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Dormant season foliar sprays of broad-spectrum insecticides: An effective component of integrated management for *Diaphorina citri* (Hemiptera: Psyllidae) in citrus orchards

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A B S T R A C T

Diaphorina citri Kuwayama (Hemiptera: Psyllidae), is a global pest of citrus and vector of *Candidatus* Liberibacter, a bacteria that causes huanglongbing or greening, a devastating disease of citrus. Mature citrus trees are dormant in winter and produce most new shoots in spring, followed by sporadic canopy growth in summer and fall. Young shoots are required for oviposition and nymphal development, but adults can survive and overwinter on hardened leaves. Surviving adults reproduce in spring shoots and their progeny are probably responsible for a large portion of disease spread as they disperse to search for food. Therefore, foliar sprays of broad-spectrum insecticides applied to mature trees in winter were evaluated in a commercial citrus orchard as tactic to reduce pest populations and insecticide use in spring and summer when beneficial insects are most active. A single spray of chlorpyrifos (2.8 kg a.i. ha^{-1}) in January 2007 reduced adult psyllids an average of 10-fold over six months compared to untreated trees. The following year, differences with the untreated control averaged 15-fold for over five months following a single spray of chlorpyrifos, fenpropathrin (0.34 kg a.i. ha^{-1}), or oxamyl (1.12 kg a.i. ha^{-1}) applied in January. Spiders, lacewings and ladybeetles were equally abundant during the growing season in both treated and untreated trees both years ($P = 0.05$). Thus foliar sprays of broad-spectrum insecticides before spring growth suppressed *D. citri* for five to six months, with no detectible impact on key natural enemies. This tactic has been widely adopted to control the psyllid in Florida, in some cases area-wide. Additional sprays during the growing season should be based on scouting and targeted at adults before anticipated new flush.

1. Introduction

Diaphorina citri Kuwayama (Hemiptera: Psyllidae), also known as the Asian citrus psyllid (ACP), is an economically important insect pest in many citrus-growing regions of the world (Halbert and Manjunath, 2004). A different species, *Trioza erytreae* (Del Guercio), is present in South Africa and some neighboring countries with only the Mediterranean and Australia still psyllid free among major citrus-growing regions. Feeding by *D. citri* nymphs and adults on young citrus shoots causes reduced and distorted leaf development. Of much greater concern is the ability of the pest to transmit phloem-limited bacteria of genus *Candidatus* Liberibacter, the causal organism of huanglongbing (HLB) also known as citrus greening disease (Garnier et al., 2000; Halbert and Manjunath, 2004; Bové, 2006). HLB is one of the world's most devastating diseases of citrus, responsible for the decline of most trees in the disease infected regions (Roistacher, 1996; Halbert and Manjunath, 2004; Bové, 2006).

In the United States, *D. citri* was first discovered in Palm Beach County, Florida on hedges of orange jasmine, *Murraya paniculata* (L.) Jack. (Rutaceae) in 1998 (Halbert, 1998). The psyllid is now well established in citrus producing regions of the state (Michaud, 2002; Tsai et al., 2002; Halbert and Manjunath, 2004; Qureshi et al., 2009) and is also present in Texas, Louisiana, Mississippi, Georgia, South Carolina, Hawaii and southern California (French et al., 2001; FDQO-CG-ACP, 2008). First identification in the USA of the Asian form of citrus greening disease caused by the bacterium *Candidatus* Liberibacter asiaticus, was in south Miami Dade during August 2005 (Halbert, 2005). The disease now occurs throughout the state with highest incidence in the east coast and southwest regions and threatens a citrus industry valued at $1.3 billion in direct sales alone (Anonymous, 2008; FDACS-DPI, 2008). HLB was also identified from Louisiana in 2008 and from South Carolina and Georgia in 2009 (http://www.aphis.usda.gov/plant_health/plant_pest_info/

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citrus_greening/index.shtml, September 12, 2009). Other than plant movement by people, *D. citri* is the primary cause of the spread of the citrus greening disease (Halbert and Manjunath, 2004); therefore, effective and sustainable pest and disease management strategies are required to reduce spread.

Insecticidal control of the vector along with removal of HLB infected trees are the tactics being employed to slow the spread of HLB in Florida citrus orchards (Qureshi and Stansly, 2007, 2008; Rogers et al., 2008; Brlansky et al., 2009). However, biological control has always been an important component of citrus insect pest management in Florida (McCoy, 1985) including *D. citri*. Lady-beetles, lacewings and spiders are all well-known predators of citrus psyllids (Van den Berg et al., 1992; Shivankar et al., 2000; Michaud, 2002, 2004; Qureshi and Stansly, 2008, 2009). These and other predators were observed to inflict 80–100% mortality to *D. citri* immatures and were abundant during spring and summer, though largely absent during winter in concert with citrus growth patterns and psyllid abundance (Michaud, 2004; Qureshi and Stansly, 2007, 2009). *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae), a species-specific ecto-parasitoid of *D. citri* that was imported from Taiwan and southern Vietnam, is well established in Florida. However, parasitism rates in Florida are variable and appear to be generally low compared to other regions where intentional or accidental introduction of the parasitoid occurred (Étienne and Aubert, 1980; Étienne et al., 2001; Pluke et al., 2008; Qureshi et al., 2009). While augmentation of natural enemy populations remains an option for the future, biological control alone has not been sufficient to halt the spread of greening in Florida. Nevertheless, natural enemies provide significant mortality to psyllid populations and warrant conservation (Qureshi and Stansly, 2009).

