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TOXICITY OF 6 MITICIDES TO THE ASIAN CITRUS PSYLLID, *DIAPHORINA CITRI* (HEMIPTERA: LIVIIDAE)

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ABSTRACT

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is an important pest of citrus. Research into strategies to control ACP is ongoing at many facilities, including at the USDA-ARS U.S. Horticultural Research Laboratory (USHRL) in Fort Pierce, Florida. The USHRL maintains colonies of ACP, but their survival is often threatened by mites which render host plants unsuitable for ACP. Our objective was to identify miticides/insecticides that could be used to control mite outbreaks with minimal or no adverse affect on ACP. We tested the following 6 miticides in greenhouse trials for their toxicity to each life stage of ACP and also investigated sublethal effects on development and oviposition of ACP: bifenazate (Acramite 50 WS), spirodiclofen (Envidor 2 SC), dicofol (Kelthane MF), pyridaben (Nexter), petroleum oil, and chlorfenapyr (Pylon). The miticides differed in their toxicity when applied directly to ACP. Bifenazate was the only miticide that was not toxic to any life stage of ACP, whereas pyridaben and chlorfenapyr, which are also labeled as insecticides, were toxic to all life stages of ACP. Petroleum oil and dicofol killed adult and nymphal ACP, but were nontoxic to eggs. Spirodiclofen was nontoxic to adults, but reduced nymphal survivorship and killed eggs. The duration of residual activity against adult ACP also was widely variable: dicofol residues were only toxic for up to 10 days, whereas chlorfenapyr residues were still toxic after 36 days. We recommend using dicofol, pyridaben, petroleum oil, and chlorfenapyr to maintain clean plants prior to colonization by ACP and then rotating bifenazate and spirodiclofen, if maintaining adult ACP, or bifenazate, dicofol, and petroleum oil, if maintaining eggs. Bifenazate is the only product safe for maintaining nymphal ACP. Our results are useful for research facilities that wish to maintain colonies of ACP and control mites and may be useful for citrus growers and researchers that wish to kill ACP and mites with a single treatment.

Key Words: bifenazate, chlorfenapyr, dicofol, petroleum oil, pyridaben, spirodiclofen

RESUMEN

El psílido asiático de los cítricos (PAC), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), es una plaga importante de los cítricos. Se estan realizando investigaciones sobre las estrategias para el control de PAC en muchas instalaciones, incluyendo el Laboratorio de Investigacion de Horticultura (USHRL) de USDA-ARS en Fort Pierce, Florida. El USHRL mantiene colonias de PAC, pero su supervivencia está amenazada frecuentemente por los ácaros que hacen las plantas hospedantes no aptas para el PAC. Nuestro objetivo fue identificar los acaricidas / insecticidas que podrían ser utilizados para controlar las infestaciones de ácaros sin o con mínimos efectos adversos sobre los PAC. Hemos probado los siguientes 6 acaricidas en ensayos de invernadero por su toxicidad hacia cada estadio de los PAC y también se investigó los efectos subletales sobre el desarrollo y la oviposición de PAC: bifenazato (Acaramite® 50 WS), espirodiclofeno (Envidor® 2 SC), dicofol (Kelthane® MF), piridaben (Nexter®), aceite de petróleo, y clorfenapir (Pylon®). Los acaricidas varían en su toxicidad cuando fueron aplicados directamente a los PAC. Bifenazato fue el único acaricida que no era tóxico para cualquier estadio de los PAC, mientras que piridaben y clorfenapir, que también son registrados como insecticidas, fueron tóxicos para todos los estadios de PAC. El aceite de petróleo y dicofol mató los adultos y las ninfas de PAC, pero no fueron tóxicos para los huevos. La duración de la actividad residual contra los adultos de PAC también fue muy variable: los residuos de dicofol sólo fueron tóxicas para un máximo de 10 días, mientras que los residuos de chlorfenapyr fueron tóxicos después de 36 días. Recomendamos el uso de dicofol, piridaben, aceite de petróleo, y clorfenapir para mantener limpias las plantas antes de la colonización por los PAC y luego alternando el uso de bifenazato y espirodiclofeno para mantener los adultos de PAC, o bifenazato, dicofol, y aceite de petróleo para mantener los huevos. El bifenazato es el único producto seguro para mantener las ninfas de PAC. Nuestros resultados son útiles para las instalaciones de investigación que desean mantener colonias de PAC y control de los ácaros y puede ser útil para los productores de cítricos y los investigadores que desean matar los PAC y ácaros con un solo tratamiento.

