

LABORATORY AND FIELD EVALUATIONS OF SILWET L-77 AND KINETIC ALONE AND IN COMBINATION WITH IMIDACLOPRID AND ABAMECTIN FOR THE MANAGEMENT OF THE ASIAN CITRUS PSYLLID, *DIAPHORINA CITRI* (HEMIPTERA: PSYLLIDAE)

RAJAGOPALBABU SRINIVASAN<sup>1,3</sup>, MARJORIE A. HOY<sup>1</sup>, RAGHUWINDER SINGH<sup>1</sup> AND MICHAEL E. ROGERS<sup>2</sup>

<sup>1</sup>Department of Entomology and Nematology, P.O. Box 110620, Building 970, Natural Area Drive, University of Florida, Gainesville, FL 32611

<sup>2</sup>Citrus Research and Education Center, University of Florida, Lake Alfred, FL 33850

<sup>3</sup>Presently at Aberdeen Research and Extension Center, University of Idaho, Aberdeen, ID 83210  
E-mail: babu@uidaho.edu

ABSTRACT

Silwet L-77, an organosilicone adjuvant, was used to enhance coverage of an entomopathogenic fungus in field trials conducted in a central Florida citrus research grove. The results indicated that Silwet L-77, by itself, was toxic to nymphs of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). Laboratory bioassays were conducted to confirm the toxicity of the adjuvant to eggs, nymphs, and adults of *D. citri*. Silwet L-77 at 0.05% (500 ppm) killed all nymphs, but was not as effective against eggs and adults. However, Silwet L-77, when combined with a reduced rate (one-tenth the lowest label rate = LLR) of imidacloprid, killed >90% of eggs and adults in laboratory tests. In a subsequent field trial, the combination of Silwet L-77 and one-tenth the LLR of imidacloprid gave good control of nymphs, but exhibited weak residual toxicity to adults when compared to imidacloprid at the LLR. Additional trials were then conducted with Silwet L-77 and Kinetic, another organosilicone adjuvant, alone and in combination with different rates of imidacloprid or abamectin using potted citrus trees in the greenhouse. Combining Silwet L-77 or Kinetic with one-fourth or one-half the LLR of imidacloprid killed as many eggs, nymphs, or adults as the LLR of imidacloprid. The combination of Silwet L-77 or Kinetic with one-fourth or one-half the LLR of abamectin killed as many eggs and nymphs as the LLR, but did not control adults as well. The results are discussed in terms of managing the Asian citrus psyllid in Florida now that citrus greening disease is endemic.

Key Words: organosilicone adjuvants, Silwet L-77, Kinetic, Asian citrus psyllid, reduced rates of insecticides, imidacloprid, abamectin

RESUMEN

El Silwet L-77, Un adyuvante orgánico, de silicon, fue usado para mejorar la cobertura de un hongo entomopatógeno en pruebas de campo realizadas en un huerto de cítricos de investigación en el centro de la Florida. Los resultados indican que el Silwet L-77, por si solo, fue tóxico a las ninfas del sílido asiático de los cítricos, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). Se realizaron bioensayos de laboratorio para confirmar a toxicidad del adyuvante a los huevos, ninfas y adultos de *D. citri*. El Silwet L-77 en una concentración de 0.05% (500 ppm) mató todas las ninfas, pero no fue efectivo contra los huevos y adultos. Sin embargo, Silwet L-77 mató >90% de los huevos y adultos en pruebas del laboratorio cuando fue combinado con una tasa reducida (una décima parte de la menor tasa indicada por la etiqueta = MTE) del imidacloprid. En pruebas de campo subsecuentes, la combinación de Silwet L-77 y una décima parte del MTE de imidacloprid resultó en un buen control de las ninfas, pero mostró una débil toxicidad residual a los adultos en comparación a imidacloprid al nivel de MTE. Se realizaron pruebas adicionales con Silwet L-77 y Kinetic, otro adyuvante orgánico, de silicon, solo y en combinación con diferentes tasas de imidacloprid o abamectin usando árboles de cítricos sembrados en macetas en el invernadero. Silwet L-77 o Kinetic en combinación con un cuarto o la mitad del MTE de imidacloprid mató tantos huevos, ninfas o adultos como la MTE de imidacloprid. Silwet L-77 o Kinetic en combinación con un cuarto o la mitad de la MTE de abamectin mató tantos huevos y ninfas como la de MTE, pero no controló los adultos tanto como los huevos y ninfas. Los resultados estan discutidos en terminos de manejo del sílido asiático de los cítricos en Florida ahora que la enfermedad de enverdecimiento es considerada endémica.