The advent of HLB has greatly intensified insecticide use in Florida citrus (Rogers, 2008; Rogers et al., 2008). The systemic insecticides, thiamethoxam and imidacloprid are allowed in Florida citrus but are subject to rate restrictions that limit their use to young trees. Aldicarb is also labeled for use in Florida citrus during the dry season from 15th November through April, but effectiveness is sometimes compromised by lack of rain required for activation of the insecticide (Qureshi and Stansly, 2008). Therefore, foliar insecticides are required to suppress *D. citri* in most Florida citrus. The inability of foliar sprays to provide anything but short-term control of psyllid when directed against immature stages on new shoots is amply documented (Qureshi and Stansly, 2007; Stansly et al., 2008b,c,d). This is probably due to the protection afforded to eggs and young nymphs by the tightly appressed emergent leaves of young shoots (= flush) which are nevertheless attractive to a diverse and effective fauna of natural enemies of the psyllid (Qureshi and Stansly, 2007, 2008, 2009). Therefore, application to flushing trees is generally contraindicated.

Reproduction of *D. citri* is totally dependent on availability of young shoots containing feather stage to recently expanded tender leaves. Female psyllids must feed on tender shoots to mature eggs and prefer opening buds and shoots for oviposition. During the following 2–3 weeks, shoot and leaf tissues are still tender and are utilized by nymphs and newly emerged adults respectively to complete development and mature eggs. Adults can also feed and survive on the fully developed leaves for several months (Tsai et al., 2002; Qureshi and Stansly, 2008). Flush production is influenced by weather, plant age and variety (Knapp et al., 1995). However, the typical pattern of shoot production in mature citrus trees in Florida is comprised of a growing season starting with a major flush in late winter and early spring, a relatively small flush in the early summer and some minor flushes during late summer and fall, followed by a dormant winter season in late fall and early winter with little or no new foliage growth (Hall and Albrigo, 2007; Qureshi et al., 2009).

Over-wintering psyllid adults survive by feeding on mature leaves until the appearance of spring flush to commence oviposition. There is little or no flush or suitable host to attract psyllid natural enemies in winter and most parasitoids are protected inside their hosts. However, if allowed to enter the spring flush without insecticidal intervention, survival of the first generations of *D. citri* is high due to abundance of flush that not only provides unlimited food but also cover from natural enemies (Qureshi and Stansly, 2009). Highest numbers of *D. citri* adults testing positive for *Ca.* Liberibacter asiaticus were caught in suction traps in April soon after spring flush (Manjunath et al., 2008). Therefore, over-wintering populations of *D. citri* should be suppressed to reduce the spread of psyllid and citrus greening in the spring and afterward.

Many of the insect and mite pests found in Florida citrus groves are under biological control where they merely co-exist with other consistently injurious species (McCoy, 1985; McCoy et al., 2009). Therefore, repeated sprays of broad-spectrum insecticides during spring and summer (Rogers, 2008) when most pests and beneficial insects are common will likely select for resistance and result in secondary pest outbreaks. Therefore, it would be beneficial to confine broad-spectrum insecticides to the dormant winter season as much as possible. In this two year study, the impact of foliar sprays of some currently recommended broad spectrum insecticides applied to citrus trees during the dormant winter period was evaluated by monitoring the populations of *D. citri* and its natural enemies during the following spring and summer. Our objective was to provide proof of concept for the dormant spray tactic to control ACP, not to compare the efficacy of currently available insecticides per se, which has been well documented elsewhere (Qureshi and Stansly, 2007; Stansly et al., 2008a,b,c,d).

2. Materials and methods

Experiments in both years were conducted in a commercial citrus grove of Barron Collier Company in Immokalee, FL in two equal size adjacent blocks of 27 ha each planted in 1982 with 'Hamlin' orange trees originally on 'sour orange' rootstock at a density of 373 trees ha^{-1} on double-row raised beds. Trees subsequently lost to citrus tristeza disease had been replaced by the same variety grafted to 'Carrizo' citrange rootstock.