Palabras Clave: bifenazato, clorfenapir, dicofol, aceite de petróleo, piridaben, espirodiclofeno

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), is an important pest of citrus because it transmits bacteria (*Candidatus* Liberibacter spp.) strongly implicated in huanglongbing (HLB; citrus greening disease), which is the world's most serious disease of citrus (McClean & Schwartz 1970; Bové 2006). A 3-component management program against HLB was originally recommended in Florida: chemical control of ACP, removing HLB-infected trees, and planting disease-free nursery stock (Gottwald 2007; Hall & Gottwald 2011). The effectiveness of this and other management programs in negating incidence and spread of HLB is unknown, but an integrated pest management approach is needed to provide more economical, environmentally safe, and sustainable control of ACP, and ultimately HLB. Strategies that eventually may complement or replace chemicals for controlling ACP include host plant resistance, augmentative or classical biological control, cultural control, or molecular methods to silence genes of ACP to induce mortality, block transmission of the pathogen, or otherwise render ACP a less efficient vector (reviewed in Hall et al. 2013). Research into these strategies is ongoing worldwide, including at the USDA-ARS U.S. Horticultural Research Laboratory (USHRL) in Fort Pierce, Florida. Colonies of ACP currently are maintained at the USHRL on *Citrus macrophylla* Wester (Hall et al. 2010) to support this research.

The twospotted spider mite (TSSM; *Tetranychus urticae* Koch), sixspotted mite [*Eotetranychus sexmaculatus* (Riley)], broad mite [*Polyphagotarsonemus latus* (Banks)], and citrus red mite [*Panonychus citri* (McGregor)] are the most common intruding pests on citrus plants maintained in the greenhouse at the USHRL and can hinder or prevent production of ACP. Mites often colonize citrus plants in greenhouses prior to being inoculated with ACP for rearing or experiments and mite outbreaks also occur on citrus plants already colonized by ACP. TSSM is the most abundant mite in the greenhouse at the USHRL and produces copious amounts of webbing, which ensnares nymphal and adult ACP and inhibits feeding and oviposition. TSSM also feeds on cell chloroplasts under the leaf epidermis, damaging or killing the leaves on which ACP feed and develop. Predatory mites can be released to control herbivorous mites, but the proper methods, efficacy, and impact of this strategy on ACP are unknown. Herbivorous mites can be successfully controlled in greenhouses using commercial miticides (Brodsgaard & Albajes 1999; Cloyd et al. 2009). Regular applications of miticides are necessary to control TSSM because a single female completes a generation in 2 to 3 weeks and can lay more than 100 eggs (Gerson & Weintraub 2012), so their population quickly rebounds after halting applications of miticides. However, ACP are relatively susceptible to direct sprays or residues of a number of toxins used on citrus in greenhouses (MLR, personal observation). Therefore, this study arose out of the necessity of identifying miticides with little or no toxicity to ACP. Specifically, we tested whether 6 commonly-used miticides/insecticides are lethal to adults, nymphs, and eggs of ACP or have sub-lethal effects on development or oviposition.

Materials and Methods

ACP for all experiments presented here were obtained from a colony established at the USHRL during early 2000 (Hall et al. 2010) and maintained on *Citrus macrophylla* Wester using similar procedures described by Skelley & Hoy (2004). The following experiments were conducted from mid-Apr to mid-Sep 2012, which is when mite populations peak in greenhouses in South Florida and miticides are applied frequently.

Mortality of Adults Sprayed with Miticide

We collected 70 female ACP that had been adults for less than 24 h to standardize their age and minimize the probability that they had mated (Wenninger & Hall 2007). On each of 35 'Duncan' grapefruit (*Citrus paradisi* Macf.) trees, one female ACP was caged on the abaxial side of a leaf and a second female was caged on the adaxial side of a second leaf with clip cages. Clip cages were round plastic tubes (21 × 27 mm) attached to aluminum clips and had a top opening covered by screen. ACP acclimated to plants for 48 h in a greenhouse, which averaged ~30 °C, ~56% relative humidity (RH), and 14 hr photoperiod, before we sprayed them with miticides.

