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TOXICITY OF ORGANOSILICONE ADJUVANTS AND SELECTED PESTICIDES TO THE ASIAN CITRUS PSYLLID (HEMIPTERA: PSYLLIDAE) AND ITS PARASITOID *TAMARIXIA RADIATA* (HYMENOPTERA: EULOPHIDAE)

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ABSTRACT

The acute toxicity of the adjuvants Silwet L-77 and Kinetic, alone and in combination with petroleum oil and copper hydroxide, to the Asian citrus psyllid *Diaphorina citri* Kuwayama was evaluated in screenhouse bioassays. In addition, the acute and residual toxicity of Silwet L-77 and Kinetic, alone and in combination with petroleum oil, copper hydroxide, imidacloprid, and abamectin, to the parasitoid *Tamarixia radiata* (Waterston) were evaluated under laboratory conditions. In screenhouse trials, Silwet L-77 (0.05%) was more insecticidal than Kinetic (0.05%) and increased the toxicity of both petroleum oil and copper hydroxide to *D. citri*. Petroleum oil at reduced rates (0.5 and 1%) in combination with Silwet L-77 or Kinetic was less effective in reducing *D. citri* populations than petroleum oil at 2% in combination with these adjuvants. Petroleum oil at 2% plus Silwet L-77 was the most toxic combination to *D. citri* eggs, young (first- and second- instars) and mature nymphs (third- to fifth-instars), and adults (81, 83, 74, and 55% mortality, respectively). Copper hydroxide was only toxic to young nymphs when combined with Silwet L-77 (64.9% mortality). Under laboratory conditions, survival of *T. radiata* was reduced by the residual effects of imidacloprid (>95% mortality) and by the acute toxicity of abamectin (>91% mortality). Silwet L-77 and Kinetic alone, and petroleum oil and copper hydroxide alone or in combination with these adjuvants, had low residual and acute toxicity to the parasitoid and appear to be compatible with the biological control of *D. citri* by *T. radiata*. The results of this study suggest that Silwet L-77 may be used in a citrus IPM program in combination with petroleum oil or copper hydroxide to increase psyllid control while spraying to suppress other insect pests or plant diseases. Field trials should be conducted to evaluate the effectiveness of these products against *D. citri* and their impact on *T. radiata* populations.

Key Words: Silwet L-77, Kinetic, *Diaphorina citri*, *Tamarixia radiata*, petroleum oil, copper, imidacloprid, abamectin, acute toxicity, residual effects

RESUMEN

La toxicidad aguda de los adyuvantes Silwet L-77 y Kinetic, solo o en combinación con aceite petróleo y hidróxido de cobre, al sílido asiático de los cítricos, *Diaphorina citri* Kuwayama fue evaluada en bioensayos hechos en la casa de tamizado. Además, la toxicidad aguda y residual de Silwet L-77 y Kinetic, solos o en combinación con aceite de petróleo, hidróxido de cobre, imidacloprid y abamectin, al parasitoide *Tamarixia radiata* (Waterston) fueron evaluadas baja condiciones del laboratorio. En las pruebas en la casa de tamizado, Silwet L-77 (0.05%) fue mas como un insecticida que Kinetic (0.05%) y aumento la toxicidad del aceite de petróleo y hidróxido de cobre a *D. citri*. Tasas reducidas (0.5 y 1%) del de aceite petróleo en combinación con Silwet L-77 o Kinetic fueron menos efectivas en reducir poblaciones de *D. citri* que el aceite de petróleo al 2% en combinación con estos adyuvantes. El aceite de petróleo al 2% mas Silwet L-77 fue la combinación mas toxica a los huevos, las ninfas inmaduras (jóvenes) del primero y segundo estadio, las ninfas inmaduras (maduras) del tercer a quinto estadio y los adultos (con una mortalidad 81, 83, 74 y 55%, respectivamente). El hidróxido de cobre solamente fue toxico a los inmaduros jóvenes cuando fue combinado con Silwet L-77 (mortalidad de 64.9%). Bajo condiciones de laboratorio, el sobrevivencia de *T. radiata* fue reducida por los efectos residuales de imidacloprid (mortalidad >95%) y por la toxicidad aguda de abamectin (mortalidad >91%). El Silwet L-77 y Kinetic solo, y el aceite de petróleo y el hidróxido de cobre solo o en combinación con estos adyuvantes, tenían una baja toxicidad residual y aguda al parasitoide y aparece ser compatible con el control biológico de *D. citri* por *T. radiata*. Estos resultados sugirieron que Silwet L-77 puede ser usado en un programa de MIP en los cítricos en combinación con el aceite de petróleo o hidróxido de cobre para aumentar el control del silido mientras rocían el cultivo para suprimir las otras plagas de insectos o enfermedades. Se debe realizar pruebas del campo para evaluar la efectividad de estos productos contra *D. citri* y su impacto sobre poblaciones de *T. radiata*.

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The Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) is the vector of citrus greening disease (also known as huanglongbing or HLB) caused by the bacterium *Candidatus* Liberibacter asiaticus. Greening disease is one of the most important citrus diseases in the world (Bové 2006), causing mottling and leaf chlorosis, twig dieback, reduced production, and eventual death of the trees in 5-8 years. Infected trees produce misshapen, poorly colored, and bitter-tasting fruits, not usable for consumption (Halbert & Manjunath 2004). *Diaphorina citri* was first found in Florida in Jun 1998 (Hoy & Nguyen 1998) and is now established in Florida and Texas (French et al. 2001). Although the Asian citrus psyllid causes direct feeding damage to citrus (Mead 1976), its economic importance is due to transmission of the bacterium. Citrus greening was detected in Florida during 2005 and is now found in 30 counties (Florida Department of Agriculture and Consumer Services 2008). An eradication program for citrus greening disease was not developed because of its wide distribution when detected.

