

Costs and benefits of frequent low-volume applications of horticultural mineral oil for management of Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae)



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ABSTRACT

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), vectors a pathogen that causes huanglongbing (HLB) in Florida citrus. The need to suppress ACP populations has resulted in greatly increased insecticide use in Florida. Horticultural mineral oils (HMOs), typically applied as 1–2% v/v aqueous emulsions at 937 L water ha⁻¹, are also used for insect pest management for citrus in Florida. Low-volume applications of other insect control products can reduce costs and application time and are effective for ACP control. The efficacy of low-volume applications of HMOs for ACP has not been tested. We initiated a three-year trial in February 2011 in a commercial Valencia orange grove in Lee County, Florida to compare low-volume (18.7 L ha⁻¹) sprays of HMO applied every two weeks to a grower standard (GS) (mixes of insecticide and HMO) and an untreated control. HMO and GS treatments significantly reduced ACP adult and nymph populations. Yields were greater for HMO-treated than untreated trees in the final study year. GS and HMO treatments reduced fruit drop in 2013. Fruit quality was generally unaffected by treatments. ACP suppression, higher yields and eventual production gains indicated that frequent, low-volume application of HMO may be a viable alternative for suppressing ACP populations.

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1. Introduction

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Sternorrhyncha: Psyllidae) became the key pest of Florida citrus with the detection of huanglongbing (HLB) (citrus greening) in 2005 (Halbert, 2005). ACP adults transmit, and nymphs principally acquire the causal agent of HLB, '*Candidatus Liberibacter asiaticus*' (Clas) (Pelz-Stelinski et al., 2010; Xu et al., 1988). This disease reduces yield, is incurable, and was estimated to cost the Florida citrus industry \$4.5 billion from 2007 through 2011 (Hodges and Spreen, 2012). Prior to detection of HLB in Florida, control of adult ACP with synthetic insecticides was not

considered necessary for mature healthy citrus trees (Childers and Rogers, 2005). Since detection of HLB in Florida, control of *D. citri* with synthetic, broad-spectrum insecticides has become prevalent and many growers spray monthly. Evidence for resistance to neonicotinoids, pyrethroids, and organophosphates has emerged in some Florida ACP populations (Tiwari et al., 2011).

Petroleum-based horticultural mineral oils (HMOs) control citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), red scale, *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae), chaff scale, *Parlatoria pergandii* Comstock spiny whitefly, *Aleurocanthus spiniferus* (Quaintance) (Hemiptera: Aleyrodidae) and ACP in citrus (Beattie et al., 2000; Davidson, 1991; Rae et al., 1996, 1997, 2000). Most HMOs are relatively inexpensive, have low mammalian toxicity, little residual activity, and are relatively harmless to beneficial insects (Stansly et al., 2002). HMO-associated ACP population suppression is comparable to that seen for conventional insecticides; this effect was attributed to reduced oviposition (Rae et al., 1997). Reduced HLB infection has been

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attributed to suffocation of ACP nymphs, reduced oviposition and adult mortality associated with application of HMO to run-off (Leong et al., 2012). Resistance has not been reported. Most HMO products are Organic Materials Review Institute (OMRI)-approved for organic citrus production (OMRI, 2015).

HMOs are typically applied at rates of 1–2% HMO in 937 L water ha⁻¹ in Florida. Low-volume (typically < 100 L ha⁻¹) applications of other pest control products have become important for ACP control in Florida citrus (Atwood and Stelinski, 2008). However, tests of low-volume HMO have yielded inconsistent results for other pests: for example, *Ceroplastes sinensis* del Guercio (Hemiptera: Coccidae) (Beattie et al., 1991; Beattie and Kaldor, 1990). Cases of successful control of citrus pests with low-volume HMOs, and potential time and cost-savings necessitate testing their efficacy for ACP control in Florida citrus.

We compared high frequency, low-volume HMO, a grower standard (GS) program based on mixes of broad-spectrum insecticides and HMO, and an untreated control in a commercial orange grove over three growing seasons. We tested four major hypotheses: 1) that effective ACP control can be achieved with high-frequency, low-volume HMO without recourse to other insecticidal substances; 2) ACP control will result in reduced HLB incidence; 3) ACP control will increase agronomic performance of treated trees; and 4) that ACP control can be achieved at a lower cost with HMO than GS treatments.

2. Methods and materials

2.1. Study site and treatment application

This study was a 4.4 ha block within a 31 ha commercial citrus grove (26.704 N, -81.750 W), in Lee County, Florida. The original planting of sweet orange, *Citrus sinensis* (L.) Osb. 'Valencia', on 'Swingle' citrumelo [*C. paradise* Macf. × *Poncirus trifoliata* (L.) Raf.] rootstock (tree density 270 ha⁻¹, bed spacing 8.23 × 4.57 m) was ca. 25 years old at the start of the study. During the course of the current study, trees were hedged and topped late January to early February of each year. Tree heights were approximately 8 m. Tree beds were mowed four times per year and irrigated with micro sprinklers as required.

Three treatments were applied in a 3 × 3 Latin square of nine 0.49 ha plots. Treatments included: mixes of insecticides and HMO,

typical of grower standard practices (GS) (Table 1), an untreated control and HMO alone. The oil used was a narrow-range petroleum distillate nC23 HMO with 2% inert emulsifier, 99% unsulfonated residues and 50% distillation temperature of 224 °C (435 °F) at 1.33 kPa (Purespray Green®, Petro-Canada Lubricants, Inc., Mississauga, Ontario, Canada). HMO sprays were applied every two weeks at 18.7 L ha⁻¹ from 3 March 2011 to 3 November 2011. HMO was applied as a 50% aqueous emulsion at 18.7 L ha⁻¹ every two weeks from 10 February 2012 to 2 November 2012 and from 8 February 2013 to 15 November 2013. A Proptec™ rotary atomizer P400D sprayer (Curtec Sprayers, Vero Beach, Florida) was used to administer HMO treatments. GS treatments (Table 1) included HMO at 18.7 L ha⁻¹ and were applied as a 2% aqueous emulsion with a Rears Mfg. Co.™ (Eugene, OR) low-profile air-blast sprayer at 937 L ha⁻¹. Timing and products used for GS treatments were at the grower's discretion (Table 1, Fig. 1) and typically occurred when we advised him that ACP adult counts exceeded a nominal threshold of 0.2 per tap sample (as per Qureshi and Stansly, 2007). Although timing of HMO and GS applications differed, we believe that these regimens allowed for an appropriate comparison of a high-frequency, low-volume HMO application and common practices for insecticide application for ACP in Florida. For the six months prior to initiation of the study, insecticides were applied to the entire trial site monthly. A foliar nutritional program consistent with current recommendations (Stansly et al., 2014) was followed on all treatments for the six months prior to initiation of and over the course of the study. All treatments including controls received a dormant spray of insecticide without HMO during winter months (Table 1) as recommended in Florida for ACP control (Qureshi and Stansly, 2010).

