) or within columns (B) are not statistically different (LSD, $P < 0.05$). No letter after a mean in (A) indicates a significant interaction term.

ANOVAS: 7a, 2010, Kg Juice per Box; F = 4.63; d.f. 6,9; P = 0.020 (model); 0.0003 (Interaction); P = 0.037 (Nutritional); P = 0.19 (Insecticide); Acid; F = 5.36; P = 0.013 (model); P = 0.050 (Interaction); P = 0.006 (Nutritional); P = 0.050 (Insecticide); Brix: F = 1.73; d.f. 6,9; P = 0.221; P = 0.059 (Interaction); P = 0.053 (Nutritional); P = 0.50 (Insecticide); Ratio: F = 3.24; d.f. 6,9; P = 0.055 (model); P = 0.889 (Interaction); P = 0.13 (Nutritional); P = 0.016 (Insecticide); Kg Solids Per Box: F = 4.63; P = 0.020 (model); P = 0.003 (Interaction); P = 0.037 (Nutritional); P = 0.187 (Insecticide); 2012: Kg Juice per Box: F = 0.56; d.f.=6,25; P = 0.760 (model); P=0.695 (interaction); P=0.34 (Nutritional); P=0.933 (Insecticide); Acid: F = 3.89; P= 0.007 (model); P = 0.448 (Interaction); P = 0.899 (Nutritional); P = 0.014 (Insecticide); Brix: F = 4.70, P = 0.0025 (model); P = 0.104 (Interaction); P = 0.13 (Nutritional); P = 0.036 (Insecticide); Ratio: F = 2.00; P=0.10 (model); P = 0.441 (Nutritional); P = 0.133 (Insecticide); Kg Solids per Box: F = 1.92; P = 0.12 (model); P = 0.74 (Nutritional); P = 0.74 (Insecticide);
7B: 2010 Kg Juice per Box: F=3.32, P=0.052, df=6,9 (model); F=3.48, P=0.064 (Treatment); Acid: F=5.4, P=0.013 (model); F= 7.68, P = 0.008 (Treatment), Brix: F=1.73, P = 0.22 (model), F= 3.38, P=0.068 (Treatment); Ratio: F=3.24, P=0.56, (model), F=0.38, P=0.050 (Treatment), KgSoBox F=4.63, P = 0.20 (model), F=7.86, P = 0.007 (Treatment). 2012: Kg Juice per Box: F=0.56, P=0.76, df=6,25 (model); F=0.38, P= 0.77 (Treatment); Acid: F=3.9, P=0.007 (model); F= 7.68, P = 0.008 (Treatment), Brix: F=4.7, P = 0.003 (model), F=2.83, P=0.058 (Treatment); Ratio: F=2.0, P=0.10, (model), F=.16, P=0.35 (Treatment), KgSoBox F=1.92, P = 0.12 (model), F=0.95, P = 0.43 (Treatment

Table 8. Net change in production (kg.s./ha), revenue ($/ha) for three treatments delivered-in fruit prices, and cost for enhanced foliar nutrition (EFN) = systemic acquired resistance (EFN+SAR) or EFN alone by treatment during 2011-12 season.

Treatment	Production Total (kg.s./ha)	Production Gains (kg.s./ha)	Added Revenue ($/ha)			Added Cost($/ha)
			$3.85/kg.s. ($1.75/p.s.)	$3.30/kg.s. ($1.50/p.s.)	$2.75/kg.s. ($1.25/p.s.)	Insect +SAR
Untreated	1,642	-	-	-	-	$0
Insecticide	1,887	245	$943	$809	$674	$689[1]
Nutrition	2,097	425	$1,636	$1,403	$1,169	$1,588
Insecticide+nutrition	2,173	531	$2,044	$1,752	$1,460	$2,229[2]