The highest concentrations of 6 miticides/insecticides (hereafter "miticides"), bifenazate (Acramite 50 WS), spirodiclofen (Envidor 2 SC), dicofol (Kelthane MF), pyridaben (Nexter), petroleum oil, and chlorfenapyr (Pylon) (Table 1) were prepared separately in 180 mL Nalgene aerosol spray bottles (#2430-0200, Fisher Scientific, Pittsburgh, PA) by mixing the miticides with 100 mL of tap water. The amount of product per 100 mL of tap water was calculated from the labeled rate per 100 gal of water. These miticides were selected because they are labeled for, and commonly used in, greenhouses, have a relatively short restricted entry interval (12 h), and some are labeled specifically for mites and not insects, such as bifenazate, spirodiclofen, and dicofol (Table 1). Plants were separated into 7 groups of 5 plants each and all ACP within a group (n = 10 ACP per group) were sprayed with a single miticide or tap water (control). We sprayed ACP through the mesh top of the clip cages until the entire leaf surface was wet using the aforementioned aerosol

TABLE 1. MITICIDES/INSECTICIDES SCREENED FOR TOXICITY TO *DIAPHORINA CITRI*.

Trade name	Class	Active ingredient	Miticide / insecticide	Highest application rate (product/100 mL water)	Moderate application rate (product/100 mL water)	Manufacturer
Acramite 50 WS	Carboxylic acid ester	Bifenazate (50%)	M	0.237 g	0.185 g	Chemtura Corporation, Middlebury, CT
Envidor 2 SC	Tetronic acids	Spirodiclofen (22.3%)	M	0.26 mL	0.211 mL	Bayer CropScience LP, Research Triangle Park, NC
Kelthane MF	Organochlorine	Dicofol (42%)	M	0.26 mL	0.211 mL	Dow AgroSciences LLC, Indianapolis, IN
Nexter	Pyridazinone	Pyridaben (75%)	M / I	0.105 g	0.079 g	Gowan Company, Yuma, AZ
Petroleum oil	Oil	Petroleum oil (98%)	M / I	1.04 mL	0.792 mL	BASF Corporation, St. Louis, MO
Pylon	Pyrroles	Chlorfenapyr (21.4%)	M / I	0.09 mL	0.053 mL	OHP, Inc., Mainland, PA

spray bottle. Each adult ACP was checked daily until their death to determine whether these miticides reduced the lifespan of adults. This experiment was repeated in its entirety with novel plants and ACP, but using moderate concentrations of the miticides mixed with 100 mL of tap water (Table 1). The moderate concentration for each product was calculated by halving the highest concentration to find the lowest concentration, and then using the midpoint between the high and low concentration as the moderate concentration.

We used separate negative binomial models (PROC GENMOD, SAS Institute, 2011) to test for differences in adult lifespan among high and moderate applications of the miticides. The side of the leaf on which ACP were caged was included in the original statistical model, but was removed because it did not influence longevity of ACP. The LSMEANS statement was then used to estimate separation between pairs of means (SAS Institute 2011; Sokal and Rohlf 1995). The final number of ACP used for statistical analyses of this experiment, and the following experiments, was sometimes reduced because individuals escaped.

Sublethal Effects of Miticides on Oviposition

We sprayed 10 flushing 'Ridge pineapple' (*C. sinensis* L. Osbeck) seedlings each with tap water or the highest labeled concentration of bifenazate or spirodiclofen (N = 30) until thoroughly wet and allowed them to dry for 2 h in a greenhouse. We used these 2 miticides for this experiment because the other miticides were toxic to adults (see Results). We separated 60 female and 30 male ACP into 30 groups, each consisting of 2 females and one male. We chose ACP that had been adults for approximately 6 days to ensure that they had reached reproductive maturity (Wenninger & Hall 2007). Each group of 3 adults was briefly anesthetized with CO_2, placed onto filter paper in a Petri dish, and sprayed with miticide or tap water using an aerosol spray bottle. Each group was then transferred using a small brush moistened with tap water to a seedling that had been sprayed with the same treatment. We enclosed plants with plastic cylinders (37 × 255 mm) that had one top opening and 4 circular side openings (25 mm diameter) that were covered with screen. The open bottom of each cylinder was pressed into the Cone-tainer (Stuewe and Sons, Corvalis OR) to prevent adult ACP from escaping. The plants were placed in an environmental chamber operating at ~26.8 °C, 89% relative humidity, and 14 h daily illumination. The number of eggs on each plant was counted 6 days after inoculation under a dissection microscope. We did not repeat this experiment with moderate concentrations of the miticides because high concentrations did not reduce oviposition (see Results).