2.1. 2007

Each of the two 27 ha blocks of citrus was considered a replicate. Within each block, two side by side 6.1 ha plots, were randomly assigned to either the spray application treatment or the untreated control. Treatment plots were sprayed on 15th January with chlorpyrifos 2.8 kg a.i. ha^{-1} (Lorsban 4 E, Dow AgroSciences LLC, Indianapolis, IN) using a tractor mounted Air-O-Fan air blast sprayer operating at a pressure of 1241 kPa with 8 Albuz, blue nozzles per side delivering a medium quality spray at 936 L ha^{-1}. No other insecticides were applied anywhere in the blocks through August. Applications of broadcast fertilizer were made in January, March and October. Standard cultural practices were followed uniformly throughout the grove.

Flush density and populations of *D. citri* and generalist predators were monitored on 22nd January, 8th and 29th March, 12th and 25th April, 29th May, 22nd June, 24th July and 27th August. Bedsides of the four trees at each of eight non-systematically selected locations in treated and untreated plots were sampled. Shoot density was estimated by using a quadrat frame made from PVC pipe to sample a volume of 39 dm^3 (36 × 36 × 30 cm) of tree canopy (Qureshi et al., 2009). The frame was placed at a non-systematically chosen location in the outer tree canopy about 1–2 m above ground and shoots containing feather stage to recently expanded leaves were counted to a depth of 30 cm. Density of *D. citri* adults and predators was estimated from a "tap" sample made by counting the number falling on a 22 × 28 cm laminated white sheet held on a clipboard horizontally under non-systematically selected branches which were tapped three times by hand (Qureshi and Stansly, 2007). Depending upon availability, 10 or fewer non-systematically selected shoots were examined per tree and the number infested with eggs or nymphs of *D. citri* was recorded to estimate infestation rates. Eight to 10 shoots tree^{-1} were examined on average, except on 12th April when shoot density was estimated at only one per frame. Shoots were even scarcer on 27th August and not sampled. On other dates, one of the infested shoots from each tree was collected and examined in the laboratory under a stereoscopic microscope to count the eggs and nymphs of *D. citri*.

2.2. 2008

The total area of 54 ha was divided into 24 plots of 2–3 ha, each containing seven-beds and 14 tree rows. Plots were assigned randomly to five treatments and an untreated control using a randomized complete block design with 4 replications. The additional treatments were included to determine whether broad-spectrum insecticides in 3 different chemical classes (organophosphate, carbamate and pyrethroid) could be used as dormant sprays and also whether two dormant sprays were more effective than a single spray. Procedures for spray applications were the same as described above. Sprays applied in designated plots on 16–17th January included treatments of the carbamate oxamyl at 0.56 and 1.12 kg a.i. ha^{-1} (Vydate 2 L, DuPont Company, Dupont Crop Protection, Newark, DE), the organo-phosphate chlorpyrifos at 2.8 kg a.i. ha^{-1} (Lorsban 4 E, Dow AgroSciences LLC, Indianapolis, IN) and the pyrethroid fenpropathrin at 0.34 kg a.i ha^{-1} (Danitol 2.4 EC, Valent USA Corporation, Walnut Creek, CA). Fenpropathrin was applied to two plots in each replicate, one of which received a second application of chlorpyrifos at 2.8 kg a.i. ha^{-1} on 12th February. No other insecticides were applied anywhere in the blocks through July. Applications of broadcast fertilizer were made in January, April and October. Standard cultural practices were followed uniformly throughout the grove.

Flush and *D. citri* populations were monitored on 7th February, 10th March, 10th April, 11th May, 4th June and 1st July. Three central beds per plot were used and four trees sampled at each of the two non-systematically selected locations per bed for a total of 24 trees plot^{-1}. The same procedure was used as in the 2007 experiment except that nymphal density was rated in the field on a scale of 0–5: 0 = none, 1 = 1–10, 2 = 11–20, 3 = 21–30, 4 = 31–40 and 5 = 41+ nymphs per shoot. An average of 6 (10th March), 6 (10th April), 5 (11th May), 9 (4th June) and 7 (1st July) shoots per tree were examined to estimate infestation rates. Shoots were very scarce and not sampled on 7th February.

In addition to the designed experiments at the commercial grove, several blocks of different citrus varieties were monitored that had been treated in January of 2007 and 2008 at the experimental orchard of the Southwest Florida Research and Education Center (SWFREC), Immokalee, FL (Table 1). The objective was to control psyllids and not to conduct a replicated trial, although one block was left untreated to compare with dormant season treated blocks for psyllid populations. These blocks contained 12–13 yr old trees planted at a density of 373 per hectare on double-row raised beds. Blocks, area, varieties and application rates of insecticides are given in Table 1. Foliar applications were made using a tractor mounted Durand Wayland 3P-10C-32 air blast sprayer (Qureshi and Stansly, 2007). Aldicarb (Temik 15 G, Bayer CropScience LP, Research Triangle Park, NC) a systemic insecticide was applied in the soil using a modified Gandy granular applicator (Qureshi and Stansly, 2008). Applications of broadcast fertilizer and KeyPlex 445 a formulation of micronutrients were made in April and June, respectively. Although, treated and untreated blocks were monitored monthly over a two-year period, data are presented only through May due to subsequent insecticide applications made in some blocks for various reasons. Standard cultural practices were followed uniformly throughout the grove.