Current management measures for greening disease in Florida citrus groves include soil application of systemic insecticides (imidacloprid and aldicarb) and multiple applications (up to 8-18) of broad-spectrum foliar insecticides (including fenpropathrin, imidacloprid, abamectin, dimethoate, carbaryl, and chlorpyrifos). Insecticides are applied during the dormant foliar season, when new flushes required for psyllid female oviposition and nymphal development are rare, with the goal of reducing adult psyllid populations prior to the first flush cycle and during the flushing season (Rogers 2008; University of Florida-IFAS Extension 2008). Trees are visually inspected and removed if infected. These approaches to managing greening disease have increased the costs of citrus production, may lead to development of insecticide resistance in *D. citri*, and are likely to be disruptive to natural enemies of psyllids (and other citrus pests), such as ladybeetles, lacewings, spiders (Michaud 2004), and parasitic wasps including the specialist parasitoid of the Asian citrus psyllid, *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) (Hoy & Nguyen 1998; Hoy et al. 1999). Repeated sprays of these broad-spectrum insecticides over several years could cause secondary outbreaks of whiteflies, aphids, armored scales, and mealybugs previously held below damage thresholds by a complex of beneficial insects (University of Florida-IFAS Extension 2008). *Tamarixia radiata* was imported as a part of a classical biological control program and is now established in Florida (Hoy et al. 1999; Skelley & Hoy 2004).

Insecticides are often used in combination with organosilicone adjuvants to facilitate the wetting and the spread of droplets on leaves, resulting in a more uniform distribution of active ingredients (Foy 1989; Pollicello et al. 1995). Adjuvants may also show insecticidal activity to several pests, although the mechanism by which organosilicones are toxic to insects and mites has not been adequately determined (Srinivasan et al. 2008). Petroleum oil and organosilicone adjuvants might be suitable for citrus IPM programs if they are effective in suppressing the pests while allowing their natural enemies to persist. Treatments with petroleum oil are compatible with the IPM program developed for the citrus leafminer *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), because petroleum oil has short residual activity and allows survival of its parasitoid *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae) (Villanueva-Jimenez & Hoy 1998).

Recent laboratory, greenhouse, and small-scale field studies demonstrated the effectiveness of reduced rates of imidacloprid and abamectin in combination with Silwet L-77, a nonionic organosilicone surfactant, to control *D. citri* eggs, young nymphs, and adults (Srinivasan et al. 2008). However, they did not evaluate toxicity of petroleum oil in combination with Silwet L-77 or Kinetic to psyllid immatures and adults and did not evaluate the effects of these products on any natural enemies of *D. citri*. Because imidacloprid and abamectin are toxic to several beneficial arthropods, multiple applications could disrupt biological control of citrus pests and frequent applications could select for resistant populations (Villanueva-Jimenez & Hoy 1998; Grafton-Cardwell et al. 2001; Liburd et al. 2004; Toscano et al. 2004). To further investigate *D. citri* management strategies that are compatible with biological control by *T. radiata* (and, potentially, other natural enemies), we investigated the acute toxicity of 3 rates of petroleum oil alone and in combination with Silwet L-77 and Kinetic to all life stages of *D. citri* (eggs, young or mature nymphs, and adults). We tested petroleum oil because it is considered more compatible with IPM programs than imidacloprid and abamectin, due to its moderate or low toxicity to beneficial arthropods and its short residual effects (Beattie & Hardy 2004; Erkilic & Uygun 1997). Furthermore, petroleum oil applications do not increase the likelihood of pesticide resistance (Thomson 2001). Bioassays using *D. citri* were conducted inside a screenhouse to evaluate the effectiveness of treatments with natural photoperiods and temperatures. In addition, we investigated, for the first time, the susceptibility of *T. radiata* adults when parasitoids were sprayed directly (acute toxicity) or exposed to the residues (residual toxicity) of these adjuvants alone and in combination with petroleum oil, copper hydroxide, abamectin, and imidacloprid under laboratory conditions.

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Materials and Methods

Rearing of *D. citri* and *T. radiata*

Diaphorina citri and *T. radiata* were reared according to a method modified from Skelley & Hoy (2004). The psyllid colony was maintained with potted citrus trees, approximately 30-50 cm tall. The trees were pruned, fertilized, watered, and placed in PVC (Poly Vinyl Chloride) frame cages ($60 \times 60 \times 60$ cm) covered with organdy cloth in a greenhouse maintained at 21-42°C, 20-79% RH, under a 16L:8D photoperiod. After 10-14 d, the trees had new tender growths (= flushes) 0.5-1 cm long, and were exposed to ovipositing *D. citri* females (approximately 5 per tree). Females were aspirated out with a vacuum pump after 72 h.

The parasitoid *T. radiata* was reared on potted citrus trees as described above for the *D. citri* colony. Ten trees were placed in wooden-framed cages covered with organdy cloth ($114 \times 94 \times 76$ cm) held in a greenhouse at 20-38°C, 22-81% RH, under a 16L:8D photoperiod. Approximately 50 ovipositing *D. citri* females were released in the cage and allowed to oviposit for 3-5 d, then removed with a vacuum pump. Adults of *T. radiata* (50 females and 25 males) were collected and released into the cage when the psyllid population reached the third instar. Adults of *D. citri* that emerged from unparasitized nymphs were collected as a by-product and were used to maintain the psyllid colony.

Effects of Adjuvants With and Without Petroleum Oil and Copper Hydroxide on *D. citri*