We used a negative binomial model (PROC GENMOD, SAS Institute, 2011) to test for differences in the number of eggs laid among treatments. The number of adults surviving at the end of the 6-day oviposition period was included as a covariate because the number of eggs laid can be correlated with the number of adults on a plant (MLR, unpublished data). The LSMEANS statement was then used to estimate separation between pairs of means (SAS Institute 2011; Sokal & Rohlf 1995).

Residual Activity of Miticides against Adult ACP

One hundred 'Valencia' sweet orange (*C. sinensis*) trees approximately 70-105 cm tall were separated into 5 groups of 20 trees each in a greenhouse. One group each was sprayed with tap water or the highest labeled concentration of a miticide that kills adult ACP when sprayed directly: dicofol, pyridaben, petroleum oil, and chlorfenapyr. Bifenazate and spirodiclofen were not used because they do not kill adults (see Results). Plants dried for one day and then half the plants in each treatment were watered from above the leaf canopy and the other half were watered below the leaf canopy to test whether rinsing the plants with water removes chemical residues. We then used clip cages to cage 80 adult, virgin ACP of mixed gender on the leaves of 16 plants of each treatment (5 ACP per plant and 8 cages per watering regime). We used the watering regime previously described on each day following application of tap water or miticide, but did not water above leaves that had ACP caged on them. Percent mortality of ACP was determined 72 h after confining them to plants. New ACP were transferred to new leaves on the plants 4, 7, 11, 15, 22, 29, and 36 days following application of miticides and mortality was checked 72 h after each transfer. Plants were removed from the experiment when the miticide with which they were sprayed no longer caused higher mortality to ACP than the control.

We tested whether percent mortality of ACP was influenced on each transfer date by the miticide treatment, watering regime, and interaction of miticide and watering regime by using separate non-parametric repeated measures analyses: the F-approximation of the Friedman test (Ipe 1987) and the associated Rank Sum multiple comparison test (PROC GLIMMIX, SAS Institute 2011).

Mortality of Nymphs Sprayed with Miticide

Toxicity of miticides to nymphs was investigated using leaf disks embedded on agar. We excised mature leaves from young 'Ridge pineapple' trees maintained in a greenhouse and cut 30 circular disks from these leaves using a 2.34 cm diameter copper pipe with sharpened edges. Each leaf disk, adaxial side up, was embedded on agar (7 g / 500 ml water) in a small Petri dish (suspension culture dish, 35 mm × 10 mm, non-treated polystyrene, #430588, Corning Inc., Corning, NY) following the methods of Hall et al. (2010) and Hall & Richardson (2012). 5 nymphs in the fifth instar were transferred from a flush shoot to a filter paper disk using a small brush moistened with tap water. Nymphs on 10 disks each (N = 30 disks, 150 ACP) were misted until wet with tap water or the highest labeled concentrations of bifenazate or spirodiclofen. We used bifenazate and spirodiclofen for this experiment because the other miticides are toxic to adults and nymphs are usually as, or more, susceptible to insecticides than adults (Hall & Richardson 2012). We then transferred the nymphs to the leaf disks, capped the Petri dish with the lid, and secured the lid using laboratory sealing film (#6600 1026, Whatman International Ltd, Maidstone, England) to prevent nymphs from escaping. Nymphs were not sprayed directly on leaf disks to prevent drowning in the chemical or water droplets. The Petri dishes were placed on a tray in an environmental chamber operating at ~24 °C, 89% relative humidity, and 14 h daily illumination. The water associated with the agar generally maintains relative humidity in the Petri dishes at 98 to 100% (DGH, unpublished data). The percentage of dead ACP in each Petri dish was calculated after 48 h. We did not repeat this experiment with moderate concentrations of the miticides because high concentrations did not kill nymphs (see Results).

We tested whether percent mortality of ACP differed among miticide treatments using the F-approximation of the Friedman test and the associated Rank Sum multiple comparison test (PROC GLMMIX, SAS Institute 2011).

Number of ACP Reaching Adulthood when Sprayed with Miticide during the Nymphal Stage

We caged 18 flushing 'Duncan' grapefruit trees individually in bugdorms (MegaView Science Co., Ltd., Taichung, Taiwan) in a greenhouse that averaged ~27 °C, ~71% relative humidity, and ~13 h 40 minutes of daily illumination. We released 20 adult ACP into each cage, with no regard to gender. Adult ACP were removed from the cages after 3 days and then 5 days after removal of the adults we sprayed 6 plants each with tap water or a moderate concentration of bifenazate or spirodiclofen when nymphal ACP were in the third or fourth instar. No attempt was made to determine the initial number of nymphs on each plant because handling the plants and nymphs causes high mortality of nymphs. The initial date nymphs developed into adults in each cage was noted in order to test whether miticides prolonged development of ACP. All ACP that survived to adulthood also were collected, counted, and sexed.