2.2. Flush densities and ACP egg and nymph densities

Sampling was conducted every two weeks from 1 March 2011 to 4 December 2013. Each plot incorporated two sampling sites chosen at random with two sub-samples taken from each. When flush density was high (>1 per 0.03 m³), the numbers of young shoots inside a randomly-placed, 1 ft³ (0.03 m³: 30.5 cm × 30.5 cm × 30.5 cm) pipe-framed sampling cube were counted. When flush density was low, young shoots were counted within a 10 ft³ (0.28 m³: 152.4 cm × 61.0 cm × 30.5 cm) volume of canopy. If 10 young shoots were not found within the initial

Table 1
Comparison of 2014 US dollar materials costs of specific insecticide products applied on specific dates. *Dormant sprays were applied to all treatments. Insecticide applications included nC23 HMO (Purespray Green®, Petro-Canada Lubricants, Inc., Mississauga, Ontario, Canada) at 18.7 L (\$28.80) ha⁻¹. In addition to SI units, US measures of mass and volume are also presented.

Date	End-use product	Active ingredient	Manufacturer	Retail cost	Rate	Cost			
						Per acre	Per ha	Per acre	Per ha
23-Mar-11	Sevin XLR	Carbaryl	Bayer CropScience	\$51.60/1 gal	3.79L	0.75 gal	7.02 l	\$38.70	\$95.63
8-Apr-11	Azatin XL	Azadirachtin	OHP Inc.	\$201.67/32 oz	0.95 L	6 fl oz	0.44 l	\$37.81	\$93.43
21-Apr-11	Actara WG	Thiamethoxam	Syngenta Crop Protection	\$92.70/30 oz	2.10 kg	5.5 oz	0.39 kg	\$17.00	\$42.01
14-Jun-11	Agri-mek	Abamectin	Syngenta Crop Protection	\$91.00/32 oz	0.95 L	10 fl oz	0.73 l	\$28.44	\$70.28
30-Jun-11	Dimethoate	Dimethoate	Helena Chemical	\$51.37/1 gal	3.79 L	20 fl oz	1.46 l	\$8.03	\$95.63
29-Jul-11	Delegate WG	Spinetoram	Dow Agrosciences	\$229.32/26 oz	1.82 kg	4 oz	0.28 kg	\$35.28	\$87.18
10-Jan-12	Danitol*	Fenpropathrin	Valent USA	\$160.21/1 gal	3.79 L	16 fl oz	1.17 l	\$20.03	\$49.50
12-Mar-12	Micromite 80 WGS	Diiflubenzuron	Chemtura Corporation	\$100.00/1.95 lb	0.88 kg	6.25 oz	0.44 kg	\$20.03	\$49.50
17-May-12	Agri-mek	Abamectin	Syngenta Crop Protection	\$92.70/30 fl oz	0.89 L	16 fl oz	1.17 l	\$49.44	\$122.17
11-Jul-12	Delegate WG	Spinetoram	Dow Agrosciences	\$229.32/26 oz	1.82 kg	3 oz	0.21 kg	\$26.46	\$65.38
10-Aug-12	Dimethoate	Dimethoate	Helena Chemical	\$51.37/1 gal	3.79 L	16 fl oz	1.17 l	\$6.42	\$15.86
7-Nov-12	Imidan 70 W	Phosmet	Gowan	\$11.00/1 lb	0.45 kg	1 lb	1.12 kg	\$11.00	\$27.18
10-Jan-13	Danitol*	Fenpropathrin	Valent USA	\$160.21/1 gal	3.79 L	16 fl oz	1.17 l	\$20.03	\$49.50
9-Apr-13	Delegate WG	Spinetoram	Dow Agrosciences	\$229.32/26 oz	1.82 kg	4 oz	0.28 kg	\$35.28	\$87.18
1-Jul-13	Agri-mek	Abamectin	Syngenta Crop Protection	\$91.00/32 fl oz	0.95 L	15 fl oz	1.10 l	\$42.66	\$105.42
7-Oct-13	Gladiator	Zeta-cypermethrin + Abamectin	FMC	\$93.16/1 gal	3.79 L	19 fl oz	1.39 l	\$13.83	\$34.17
5-Dec-13	Danitol*	Fenpropathrin	Valent USA	\$160.21/1 gal	3.79 L	19 fl oz	1.39 l	\$23.78	\$58.76