Screenhouse experiments were undertaken to evaluate the acute toxicity of Silwet L-77 (99.5% polyalkyleneoxide modified heptamethyltrisiloxane; Helena Chemical Co., Collierville, TN) and Kinetic (99% proprietary blend of polyalkyleneoxide modified polydimethylsiloxane and nonionic surfactants; Helena Chemical Co., Collierville, TN) alone and in combination with agrochemicals to psyllid eggs, young or mature nymphs, and adults. The screenhouse bioassays described here were conducted under variable environmental conditions between May and Oct 2007 to mimic grove conditions. Fifteen treatments were tested: water; Silwet L-77 at 0.05% (v:v); Kinetic at 0.05% (v:v); petroleum oil 435 (Growers 435, Growers Fertilizer Co., Lake Alfred, FL) at 0.5, 1, and 2% (v:v); copper hydroxide (Kocide 2000, DuPont, Wilmington, DE) at the recommended lowest label rate (LLR) (2.4 g/L of water) for suppression of citrus canker in Florida; petroleum oil 435 at 0.5% + Silwet L-77 at 0.05%; petroleum oil 435 at 1% + Silwet L-77 at 0.05%; petroleum oil 435 at 2% + Silwet L-77 at 0.05%; petroleum oil 435 at 0.5% + Kinetic at 0.05%; petroleum oil 435 at 1% + Kinetic at 0.05%; petroleum oil 435 at 2% + Kinetic at 0.05%; copper hydroxide at the LLR+ Silwet L-77 at 0.05%; and copper hydroxide at the LLR + Kinetic at 0.05%. The recommended LLR was calculated for 935 L per ha (100 gals/acre). Petroleum oil was evaluated at rates lower than 2% (Childers & Rogers 2005) to determine whether the adjuvants improved its toxicity to *D. citri*. Because an unnamed organosilicone surfactant tested at 0.2% (v:v) was reported to enhance the spread of citrus bacterial spot caused by *Xanthomonas axonopodis* pv. *citrumelo* (Gottwald et al. 1997), questions were raised as to whether Silwet L-77 might enhance the rate of infection with citrus canker (*Xanthomonas axonopodis* pv. *citri*). Because copper hydroxide is often used to protect citrus trees from citrus canker, it was combined with these adjuvants to evaluate the toxicity of this formulation to *D. citri*. All products were mixed with purified water (Barnstead Nanopure II system, Dubuque, IA) at a pH of 6.8 at 24.8°C. The experiments were carried out at the Department of Entomology and Nematology, University of Florida, Gainesville.

The protocol to evaluate the acute toxicity of the pesticide solutions on *D. citri* eggs, young or mature nymphs, and adults was similar to that of Srinivasan et al. (2008), except that each shoot was treated separately, and the untreated canopy was shielded with a plastic bag. The experimental design was modified because they did not test treatments on mature nymphs of *D. citri* and they evaluated only the residual toxicity of imidacloprid and abamectin to psyllid adults. To investigate the toxicity of treatments to psyllid eggs, potted citrus trees with at least 5 flushes 0.5-1 cm long that were infested with eggs were selected. On each tree, 5-6 shoots were sprayed with a different Preval® gun sprayer (Precision Valve Corp., Yonkers, NY) for each treatment. Shoots were sprayed from a distance of 70-80 cm for 10 s around each shoot, which covered approximately 90% of the leaf surface. Each shoot had 28-369 eggs (mean ± SD = 110 ± 57) and was considered a replicate. The trees were placed into cages inside a covered screenhouse for 9 d at 13-32°C, 27-94% RH, under a natural photoperiod during May 2007. The numbers of live nymphs and the numbers of unhatched eggs were scored with use of a dissecting microscope. Dead first-instars were considered as uneclosed eggs, because hatching larvae might be intoxicated if the treatment penetrated the egg shell or the larvae encountered a lethal concentration on the egg shell.

To assess the acute toxicity of the treatments to first- and second-instars (= young) *D. citri*, the potted citrus trees were infested as described for eggs. After 72 h, the trees were placed into cages inside the same screenhouse and left undisturbed for 6 d at 11-30°C, 19-96% RH, under a natural photoperiod during May 2007 to allow development to the nymphal stage. Shoots were sprayed

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using the same method as for the eggs. Each shoot had 33-390 nymphs (mean ± SD = 143 ± 72). After 4 d at 12-31°C and 18-94% RH under a natural photoperiod, live and dead nymphs were scored under a dissecting microscope. Motion, posture, presence of waxy excretions, dehydration of the body were considered to determine whether the nymphs were dead or live. To estimate the percentage distribution of the different instars at the time of the treatment, 6 flushes were sampled separately from shoots used to evaluate the treatments. The nymphs in each instar were scored separately to assess the instar densities across the tree. Each flush had 55-164 nymphs (mean ± SD = 113 ± 45).

Another experiment was conducted with third- to fifth-instars to verify whether the treatments were effective on 'large nymphs'. The same methodology as for young nymphs was used, except that the trees were left undisturbed for 10 d after the oviposition period at 18-34°C and 27-95% RH under a natural photoperiod during Jun 2007. Each shoot had 20-184 nymphs (mean ± SD = 79 ± 34). The treatments were based on the results of the toxicity bioassay on young nymphs, and chosen on the assumption that ineffective treatments for young nymphs were likely to be ineffective against larger nymphs. The 9 treatments were: water; Silwet L-77 at 0.05%; Kinetic at 0.05%; petroleum oil 435 at 2%; copper hydroxide at the LLR; petroleum oil 435 at 2% + Silwet L-77 at 0.05%; petroleum oil 435 at 2% + Kinetic at 0.05%; copper hydroxide at the LLR+ Silwet L-77 at 0.05%; and copper hydroxide at the LLR + Kinetic at 0.05%. Two days after treatment, alive and dead nymphs were recorded with use of a dissecting microscope, based on the same criteria as described above for young nymphs (post-treatment conditions: 21-36°C, 26-94% RH, natural photoperiod). The shoots sampled to estimate the percentage distribution of nymphs had 63-163 nymphs (mean ± SD = 96 ± 36).

Acute toxicity to adults was assessed on potted citrus trees with only young flushes approx 3-5 cm long that fit inside Plexiglas cylinders (45.5 cm tall, 12.6 cm outside diameter). The top of the cylinder and 4 side holes (5.4 cm in diameter) were covered with mesh for air circulation. The soil surface was lined with plastic wrap and 2 paper coffee filters. The coffee filters were taped to the pot to facilitate the count of psyllid adults. Psyllid adults approximately 3 weeks old were exposed to the trees for 4-6 h within a cage in the greenhouse. Trees were infested with 20-98 adults (mean psyllids per tree ± SD = 51 ± 19). Different sprayers for each treatment were used to spray the trees containing adults; sprayers were held 70-80 cm from the trees and sprays applied for 15 s around the canopy. The trees were set inside the cylinders and placed into a roofed screenhouse. Alive and dead adults inside cylinders were counted after 72 h. Motion and maintenance of their typical posture (with the head touching the surface and the abdomen raised to a 30-45 degree angle) were considered to determine whether the adults were dead or live. The experiment was conducted 8 times on 4 different dates during Jul, Aug, and Oct 2007. During the experiments, the average minimum and maximum temperatures (mean ± SD) were 22 ± 3.1°C and 33 ± 2.9°C, respectively. The mean RH (± SD) averaged 42 ± 8.6% (minimum) and 92 ± 4.5% (maximum). Daily weather data were obtained from the weather station maintained by the Department of Agricultural and Biological Engineering, University of Florida, Gainesville, approximately 800 m away. Temperature and RH inside and outside the cylinders were recorded with a Traceable® Digital Thermometer (Fisher Electronics, Pittsburgh, PA). The mean minimum and maximum temperatures inside the cylinders were higher (0.18°C and 0.48°C, respectively) than the environment temperature. The minimum and maximum RHs inside the cylinder were higher by 7.8 and 0.2%, respectively. Data expressed as percentages were evaluated with one-way analysis of variance with Proc GLIMMIX (SAS Institute 2002), and a Tukey-Kramer test was used to separate means ($\alpha < 0.05$).

