



Single and Multiple Modes of Action Insecticides for Control of Asian Citrus Psyllid and Citrus Leafminer

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Abstract. Control of Asian citrus psyllid *Diaphorina citri* Kuwayama and citrus leafminer *Phyllocnistis citrella* Stainton is important to reduce the spread and severity of huanglongbing (HLB) (citrus greening) and citrus canker diseases, respectively. Insecticides are critical for the management of these pests. We therefore conducted two replicated experiments using spray treatments containing single or multiple modes of action (MoA) insecticides to reduce the incidence of these two pests in bearing citrus. Tank mixing in 47 L·ha⁻¹ (5 gal/acre) of water with synthetic plant terpenes (Requiem 25 EC, Unknown MoA) or adjuvant petroleum oil (PureSpray Green, Unknown MoA) did not improve the effectiveness of the pyrethroid zeta-cypermethrin (Mustang Max 0.15 EC, MoA 3A) against *D. citri*. Its control with flupyradifurone (Sivanto 200 SL MoA 4D) and PureSpray Green in 935 L·ha⁻¹ (100 gal/acre) water was similar to Mustang Max 0.15 EC and Requiem 25 EC, but mixtures did not provide better control than Mustang Max 0.15 EC alone. *Phyllocnistis citrella* was controlled only with Sivanto 200 SL and PureSpray Green and Requiem 25 EC alone. The addition of cyantraniliprole (group 28 MoA in A16971 premixed with thiamethoxam MoA 4A), pymetrozine (Fulfill 50 WDG, MoA 9B), or abamectin (Agri-Mek SC, MoA 6) did not improve and in many cases reduced the performance of thiamethoxam (Actara 25 WG, MoA 4A) against *D. citri* and *P. citrella*. These results demonstrated no advantage to single applications of multiple MoAs over the most effective active ingredients when applied alone for control of *D. citri* or *P. citrella*. Therefore, rotations of these active ingredients would be preferable to mixtures to avoid selection for resistance against multiple MoAs by any one application.

The Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) and citrus leafminer *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) are two important pests of citrus. *Diaphorina citri* vectors putative pathogens of HLB or citrus greening disease (Halbert and Manjunath, 2004). Nymphs are considered more efficient at acquisition and adults at the transmission and spread of these pathogens. Feeding damage by the larvae of *P. citrella* exacerbates the spread of citrus canker disease, another serious threat to the citrus industry (Chagas et al., 2001; Sohi and Sandhu, 1968). Control of *D. citri* and *P. citrella* is critical because related diseases are now endemic in Florida.

Most insecticide applications in Florida citrus target *D. citri* because of the severity and devastation of HLB. Soil drenches of neonicotinoid insecticides, with MoA 4A and cyantraniliprole (MoA 28), provide extended protection from *D. citri* and *P. citrella* lasting

6–8 weeks, but are limited to young trees because of rate restrictions (Qureshi et al., 2014a; Rogers and Shawer, 2007; Stansly and Kostyk, 2012, 2013, 2014). Both *D. citri* and *P. citrella* need developing shoots (flush) to develop and reproduce. However, mature trees generally do not flush in winter. Sprays of broad-spectrum insecticides in winter have been shown to provide significant suppression of overwintering *D. citri*, reducing numbers accessing spring growth, and are used area-wide (Qureshi and Stansly, 2010a; Stansly et al., 2009). *Phyllocnistis citrella* is thought to overwinter as larvae or pupae in scarce flush when most trees are not producing new growth attractive to both the pests to develop and reproduce (Hall and Albrigo, 2007; Lim et al., 2006; Qureshi et al., 2009). During the growing season, trees produce new growth used by both species to reproduce and increase their populations. Several natural enemies of *D. citri* and *P. citrella* are also common during the growing season and contribute to their control (Qureshi and Stansly, 2009, 2010a; Xiao et al., 2007). However, additional measures to control both *D. citri* and *P. citrella* are warranted.