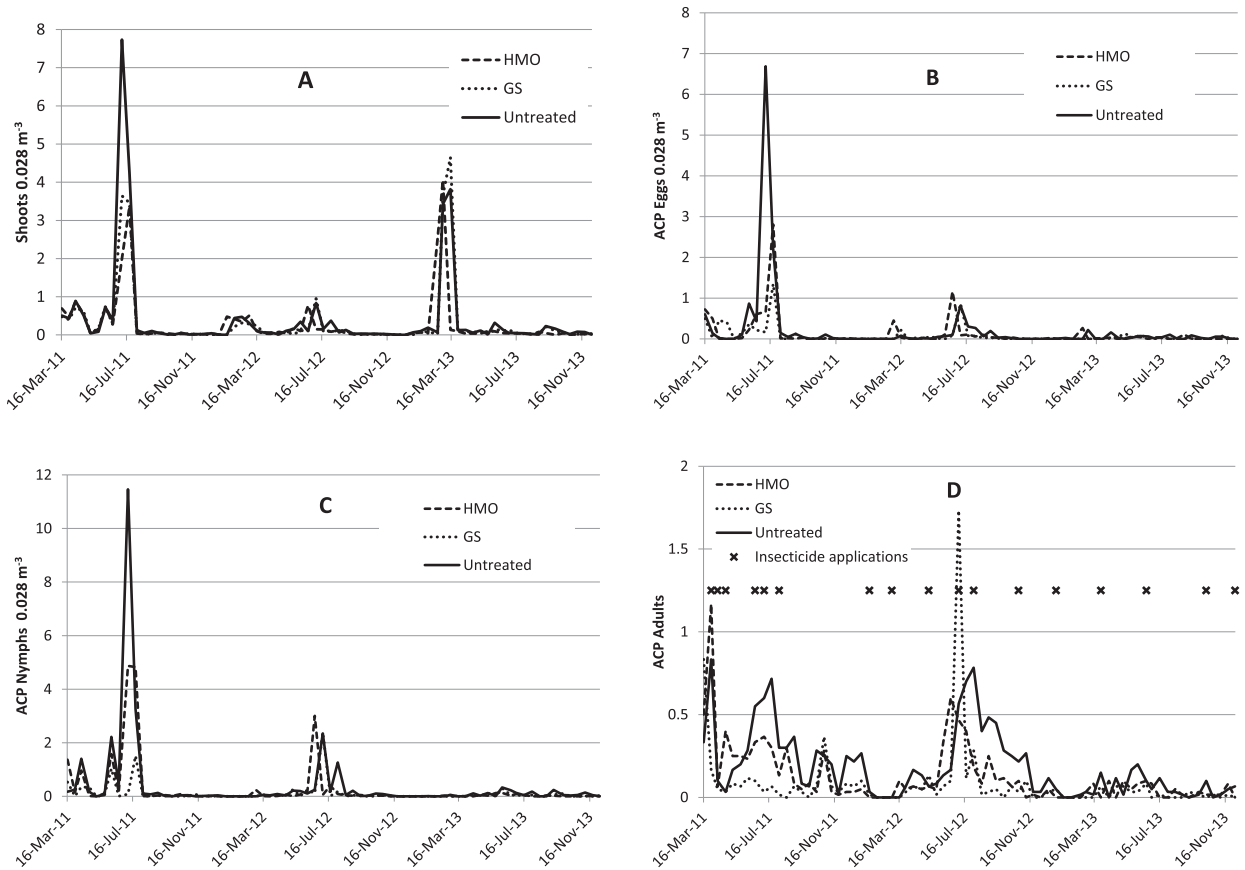


Fig. 1. Mean Citrus flush densities (A), *Diaphorina citri* (ACP) egg (B) and nymph (C) densities and adult counts with associated insecticide application dates (D) by sampling date in untreated, horticultural mineral oil (HMO) - treated and grower standard (GS) - treated Valencia orange trees.

sampling volume, sampling continued to a maximum of 10 trees per plot.

Cumulative volume of samples needed to find 10 shoots was used to calculate flush density, expressed as shoots 0.028 m^{-3} . Counts of ACP eggs and nymphs on infested shoots were conducted in the laboratory using a stereoscopic microscope. ACP egg and nymph densities, expressed as individuals 0.028 m^{-3} , were calculated using insect counts per canopy volume sampled during flush evaluations.

2.3. ACP adult counts

Adult sampling was conducted on the same dates as flush assessments. Five randomly-selected trees in two locations within the middle bed of each plot were assessed using a stem-tap sampling method where a randomly chosen branch is struck 3 times with a short length of PVC pipe and insects falling onto a plastic clipboard are quickly counted (Qureshi and Stansly, 2007).

2.4. Yield, fruit drop and juice quality

All ripe fruit from experimental plots were harvested the week of 7 February 2011. All ripe fruit from 10 trees that were considered average for each plot were harvested during the weeks of 27 February 2012, 1 March 2013 and 21 April 2014. In 2012, 2013, oranges from 10 trees were placed in commercial 10-box bins; masses of filled bins were assessed with a Gator Deck Scale (Scale Systems, Novi, MI) and corrected for the mean mass of four bins. In 2014,

each bag of fruit picked from 10 trees was weighed with a produce scale. Masses were corrected for mean mass of harvest bags. Masses of fruit per plot were calculated based on these masses and numbers of trees per plot. One 17.6 L sample (bag of fruit pooled from the ten trees that were harvested per plot) was taken for quality analysis each year. Samples were analyzed by the University of Florida Citrus Quality Laboratory in Lake Alfred FL. Juice was de-aerated under vacuum for 2–3 min. Soluble solids content was measured by hydrometer, brix by refractometer and titratable acidity was measured as citric acid (pH endpoint 8.2).

2.5. Incidence of HLB and PCR analysis of plant samples

A small number (96: 1.2%) of trees from the entire grove were visually identified as symptomatic of HLB in June 2010. For real-time polymerase chain reaction (PCR) analysis, two groups of five randomly selected leaf-petiole units per cardinal point per tree were collected and transported on ice to the laboratory. Samples were collected April 2011 (190), and March 2013. In 2013, 178 trees were sampled: 88 from the group to be harvested, 90 from other randomly-selected trees. Approximately 6 trees per plot, per group were sampled. Samples were processed and analyzed with Applied Biosystems 7500 system SDS software version 1.2 (Stansly et al., 2014). The cycle threshold (Ct) value represents the minimum DNA amplification cycles required for signal detection and is considered indicative of target DNA titer (Li et al., 2006). A sample was considered to be negative for *Ca. L. asiaticus* if the Ct-value exceeded 36 as per Stansly et al. (2014).

2.6. Economic analysis

An evaluation of economic benefits of applications was conducted using materials and application costs of GS and HMO treatments, and annual yield and fruit quality data. After HLB detection in Florida, grower costs increased to approximately \$1695 per acre (\$4187 ha⁻¹) (Muraro, 2006, 2012b) or \$15.51 per tree (at 270 trees per ha). A comparison was made of annual mean treatment yields and recent estimates of yield-based break-even points for profitability (Muraro, 2012b).

A marginal analysis in which mean annual treatment yields and kg solids were related to input costs associated with experimental ACP control inputs was also conducted. The 2011 harvest results were not included as ACP control was not undertaken prior to that harvest. Costs of materials by treatment were based on quotes from chemical supply company sales representatives July 2014 (Table 1). Analyses were conducted both with and without incorporating the cost of harvest and transport (hereafter referred to as the delivered-in cost): \$2.84 per 40.82 kg of harvested oranges (Muraro, 2012a). Application costs for treatments were based on State averages of \$7.50 per acre (\$18.53 per ha) for low-volume HMO applications and \$27.89 per acre (\$68.92 per ha) for 100 gallon per acre (935 L ha⁻¹) air-blast insecticide applications (Muraro, 2012b) (Table 2).