Silwet L-77, an organosilicone adjuvant, was applied together with entomopathogenic fungal spores in a field trial during 2006 to assess the effectiveness of the fungus to manage the Asian citrus psyllid, *Diaphorina citri* Kuwayama, in a citrus grove in Florida (Hoy et al. unpublished). The results indicated that Silwet, by itself, induced high rates of mortality of *D. citri* nymphs.

A subsequent literature review indicated that trisiloxanes, a subclass of organosilicones, include non-ionic surfactants that have an extraordinary ability to reduce the surface tension of water (Witco 1997). The reduction in surface tension allows water to interact with hydrophobic or waxy surfaces and enhances the spreading of water. These surfactants are used as agricultural spray adjuvants to enhance the spreading of spray droplets on leaves and result in increased pest control (Pollicello et al. 1995). The review also revealed that Silwet L-77 is toxic to arthropod pests, including two-spotted spider mites, aphids, citrus leafminers, tephritid fruit flies, and armyworms (Chandler 1995; Imai et al. 1995; Purcell & Schroeder 1996; Shapiro et al. 1998; Cowles et al. 2000; Tipping et al. 2003; Neven et al. 2006). The mode of action or toxicity mechanism(s) of Silwet L-77 have not been identified, but Cowles et al. (2000) hypothesized that trisiloxane surfactants could act like hyperactive soaps, thereby permitting water to infiltrate the respiratory system of insects to disrupt essential gaseous exchange. Besides drowning, other mechanisms could be involved. Chandler (1995) observed mortality of armyworm larvae upon topical application, and these compounds are believed to interact with nerve and cell membranes to disrupt their functions (Puritch 1981).

The Asian citrus psyllid is an economically important pest because it is a vector of citrus greening disease, also known as Huanglongbing, yellow shoot or yellow dragon (Capoor et al. 1974; Aubert 1987; da Graca 1991). Greening disease is caused by a bacterium (Alpha Proteobacterium, Rhizobiales, Family Rhizobiaceae, *Candidatus Liberibacter asiaticus* Garnier) that is transmitted by the psyllid. Greening disease is considered the most serious citrus disease in the world and the bacterium is listed as a select agent under the Agricultural Bioterrorism Protection Act of 2002. Greening disease was confirmed to be in Florida during Aug 2005, and now is widely distributed in commercial orchards and in residential trees (Florida Dept. of Agriculture and Consumer Services 2006). As a result of its wide distribution when detected, eradication efforts were not implemented and the goal now is to reduce the rate of spread of the pathogen in groves where present and to develop long-term management methods. The psyllid is established in Texas (French et al. 2001) and could invade other citrus-growing regions in the USA (i.e., Arizona and California).

The tools available to manage *D. citri* and greening disease at this time in Florida include removing infected trees, replanting with clean nursery stock, and attempting to suppress psyllid populations (Stansly & Rogers 2006; Rogers & Timmer 2007). Insecticides, such as imidacloprid and abamectin, are being applied to suppress psyllid populations but the required multiple applications (up to 6-8) are expensive, disruptive to natural enemies, and may lead to the development of insecticide resistance in the psyllid. With up to 6-8 sprays a year predicted to be used to suppress psyllid populations in Florida, at least until other greening disease management methods are developed, the costs will be substantial in both economic and ecological terms. Thus, there is a need for citrus growers to suppress psyllids in the least expensive, yet most effective, manner possible. Because Silwet L-77, applied at the rate of 0.05% would cost approximately \$2.00 per acre (0.4 ha), the use of organosilicone adjuvants as substitutes for chemical pesticides or as adjuvants to allow use of lower rates of such pesticides could result in substantial savings in managing psyllid populations.