Acute and Residual Toxicity of Adjuvants and Selected Pesticides to *T. radiata* Adults

The acute and residual toxicities of adjuvants alone and in combination with petroleum oil, copper hydroxide, abamectin, and imidacloprid were tested in the laboratory with clip cage bioassays (Villanueva-Jimenez & Hoy 1998).

Residual Toxicity. Potted citrus trees with leaves 1 month old were sprayed to cover approximately 90% of the leaf surface. Silwet L-77 and Kinetic were tested alone (0.05%) and in combination with petroleum oil 435 at 2%; copper hydroxide at the LLR; abamectin (Agrimek 0.15 EC; Syngenta Crop Protection, Greensboro, NC) at the LLR; and imidacloprid (Provado 1.6 Flowable; Bayer Cropscience, Research Triangle Park, NC) at the LLR. Water was used as control. Trees were allowed to dry for about 30 min. Two treated leaves were pruned randomly from each treatment and placed at the top (with the abaxial surface downward) and the bottom (with the adaxial surface upward) of an acrylic ring (38 mm outside diameter, 10 mm tall). Foam tape (M-D Building Products, Oklahoma City, OK) 6 mm wide was placed on both edges of the ring to seal the clip cage. The treated surface of the clip cage chamber represented ca. 64.3% of the internal area. The rings had 2 mesh-covered windows 5 mm in diameter to reduce RH inside the chamber and provided a method for feeding *T. radiata* by placing a water-soaked rolled paper outside the mesh win-

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dow and a honey-soaked strip inside the chamber. The clip cage was held together by 2 pieces of circular cardboard on the top and bottom (40 mm diam) with 4 hair clips (Villanueva-Jimenez & Hoy 1998). To place *T. radiata* into the clip cage, 10 adult females 3-4 d old were chilled inside a 50-mL conical centrifuge tube in crushed ice for 5 min and gently tapped inside the clip cage. The clip cage was placed inside a plastic bag with a wet paper towel to ensure survival of the parasitoids throughout the experiment (McFarland & Hoy 2001). The plastic bags were placed inside a growth chamber at 24 ± 1°C under a 16L:8D photoperiod. Adult mortality was evaluated after 24 h. The insects were touched gently with an insect pin and considered alive if they walked, jumped, or flew away, and dead if they did not move or only moved antennae, wings, or legs. When the mortality in the water control was >15%, the entire replicate was eliminated. The bioassay was replicated 6 times on 3 different dates. The percentage of mortality data were arcsine $\sqrt{P}$ transformed before statistical analysis to approximate a normal distribution. Data were subjected to one-way analysis of variance (ANOVA) with Proc GLM (SAS Institute 2002), and significant differences among means were evaluated by Fisher's Least Significance Difference (LSD) test (α = 0.05%). When water-control mortality was detected, data were corrected by Abbott's formula for control response (Abbott 1925).

The position of *T. radiata* adults inside the clip cage chamber was observed to determine whether the treatments were repellent, and the parasitoid preferred to move or settle on the untreated surfaces. The relative position (inner surface of the ring or treated leaves) of the parasitoid was recorded after 2 and 6 h, in 3 replicates per treatment. Data expressed as number of *T. radiata* on the leaves over total parasitoids inside the clip cage were evaluated with analysis of variance (Proc GLIMMIX) (SAS Institute 2002).

Acute (Direct) Toxicity. The bioassays were performed as for the residual toxicity test, except that untreated foliage was used and *T. radiata* were treated directly by placing 10 females 3-4 d old into a Petri dish upon crushed ice for 5 min (Villanueva-Jimenez & Hoy 1998). The adult females then were sprayed with a gun sprayer from a distance of 1 m for 5 s and placed inside the clip cage chamber. Twelve treatments was tested: water, oil 435 at 2%, abamectin at the LLR, copper hydroxide at the LLR, Silwet L-77 (0.05%) and Kinetic (0.05%) alone and in combination with petroleum oil 435 at 2%, abamectin at the LLR, and copper hydroxide at the LLR. Because imidacloprid alone and in combination with both adjuvants killed almost all parasitoids in the residual toxicity bioassay (see Results), acute toxicity of these products was not tested. Data were analyzed as for the residual toxicity test.

RESULTS

Effects of Adjuvants With and Without Petroleum Oil on *D. citri*

Eggs. Silwet L-77 and Kinetic alone caused only 20.4 and 16.5% mortality, respectively, to *D. citri* eggs (Table 1). Mortality of psyllid eggs sprayed with petroleum oil at 0.5, 1, and 2% ranged from 61.5 to 66.7%, but the differences were not significant. When petroleum oil at 1 and 2% was combined with 0.05% Silwet L-77, the toxicity increased from 66.3 to 78.7%, and from 61.5 to 81.3%, respectively, (F = 122.37; df = 11, 57; P < 0.0001) (Table 1). Petroleum oil at 0.5, 1, and 2% in combination with Kinetic was less effective (52.6, 57.7, and 52.3%, respectively) in killing psyllid eggs than petroleum oil alone at the same rates. Mortality rates of eggs sprayed with petroleum oil at 1 and 2% plus Silwet L-77 were significantly higher than when petroleum oil in combination with Kinetic was applied.