2.7. Statistical analyses

ACP egg and nymph densities, adult counts and flush densities per plot over all sampling dates and annual evaluations of treatment effects on fruit yield, drop and quality were compared using repeated measures analysis with the Mixed procedure in SAS (SAS Institute, 2012). Treatment and sampling date were fixed factors; row and column were random factors. Flush densities and ACP egg and nymph densities and adult count data were transformed by $\sqrt{(x + 0.05)}$ to stabilize variances. When main effects were significant (at $\alpha = 0.05$), pairwise comparisons (*t*-tests) were made using a Tukey test controlling for overall experiment-wise error rates (pdiff adjust = Tukey). Relationships between treatment-specific ACP eggs, nymphs, and adults with flush densities, yield, drop and quality measures were evaluated with Pearson correlation statistics and compared with Fisher's *z*-transformations using the Corr procedure (SAS Institute, 2012).

We identified trends in time series for treatment-specific flush and ACP populations using the SAS generalized additive models

(GAM) non-parametric regression procedure (SAS Institute, 2012). This technique incorporates smoothing splines to fit complex data to smoothed curves through data points and allows separation of underlying patterns from sampling interval-to-interval noise. A generalized cross validation (GCV) function was specified to approximate expected prediction errors. Model fit was evaluated in two ways. First, the Aikake information criterion (AIC statistic), calculated as the model deviance + $2 \times$ model-specified degrees of freedom, was compared among separate models specifying a range (4–36) of degrees of freedom. A lower AIC value indicates better model fit. Secondly, as model selection based on minimizing AIC may 'over fit' data, visual inspection of models was conducted. Recommendations for visual inspection include varying model degrees of freedom until the noise of point-to-point sampling is incorporated without excessive jaggedness: up to $df = n/3 - n/2$ (Fewster et al., 2000). We chose models with $df = n/3$ (24) for most tests as these met the criteria. The model for ACP nymphs from GS-treated trees required $df = 34$ for adequate fit. When significant linear components of trends were apparent, slopes and intercepts were compared with *t*-tests.

Ct-values of 40 were excluded from subsequent analyses. Due to a large number of zeros in the data set for 2011, these data were analyzed with the SAS Genmod procedure, specifying a zero-inflated Poisson distribution and the zeromodel option. Mean 2013 Ct-values per plot were subject to the SAS mixed procedure specifying treatment and sample protocol (randomly selected or harvested tree) as fixed factors; row and column were random factors. Relationships of Ct values, HLB incidence and ACP adult counts were assessed with the SAS Corr procedure, specifying the Spearman option (SAS Institute, 2012) for 2011 and 2013 data.

3. Results

3.1. Densities of flush and ACP eggs and nymphs

Peaks in flush densities occurred March and July 2011, February, June and July 2012 and February 2013; low flush densities were apparent in September 2011–January 2012, September–December 2012 and in November 2013 (Fig. 1A). A significant effect of sampling date was apparent ($F_{71, 430} = 56.10$; $P < 0.001$) as was a significant treatment by sampling date interaction ($F_{142, 430} = 1.54$; $P < 0.001$). Trees in both HMO- and GS-treated plots had fewer flush shoots when compared to untreated trees ($P = 0.003$ and $P = 0.006$, respectively) (Table 3). However, significant ($P < 0.05$) differences

Table 2
Applications and associated costs of horticultural mineral oil (HMO) and insecticide + HMO (GS) treatments (US dollars ha⁻¹) relative to the untreated control (UTC).

	Treatment	2011–2012	2012–2013	2013–2014	Sum
HMO applications	HMO	17	19	17	53
	GS	6	5	3	14
	UTC	0	0	0	0
Insecticide applications	HMO	1	1	1	3
	GS	7	6	4	17
	UTC	1	1	1	3
Cumulative material costs (\$)	HMO	539.10	596.70	539.10	1674.90
	GS	630.64	473.59	371.92	1476.15
	UTC	49.50	49.50	49.50	148.50
Cumulative application costs (\$)	HMO	383.98	421.04	383.98	1189.00
	GS	482.42	413.51	275.67	1171.60
	UTC	68.92	68.92	68.92	206.75
Total costs (\$)	HMO	923.08	1017.74	923.08	2863.90
	GS	1113.06	887.10	647.59	2647.75
	UTC	118.42	118.42	118.42	355.25
Differential (\$)	HMO	804.66	899.33	804.66	2508.64
	GS	994.65	768.68	529.17	2292.50
	UTC	0.00	0.00	0.00	0.00

Table 3

Comparisons of treatment effects on flush densities (shoots 0.028 m⁻³) and *Diaphorina citri* (ACP) egg and nymph densities (insects 0.028 m⁻³) and adult counts per stem tap sample on Valencia orange trees treated with horticultural mineral oil (HMO), a grower standard (GS) (insecticide) and untreated. Like-lettered groups are not significantly different at $\alpha = 0.05$. Grouping comparisons are by variable.

Variable	Treatment	Mean (\pm S.E.M.)		F	df	P
Flush density	Untreated	0.409 (\pm 0.081)	a	6.94	2, 430	0.001
	HMO	0.292 (\pm 0.054)	b			
	GS	0.331 (\pm 0.072)	b			
ACP eggs	Untreated	0.204 (\pm 0.079)	a	3.42	2, 430	0.034
	HMO	0.121 (\pm 0.029)	ab			
	GS	0.081 (\pm 0.020)	b			
ACP nymphs	Untreated	0.376 (\pm 0.105)	a	13.8	2, 430	<0.001
	HMO	0.268 (\pm 0.094)	b			
	GS	0.118 (\pm 0.026)	b			
ACP adults	Untreated	0.184 (\pm 0.019)	a	29.5	2, 386	<0.001
	HMO	0.128 (\pm 0.018)	b			
	GS	0.082 (\pm 0.021)	c			

were limited to summer 2011 (Fig. 1A).

ACP egg densities varied by treatment ($F_{2, 430} = 3.42$; $P = 0.034$) and were significantly lower on GS-treated than untreated trees ($P < 0.05$) (Table 3). A significant effect of date was detected ($F_{71, 430} = 9.55$; $P < 0.001$) with peak densities coinciding with peaks in flush densities for all treatments (Fig. 1B).

ACP nymph densities were influenced by treatment ($F_{2, 430} = 13.82$; $P < 0.001$). Fewer nymphs were found on GS- and HMO-treated than on untreated trees ($P < 0.001$ and $P = 0.001$, respectively) (Table 3). A significant effect of date was also detected ($F_{71, 430} = 15.19$; $P < 0.001$) with peaks in nymph densities coinciding with peaks in flush densities (Fig. 1C).