Sprays of insecticides have greatly increased in Florida since the advent of HLB to reduce populations of *D. citri* as well as other pests such as *P. citrella* (Monzo and Stansly, 2015; Rogers et al., 2014). The increased use of insecticides has negatively

impacted biological control which has always been an important component of citrus insect pest management in Florida and other regions (McCoy, 1985; Michaud, 2004; Monzo et al., 2014; Qureshi and Stansly, 2007, 2009, 2010a, 2010b; Qureshi et al., 2014b; Van den Berg et al., 1992), resulting in increases in secondary pest outbreaks. Such intense use of insecticides may also accelerate selection for pest resistance already documented in some studies (Kanga et al., 2014; Tiwari et al., 2011).

Insecticidal sprays used against *D. citri* contain single or multiple MoA toxins. The latter is achieved by using premixed products or tank mixing multiple products with different MoAs to enhance efficacy and target multiple pests. However, it is important to investigate the effectiveness of the single and multiple MoA treatments for their impact on the populations of *D. citri* and other pests such as *P. citrella*. Low-volume applications tested here have been used for *D. citri* control in Florida but with variable results depending, in part, on the product tested (Attwood and Stelinski, 2008; Qureshi et al., 2014a). Findings are reported from the experiments designed to evaluate the effectiveness of labeled and experimental insecticides sprayed alone and in mixtures against *D. citri* and *P. citrella*.

Materials and Methods

Two experiments were conducted in the citrus orchards during the growing season to assess the lethal effects of foliar sprays containing single MoA insecticides, multiple MoA insecticides, or both premixed or tank mixed for control of *D. citri* and *P. citrella*. Information on the chemicals, their MoAs, and manufacturers is provided in Table 1.

Experiment 1. The experiment was conducted in a block of 6-year-old sweet orange *Citrus sinensis* (L.) Osbeck ‘Valencia’ and ‘Hamlin’ orange trees planted at a density of 326 trees/ha (132 trees/acre) at the Southwest Florida Research and Education Center in Immokalee, FL. Seven spray treatments containing one or two insecticides (Table 2) and an untreated control were assigned to a randomized complete block design (RCBD) with four replicates in seven-tree plots over four rows separated by two untreated buffer rows. There was one untreated buffer tree between treatment plots within a row. Treatments were applied on 3 June 2014 to both sides of the trees. Five treatments were applied at low volume using a Proptec rotary atomizer sprayer operating at 5 mph and equipped with a peristaltic pump delivering a final application volume at 47 L·ha⁻¹ (5 gal/acre). Ground and aerial applications against *D. citri* are becoming more and more common in Florida. The two high-volume treatments were applied at 935 L·ha⁻¹ (100 gal/acre) using a Durand Wayland AF100-32 air blast speed sprayer operating at 1.9 mph and 350 psi equipped with four John Bean ceramic nozzles no. 4, 4, 4, 2.5 on each side. Ten randomly selected shoots per plot were collected and examined under a stereomicroscope

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in the laboratory to count dead and live *D. citri* nymphs, and three fully expanded leaves on each shoot were examined to count *P. citrella* larvae at 3, 7, 14, 20, and 27 d after treatment (DAT). Density of *D. citri* adults was estimated using a “stem tap” sample taken from each of the six central trees in each plot on the above dates and 34 and 41 DAT. Adults were counted that fell on a clipboard covered with a 22 × 28 cm (8½ × 11 inches) laminated white sheet held horizontally under randomly chosen branches which were struck three times with a length of PVC pipe (Qureshi and Stansly, 2007; Qureshi et al., 2009). Two tap samples were conducted per tree on each observation.

Experiment 2. This experiment was conducted in a commercial grove near LaBelle, FL. The experimental block consisted of 7-year-old sweet orange *Citrus sinensis* (L.) Osbeck ‘Hamlin’ trees planted at a density of 326 trees/ha (132 trees/acre). Five spray treatments of single or multiple MoA insecticides (Table 4) and an untreated control were randomly distributed in an RCBD with four replicates in five-tree plots over four rows separated by one untreated buffer row. There was one untreated buffer tree between treatment plots within a row. Treatments were applied on 12 Aug. 2014 to both sides of the trees with a Durand Wayland AF100-32 air blast speed sprayer operating at 1.9 mph and 350 psi equipped with three John Bean ceramic nozzles 3, 4, 5 on each side delivering final application volume at 935 L·ha⁻¹

(100 gal/acre). Sampling was conducted at 3, 7, 14, 21, and 28 DAT using the procedure described in experiment 1 except that four tap samples were conducted per tree on three trees per plot because of less number of total trees per plot.