Young Nymphs. The bioassay on young nymphs was conducted when nymphs were predominantly first or second instars (20.9 and 64.7%, respectively). Silwet L-77 sprayed on trees infested with *D. citri* nymphs killed 69.7%, while Kinetic alone was significantly less effective (46.2% mortality) (F = 101.09; df = 11, 60; P < 0.0001) (Table 1). Toxicity of petroleum oil to young nymphs increased with the rate applied. Petroleum oil at 0.5% caused 34.5% mortality, which was significantly lower than at 1% (56.5%) or at 2% (84.7%). Silwet L-77 increased significantly the toxicity of petroleum oil to young *D. citri* nymphs when applied at 0.5% from 34.5 to 57.2%, and at 1% from 56.5 to 68.2%. However, adding Silwet L-77 or Kinetic did not improve the efficacy of petroleum oil at 2% because it was effective by itself to young nymphs (84.7% mortality). When Kinetic was combined with 0.5% petroleum oil, mortality of nymphs increased significantly from 34.5 to 56.2%, but not at the other rates tested. Petroleum oil at 2% was the most effective treatment in suppressing young nymphs (84.7%).

Mature Nymphs. Infested trees used to test the toxicity of petroleum oil and adjuvants to mature nymphs were sprayed when 94.5% of the nymphs were either third, fourth or fifth instars. Mortality of these larger *D. citri* nymphs on Silwet L-77-treated trees was 64.2%, which was higher than on Kinetic-treated trees (35.2%) (F = 56.95; df = 5, 28; P < 0.0001) (Table 1). When petroleum oil at 2% was applied in combination with an adjuvant, only Silwet L-77 significantly increased the mortality of mature nymphs from 44.8 to 74.3%. These tests indicate that Silwet L-77 is better than Kinetic in suppressing young and mature nymphs, and that Silwet L-77 significantly improves the efficacy of petroleum oil at 2% against large nymphs.

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TABLE 1. ACUTE TOXICITY OF PETROLEUM OIL 435 AT DIFFERENT RATES, ALONE AND IN COMBINATION WITH SILWET L-77 OR KINETIC, TO IMMATURES AND ADULTS OF *D. CITRI* UNDER SCREENHOUSE CONDITIONS IN GAINESVILLE, FL, DURING MAY-OCT 2007.

Treatments[a]	Mean mortality of *D. citri* (% ± SE)			
	Eggs[b]	Young nymphs[b]	Mature nymphs[b]	Adults[b]
Water	7.1 ± 0.8 f	3.1 ± 0.5 g	7.1 ± 1.0 e	0.8 ± 0.8 e
Silwet L-77 (0.05%)	20.4 ± 1.7 e	69.7 ± 1.9 c	64.2 ± 8.7 b	30.6 ± 5.3 b
Kinetic (0.05%)	16.5 ± 1.6 e	46.2 ± 5.8 ef	35.2 ± 3.8 d	5.9 ± 1.2 de
Oil 0.5%	66.7 ± 4.8 b	34.5 ± 5.6 f	—	2.7 ± 0.9 e
Oil 1%	66.3 ± 4.3 b	56.5 ± 5.9 d	—	4.9 ± 1.3 de
Oil 2%	61.5 ± 4.3 bc	84.7 ± 2.6 a	44.8 ± 2.6 c	17.5 ± 3.4 c
Oil 0.5% + Silwet L-77 (0.05%)	60.3 ± 7.8 bcd	57.2 ± 4.6 d	—	38.4 ± 5.0 b
Oil 1% + Silwet L-77 (0.05%)	78.7 ± 4.7 a	68.2 ± 5.3 c		35.2 ± 3.8 b
Oil 2% + Silwet L-77 (0.05%)	81.3 ± 4.9 a	83.0 ± 3.1 ab	74.3 ± 5.3 a	54.7 ± 6.1 a
Oil 0.5% + Kinetic (0.05%)	52.6 ± 5.1 cd	56.2 ± 6.0 ed	—	4.6 ± 0.6 de
Oil 1% + Kinetic (0.05%)	57.7 ± 4.8 cd	49.3 ± 5.0 e	—	8.2 ± 2.1 d
Oil 2% + Kinetic (0.05%)	52.3 ± 5.0 cd	76.0 ± 3.1 bc	41.5 ± 5.5 cd	34.5 ± 5.4 b

[a]Screenhouse conditions: eggs (13-32°C, RH 27-94%, during May 2007); young nymphs (11-31°C, RH 18-96%, during Jun 2007); mature nymphs (18-36°C, RH 26-95%, during Jul 2007); adults (22 ± 3.1 - 33 ± 2.9°C, RH 42 ± 8.6 - 92 ± 4.5%, during Jul, Aug, and Oct 2007), all under a natural photoperiod.

[b]Significant differences compared with Proc GLIMMIX ($P < 0.0001$), treatment means with the same letter within a column are not different by Tukey-Kramer test ($\alpha < 0.05$).

Adults. Silwet L-77 applied to *D. citri* adults caused 30.6% mortality, which was more effective than Kinetic (5.9% mortality) ($F = 58.35$; $df = 11, 84$; $P < 0.0001$) (Table 1). Mortality of adults sprayed with petroleum oil at 0.5, 1, and 2% was 2.7, 4.9, and 17.5%, respectively, indicating that petroleum oil alone is not effective in suppressing *D. citri* adults. Combining Silwet L-77 with petroleum oil at 0.5, 1, and 2% significantly increased the mortality of adults to 38.4, 35.2, and 54.7%, respectively. Kinetic applied in combination with petroleum oil improved the toxicity of that insecticide only at the rate of 2%, from 17.5 to 34.5%. These data suggest that Silwet L-77 is better than Kinetic at increasing the efficacy of petroleum oil to *D. citri* adults.

Effects of Adjuvants With and Without Copper Hydroxide on *D. citri*

Mortality of *D. citri* eggs on trees sprayed with copper hydroxide at the LLR alone and in combination with Kinetic was not different, ranging from 14.1 to 14.7% (Table 2). However, when Sil-

TABLE 2. ACUTE TOXICITY OF COPPER HYDROXIDE, ALONE AND IN COMBINATION WITH SILWET L-77 AND KINETIC, TO IMMATURES AND ADULTS OF *D. CITRI* UNDER SCREENHOUSE CONDITIONS IN GAINESVILLE, FL, DURING MAY-OCT 2007.