3.2. ACP adult counts

Adult counts differed by treatment ($F_{2, 386} = 29.53$; $P < 0.001$) and were greatest on untreated trees, followed by HMO trees and then GS trees (Table 3). The effect of sampling date was also significant ($F_{64, 386} = 6.59$; $P < 0.001$). Peaks in ACP adult counts occurred in March 2011, June–July 2011, and August 2012 (Fig. 1D).

3.3. Relating flush and ACP eggs, nymphs and adults

All correlations were significant except flush with adult ACP for

trees treated with HMO or GS (Table 4). The correlations flush – eggs, flush – nymphs and eggs – nymphs were greatest for untreated trees, with the flush–nymph correlation least for GS (Table 4). The nymph–adult correlation was greatest for GS-treated trees while there were no significant differences among treatments for egg – adult counts. Although treatment differences for flush – adult counts were not detected, this correlation was only significant for untreated trees (Table 4).

3.4. Trends in flush density and ACP eggs, nymphs and adults over time

Flush densities declined significantly over the course of this study for untreated trees (Table 5). However, the rate of decline was very low (slope = -0.0007) and did not differ significantly from HMO or GS treatments ($P > 0.05$ for both comparisons). The rate of decline and intercept was greater for ACP eggs in untreated compared to GS-treated trees ($P = 0.041$ and 0.040 respectively). Greater intercepts and rates of decline were also found for both ACP nymphs and adults in HMO and untreated trees compared to GS-treated trees ($P < 0.05$ for all assessments).

3.5. Yield, fruit drop and juice quality

Yields differed by harvest year ($F_{3, 8} = 25.38$; $P < 0.001$) and were lowest in 2011 (98.95 ± 10.74 kg/tree). Yields significantly ($P < 0.05$) increased in 2012 (239.13 ± 10.57 kg/tree) and again in 2013 (339.05 ± 115.22 kg/tree) but decreased significantly ($P < 0.05$) from 2013 to 2014 (218.74 ± 15.53 kg/tree). HMO treatment yield was higher ($P < 0.05$) than the GS treatment in 2012 and more ($P < 0.05$) than untreated trees in 2014 (Table 6). Yields from GS-treated and untreated trees increased ($P < 0.05$) from 2012 to 2013 but fell ($P < 0.05$) in 2014; HMO-treated tree yields remained relatively constant from 2012 to 2014 (Table 6).

Three year (2012–2014) mean yields were numerically greatest for HMO treatments (271.32 ± 15.05 kg/tree), followed by GS (263.92 ± 25.23 kg/tree), and untreated trees (261.68 ± 27.41 kg/tree). In 2013, fruit drop was greater for untreated than either HMO- or GS-treated trees ($P < 0.05$ for both comparisons) (Table 6). Fruit drop was numerically greatest from untreated trees in 2014 (Table 6).

No treatment effect on juice (kg) per box was detected ($F_{2,$

Table 4

Comparison of correlation analyses of mean *Diaphorina citri* egg and nymph densities (insects 0.028 m⁻³), adult counts and flush densities (shoots 0.028 m⁻³) on 'Valencia' orange trees treated with horticultural mineral oil (HMO), a grower standard (GS) (insecticide) and untreated. There are no significant differences between like-lettered groups within variable–variable groups.

Variable	With Variable	Treatment	n	Sample Corr.	Fisher's z	95% C.I.	p		
Flush	Eggs	Untreated	72	0.846	1.242	0.761	0.900	<0.001	a
		HMO	72	0.587	0.673	0.407	0.719	<0.001	b
		GS	72	0.419	0.446	0.204	0.590	<0.001	b
Flush	Nymphs	Untreated	72	0.823	1.166	0.728	0.884	<0.001	a
		HMO	72	0.578	0.660	0.397	0.712	<0.001	b
		GS	72	0.301	0.311	0.073	0.497	0.010	c
Flush	Adults	Untreated	72	0.291	0.300	0.062	0.488	0.013	a
		HMO	72	0.060	0.060	-0.174	0.287	0.617	a
		GS	72	0.161	0.162	-0.075	0.378	0.177	a
Eggs	Nymphs	Untreated	72	0.981	2.318	0.969	0.988	<0.001	a
		HMO	72	0.822	1.163	0.727	0.884	<0.001	b
		GS	72	0.859	1.288	0.780	0.908	<0.001	b
Eggs	Adults	Untreated	72	0.414	0.441	0.199	0.587	<0.001	a
		HMO	72	0.421	0.448	0.207	0.592	<0.001	a
		GS	72	0.519	0.574	0.323	0.668	<0.001	a
Nymphs	Adults	Untreated	72	0.410	0.436	0.195	0.584	<0.001	b
		HMO	72	0.369	0.387	0.148	0.552	0.001	b
		GS	72	0.757	0.990	0.634	0.840	<0.001	a

Table 5
Generalized additive models (GAM) non-parametric regression analyses of deviance and linear trend assessment of relationships between sampling date and mean flush densities (shoots 0.028 m^{-3}), *Diaphorina citri* egg and nymph densities (insects 0.028 m^{-3}) and adult counts on 'Valencia' orange trees treated with horticultural mineral oil (HMO), a grower standard (GS) (insecticide) and untreated.

Variable	Treatment	χ^2	p	Parameter	Estimate	Standard error	t	p
Flush	HMO	81.24	<0.001	Intercept	15.97	8.76	1.82	0.075
				Linear	-0.00038	0.00021	-1.79	0.080
	GS	91.63	<0.001	Intercept	14.75	10.54	1.4	0.168
				Linear	-0.00035	0.00026	-1.37	0.178
	Untreated	93.31	<0.001	Intercept	29.12	13.63	2.14	0.038
				Linear	-0.00070	0.00033	-2.11	0.041
Eggs	HMO	56.88	<0.001	Intercept	14.71	5.04	2.92	0.005
				Linear	-0.00035	0.00012	-2.9	0.006
	GS	37.46	0.0394	Intercept	9.89	3.00	3.29	0.002
				Linear	-0.00024	0.00007	-3.27	0.002
	Untreated	80.17	<0.001	Intercept	28.68	10.06	2.85	0.007
				Linear	-0.00069	0.00024	-2.83	0.007
Nymphs	HMO	83.02	<0.001	Intercept	37.99	10.51	3.61	<0.001
				Linear	-0.00092	0.00026	-3.59	<0.001
	GS	49.06	0.0457	Intercept	10.06	5.49	1.83	0.074
				Linear	-0.00024	0.00013	-1.81	0.077
	Untreated	66.20	<0.001	Intercept	49.44	18.46	2.68	0.010
				Linear	-0.00119	0.00045	-2.66	0.011
Adults	HMO	88.34	<0.001	Intercept	12.19	2.00	6.11	<0.001
				Linear	-0.00029	0.00005	-6.05	<0.001
	GS	55.91	<0.001	Intercept	5.83	3.09	1.89	0.066
				Linear	-0.00014	0.00008	-1.86	0.069
	Untreated	167.6	<0.001	Intercept	12.55	1.77	7.1	<0.001
				Linear	-0.00030	0.00004	-6.99	<0.001

Table 6
Comparisons of yield per tree (kg) and fruit drop from 'Valencia' orange trees treated with horticultural mineral oil (HMO), a grower standard (GS) (insecticide) and untreated. Like-lettered groups are not significantly different. Pairwise comparisons are within years.