We compared date of adult emergence and abundance of male, female, and total ACP among miticide treatments using separate negative binomial models (PROC GENMOD, SAS Institute, 2011). The number of flush shoots in each cage was included as a covariate because the number of eggs laid on a plant is often correlated with the number of flush shoots (MLR, unpublished data). The LSMEANS statement was then used to estimate separation between pairs of means (SAS Institute 2011; Sokal & Rohlf 1995).

Number of ACP Reaching Adulthood when Sprayed with Miticide during the Egg Stage

We caged 21 flushing 'Duncan' grapefruit trees individually in bugdorms in a greenhouse that averaged ~27°C, ~71% relative humidity, and ~13 h 40 minutes of daily illumination. We released 20 adult ACP into each cage. After 3 days we removed adult ACP from the cages and sprayed 3 plants each with the highest labeled concentration of miticide or tap water. The initial date nymphs developed into adults in each cage was noted in order to test whether miticides prolonged development of ACP. All ACP that survived to adulthood also were collected, counted, and sexed. This experiment was repeated in its entirety for a total of 6 replications per treatment. We also repeated this experiment with moderate concentrations of spirodiclofen, pyridaben, and chlorfenapyr because these 3 miticides are toxic at the maximum labeled concentration (see Results) and we wanted to determine if they also were toxic at moderate concentrations.

We compared date of adult emergence and abundance of male, female, and total ACP among miticide treatments using separate negative binomial models and the LSMEANS statement, as discussed for the previous experiment.

Results

Mortality of Adults Sprayed with Miticide

The lifespan of adult ACP was influenced by miticide when applied at the high (χ^2 = 68.7, df = 6,62, P < 0.001) or moderate rates (χ^2 = 31.7, df = 6,60, P < 0.001). Dicofol, pyridaben, petroleum oil, and chlorfenapyr shortened the lifespan of ACP compared to the control, whereas bifenazate and spirodiclofen did not (Fig. 1a, b).

Sublethal Effects of Miticides on Oviposition

The number of eggs laid per plant was influenced by the total number of adult ACP remaining on the plants after 6 d, but not the miticide (adults ACP, χ^2 = 18.6, df = 1,26, P < 0.001; miticide, χ^2 = 5.1, df = 2,26, P = 0.08). The mean (± SEM) number of eggs per adult ACP was 116 ±

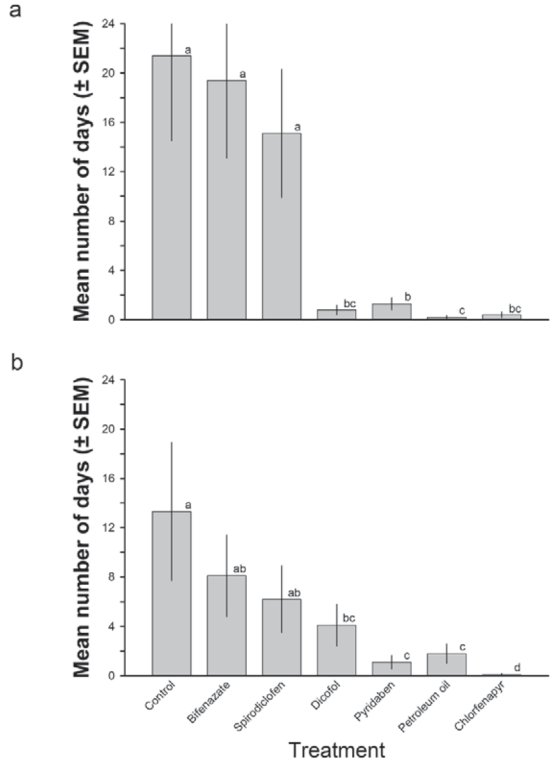

Fig. 1. Mean (± SEM) lifespan of adult *Diaphorina citri* on plants sprayed with a) the highest and b) moderate labeled concentration of 6 miticides or tap water (control). Means with different letters are significantly different (means separation test, P < 0.05).

46 when sprayed with tap water, 93 ± 49 when sprayed with bifenazate, and 90 ± 47 when sprayed with spirodiclofen.