Data were analyzed using SAS Systems for Windows, version 9.4 (SAS Institute, 2012). The Shapiro–Wilk W test and normality plots did not validate the assumptions of parametric analysis using the Univariate procedure. Data were log transformed to reduce heterogeneity of variances and analyzed by using the nonparametric Kruskal–Wallis test at $P < 0.05$ for significant effects. Actual means are presented.

Results

Experiment 1. Effects of treatments on *Diaphorina citri* and *Phyllocnistis citrella*. There was no significant difference in dead nymphs between treatments of Mustang Max 0.15 EC alone and Mustang Max 0.15 EC plus Requiem 25 EC at 3 DAT; however, significantly more dead *D. citri* nymphs compared with the untreated control were observed with all treatments ($\chi^2 = 57.23$, $df = 7$, $P < 0.0001$) (Table 2). In contrast, no more dead nymphs than the untreated control were found as a result of any treatments that included Requiem 25 EC at 7 DAT and less with Mustang Max 0.15 EC plus Requiem 25 EC than Mustang Max 0.15 EC alone ($\chi^2 = 24.04$, $df = 7$, $P = 0.0011$). The number of live nymphs did not differ

significantly between treatments of Mustang Max 0.15 EC alone and Mustang Max 0.15 EC plus Requiem 25 EC; however, fewer were observed in response to all treatments compared with the untreated control on all sample dates through 27 DAT ($\chi^2 = 41.90$, $df = 7$, $P < 0.0001$) except for Requiem 25 EC alone at either volume after 7 DAT.

Treatment effects on *P. citrella* populations were significant at 3 DAT ($\chi^2 = 64.08$, $df = 7$, $P < 0.0001$) and 7 DAT ($\chi^2 = 26.01$, $df = 7$, $P = 0.0005$) although only Sivanto 200 SL plus PureSpray Green and Requiem 25 EC applied alone at high volume provided significant reduction in larvae compared with the untreated control at either 3 or 7 DAT (Table 2).

Significant reduction in adult *D. citri* populations was observed with all treatments compared with the untreated control at all observation dates from 7 through 27 DAT ($\chi^2 = 24.40$, $df = 7$, $P < 0.0010$, Table 3) except at 3 DAT for Requiem 25 EC alone at either volume or with Mustang Max 0.15 EC and at 14 DAT for Requiem 25 EC alone at low volume. Overall treatment effect was still significant at 34 DAT ($\chi^2 = 24.97$, $df = 7$, $P = 0.0008$), but only Mustang Max 0.15 EC plus Requiem 25 EC at either rate and Sivanto 200 SL plus PureSpray Green were providing reduction compared with the untreated control. However, tank mixing with Requiem 25 EC or PureSpray Green did not improve the effectiveness of Mustang Max 0.15 EC on any date. No phytotoxicity was observed with any treatment.

Table 1. Details of insecticides and adjuvants sprayed on citrus trees infested with *Diaphorina citri* Kuwayama and *Phyllocnistis citrella* Stainton.

Brand name formulation (I, A) ²	Chemical name	IRAC MoA ³	Manufacturer
Mustang Max 0.15 EC (I)	Zeta-cypermethrin	3A	FMC Corporation (PA)
Requiem 25 EC (I)	Chenopodium	Unknown	Bayer CropScience (NC)
Sivanto 200 SL (I)	Flupyradifurone	4D	Bayer CropScience (NC)
PureSpray Green (A)	petroleum oil	Unknown	Petro-Canada (ON)
Fulfil 50 WDG (I)	Pymetrozine	9B	Syngenta Crop Protection (NC)
Agri-Mek SC (I)	Abamectin	6	Syngenta Crop Protection (NC)
Actara 25 WG (I)	Thiamethoxam	4A	Syngenta Crop Protection (NC)
A16971 ⁴ (I)	Thiamethoxam + cyantraniliprole	4A + 28	Syngenta Crop Protection (NC)
Dyne-Amic (A)	Nonionic surfactant	Unknown	Helena Chemical Company (TN)

²I = insecticide, A = adjuvant.