Treatments[a]	Mean mortality of *D. citri* (% ± SE)			
	Eggs[b]	Young nymphs[b]	Mature nymphs[b]	Adults[b]
Water	7.1 ± 0.8 d	3.1 ± 0.5 f	7.1 ± 1.0 e	0.8 ± 0.8 c
Silwet L-77 (0.05%)	20.4 ± 1.7 b	69.7 ± 1.9 a	64.2 ± 8.7 a	30.6 ± 5.3 a
Kinetic (0.05%)	16.5 ± 1.6 bc	46.2 ± 5.8 c	35.2 ± 3.8 c	5.9 ± 1.2 bc
Copper hydroxide (LLR)	14.1 ± 2.2 c	18.8 ± 3.6 e	15.1 ± 3.6 e	0.4 ± 0.4 c
Copper hydroxide (LLR) + Silwet L-77 (0.05%)	37.3 ± 6.3 a	64.9 ± 4.4 b	46.7 ± 4.8 b	34.5 ± 5.1 a
Copper hydroxide (LLR) + Kinetic (0.05%)	14.7 ± 2.1 cd	30.1 ± 6.1 d	22.6 ± 2.8 d	6.6 ± 2.1 b

[a]LLR indicates the lowest label rate. Screenhouse conditions: eggs (13-32°C, RH 27-94%, during May 2007); young nymphs (11-31°C, RH 18-96%, during Jun 2007); mature nymphs (18-36°C, RH 26-95%, during Jul 2007); adults (22 ± 3.1 - 33 ± 2.9°C, RH 42 ± 8.6 - 92 ± 4.5%, during Jul, Aug, and Oct 2007), all under a natural photoperiod.

[b]Significant differences compared with Proc GLIMMIX ($P < 0.0001$), means with the same letter within a column are not different by Tukey-Kramer test ($\alpha < 0.05$).

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wet L-77 was added to copper hydroxide at the LLR, mortality increased from 14.1 to 37.3% (F = 31.45; df = 5, 29; P < 0.0001). Copper hydroxide at the LLR caused 18.8 and 15.1% mortality of young and mature nymphs, respectively. When Kinetic was added to copper hydroxide, mortality rates of young (F = 172.52; df = 5, 30; P < 0.0001) and mature nymphs (F = 69.70; df = 5, 29; P < 0.0001) increased significantly to 30.1 and 22.6%, respectively. However, these rates were significantly lower than mortality of nymphs exposed to copper hydroxide at the LLR plus Silwet L-77, which were 64.9 and 46.7%, respectively (Table 2).

Copper hydroxide at the LLR killed very few *D. citri* adults (0.4%). When it was combined with Kinetic the mortality rate increased significantly to 6.6%, which was significantly lower than the mortality caused by copper hydroxide at the LLR in combination with Silwet L-77 (34.5%) (F = 45.69; df = 5, 42; P < 0.0001) (Table 2). However, the 34.5% mortality observed with the Silwet L-77 plus copper hydroxide combination was caused primarily by Silwet L-77 because there were no significant differences in mortality between copper hydroxide at the LLR plus Silwet L-77 and Silwet L-77 alone. Adding Silwet L-77 to copper hydroxide or petroleum oil caused these pesticides to uniformly cover the leaf surface rather than forming small separated droplets.

Acute and Residual Toxicity of Adjuvants and Selected Pesticides to *T. radiata* Adults

Residual Toxicity. *Tamarixia radiata* adult females did not appear to show any preference between treated and untreated surfaces inside the clip cages sprayed with water, Silwet L-77, and Kinetic alone and in combination with copper hydroxide at the LLR, petroleum oil at 2%, and abamectin at the LLR (data not shown). There was no significant difference in the proportion of *T. radiata* settling or walking on the leaf surface (treated with water, Silwet L-77, and Kinetic alone and in combination with petroleum oil, copper hydroxide, and abamectin) or on the inner surface of the untreated ring (F = 1.07; df = 11, 59; P = 0.4039). However, after 2 h, parasitoids exposed to leaves previously treated with imidacloprid alone and in combination with Silwet L-77 or Kinetic had died; thus these data were not evaluated by analysis of variance to determine the preferred position of *T. radiata* within the clip cages.

Mortality of *T. radiata* exposed to treated foliage was different among treatments (F = 55.05; df = 14, 75; P < 0.0001) (Table 3). Silwet L-77 and water did not affect the survival of the parasitoid, but Kinetic was significantly more toxic, resulting in the death of 13.3% of adults after 24 h. Leaves treated with 2% petroleum oil in combination with Silwet L-77 or Kinetic

TABLE 3. RESIDUAL AND ACUTE TOXICITY OF PETROLEUM OIL 435, ABAMECTIN, IMIDACLOPRID, AND COPPER HYDROXIDE ALONE AND IN COMBINATION WITH SILWET L-77 AND KINETIC, TO ADULT FEMALES OF *T. RADIATA* USING CLIP CAGES.

Treatments[a]	Mortality of *T. radiata* (mean % ± SE)	
	Residual toxicity[b]	Acute toxicity[b]
Water	0 e	1.7 ± 1.7 b
Silwet L-77 (0.05%)	0 e	0 b
Kinetic (0.05%)	13.3 ± 4.9 c	1.9 ± 1.9 b
Oil 2%	11.7 ± 4.0 c	1.7 ± 1.7 b
Oil 2% + Silwet L-77 (0.05%)	8.3 ± 3.1 cd	1.7 ± 1.7 b
Oil 2% + Kinetic (0.05%)	15.0 ± 5.6 c	0 b
Abamectin (LLR)	23.3 ± 11.2 c	93.1 ± 5.0 a
Abamectin (LLR) + Silwet L-77 (0.05%)	15.0 ± 4.3 c	96.7 ± 3.3 a
Abamectin (LLR) + Kinetic (0.05%)	91.7 ± 3.1 b	96.7 ± 2.1 a
Imidacloprid (LLR)	100 a	—
Imidacloprid (LLR) + Silwet L-77 (0.05%)	95.0 ± 3.4 ab	—
Imidacloprid (LLR) + Kinetic (0.05%)	100 a	—
Copper hydroxide (LLR)	5.0 ± 2.2 cde	1.7 ± 1.7 b
Copper hydroxide (LLR) + Silwet L-77 (0.05%)	1.7 ± 3.1 de	3.3 ± 3.3 b
Copper hydroxide (LLR) + Kinetic (0.05%)	6.7 ± 5.6 cde	0 b

[a]LLR indicates the lowest label rate. Laboratory conditions: 24 ± 1°C, 16L:8D photoperiod

[b]Significant differences compared with Proc GLM (P < 0.0001), treatment means with the same letter within a column are not different by Fisher's LSD test (α < 0.05). Imidacloprid caused high residual mortality and was not tested for acute toxicity.