Four trees were sampled at each of the eight non-systematically selected locations per block. Procedures were similar to the ones described for the commercial grove experiment 2007 except that tap samples were increased from one to four per tree, one each on the north, south, east and west sides between December 2007 and December 2008 due to the low populations of *D. citri*. Only adult data are presented.

2.3. Statistical analysis

Estimates of adults per tap sample and nymphs per 39 dm^3 canopy volume were analyzed. Nymphs per 39 dm^3 were obtained by multiplying mean shoot density per sample by the proportion of shoots infested and the number of nymphs per shoot (Hall et al., 2008; Pluke et al., 2008). Average nymphal density per shoot represented by each rating scale was used for 2008 data. There were only two cases when nymphal density was rated 5 for which no range was specified so an arbitrary value of 50 was used.

The Shaprio Wilk *W* test ($P = 0.05$) and normality plots were used to test data for assumptions for parametric analysis using the univariate procedure (Shapiro and Wilk, 1965; Shapiro et al., 1968; SAS Institute, 2004). Data did not conform to these assumptions despite appropriate transformations used to reduce heterogeneity of variance. Therefore, the nonparametric Kruskal–Wallis test was used to analyze treatment effects, followed by pairwise treatment comparisons made with the Mann–Whitney *U*-test at a significance level of 0.05 (Hollander and Wolfe, 1973; SPSS, 2004). Actual means and their standard error (SE) are presented for all data. No statistical analysis was conducted of data from SWFREC which was unreplicated.

3. Results

3.1. Designed experiments

3.1.1. 2007

The single spray of chlorpyrifos made to citrus trees in January resulted in significantly fewer *D. citri* adults in the treated trees than untreated trees on all sampling dates through July with overall means of 0.1 (SE = 0.02) and 1 (SE = 0.07) per tap sample, respectively ($H = 144$, $df = 1$, $P < 0.0001$, Fig. 1A). No adults were observed in the tap samples conducted in the treated trees compared to a mean of 0.3 (SE = 0.1) per tap sample in the untreated trees through 12th April (22nd January: $H = 4.1$, $df = 1$, $P = 0.043$; 8th March: $H = 8.5$, $df = 1$, $P = 0.004$; 29th March: $H = 13.1$, $df = 1$, $P = 0.0003$; 12th April: $H = 28.9$, $df = 1$, $P < 0.0001$; Fig. 1A). On 25th April, 46-fold fewer adults were observed in treated trees compared to untreated trees ($H = 40.4$, $df = 1$, $P < 0.0001$). Adult density peaked at 3.06 per tap sample in untreated trees on 29th May, 11-fold more than treated trees ($H = 48.6$, $df = 1$, $P < 0.0001$). Numbers declined consistently after that in both treated and untreated trees. However, a significant difference of 8 and 3-fold in adult numbers was still observed between treated and untreated trees on 22 June ($H = 38.4$, $df = 1$, $P < 0.0001$) and 24 July ($H = 13.9$, $df = 1$, $P = 0.0002$), respectively. Adults averaged <1 per tap sample on 27th August and did not differ between treated and untreated trees ($H = 0.527$, $df = 1$, $P = 0.468$).

Table 1
Mean (SEM) number of *Diaphorina citri* adults per tap sample on dormant season treated and untreated citrus trees at SWFREC grove, Immokalee, FL 2007–2008.

Blocks (No.)	Area (ha)	Varieties[a]	Dormant Season Treatments Jan. (07–08)	Rate (Kg/ha)	Application method	*Diaphorina citri* adults per tap sample					
						2007			2008		
						March	April	May	March	April	May
1	2	Valencia	Untreated		None	0.19 (0.11)	0.28 (0.10)	1.94 (0.30)	0.45 (0.09)	0.13 (0.04)	0.16 (0.07)
1	1.8	Valencia	Fenpropathrin	0.34	Spray	0.00 (0.00)	0.00 (0.00)	0.03 (0.03)	0.00 (0.00)	0.05 (0.02)	0.04 (0.03)
2	2	Valencia + Grapefruit	Fenpropathrin + Aldicarb	0.34 + 5.6	Spray + Soil	0.00 (0.00)	0.00 (0.00)	0.02 (0.02)	0.00 (0.00)	0.00 (0.00)	0.04 (0.01)
4	9.3	Hamlin + Valencia + Grapefruit	Chlorpyrifos + Aldicarb	2.8 + 5.6	Spray + Soil	0.00 (0.00)	0.00 (0.00)	0.02 (0.02)	0.00 (0.00)	0.01 (0.00)	0.02 (0.01)
2	2.2	Grapefruit + Navel	Aldicarb	5.6	Soil	0.00 (0.00)	0.00 (0.00)	0.05 (0.03)	0.00 (0.00)	0.02 (0.01)	0.00 (0.00)

[a] Varieties planted in separate blocks.