³Insecticide Resistance Action Committee, Mode of Action, <http://www.irac-online.org/>.

⁴Experimental premix.

Table 2. Mean number of *Diaphorina citri* Kuwayama nymphs and *Phyllocnistis citrella* Stainton larvae per shoot in 6-year-old ‘Valencia’ and ‘Hamlin’ orange trees that were untreated or treated with foliar sprays containing single or multiple Mode of Action insecticides and adjuvants on 3 June 2014 at Southwest Florida Research and Education Center, Immokalee, FL.

Treatment/formulation	Rate L·ha ⁻¹ (oz/acre)	Application volume L·ha ⁻¹ (gal/acre)	<i>D. citri</i> dead nymphs/shoot days after treatment		<i>D. citri</i> live nymphs/shoot days after treatment					<i>P. citrella</i> larvae/shoot days after treatment	
			3	7	3	7	14	20	27	3	7
Untreated control			0.08 c	0.05 d	6.60 a	10.88 a	9.13 a	12.05 b	8.90 a	1.28 a	1.23 a
Requiem 25 EC	4.7 (64)	47 (5)	1.83 b	0.38 d	2.65 bc	4.08 bc	5.18 a	17.00 ab	7.68 ab	1.07 a	0.98 a
Mustang Max 0.15 EC	0.3 (4)	47 (5)	4.93 a	2.50 a	2.05 bc	2.25 d	0.43 cb	4.55 c	0.37 c	1.42 a	1.05 a
Mustang Max 0.15 EC + PureSpray Green	0.3 + 9.3 (4 + 128)	47 (5)	6.98 a	1.80 abc	2.03 bc	10.40 ab	1.50 cb	3.12 c	0.75 c	1.28 a	0.88 a
Mustang Max 0.15 EC + Requiem 25 EC	0.3 + 2.3 (4 + 32)	47 (5)	4.80 a	0.68 bcd	1.90 bc	3.75 cd	0.08 c	4.75 c	0.53 c	1.20 a	0.95 a
Mustang Max 0.15 EC + Requiem 25 EC	0.3 + 4.7 (4 + 64)	47 (5)	8.05 a	1.00 cd	2.00 bc	0.30 e	0.75 cb	2.40 c	1.14 c	1.10 a	1.13 a
Requiem 25 EC	4.7 (64)	935 (100)	4.73 ab	0.73 bcd	3.13 b	2.65 de	7.65 a	22.75 a	4.10 ab	0.25 b	0.50 b
Sivanto 200 SL + PureSpray Green	0.7 + 9.3 (10 + 128)	935 (100)	6.45 a	1.60 ab	0.70 c	2.45 de	1.93 b	2.50 c	4.53 bc	0.10 b	0.40 b

Means in a column sharing common letters are not significantly different ($P > 0.05$).

Experiment 2. Effects of treatments on *Diaphorina citri* and *Phyllocnistis citrella*. Significant reduction of live *D. citri* nymphs was observed with all treatments on all observation days through 14 DAT ($\chi^2 = 68.43$, $df = 5$, $P < 0.0001$) except at 14 DAT for the only treatment that did not include thiamethoxam: Fulfill 50 WDG 0.4 L-ha⁻¹ (5.5 oz/acre) (Table 4). None of the mixed treatments provided better control of nymphs compared with thiamethoxam (Actara 25 WG 0.4 L-ha⁻¹, 5.5 oz/acre) alone. Similar treatment effects were seen against adults with Fulfill 50 WDG alone being the weakest treatment, and none were better than thiamethoxam (Actara 25 WG) alone which was the only treatment still effective at 28 DAT ($\chi^2 = 14.18$, $df = 5$, $P = 0.0145$).