In order to explore this possibility, laboratory, greenhouse, and field experiments were conducted with Silwet L-77 and another organosilicone adjuvant, Kinetic, alone and in combination with different rates of imidacloprid and abamectin.

#### MATERIALS AND METHODS

Field Trial during Aug 2006 Demonstrating Efficacy of Silwet L-77 as an Insecticide

The 3-year-old block of citrus trees to be sprayed was located at the Water ConservII Research Site, in Orange County, near Winter Garden, Florida and consisted of Minneola (cross LB 89) trees planted 3.8-m apart in the row with rows 6.1-m apart. The 20 experimental trees were approximately 1.2 to 1.5 m tall, and the treatments were randomly chosen by coin toss. Trees initially contained flush less than 4 cm long with eggs and small psyllid nymphs. Ten trees were treated with 500 mL of Silwet L-77 surfactant (99.5% polyalkyleneoxide modified heptamethyltrisiloxane, Helena Chemical Co., Collierville, TN) at a rate of 0.05% (v:v), or 500 ppm. Ten trees also were treated with 500 mL of water each using a 12-gal (45.5 L) battery-operated sprayer (Scorpion sprayer, AgSouth, LLC, Union City, TN) calibrated to deliver 1.0 gpm (3.8 L per min) at 60 PSI (27.2 kg/6.45 square cm).

A pre-treatment sample of 2 shoots was randomly collected from each tree, and each shoot was placed in a plastic bag. An hour after treatment, a post-treatment sample of 2 shoots per tree was collected from all trees, as described for the pre-treatment samples. Plastic bags contain-

ing shoots were stored in an ice chest with ice packs for the trip to Gainesville. At Gainesville, the number of dead or live psyllid nymphs per shoot and the shoot length was recorded. Data were analyzed by Proc ANOVA (version 9.1) (SAS Institute 1996), and means were separated with Fisher's least significant difference (LSD) at the 5% significance level.

#### Laboratory Studies with Silwet L-77 Using Eggs, Nymphs, or Adults of *D. citri*

A series of laboratory trials were conducted following this initial field trial to evaluate the toxicity of Silwet L-77 alone and in combination with imidacloprid (Provado 1.6 Flowable, Bayer Crop-Science, Research Triangle Park, NC) against eggs, nymphs, and adults of *D. citri*.

Silwet L-77 at 0.05%, imidacloprid at one-tenth the lowest label rate (LLR), imidacloprid at the LLR (1 oz/10 gal of water or 0.8 mL/L of water) and Silwet L-77 at 0.05% with one-tenth the LLR of imidacloprid were used to evaluate toxicity to eggs of *D. citri*. Potted citrus trees with at least 6 new flushes per tree were selected. The trees were placed inside nylon fabric cages at the University of Florida, Department of Entomology and Nematology greenhouse, Gainesville at 18-36°C, 35-80% RH under a 16L:8D photoperiod. Ovipositing females were released into the cage (approximately 5 females per tree) for a period of 72 h. Branches of a single tree containing eggs were hand dipped into treatment solutions for 10 s. Because the branches were hand dipped, each branch was considered a replicate. Water-treated trees served as controls. The trees were left undisturbed for a period of 5-7 d and the number of unhatched eggs and dead or live nymphs on each shoot was counted and recorded under a dissecting microscope. Percentage mortality data were subjected to arcsine-square root transformation when needed to mitigate the skewness of the data and meet the requirements of normality. Analysis of variance was estimated using Proc ANOVA (SAS Institute 1996) to detect differences among treatments, if any, and treatment means were separated using Fisher's LSD.