Overall nymphal density was lower on the treated trees compared to untreated trees, averaging 20 (SE = 3) and 67 (SE = 9) nymphs per 39 dm^3, respectively ($H = 39.58$, $df = 1$, $P < 0.0001$, Fig. 1B). Nymphal density differed between treated and untreated trees on four samplings conducted in spring (8th March: $H = 13.5$, $df = 1$, $P = 0.0002$; 29th March: $H = 24.1$, $df = 1$, $P < 0.0001$; 12th April: $H = 28.9$, $df = 1$, $P < 0.0001$; 25th April: $H = 25.4$, $df = 1$, $P < 0.0001$; Fig. 1B) and averaged only 0.1 (SE = 0.1) nymphs per 39 dm^3 in treated trees compared to 39 (SE = 23) per 39 dm^3 in untreated trees. The greatest reduction, 335-fold, was seen on 25th April. Most nymphs were seen on 24th July when they averaged 67 (SE = 12) and 212 (SE = 45) per 39 dm^3 in treated and untreated trees, respectively, still significantly different between the two treatments ($H = 11.8$, $df = 1$, $P = 0.0006$). Differences were not significant on 29th May and 22nd June ($H = 1.9$, $df = 1$, $P = 0.163$ and $H = 1.9$, $df = 1$, $P = 0.163$, respectively), nor on 22nd January ($H = 0.1$, $df = 1$, $P = 0.820$) one week after treatment.

Ladybeetles [*Olla v-nigrum* (Mulsant), *Curinus coeruleus* (Mulsant), *Harmonia axyridis* (Pallas) and *Cycloneda sanguinea* (L.)] and lacewings (*Ceraeochrysa* sp. and *Chrysoperla* sp.) were equally abundant in treated and untreated trees over all sample dates ($H = 1.86$, $df = 1$, $P = 0.17$ and $H = 0.17$, $df = 1$, $P = 0.68$, respectively) and on respective dates [$H \leq 3$, $df = 1$, $P > 0.05$ for ladybeetles comparisons between treated and untreated trees on individual dates (Fig. 2A); $H \leq 2$, $df = 1$, $P > 0.05$ for lacewings comparisons between treated and untreated trees on individual dates (Fig. 2C)]. Spiders were more abundant in untreated trees on January 22nd

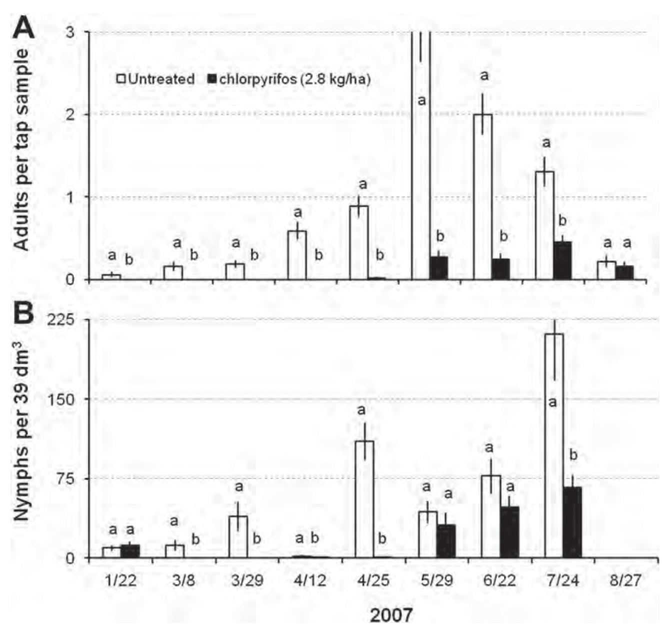

Fig. 1. Mean (SEM) number of *Diaphorina citri* adults per tap sample (A) and nymphs per 39 dm^3 of tree canopy (B) in Hamlin orange trees untreated and treated with a foliar spray of chlorpyrifos on 15th January 2007 at a commercial grove in Immokalee, FL. No other insecticides were used in the experimental blocks through August. Shoots were scarce and not sampled on 27th August to estimate nymphal density. Treated and untreated trees represented by columns with same letter were not significantly different (Kruskal–Wallis test, $P > 0.05$).