Residual Activity of Miticides against Adult ACP

All the miticides had some residual activity against adult ACP (Tables 2 and 3). Rinsing the plants with tap water had no effect 1 day after application of miticides, and all miticides were toxic to ACP (Tables 2 and 3). Dicofol residues no longer caused higher mortality to ACP than the control by day 4 on plants that were rinsed and day 7 on plants that were not rinsed (Table 3). Pyridaben residues no longer caused higher mortality to ACP than the control on plants that were not rinsed by day 4, but were toxic up to 36 days on plants that were rinsed (Table 3). Petroleum oil residues no longer caused higher mortality to ACP than the control by day 7 on plants that were rinsed and 22 days on plants that were not rinsed (Table 3). Chlorfenapyr residues only started to decrease in toxicity 29 days after application and mortality of ACP on plants that were not rinsed was still higher than the control after 36 days (Table 3).

TABLE 2. RESULTS OF NON-PARAMETRIC REPEATED MEASURES ANALYSIS THAT TESTED DIFFERENCES IN PERCENT MORTALITY OF *DIAPHORINA CITRI* AMONG RESIDUES OF 4 MITICIDES UP TO 36 DAYS FOLLOWING APPLICATION. HALF THE HOST PLANTS WERE RINSED WITH TAP WATER DAILY AND THE OTHER HALF WERE NOT RINSED TO TEST WHETHER THIS INFLUENCED RESIDUAL ACTIVITY.

	Independent variable		
Days post-application	Miticide	Rinsed	Miticide × Rinsed
1	$F = 26.7$, df = 4, $P < 0.001$	$F = 26.7$, df = 1, $P < 0.001$	$F = 0.8$, df = 4, $P = 0.53$
4	$F = 15.8$, df = 4, $P < 0.001$	$F = 0.53$, df = 1, $P = 0.47$	$F = 3.65$, df = 4, $P = 0.009$
7	$F = 13.8$, df = 4, $P < 0.001$	$F = 2.46$, df = 1, $P = 0.12$	$F = 1.65$, df = 4, $P = 0.20$
11	$F = 9.11$, df = 3, $P < 0.001$	$F = 0.00$, df = 1, $P = 0.98$	$F = 0.00$, df = 1, $P = 0.98$
15	$F = 50.4$, df = 3, $P < 0.001$	$F = 0.56$, df = 1, $P = 0.46$	$F = 2.88$, df = 1, $P = 0.10$
22	$F = 21.1$, df = 3, $P < 0.001$	$F = 4.18$, df = 1, $P = 0.05$	$F = 4.18$, df = 1, $P = 0.05$
29	$F = 5.03$, df = 2, $P < 0.001$	$F = 0.17$, df = 1, $P = 0.69$	$F = 1.13$, df = 1, $P = 0.29$
36	$F = 3.68$, df = 2, $P = 0.04$	$F = 0.04$, df = 1, $P = 0.85$	NA

Mortality of Nymphs Sprayed with Miticide

Percent mortality (± SEM) of nymphs sprayed with the highest labeled concentration of miticides averaged 16 ± 6.5% for bifenazate and 16 ± 8.3% for spirodiclofen, which was not different than the mortality of nymphs sprayed with tap water (11 ± 6.0%; $F_{2,27} = 0.76$, $P = 0.76$).

Number of ACP Reaching Adulthood when Sprayed with Miticide during the Nymphal Stage

The number of males, number of females, and total number of ACP reaching adulthood after being sprayed with miticides as nymphs were all similarly influenced by the miticides (females, $\chi^2 = 34.4$, df = 2,18, $P < 0.001$; males, $\chi^2 = 28.1$, df = 2,18, $P < 0.001$; total adults, $\chi^2 = 27.6$, df = 2,18, $P < 0.001$), so only the results for total number of ACP reaching adulthood are discussed. The mean number of ACP surviving to adulthood when sprayed with spirodiclofen was 5.6 ± 1.4, which was significantly lower than the mean for bifenazate (81.0 ± 14.3) and the control (97.4 ± 17.0). However, neither miticide delayed development of ACP ($\chi^2 = 0.27$, df = 2,18, $P = 0.88$).