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caused 8.3 and 15% mortality, respectively, which were not different from the mortality (11.7%) caused by 2% petroleum oil alone (Table 3). Mortality of *T. radiata* adults exposed to leaves treated with abamectin at the LLR (23.3%) or abamectin plus Silwet L-77 (15%) was not significantly different. When Kinetic was added to abamectin at the LLR, the mortality rate increased from 23.3 to 91.7%, suggesting that Kinetic increases significantly the toxicity of abamectin to *T. radiata* adults (Table 3). *Tamarixia radiata* adults exposed to that treatment appeared intoxicated; when touched with an insect pin, parasitoids moved wings, legs, or antennae, but could not walk, jump or fly.

Imidacloprid at the LLR alone and in combination with Silwet L-77 or Kinetic caused mortality of *T. radiata* adults ranging from 95 to 100% (Table 3). Imidacloprid alone or in combination with Silwet L-77 or Kinetic was significantly more toxic to the parasitoid than abamectin alone or with these adjuvants. Mortality of parasitoids exposed to foliage treated with copper hydroxide at the LLR was 5% (Table 3), and when Silwet L-77 or Kinetic was added to copper hydroxide, the mortality of *T. radiata* did not increase significantly (1.7 and 6.7%, respectively). Toxicity of copper hydroxide alone or in combination with Silwet L-77 or Kinetic to adults of *T. radiata* was not significantly different from that caused by water.

Acute (Direct) Toxicity. Mortality of *T. radiata* adult females sprayed with Silwet L-77 or Kinetic alone was 0 and 1.9%, respectively, which was not different from mortality of adults treated with water (1.7%) (Table 3). Petroleum oil at 2% and copper hydroxide at the LLR alone and in combination with Silwet L-77 or Kinetic caused 0 to 3.3% mortality of *T. radiata*, which were not significantly different from the water control. Abamectin at the LLR alone and in combination with Silwet L-77 or Kinetic killed 91.7 to 100% of *T. radiata* adults ($F = 102.39$; $df = 11, 60$; $P < 0.0001$), suggesting that abamectin is very toxic to this parasitoid when applied directly, but that petroleum oil at 2% and copper hydroxide alone and in combination with Silwet L-77 or Kinetic do not affect its survival.

Discussion

The insecticidal and miticidal effects of surfactants and soaps have been known for a long time, although the mode of action is not clear (van der Meulen & van Leeuwen 1929; Wilcoxon & Hartzell 1931). Silwet L-77 has been reported to be effective in controlling mites, aphids, tephritids, mealybugs, armyworms, and citrus leafminer (Dentener & Peetz 1992; Imai et al. 1994, 1995; Chandler 1995; Purcell & Schroeder 1996; Wood et al. 1997; Shapiro et al. 1998; Cowles et al. 2000; Tipping et al. 2003). Under laboratory, greenhouse, and field conditions, Silwet L-77 alone was toxic to young *D. citri* nymphs and increased the toxicity of imidacloprid and abamectin at lower-than-label rates (Srinivasan et al. 2008).

The screenhouse bioassays conducted between May and Oct 2007 show that Silwet L-77 is more toxic than Kinetic to all stages of *D. citri.* Our studies confirm the results of Srinivasan et al. (2008); both adjuvants were ineffective in killing *D. citri* eggs and Silwet L-77 was significantly more toxic than Kinetic to young nymphs. Silwet L-77 alone was as effective in killing mature nymphs as young nymphs (69.7 and 64.2%, respectively), while Kinetic killed fewer mature nymphs than young nymphs (35.2 and 46.2%, respectively). The acute toxicity of Silwet L-77 alone to *D. citri* adults caused 30.6% mortality, which was higher than the mortality caused by the residual toxicity of the same adjuvant (4.3%) (Srinivasan et al. 2008). The application of Silwet L-77 is inexpensive, estimated to cost less than $5.00 per hectare ($2.00 per acre), and could be applied to suppress psyllid nymphs. However, neither adjuvant is registered as an insecticide and can be sprayed only in combination with agrochemicals. Furthermore, Silwet L-77 is stable at pH values of 6-8 and degrades in 6 d (pH = 5) or 14 d (pH = 9) (Knoche et al. 1991). Because our experiments were carried out using purified water at a pH near neutral, Silwet L-77 should be tested under grove conditions, where water pH and quality may vary, to confirm our results.

Petroleum oil alone at all 3 rates was toxic to eggs of *D. citri* (61.5-66.7% mortality), but only the rate of 2% was effective in killing >80% of young nymphs. Petroleum oil at 2% alone was less effective against mature nymphs than against young nymphs (44.8 and 84.7% mortality, respectively). However, adding Silwet L-77 significantly increased the toxicity of petroleum oil to mature nymphs (74.3%). Silwet L-77 increased significantly the toxicity of petroleum oil at 0.5, 1, and 2% to *D. citri* eggs and young or mature nymphs, except for petroleum oil at 0.5% for eggs, and at 2% for young nymphs. Petroleum oil alone at all 3 rates showed low acute toxicity to *D. citri* adults (2.7-17.5%), but adding Silwet L-77 to petroleum oil increased the mortality to 35.2-54.7%. However, acute toxicity of petroleum oil at 2% plus Silwet L-77 caused 54.7% mortality to adults, which was lower than mortality caused by residual toxicity of imidacloprid at one-fourth and one-half the LLR in combination with Silwet L-77 or Kinetic (93-98.8%) (Srinivasan et al. 2008). Our results indicate that petroleum oil at 0.5 and 1% in combination with Silwet L-77 or Kinetic is less suitable than the 2% rate in a psyllid control program. Kinetic was less effective than Silwet L-77 in increasing the toxicity of petroleum oil to all psyllid instars.