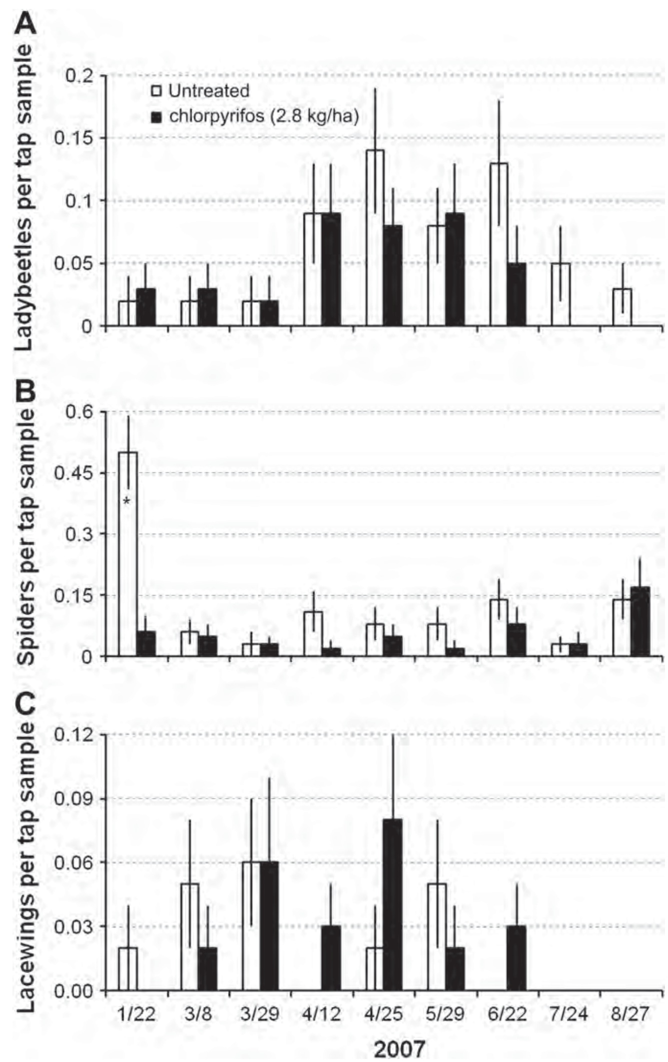

Fig. 2. Mean (SEM) number of ladybeetles (A), spiders (B) and lacewings (C) per tap sample in Hamlin orange trees untreated and treated with a foliar spray of chlorpyrifos on 15th January 2007 at a commercial grove in Immokalee, FL. No other insecticides were used in the experimental blocks through August. Treated and untreated trees did not differ for any of the three predatory groups (Kruskal–Wallis test, $P > 0.05$), except spiders on January 22nd (* = significantly different, $P < 0.05$).

soon after treatment ($H = 21.52$, $df = 1$, $P < 0.0001$) but were equally abundant in the treated and untreated trees afterward [$H \leq 3$, $df = 1$, $P > 0.05$ for comparison between treated and untreated trees on individual dates (Fig. 2B)]. Averages over all sample dates of 0.05 (SE = 0.01), 0.09 (SE = 0.01) and 0.02 (SE = 0.005) per tap sample were obtained for ladybeetles, spiders and lacewings, respectively.

3.1.2. 2008

Dormant season sprays made in January were again effective in suppressing *D. citri* populations well into the growing season, resulting in significantly fewer adults in all treated trees compared to untreated trees over all samplings through June 4 ($H = 695.67$, $df = 5$, $P < 0.0001$, Fig. 3A). Adults per tap sample averaged 1.34 (SE = 0.08) in untreated trees compared to 0.03 (SE = 0.01), 0.04 (SE = 0.01) and 0.06 (SE = 0.01) in trees treated with fenpropathrin followed by chlorpyrifos, fenpropathrin alone and the high rate of oxamyl, the three most effective treatments. No more than 0.01 (SE = 0.01) adults were observed per tap sample in February ($H = 43.37$, $df = 5$, $P < 0.0001$) and March ($H = 163.12$, $df = 5$, $P < 0.0001$) on trees receiving these treatments compared to 0.92 (SE = 0.12) in the untreated trees, a reduction of 92–100-fold (Fig. 3A). On 10th April, adults were reduced by 19, 34 and 103-fold in the trees treated with the high rate oxamyl, fenpropathrin and fenpropathrin + chlorpyrifos, respectively ($H = 298.48$, $df = 5$, $P < 0.0001$). Significant reduction of 16–40-fold was still observed in these treatments during May ($H = 162.72$, $df = 5$, $P < 0.0001$) and June ($H = 106.77$, $df = 5$, $P < 0.0001$).

Treatments significantly reduced nymphs over all samplings through 1st July ($H = 183.70$, $df = 5$, $P < 0.0001$) except the low rate oxamyl that did not differ from the untreated check (Fig. 3B). The same 3 treatments were most effective with respect to nymphs, averaging 35.9 (SE = 6.4) in untreated trees compared to 2.2 (SE = 0.5), 2.3 (SE = 0.5) and 6.1 (SE = 1.1), per 39 dm^3 for fenpropathrin + chlorpyrifos, fenpropathrin alone and the high rate of oxamyl, respectively (Fig. 3B). However, fenpropathrin alone or followed by chlorpyrifos provided 16–17-fold reduction in nymphs, compared to a 6-fold reduction with the high rate of oxamyl ($U = 2467.5$; $n = 83, 79$; $P = 0.005$ and $U = 2500.5$; $n = 89, 79$; $P = 0.001$, respectively). Greatest reduction (14–23-fold) was seen in April and June with fenpropathrin treatments followed by the high rate oxamyl. On 1st July, all treated trees except those receiving the low rate oxamyl had significantly fewer nymphs than untreated trees with greatest reduction (11–17-fold) seen following either fenpropathrin treatment ($H = 52.15$, $df = 5$, $P < 0.0001$).