Year	Treatment	Mean yield per tree (kg) (\pm S.E.M.)		Mean fruit drop per tree (\pm S.E.M.)	
2011	Untreated	85.04 (\pm 17.79)	a	—	—
	HMO	105.87 (\pm 24.64)	a	—	—
	GS	105.95 (\pm 17.76)	a	—	—
2012	Untreated	236.03 (\pm 14.89)	ab	—	—
	HMO	262.04 (\pm 13.34)	a	—	—
	GS	219.31 (\pm 14.89)	b	—	—
2013	Untreated	359.21 (\pm 32.24)	a	345.33 (\pm 29.33)	a
	HMO	304.72 (\pm 14.40)	a	170.00 (\pm 8.39)	b
	GS	353.23 (\pm 25.26)	a	182.33 (\pm 18.55)	b
2014	Untreated	189.80 (\pm 10.53)	b	220.07 (\pm 21.05)	a
	HMO	247.21 (\pm 21.42)	a	189.20 (\pm 17.82)	a
	GS	219.21 (\pm 16.04)	ab	172.70 (\pm 18.18)	a

$\delta = 4.35$; $P = 0.053$) but values were lower for all treatments in 2011 than in any other year ($P < 0.05$ for all comparisons) (Table 7). No treatment effect was apparent for acid ($F_{2, 8} = 3.54$; $P = 0.079$) although values were significantly higher for 2011 than 2012 ($P < 0.05$ for all comparisons). No significant ($P < 0.05$) treatment or

year effects were apparent for brix or ratio values (Table 7). Solids (kg per box) did not differ by treatment ($F_{2, 8} = 4.01$; $P = 0.062$) or year ($F_{3, 8} = 3.49$; $P = 0.070$) but a significant interaction was apparent ($F_{6, 8} = 8.03$; $P = 0.005$). Lesser solids (kg per box) were detected from HMO- than untreated trees in 2012 ($P < 0.05$) (Table 7).

Table 7
Mean \pm S.E.M. fruit quality measures by treatment for 'Valencia' orange trees treated with horticultural mineral oil (HMO), a grower standard (GS) (insecticide) and untreated. Pairwise comparisons are within years. There are no significant differences between like-lettered groups.

Year	Treatment	Juice (kg/box)	Acid (%w/w)	$^{\circ}$ Brix (TSS)	Ratio (Brix/Acid)	Solids (kg/box)					
2011	Untreated	16.46 \pm 1.40	a	0.96 \pm 0.02	a	7.81 \pm 2.92	a	8.26 \pm 3.14	a	1.37 \pm 0.37	b
	HMO	16.37 \pm 0.83	a	0.98 \pm 0.04	a	11.02 \pm 0.37	a	11.26 \pm 0.26	a	1.81 \pm 0.15	ab
	GS	20.18 \pm 2.29	a	0.75 \pm 0.14	a	10.82 \pm 0.30	a	15.41 \pm 2.69	a	2.19 \pm 0.26	a
2012	Untreated	23.73 \pm 0.57	a	0.76 \pm 0.05	a	11.34 \pm 0.28	a	15.05 \pm 0.56	a	2.69 \pm 0.12	a
	HMO	22.21 \pm 0.58	a	0.66 \pm 0.02	a	11.13 \pm 2.04	a	14.62 \pm 0.30	a	2.15 \pm 0.15	b
	GS	22.74 \pm 0.47	a	0.70 \pm 0.04	a	12.32 \pm 1.45	a	15.21 \pm 0.62	a	2.39 \pm 0.13	ab
2013	Untreated	24.79 \pm 0.57	a	0.81 \pm 0.07	a	11.13 \pm 0.34	a	13.86 \pm 1.04	a	2.76 \pm 0.06	a
	HMO	24.10 \pm 0.80	a	0.69 \pm 0.07	a	8.64 \pm 2.13	a	15.17 \pm 0.91	a	2.48 \pm 0.15	a
	GS	24.95 \pm 1.77	a	0.73 \pm 0.03	a	10.36 \pm 0.34	a	13.87 \pm 1.04	a	2.58 \pm 0.14	a
2014	Untreated	22.77 \pm 0.49	a	0.81 \pm 0.01	a	10.69 \pm 0.24	a	13.11 \pm 0.36	a	2.44 \pm 0.02	a
	HMO	22.39 \pm 0.26	a	0.79 \pm 0.02	a	10.41 \pm 0.25	a	14.25 \pm 0.69	a	2.33 \pm 0.06	a
	GS	22.62 \pm 0.51	a	0.75 \pm 0.02	a	10.57 \pm 0.10	a	13.28 \pm 0.57	a	2.39 \pm 0.06	a

3.6. Incidence of HLB and PCR analysis of plant samples

The numbers of HLB-symptomatic trees increased significantly ($F_{1, 17} = 15.14$; $P = 0.001$) from 2011 (0.03 ± 0.05) to 2013 (0.22 ± 0.03). Mean Ct-values decreased significantly ($F_{1, 16} = 9.78$; $P = 0.007$) from 2011 (36.09 ± 1.06) to 2013 (32.54 ± 0.63). No interaction of year and treatment was found for HLB incidence or Ct values ($P > 0.05$ for both evaluations). 2011: CT values and incidence of HLB were negatively correlated (Spearman $R = -0.791$, $n = 8$, $P = 0.019$). No significant treatment effects were detected for Ct values or HLB incidence in 2011 ($F_{2, 1} = 0.80$; $P = 0.621$ and $df = 2$; $\chi^2 = 0.10$; $P = 0.953$ respectively) or 2013 ($F_{2, 8} = 1.49$; $P = 0.282$ and $F_{2, 8} = 0.95$; $P = 0.425$ respectively). However, Ct values were numerically lower in untreated control trees in both 2011 and 2013 (Table 8). No effect of sampling method or interaction of treatment and sampling protocol was apparent for Ct-values ($F_{2, 8} = 0.49$; $P = 0.505$ and $F_{2, 8} = 0.30$; $P = 0.746$, respectively) or HLB incidence ($F_{1, 8} = 2.01$; $P = 0.194$ and $F_{2, 8} = 0.26$; $P = 0.780$, respectively). Correlation of ACP adults and Ct values approached significance only for untreated plots (Spearman $R = -0.650$; $n = 9$; $P = 0.058$).