The bioassay protocol for psyllid nymphs was modified from Cowles et al. (2000). A preliminary bioassay indicated that Silwet L-77 at 50 ppm killed all *D. citri* nymphs, so Silwet L-77 at 0, 10, 20, 30, 40, and 50 ppm were used for this experiment. Five shoots with approximately 20 nymphs per shoot (approximately 100 nymphs per concentration) were used for each treatment. The shoots with nymphs (primarily 1-3 instars) were completely dipped in different treatment concentrations for 5 s. The shoots were then placed into petri dishes lined with a filter paper to absorb excess moisture. The experiment was conducted in a growth chamber at 24°C, 77% RH under a 16L:8D photoperiod.

The number of dead or live psyllid nymphs was counted after 24 h. The death of nymphs was confirmed by touching each nymph with an insect pin. Shoots treated with water alone served as a control. Concentration-mortality analyses were conducted using Proc Probit (SAS Institute 1996).

Adults that were approximately 2 weeks old were treated with Silwet L-77 at 0.05%, imidacloprid at one-tenth the LLR, imidacloprid at the LLR, and Silwet L-77 at 0.05% + one-tenth the LLR of imidacloprid. Adults were chilled for several minutes at 5°C to inactivate them and 10-15 adults were placed on a filter paper-lined petri plate. One mL of each treatment was topically applied with a 1-mL syringe directly to psyllid adults placed on a filter paper and each treatment had 4 replicates. Controls included water or insecticide alone at the LLR. The treated psyllids were moved to a 15-mL plastic vial and a single mature citrus leaf was placed in the vial as a food source. The vials were tightly secured using cheesecloth to prevent escape. The experiment was conducted in a growth chamber at 24°C, 77% RH with a 16L:8D photoperiod. The number of dead or live psyllids was counted after 24 h. Statistical analyses were performed as for eggs.

#### Field Trial during Oct and Nov 2006

After the laboratory bioassays above indicated that Silwet L-77 alone and in combination with one-tenth the LLR of imidacloprid killed nymphs and adults of *D. citri*, a field trial was conducted to confirm whether these treatments could suppress psyllids in citrus groves. Five treatments were evaluated: water; Silwet L-77 at 0.05%; imidacloprid at one-tenth the LLR (or 1 oz/10 gal of water or 0.8 mL/1 L of water); imidacloprid at the LLR; and Silwet L-77 at 0.05% in combination with one-tenth the LLR of imidacloprid. Each treatment was replicated 10 times in a completely randomized block design using a total of 50 trees. The 3-year-old block of Mineola trees (cross LB89) was the same one used in the first field trial (described above) at the Water ConservII Research Site in Orange County. Trees initially contained a flush of growth less than 4 cm long that contained eggs and small psyllid nymphs. Individual trees were identified with flagging. A pretreatment sample of 4 shoots was randomly collected from each tree, and each shoot was placed in a separate plastic bag. An hour after treatment, a post-spray sample of 4 shoots per tree was collected from all trees, as described for the pre-treatment samples. Plastic bags containing shoots were stored in an ice chest with ice packs for the trip to Gainesville. Trees were sprayed as described above.

Four to 6 selected shoots in a node on each tree were tagged with plastic tape prior to spraying. These tagged shoots were used for subsequent sampling, assuring that sampled shoots had been

treated and were not new growth subsequent to the spray. A single shoot was sampled each week, and when the nodes no longer had any shoots, shoots of the expected size were selected from that tree. All psyllid nymphs on each shoot were counted. Sampling was conducted just before and just after treatment and each week for 6 consecutive weeks.

The effect of the treatments on shoot length and shoot growth was monitored to evaluate the possibility of phytotoxicity. Shoots taken to the laboratory to estimate the density of psyllid nymphs were measured using a caliper and the observations recorded. In order to monitor the effect of treatments on shoot growth, a single shoot on each tree was tagged at the start of the experiment and its growth was monitored over the entire sampling period.