Ladybeetles, lacewings and spiders averaged 0.02 (SE = 0.004), 0.03 (SE = 0.003) and 0.09 (SE = 0.003) per tap sample, respectively, over all sample dates (Fig. 4). No differences between treated and untreated trees were seen in numbers of lacewings ($H = 1.48$, $df = 5$, $P = 0.92$). However, ladybeetle numbers were lower than the untreated check in the trees treated with the high rate of oxamyl and fenpropathrin alone ($H = 11.94$, $df = 5$, $P = 0.04$) and spiders were low in both fenpropathrin treatments ($H = 13.31$, $df = 5$, $P = 0.02$).

3.2. SWFREC grove

Data from this grove provided an additional example of how dormant sprays functioned under different conditions. Suppression of *D. citri* was indicated by low adult numbers observed through May during both years in blocks treated during the dormant season compared to the untreated block supported the results obtained from designed experiments (Table 1). No adults were observed in tap samples conducted in the treated trees in March and April of 2007 and March 2008 and averaged between 0 and 0.05 per tap sample in treated trees in April 2008 compared to averages of 0.19–0.28 and 0.13–0.45 adults per tap sample in untreated trees in March and April of 2007 and 2008, respectively. In May, adult density was still very low in treated trees and averaged between 0 and 0.05 per tap sample compared to 0.16–1.94 per tap sample in untreated trees. Psyllid populations were relatively low in 2008 compared to 2007. An increase of 10-fold and decrease of 3-fold was observed in the adult numbers in untreated trees from March to May in 2007 and 2008, respectively.

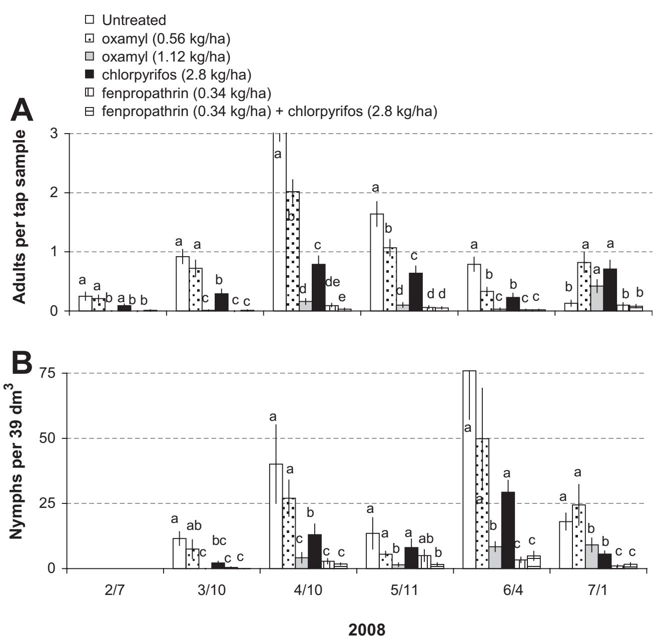

Fig. 3. Mean (SEM) number of *Diaphorina citri* adults per tap sample (A) and nymphs per 39 dm^3 of tree canopy (B) in Hamlin orange trees untreated and treated with sprays of oxamyl, chlorpyrifos and fenpropathrin on 16–17th January 2008. Fenpropathrin was applied in two plots in each replicate, one of which received a second application of chlorpyrifos on 12th February to evaluate the effect of two dormant sprays. No other insecticides were used in the experimental blocks through July. Shoots were scarce and not sampled on 7th February to estimate nymphal density. Treated and untreated trees represented by columns with same letter were not significantly different (Kruskal–Wallis test followed by Mann–Whitney *U*-test for pairwise treatment comparisons, $P > 0.05$).