Number of ACP Reaching Adulthood when Sprayed with Miticide during the Egg Stage

The number of males, number of females, and total number of ACP developing from eggs sprayed with the highest concentration of miticides were all similarly influenced by the miticides (females, $\chi^2 = 35.6$, df = 6,34, $P < 0.001$; males, $\chi^2 = 35.5$, df = 6,34, $P < 0.001$; total adults, $\chi^2 = 35.3$, df = 6,34, $P < 0.001$), so only the results for total number of ACP reaching adulthood are discussed. Spirodiclofen, pyridaben, and chlorfenapyr reduced the number of ACP surviving to adulthood, whereas bifenazate, dicofol, and petroleum oil did not (Fig. 2). Spirodiclofen, pyridaben, and chlorfenapyr were similarly toxic at moderate concentrations ($\chi^2 = 22.2$, df = 3,19, $P < 0.001$). However, the miticides did not delay development of ACP at high ($\chi^2 = 11.6$, df = 6,34, $P = 0.07$) or moderate concentrations ($\chi^2 = 1.6$, df = 3,11, $P = 0.67$).

DISCUSSION

The miticides differed in their toxicity to the 3 life stages of ACP (Table 4). Bifenazate was the only miticide that was not toxic to any life stage of ACP, whereas pyridaben and chlorfenapyr, which are also labeled as insecticides, were toxic to all life stages of ACP. Petroleum oil is labeled for mites and insects and killed adult and nymphal ACP, but was nontoxic to eggs. Dicofol is labeled only for mites, but was highly toxic to adult and nymphal ACP. Spirodiclofen was nontoxic to adults, toxic to eggs, and apparently does not kill nymphs immediately, but reduces the likelihood that they will survive to the adult stage. Spirodiclofen inhibits a key enzyme responsible for synthesis of fatty acid, which is a slower mode of action than other common miticides (Marcic 2012).

The duration of residual activity against adult ACP also was widely variable: dicofol had the shortest residual activity (fewer than 10 days), whereas chlorfenapyr was still toxic after 36 days likely because it is translaminar. Translaminar chemicals typically have good residual activity against foliar-feeding mites and insects (Lasota & Dybas 1991; Cloyd 2003), including TSSM (Abdel-Wali et al. 2012), because they penetrate leaf tissue and form a reservoir of active ingredient inside the leaf. Unlike residues of the other miticides, pyridaben residues were toxic for a longer duration on plants that were rinsed with tap water than on plants that were not rinsed. We are unsure why this occurred, but we speculate that water may have rehydrated the residues.

The miticides did not appear to have sublethal effects on development or oviposition of ACP. Bife-

TABLE 3. MEAN (± SEM) PERCENT MORTALITY OF *DIAPHORINA CITRI* CAUSED BY RESIDUES FROM 4 MITICIDES UP TO 36 DAYS FOLLOWING APPLICATION. NEW ADULT VIRGIN *D. CITRI* WERE FRESHLY EXPOSED TO PLANTS SPRAYED WITH TAP WATER OR A MITICIDE ON THE INDICATED DAYS POST-APPLICATION AND THEIR MORTALITY WAS NOTED 72 H LATER. HALF THE PLANTS WERE RINSED (R) WITH TAP WATER DAILY AND THE OTHER HALF WERE NOT RINSED (NR). TREATMENT RESIDUES WERE NO LONGER EVALUATED FOR TOXICITY AFTER MORTALITY WAS NOT SIGNIFICANTLY HIGHER THAN THE CONTROL (TAP WATER).

	Mean (± SEM) percent mortality of adult ACP subjected to the indicated treatment									
	Control		Dicofol		Pyridaben		Petroleum oil		Chlorfenapyr	
Days post-application	R	NR	R	NR	R	NR	R	NR	R	NR
1	28 (14) c	23 (10) c	88 (8) ab	82 (10) b	100 (0) a	84 (12) ab	88 (13) ab	100 (0) a	100 (0) a	100 (0) a
4	20 (8) e	29 (14) de	55 (12) cd	83 (12) ab	93 (8) ab	55 (13) cd	80 (9) bc	100 (0) a	91 (6) ab	97 (3) ab
7	40 (16) b	41 (15) b	—	35 (15) b	98 (3) a	—	68 (13) b	95 (5) a	95 (3) a	95(5) a
11	63 (13) b	58 (16) b	—	—	98 (3) a	—	—	95 (3) a	100 (0) a	100 (0) a
15	33 (13) b	13 (8) b	—	—	98 (3) a	—	—	96 (4) a	98 (3) a	100 (0) a
22	63 (13) b	30 (9) b	—	—	97 (3) a	—	—	50 (14) b	100 (0) a	100 (0) a
29	68 (11) b	55 (15) b	—	—	97 (3) a	—	—	—	80 (11) ab	95(5) a
36	41 (10) b	40 (11) b	—	—	73 (14) ab	—	—	—	50 (15) b	75(11) a

Means within a row followed by different letters are significantly different (means separation test, P < 0.05).