All treatments significantly reduced *P. citrella* compared with the untreated control through 14 DAT ($\chi^2 = 100.45$, $df = 5$, $P < 0.0001$) except Fulfill 50 WDG alone at 7 DAT which also provided significantly less reduction compared with other treatments. There was no improvement in control over thiamethoxam (Actara 25 WG) alone with any mixture containing two or more MoAs. No phytotoxicity was observed in any treatment.

Discussion

Findings from both experiments demonstrated that there was little or no advantage to single-spray applications of multiple MoA

insecticides tested over the most effective single MoA active ingredients sprayed alone for control of *D. citri* or *P. citrella*. Tank mixing with synthetic plant terpenes (Requiem 25 EC, Unknown MoA) did not improve the performance of the pyrethroid zeta-cypermethrin (Mustang Max 0.15 EC, MoA 3A) against *D. citri* and *P. citrella* except 1 week prolonged reduction of adult *D. citri*. The direct effects on nymphs are measurable for 2–3 weeks which is the time it takes for new shoots to mature and nymphs to develop into adults. The slightly prolonged effect of mixed insecticide treatments on adults compared with single insecticide could be carryover from the earlier suppression of nymphs. Petroleum-based horticultural mineral oil is a commonly used adjuvant which, when applied alone, also provides considerable control of *D. citri* and several other pests such as *P. citrella*, orange spiny whitefly, *Aleurocanthus spiniferus* (Quaintance), red scale, *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae), and chaff scale, *Parlatoria pergandii* Comstock (Hemiptera: Aleyrodidae) (Beattie et al., 2000; Davidson, 1991; Qureshi et al., 2014a; Rae et al., 1996, 1997; Tansey et al., 2015). Neither petroleum oil (PureSpray Green) nor Requiem significantly improved the performance of Mustang Max 0.15 EC against *D. citri*. Furthermore, Mustang seemed to interfere with control exerted by Requiem against *P. citrella*, which was lost when the two products were mixed.

All treatments in the second experiment were applied with Dyne-Amic nonionic surfactant which contains highly refined methylated seed oils and specialized organosilicone particles to provide effective penetration and coverage. However, the effectiveness of thiamethoxam (MoA 4A) against *D. citri* and *P. citrella* was not improved, and was even reduced, by premixing with cyantraniliprole (MoA 28) in A16971 or tank mixing with pymetrozine (Fulfill 50 WDG, MoA 9B) or abamectin (Agri-Mek SC, MoA 6). Therefore, we saw nothing gained by the tank or premixes evaluated in these trials, which furthermore incurred the additional disadvantage of exposing pest populations to multiple MoAs and thus limiting.

Insecticide resistance is a serious concern and already reported in *D. citri* against some effective MoAs (Kanga et al., 2014; Tiwari et al., 2011). Therefore, rotations of effective active ingredients would be preferable to mixtures to avoid selection for resistance against multiple MoAs by any one application. Such rotations may also use selective insecticides to help conserve the most effective MoAs (Qureshi et al., 2013; Rae et al., 1997; Tansey et al., 2015) and biological control which may suffer more mortality of predators and parasitoids from the use of multiple MoAs at one time compared with single MoA rotation over time (Qureshi and Stansly 2009, 2010a). These considerations

Table 3. Mean number of *Diaphorina citri* Kuwayama adults per tap sample in 6-year-old ‘Valencia’ and ‘Hamlin’ orange trees that were untreated or treated with foliar sprays containing single or multiple Mode of Action insecticides and adjuvants on 3 June 2014 at Southwest Florida Research and Education Center, Immokalee, FL.