3.7. Economic analysis

The values of harvested oranges in 2012, 2013 and 2014 exceeded the estimated \$4187 ha⁻¹ (\$15.51 per tree at 270 trees ha⁻¹) for combined production costs (Muraro, 2012b) in all treatments. Values of harvested solids, hereafter referred to as kg solids (kg s) or pounds solids (lb s), determined through quality analysis results (Table 7), at 270 trees per ha, were \$7,657, \$9524 and \$8663 ha⁻¹ for untreated, HMO and GS treated trees, respectively at the invented low-end value of \$2.50 per kg s (\$1.13 per lb s) in 2014. Solids per tree increased with all treatments from 2011 to 2012 and from 2012 to 2013. Untreated trees gained 12.73 and 8.84 kg s per tree, HMO treated trees gained 9.03 kg s and 4.71 kg s per tree and GS-treated trees gained 6.99 kg s and 9.63 kg s per tree from 2011 to 2012 and 2012–2013, respectively. Significant ($P < 0.01$) decreases in kg s per tree were detected from 2013 to 2014 for untreated trees (13.04 kg s) and GS-treated trees (9.45 kg s) but not HMO-treated trees ($P = 0.058$). Comparing the 2012 and 2014 harvests, greater reductions in kg s per tree were detected for untreated trees (4.19 kg s) than HMO (0.39 kg s) or GS-treated (0.21 kg s) trees. Solids were below reported State average values for all treatments in all study years (NASS, 2014a).

HMO and GS treatment-associated production gains occurred in 2014; (747 kg ha⁻¹ and 402 kg ha⁻¹ for HMO and GS-treated trees, respectively). Expenses for ACP control over those applied to the untreated control over all three years averaged \$2508 ha⁻¹ and \$2293 ha⁻¹ for HMO-, and GS-treated trees, respectively (Table 2). Input costs were comparable for GS and HMO, although production gains were greater for HMO- than GS- and for GS-treated than untreated trees in 2014. Estimates for six delivered-in prices

ranging from \$2.50 to \$5.00 per kg s are presented in Table 9. At the 2014 production differential, delivered-in price of \$2.50/kg s (\$1.13/lb s) for HMO and nearly \$3.00/kg s (\$1.36/lb s) for GS are needed to justify input costs. Calculated delivered-in prices, assuming harvesting and transport costs of \$2.84 per box (40.82 kg) of harvested oranges and 2.81 kg (6.2 lb) solids per box (Muraro, 2012a) were \$4.96/kg s (\$2.25/lb s) for 2014 (NASS, 2014b).

4. Discussion

ACP adult numbers were significantly reduced by both high volume GS and more frequent low volume HMO treatments, although GS treatments were moderately more, and more immediately, effective. Rae et al. (1997) reported reduced ACP numbers associated with sprays of HMO applied to run-off that were comparable to those for omethoate and diflubenzuron. Because our GS treatments also included an HMO component, the difference between HMO and GS treatments for ACP adult counts should be due to an additive effect of insecticides and HMO.

Reduced ACP oviposition was thought to be a response to oil films deposited on leaves by HMO sprays to run-off in the study reported by Rae et al. (1997). Reduced egg densities were seen on trees in both HMO and GS treatments in the current study could have also been influenced by the HMO component applied by low volume or mixed with insecticides respectively. Sprays of 0.25%–2% have been reported to reduce ACP feeding, possibly as a result of the ACP female's ability to detect volatile oil molecules with antennal receptors (Beattie et al., 2010).

The apparent effect of HMO and GS treatments on flush density must also be considered. Because ACP adults are attracted to young shoot material (Sétamou et al., 2008), treatment effects on adult densities may have been influenced by flush density. However, a correlation of flush density and adult numbers was detected only on untreated trees. If flush density influenced adult ACP counts, its effects were secondary to those of HMO and GS treatments.

ACP nymph densities were comparable between HMO and GS treatments and lower than on untreated trees. However, the correlation of flush with ACP nymphs was lesser for GS- than HMO-treated trees. ACP nymphs have limited mobility and do not readily abandon their home shoot (J. Tansey Pers. Obs.). Therefore reduced correlation can likely be attributed to greater nymph mortality for GS treatments. Although Rae et al. (1997) indicated that comparable results were obtained from high volume sprays of HMO and conventional insecticides, they observed numerically more 1st and 2nd instar ACP nymphs on HMO (0.5% D-C-Tron NR applied to run-off)-treated calamodin, *Citrus madurensis*, trees than on omethoate-treated trees but more 4th and 5th instar ACP nymphs on HMO- than diflubenzuron-treated trees. However, they attributed these results to flush growth.

Differences in ACP egg densities among treatments may have been due to oviposition preferences among host plants (Sétamou et al., 2008). Deterrent and/or anti-oviposition effects of both HMO and conventional insecticides have been reported for ACP (Boina et al., 2009; Yang et al., 2013). In addition, natural enemies may have influenced our results because of generally lesser impact of HMOs compared to conventional insecticides (Stansly et al., 2002). Lower correlations of eggs and nymphs on HMO- and GS-treated compared to untreated trees also suggest ovicidal effects of HMO and GS treatments.

Additive deterrent effects of HMO and insecticide components of GS treatments likely influenced adult counts. HMO deposits associated with the higher volume applications would likely have more completely covered leaf surfaces and may have influenced results. Greater nymph-adult correlation on GS-treated trees may be due to greater recruitment of ACP adults to untreated and HMO-

Table 8

Mean \pm S.E.M. Ct values by treatment for 'Valencia' orange trees treated with horticultural mineral oil (HMO), a grower standard (GS) (insecticide) and untreated. Pairwise comparisons are within years.