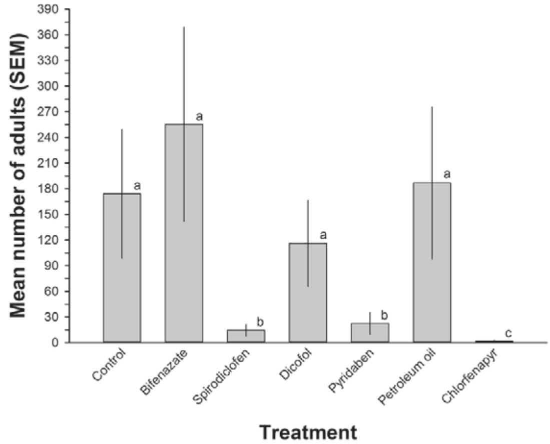

Fig. 2. Mean number (± SEM) of *Diaphorina citri* reaching adulthood when sprayed with miticide during the egg stage with the highest labeled concentration of 6 miticides or tap water (control). Means with different letters are significantly different (means separation test, P < 0.05).

nazate and spirodiclofen, which were not toxic to adult ACP, also did not reduce the number of eggs laid by adults. We also did not detect any delay in nymphal development when ACP were sprayed with the miticides during the egg or nymphal stage. However, we only measured nymphal development by noting the first day ACP reached adulthood. The average developmental period of ACP would likely be influenced by the miticides, particularly the ones that prevented most ACP from reaching adulthood.

The miticides we selected are relatively effective at the USHRL (L. Faulkner, personal communication), but TSSM is resistant to more types of pesticides than any other arthropod (Whalon et al. 2012), so the efficacy of the miticides we tested against local populations of TSSM, and other species of mites, would need to be determined before depending on particular miticides for control. Relying on only one miticide, or several miticides that have the same mode of action, can result in populations of arthropods that are resistant to the miticide (Helle 1965). Five of the miticides, depending on the life stage of ACP targeted for control, can be rotated in greenhouse environments to control ACP. However, we undertook this experiment because we wanted to find miticides that would not kill our colonies of ACP. To this end, we recommend using dicofol, pyridaben, petroleum oil, and chlorfenapyr to maintain clean plants prior to colonization by ACP and then rotating bifenazate and spirodiclofen, if maintaining adult ACP, or bifenazate, dicofol, and petroleum oil, if maintaining eggs. Bifenazate is the only product safe for maintaining nymphal ACP, and is highly toxic to TSSM

TABLE 4. TOXICITY OF DIRECT APPLICATION OF 6 MITICIDES TO EACH LIFE-STAGE OF *DIAPHORINA CITRI* AND DURATION OF RESIDUAL ACTIVITY AGAINST ADULTS.

Miticide	Adults	Nymphs	Eggs	Residual duration
Bifenazate	no	no	no	NA
Spirodiclofen	no	yes/no	yes	NA
Dicofol	yes	yes	no	3-10 days
Pyridaben	yes	yes	yes	3-35 days
Petroleum oil	yes	yes	no	6-21 days
Chlorfenapyr	yes	yes	yes	>28 days

(Liburd et al. 2007), but should be used only at the frequency and rate listed on the label so that mite populations do not develop resistance. These miticides also could be combined with other measures to control mites, such as mites that prey on herbivorous mites (Gerson & Weintraub 2012) or insecticidal soaps, which have short residues and are nontoxic to eggs of ACP (Hall & Richardson 2012).

In conclusion, our results are useful for selecting a miticide to prevent or control outbreaks of mites that have little or no activity against ACP. The results are also useful for selecting a miticide that kills ACP and mites in greenhouses or citrus groves. All the miticides, except for chlorfenapyr, are labeled and recommended as part of a pesticide rotation to control mites in citrus groves (Rogers & Dewdney 2012), so citrus producers may be able use them to simultaneously control mites and ACP. However, our ACP were reared in a laboratory environment for over 10 years and may be more susceptible to these miticides than wild ACP. In addition, whereas regular applications of petroleum oil are recommended to control ACP in citrus groves (Stansly et al. 2012), and spirodiclofen and pyridaben have been tested for control of ACP in groves (Qureshi & Stansly 2007; Stansly et al. 2012), the efficacy of the other miticides against ACP in the field needs to be verified.

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