Treatment/formulation	Rate L-ha ⁻¹ (oz/acre)	Application volume L-ha ⁻¹ (gal/acre)	<i>D. citri</i> adults/tap sample days after treatment						
			3	7	14	20	27	34	41
Untreated control			0.25 a	0.71 a	1.10 a	1.29 a	0.56 a	0.65 a	0.71 a
Requiem 25 EC	4.7 (64)	47 (5)	0.21 ab	0.27 b	0.94 a	0.58 b	0.23 bc	0.50 a	0.35 a
Mustang Max 0.15 EC	0.3 (4)	47 (5)	0.06 bc	0.08 bc	0.23 b	0.21 bcd	0.17 bcd	0.33 abc	0.38 a
Mustang Max 0.15 EC + PureSpray Green	0.3 + 9.3 (4 + 128)	47 (5)	0.04 c	0.08 bc	0.29 b	0.65 bc	0.29 b	0.46 ab	0.19 a
Mustang Max 0.15 EC + Requiem 25 EC	0.3 + 2.3 (4 + 32)	47 (5)	0.08 abc	0.00 c	0.25 b	0.17 cd	0.00 d	0.10 c	0.31 a
Mustang Max 0.15 EC + Requiem 25 EC	0.3 + 4.7 (4 + 64)	47 (5)	0.02 c	0.04 bc	0.25 b	0.13 cd	0.15 bcd	0.13 c	0.19 a
Requiem 25 EC	4.7 (64)	935 (100)	0.10 abc	0.08 bc	0.17 b	0.25 bcd	0.04 cd	0.39 abc	0.40 a
Sivanto 200 SL + PureSpray Green	0.7 + 9.3 (10 + 128)	935 (100)	0.08 abc	0.08 bc	0.04 b	0.06 d	0.21 bcd	0.15 bc	0.35 a

Means in a column sharing common letters are not significantly different ($P > 0.05$).

Table 4. Mean number of *Diaphorina citri* Kuwayama nymphs per shoot, adults per tap sample, and *Phyllocnistis citrella* Stainton larvae per shoot in 7-year-old ‘Hamlin’ orange trees that were untreated or treated with foliar sprays containing single or multiple Mode of Action insecticides and adjuvants on 12 Aug. 2014 at a commercial grove near LaBelle, FL.

Treatment/formulation ^z	Rate L·ha ⁻¹ (oz/acre)	Application volume L·ha ⁻¹ (gal/acre)	<i>D. citri</i> nymphs/shoot days after treatment			<i>D. citri</i> adults/tap sample days after treatment					<i>P. citrella</i> larvae/shoot days after treatment		
			3	7	14	3	7	14	21	28	3	7	14
Untreated control			7.00 a	7.46 a	9.97 a	0.58 a	0.63 a	0.67 a	0.38 a	0.23 ab	2.14 a	3.26 a	2.97 a
A16971	0.3 (3.75)	935 (100)	0.40 c	0.09 c	3.30 b	0.13 c	0.21 b	0.19 bc	0.27 ab	0.25 ab	0.16 c	0.06 b	0.44 c
Fulfill 50 WDG	0.4 (5.5)	935 (100)	2.80 b	3.58 b	9.78 a	0.40 b	0.54 a	0.44 ab	0.40 a	0.38 a	1.31 b	2.67 a	2.09 b
A16971 + Agri-Mek SC	0.3 + 0.2 (3.75 + 2.6)	935 (100)	0.53 c	0.00 c	0.98 cd	0.17 c	0.15 bc	0.15 c	0.06 c	0.08 bc	0.40 c	0.10 b	0.29 c
A16971 + Fulfill 50 WDG	0.3 + 0.4 (3.75 + 5.5)	935 (100)	1.08 bc	0.03 c	1.21 cb	0.19 bc	0.00 c	0.21 bc	0.08 bc	0.10 bc	0.33 c	0.22 b	0.24 c
Actara 25 WG	0.4 (5.5)	935 (100)	0.30 c	0.03 c	0.00 d	0.00 c	0.02 c	0.06 c	0.00 c	0.04 c	0.22 c	0.43 b	0.40 c

Means in a column sharing common letters are not significantly different ($P > 0.05$).

²All treatments were applied with Dyne-Amic, a nonionic surfactant at 0.9 L-ha⁻¹ (32 oz/acre).

are important for developing integrated and sustainable pest management programs.

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