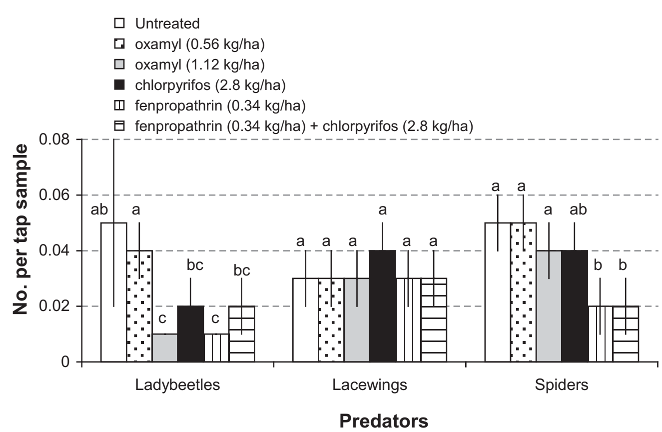

Fig. 4. Mean (SEM) number of ladybeetles, lacewings and spiders per tap sample over all sample dates in Hamlin orange trees untreated and treated with sprays of oxamyl, chlorpyrifos and fenpropathrin on 16–17th January 2008. Fenpropathrin was applied in two plots in each replicate, one of which received a second application of chlorpyrifos on 12th February to evaluate the effect of two dormant sprays. No other insecticides were used in the experimental blocks through July. Treated and untreated trees represented by columns with same letter were not significantly different (Kruskal–Wallis test followed by Mann–Whitney *U*-test for pairwise treatment comparisons, $P > 0.05$).

4. Discussion

Suppression of *D. citri* adults overwintering during the dormant winter season before bud break and prior to anticipated flush is aimed at reducing reproduction in the spring and later into the growing season. A single spray of broad-spectrum insecticide in winter consistently reduced psyllid populations for five to six months into the growing season. Effects were similar, irrespective of insecticide class and most pronounced on adults, which are the prime control target because they are directly responsible for the spread of the greening pathogen from diseased trees. By killing over-wintering adults, dormant sprays allowed fewer psyllids to enter into the spring flush, resulting in significantly lower populations throughout spring and into early summer in treated trees compared to untreated trees.

Reproduction in *D. citri* is associated with new shoot growth. Net reproductive rate averaged 379 at 25 °C for *D. citri* developing on flushing grapefruit, *Citrus paradisi* Macfadyen, under laboratory conditions (Tsai and Liu, 2000). Thus, high nymphal densities are expected under optimal field conditions. Fewer nymphs were present during spring and summer in trees receiving effective winter treatments followed presumably by predation from natural enemies that were generally in comparable numbers in both treated and untreated trees.

Biotic mortality was estimated to reduce net reproductive rate of psyllids from 5 to 27-fold during 2006–2007 in cohorts exposed to generalist predators and specialist parasitoids that attack psyllid immatures (Qureshi and Stansly, 2009). Therefore, infestation rates could be expected to be higher in the absence of predators, particularly ladybeetles, lacewings and spiders. Dormant sprays made prior to spring flush appeared to have little effect on density of predator insects migrating later into the orchards in search of aphids and other prey. Subsequent preying upon psyllids may explain, at least in part, why effects of these sprays continued to be observed well into the growing season. The effectiveness of dormant sprays in suppressing psyllids appeared to be greater in 2007, possibly due to the impact on psyllid predators from increased insecticide use in 2008 as growers became more aware of the HLB threat. A similar trend of decreased activity among psyllid predators in 2007 compared to spring 2006 was also attributed to increased insecticide use in the area (Qureshi and Stansly, 2009). Another factor may have been smaller plot size in 2008 so that psyllid migration between plots tended to obliterate differences more quickly.

Effects of dormant season treatments on *D. citri* consistently observed in this study provide strong evidence for the effectiveness of this tactic in reducing psyllid populations. Significant reduction of vector populations should ultimately contribute to reduction in the rate of HLB spread. Over-wintering psyllid adults are vulnerable during tree dormancy because of natural attrition of the population, relatively thin canopy and lack of cover, and prolonged exposure to insecticides. Furthermore, movement of psyllid adults for thermoregulation within the tree canopy is probably greater during cool weather, increasing contact with toxic residues. These factors together with the relative absence of natural enemies make the dormant winter period the best time to employ broad-spectrum insecticides against ACP in Florida citrus.

No significant advantage was observed with two dormant sprays, probably due to the short interval between applications in the one instance when this practice was evaluated. However, a spray in November or December followed by second spray at the end of January or early February might be more effective than a single dormant spray. This practice has been generally adopted in southwest Florida, with pyrethroids the usual winter choice, due to their effectiveness at that time and short (1 day) pre-harvest interval (Stansly et al., 2009a). Additional sprays during the growing season should be based on scouting and applied prior to anticipated flush. Selective materials including horticultural oils may be better suited to post-bloom applications due to relatively fewer negative impacts on beneficial insects. Organo-phosphates and carbamates could be resorted to later in the growing season when beneficial insects are not as prevalent as in spring and early summer (Qureshi and Stansly, 2009; Stansly et al., 2009a).

The dormant spray tactic is now part of an area-wide management program to reduce the incidence of Asian citrus psyllid and spread of citrus greening disease being adopted in southwest Florida (Stansly et al., 2009b). Aerially applied dormant sprays coordinated through Gulf Citrus Growers Association and monitored by Florida Department of Agriculture over 80,000 acres of citrus reduced psyllid populations by 71–88% compared to unsprayed groves in spring 2009. Thus, dormant sprays have already become a key management component being adapted area-wide to control Asia citrus psyllid in Florida.

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