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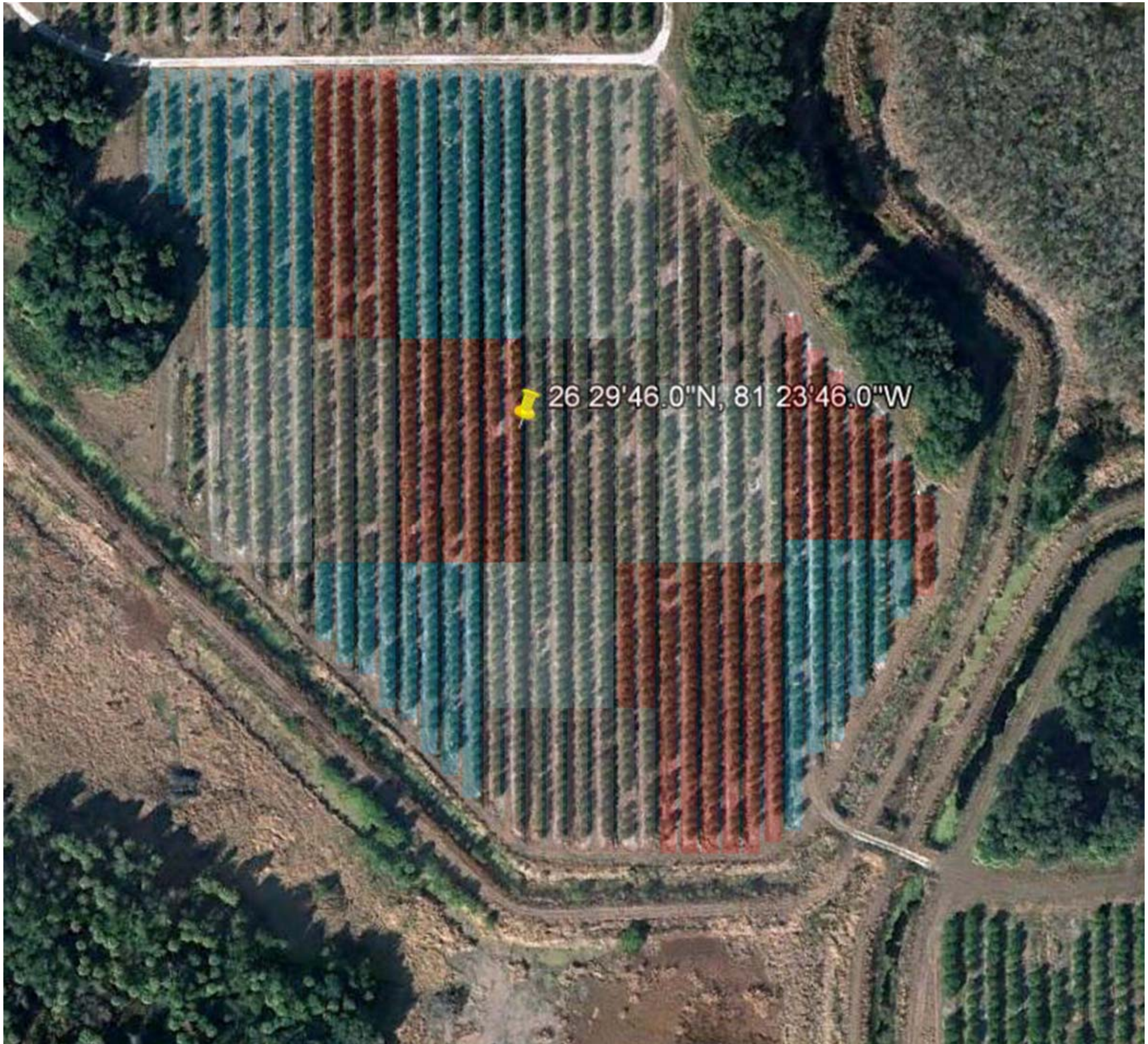
[1] Cost of insecticides only plus application.

[2] When insecticide treatment combined with nutritional treatment, insecticide materials are tanked-mixed during the 3 nutritional applications and thereby saves $48/ha ($16/app-ha x 3 app, see Table 3) in application costs.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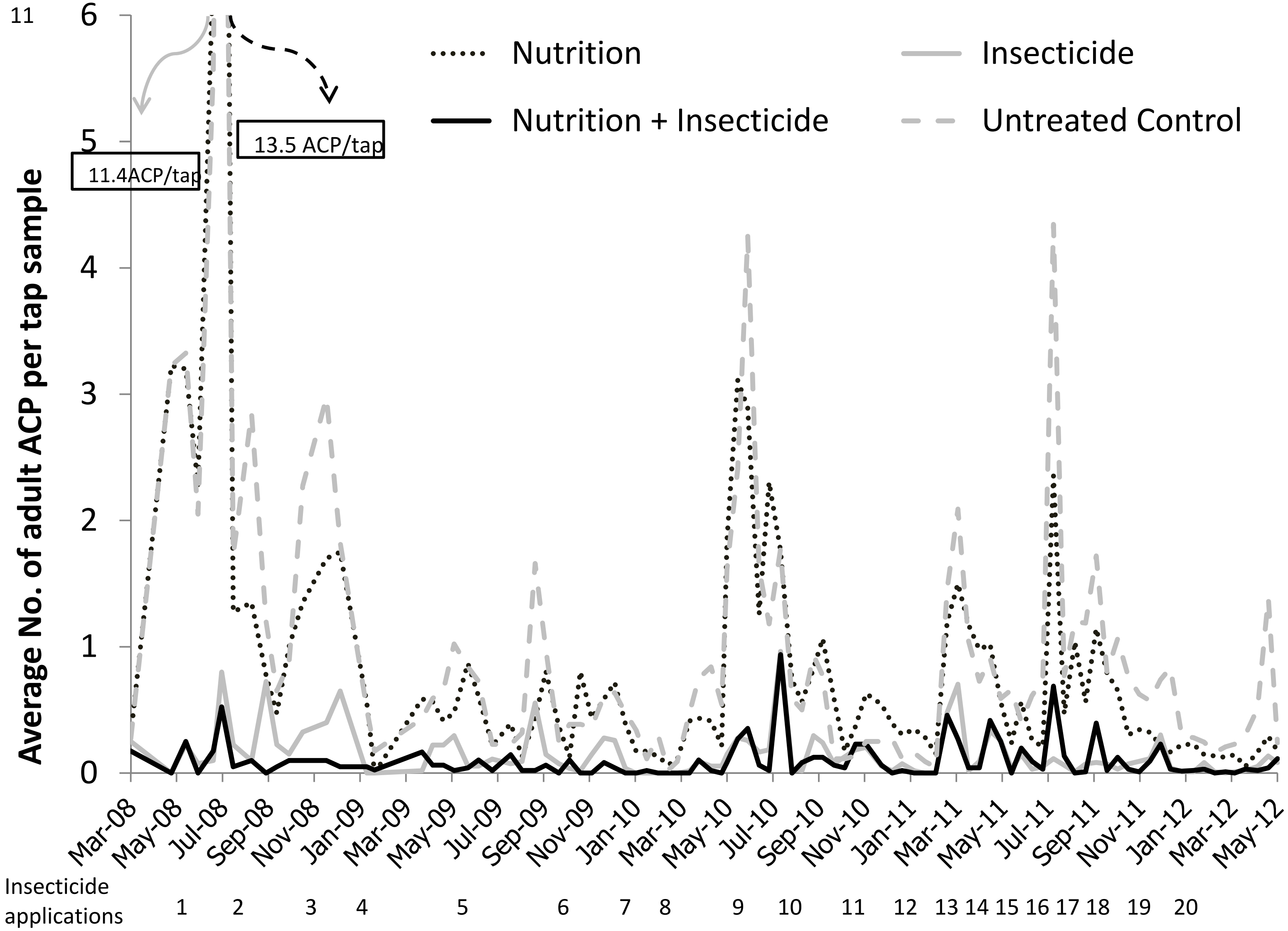