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Psyllid immatures and adults sprayed with copper hydroxide experienced low mortality, indicating that copper hydroxide is not effective in suppressing *D. citri*. However, mortality of all psyllid stages increased significantly when copper hydroxide was combined with Silwet L-77, primarily due to the acute toxicity of Silwet L-77 to *D. citri*. Kinetic was less effective than Silwet L-77 in increasing the toxicity of copper hydroxide to all *D. citri* instars. Silwet L-77 increased the coverage of copper hydroxide and petroleum oil on leaf surfaces, suggesting that the effectiveness of these pesticides in controlling diseases and insects might be enhanced.

Petroleum oil and copper hydroxide are commonly used in citrus IPM programs. Petroleum oil is sprayed against scales, mealybugs, whiteflies, citrus leafminer, aphids, citrus rust mites, and spider mites (University of Florida-IFAS Extension 2008), and no arthropod has developed resistance to petroleum oil (Thomson 2001). Rae et al. (1997) reported that petroleum oil was as effective as omethoate and diflubenzuron in suppressing *D. citri* nymphs. Copper hydroxide is applied to control citrus canker, greasy spot, melanose, and alternaria brown spot (Timmer et al. 2007). Silwet L-77 might be added to copper hydroxide or petroleum oil during the summer while spraying to control other pests or fungal diseases, which could provide the added benefit of increasing control of psyllid eggs and nymphs while minimizing application costs. However, adding adjuvants to copper hydroxide also could increase the uptake of copper by the trees and cause spray burn damage in susceptible varieties (Timmer et al. 2007). Thus, further laboratory and field trials are needed to determine whether applications of copper hydroxide in combination with Silwet L-77 affect tree growth, increase or decrease citrus canker infection rates, or disrupt pest control. Our bioassays indicate that Silwet L-77 was more insecticidal than Kinetic and more effective as an adjuvant for both petroleum oil and copper hydroxide. The different properties of Kinetic and Silwet L-77 could be due to their different composition; Kinetic is a blend of organosilicone compounds and nonionic surfactants and has a lower concentration of trisiloxanes than Silwet L-77, so Kinetic might have lower surfactant properties.

The observations of where *T. radiata* females were located inside the clip cage during the residual toxicity bioassay indicated that the agrochemicals tested did not appear to be repellent to this parasitoid. Females were observed moving and settling on both the inner surface of the acrylic ring and on the treated leaves. The laboratory bioassays showed that Silwet L-77 alone did not reduce the survival of *T. radiata* and did not increase the toxicity of any pesticide tested to this natural enemy. Kinetic alone was slightly toxic to the parasitoid and, in combination with pesticides, it significantly increased the residual toxicity of abamectin to *T. radiata*. After 24 h, adult parasitoids appeared paralyzed, due to the combined activity of Kinetic and abamectin. Abamectin alone had moderate or low residual toxicity to *Aphytis melinus* DeBach and *Rhizobius lophanthae* (Blaisdell), important natural enemies of diaspidiid scales (Bellows & Morse 1993), and the predatory mite *Metaseiulus occidentalis* (Nesbitt) (Hoy & Cave 1985). This insecticide was found to be compatible with IPM programs against the citrus mealybug, *Planococcus citri* Risso, due to its selectivity to larvae of *Cryptolaemus montrouzieri* Mulsant and adults of *Leptomastix dactylopii* Howard (Yumruktepe et al. 1996). Imidacloprid showed high residual toxicity to *T. radiata*, with mortality greater than 91%, and killed most parasitoids within 2 h. These results are consistent with other studies on the toxicity of imidacloprid to the parasitoids *A. citricola*, *A. melinus* and *Gonatocerus ashmeadi* Girault (Villanueva-Jimenez & Hoy 1998; Toscano et al. 2004).

The acute and residual toxicity of imidacloprid and abamectin alone and in combination with Silwet L-77 or Kinetic to *T. radiata* adults suggested that multiple applications of imidacloprid and abamectin will likely disrupt *T. radiata* populations, due to the residual effect of imidacloprid and the acute toxicity of abamectin. Petroleum oil and copper hydroxide alone or in combination with Silwet L-77 or Kinetic appear to be compatible with the biological control of *D. citri* by *T. radiata* due to their low acute and residual toxicity. Petroleum oil alone is compatible with other citrus natural enemies (Morse et al. 1987; Villanueva-Jimenez & Hoy 1998; Childers et al. 2001; Ulmer et al. 2006). Copper hydroxide has been shown to be moderately toxic to the parasitoids *A. citricola* and *Aprostocetus vaquitarum* Wolcott (Villanueva-Jimenez & Hoy 1998; Ulmer et al. 2006). Some studies suggested copper hydroxide stimulates populations of the citrus rust mite *Phyllocoptruta oleivora* (Ashmead) (Childers 1994), although other research suggested that copper hydroxide reduces citrus rust mite fecundity (McCoy & Lye 1995).

The aims of this study were to evaluate the efficacy of petroleum oil in conjunction with adjuvants to suppress populations of the Asian citrus psyllid and the impact of other pesticides used in citrus groves to its parasitoid *T. radiata*. Silwet L-77 and Kinetic, in combination with abamectin and imidacloprid, can be used to manage all life stages of *D. citri* (Srinivasan et al. 2008). However, our results suggest that these insecticides are harmful to *T. radiata* and would likely reduce the activity of the parasitoid in citrus groves. Petroleum oil and copper hydroxide in combination with Silwet L-77 cause moderate or high mortality to immatures and adults of *D. citri*, yet have low residual and acute toxicity to *T. radiata*. Pe-

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troleum oil in combination with Silwet L-77 may not be immediately used in Florida citrus IPM programs because of growers' current desires to reduce *D. citri* populations to levels approaching zero using long-residual products that are highly toxic to natural enemies. However, if other IPM tools become available to effectively manage citrus greening disease, petroleum oil plus Silwet L-77 could provide a less-toxic tool to suppress *D. citri* and allow natural enemies to become useful again in citrus IPM. Because copper hydroxide is being used extensively to suppress citrus canker, field trials with copper hydroxide plus Silwet L-77 should be conducted, because this combination could provide additional control of *D. citri* and have a low impact on natural enemies.

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