2011	Ct value		F	df	P
UTC	34.79 \pm 0.20	a	0.80	2, 1	0.621
GS	37.82 \pm 2.01	a			
HMO	35.67 \pm 1.61	a			
2013	Ct value		F	df	P
UTC	30.87 \pm 0.96	a	1.49	2, 8	0.282
GS	33.91 \pm 1.38	a			
HMO	32.85 \pm 1.27	a			

Table 9
Production, production differentials and revenue differentials (US Dollars) associated with horticultural mineral oil (HMO), a grower standard (GS) (Insecticide + HMO) applications relative to untreated controls for the 2014 harvest. Delivered-in prices are also presented; these incorporate \$2.82 per box (40.82 kg of harvested fruit) for picking and transport costs. Revenue differentials are presented as related to a range of specific delivered-in prices and with input costs. Kg s = kg of soluble solids determined in laboratory evaluations; lb s = pounds solids.

Without transport/picking costs (\$2.82/box)						
\$ per kg s	5.00	4.50	4.00	3.50	3.00	2.50
\$ per lb s	2.27	2.04	1.81	1.59	1.36	1.13
Marginal income per ha (\$)						
Untreated	0.00	0.00	0.00	0.00	0.00	0.00
HMO	3733.11	3359.80	2986.49	2613.18	2239.87	1866.55
GS	2010.61	1809.55	1608.49	1407.43	1206.37	1005.31
Marginal profit per ha (\$)						
Untreated	0.00	0.00	0.00	0.00	0.00	0.00
HMO	2928.45	2555.14	2181.83	1808.52	1435.21	1061.90
GS	1481.44	1280.38	1079.32	878.26	677.20	476.13
						Marginal control cost per ha (\$)
						0.00
						804.66
						529.17
With transport/picking costs (\$2.82/box)						
\$ per kg s	5.00	4.50	4.00	3.50	3.00	2.50
\$ per lb s	2.27	2.04	1.81	1.59	1.36	1.13
Marginal income per ha (\$)						
Untreated	0.00	0.00	0.00	0.00	0.00	0.00
HMO	2662.35	2289.04	1915.73	1542.42	1169.11	795.79
GS	1462.08	1261.02	1059.96	858.90	657.84	456.78
Marginal profit per ha (\$)						
Untreated	0.00	0.00	0.00	0.00	0.00	0.00
HMO	1857.69	1484.38	1111.07	737.76	364.45	-8.86
GS	932.91	731.85	530.79	329.73	128.67	-72.40
						Marginal control cost per ha (\$)
						0.00
						804.66
						529.17

treated trees from citrus adjacent the experimental site. No reduction of ACP adult counts with time was detected in GS-treated plots. This result is likely due to high adult ACP mortality at the onset of, and sustained mortality throughout the course of the experiment.

Lower flush densities in HMO- and GS-treated trees suggest either a phytotoxic effect of treatments or an effect of greater insect pressure on untreated trees. Reports of phytotoxicity in citrus from HMOs are relatively rare with the advent narrow-range high purity products and typically only occur at relatively high doses (Beattie et al., 1989; Hodgkinson et al., 2002). Citrus responds to stress with increased flushing. For example, short periods of moisture stress can promote vegetative growth flushes (Goell et al., 1981). Vegetative growth in response to insect herbivory has been documented in other plant species (McNaughton, 1983).

HLB incidence and titer, as indicated by decreasing Ct values, increased in all treatments from 2011 to 2013. Although neither Ct values nor HLB symptoms differed significantly among treatments, the relationship between ACP counts and Ct values was marginally significant only on untreated trees. These results suggest that neither HMO nor GS treatments reduced ACP numbers enough to significantly influence HLB transmission, and that relatively few ACP are required to transmit HLB. However, the marginal relationship of HLB titer and ACP counts in untreated trees suggest that the control achieved by GS and HMO treatments could influence HLB titer. These plots were relatively small (0.49 ha) and ACP moves between host plants (Sétamou et al., 2008). Controlling ACP in a relatively small area may not have prevented immigration from nearby patches or pathogen transmission throughout the study site. This suggests the importance of region-wide coordinated ACP control.

Overall increases in yields and solids from 2011 to 2012 and 2012 to 2013 were likely the result of greater management efforts including the application of foliar nutrition. Yields were greatest for HMO-treated trees in 2012 and 2014. Although yields did not differ significantly among treatments in 2013, fruit drop was significantly greater for untreated trees. Also, numerically lower Ct values in 2013 suggested that Clas titer was greatest in untreated trees. Infection influences fruit drop, although the precise relationship is

unclear (Gottwald, 2010). Reduced yields and numerically greater fruit drop in untreated trees were also apparent in 2014. Yields decreased as well in GS-treated trees from 2013 to 2014. Although this trend was seen throughout Florida's citrus growing regions (NASS, 2014a), yields were highest and least-diminished from 2013 to 2014 in HMO treatments. Yield differences between HMO- and untreated trees may well be attributable to insect control, but explaining differences between HMO- and GS-treated trees is more difficult. Further testing of yield effects of specific insecticidal modes of action with and without HMO is required.

HMO and GS treatments resulted in production gains and greater profitability than untreated trees in 2014. However, a marginally greater estimated delivered-in price was required for profitability in GS treatments. Importantly, all treatments including controls resulted in profitable crops in 2012, 2013 and 2014. We argue that ACP control and eventual yield gains justify the costs, particularly given current prices.

5. Conclusions

Based on the results of the current study, we can recommend both GS and HMO treatments for ACP control and eventual production gains relative to untreated trees. Although both treatments significantly reduced ACP populations, greater initial reductions were achieved by GS treatments. Costs exceeded measured benefits for both treatments for the first two years. Although expenses were greater for HMO than GS in 2014, greater yields resulted in greater profitability and lower break-even price for HMO-treated trees. The choice of ACP control strategy requires that both economic and ecological factors be considered. HMO is less damaging to beneficial arthropods than broad-spectrum insecticides (Stansly et al., 2002). Insect resistance to HMO has never been documented. The financial benefits of natural enemy conservation can be substantial. For example, a roughly 3.5 x return on the cost of preserving predacious beetles in 'beetle banks' near wheat fields and their effect on aphid populations has been estimated (Thomas et al., 1991). The financial benefits of natural enemies and costs of insecticide resistance are not yet known for Valencia orange production and are complicated greatly by the role of ACP as vector of HLB. However, frequent, low-

volume application of HMO may be a more sustainable ACP control strategy than conventional insecticidal control. This strategy may allow opportunities for citrus growers to maintain productive groves, conserve natural enemies, reduce the risk of insecticide resistance and participate in organic markets.

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