Average No. of adult ACP per tap sample
0
1
2
3
4
5
6
11.4ACP/tap
13.5 ACP/tap
Nutrition
Insecticide
Nutrition + Insecticide
Untreated Control
Mar-08
May-08
Jul-08
Sep-08
Nov-08
Jan-09
Mar-09
May-09
Jul-09
Sep-09
Nov-09
Jan-10
Mar-10
May-10
Jul-10
Sep-10
Nov-10
Jan-11
Mar-11
May-11
Jul-11
Sep-11
Nov-11
Jan-12
Mar-12
May-12
Insecticide applications
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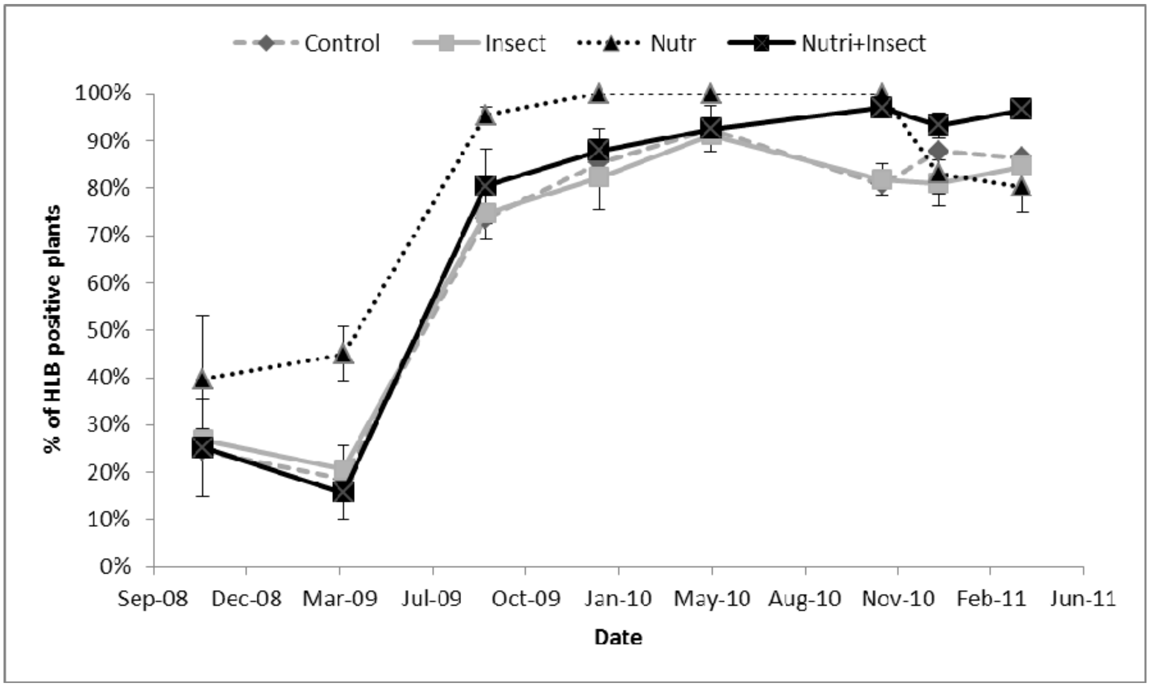
Control
Insect
Nutr
Nutri+Insect
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% of HLB positive plants
Sep-08
Dec-08
Mar-09
Jul-09
Oct-09
Jan-10
May-10
Aug-10
Nov-10
Feb-11
Jun-11
Date