Residual toxicity of the treatments was assessed by sampling mature foliage (dark green leaves that were not on new shoots) each week for 5 weeks after treatment. One mature shoot was collected from each treatment tree, and 2 leaves from each shoot were placed into 2 separate 50-mL vials. Two adults of both sexes from our laboratory colony that were approximately 2 weeks old were released into each vial and placed in a growth chamber at 23°C, and 70% RH under a 16L:8D photoperiod. Adult survival on the mature treated leaves was recorded after 48 h.

Percentage adult mortality data were subjected to arcsine-square root transformation when needed to mitigate the skewness of the data and meet the requirements of normality. Treatment differences with respect to nymphal counts, shoot length, and adult survival were estimated using Proc ANOVA (SAS Institute 1996). Treatment means were separated using Fisher's LSD.

#### Concentration-Mortality Data for Silwet L-77 and Kinetic in Laboratory Bioassays

In order to compare the efficacy of Silwet L-77 with Kinetic (proprietary blend of polyalkyleneoxide modified polydimethylsiloxane and nonionic surfactants (99%) and non-surfactant ingredients (1%) (Helena Chemical Company), laboratory bioassays were conducted using the bioassay protocol modified from Cowles et al. (2000). A preliminary bioassay indicated that Silwet L-77 and Kinetic at 50 ppm killed all nymphs so Silwet L-77 and Kinetic at 0, 10, 20, 30, 40, and 50 ppm were tested. One shoot with approximately 100 nymphs per shoot was used for each treatment. The shoots with nymphs were dipped in different treatment concentrations for 5 s. The shoots were then placed into petri plates lined with filter paper to absorb excess moisture. The experiment was conducted in a growth chamber at 24°C, 77% RH, under a 16:8D photoperiod. The dead psyllid nymphs were counted after 24 h. The death of nymphs was confirmed by touching each nymph

with an insect pin. Probit analyses were conducted as described above.

#### Greenhouse Bioassays Using Potted Citrus Trees to Compare Silwet L-77 or Kinetic Alone and in Combination with Abamectin or Imidacloprid to Eggs, Nymphs, or Adults of *D. citri*

All experiments included the same 18 treatments: untreated control, water control, Silwet L-77 at 0.05%, Kinetic at 0.05%, imidacloprid at one-fourth the LLR, imidacloprid at one-half the LLR, imidacloprid at the LLR, abamectin (Agrimek 0.15EC, Syngenta Chemical Company) at one-fourth the LLR, abamectin at one-half the LLR, abamectin at the LLR, Silwet L-77+ one-fourth the LLR of imidacloprid, Silwet L-77+ one-half the LLR of imidacloprid, Silwet L-77 + one-fourth the LLR of abamectin, Silwet L-77+ one-half the LLR of abamectin, Kinetic + one-fourth the LLR of imidacloprid, Kinetic+ one-half the LLR of imidacloprid, Kinetic + one-fourth the LLR of abamectin and Kinetic + one-half the LLR of abamectin.

To assess toxicity of these treatments to eggs of *D. citri*, pruned potted citrus trees with at least 6 new flushes per tree were selected. The trees were placed inside cages in a greenhouse at the University of Florida, Department of Entomology and Nematology, Gainesville at 18-36°C, 35-80% RH under a 16L:8D photoperiod. Ovipositing females were released into the cage (5 females per tree) for a period of 72 h. The adults were aspirated out after 72 h. The trees were sprayed using a different disposable spray gun (Preval® spray gun, Yonkers, NY) for each treatment. The spray gun was held 90 cm from the trees and moved around the tree during a 15-sec interval that produced fine droplets covering ca. 90% of the foliage. The trees were left undisturbed for a period of 5-7 d and the unhatched eggs and dead or live nymphs on each shoot were counted under a dissecting microscope and recorded. The entire experiment was conducted twice. Percentage mortality data were subjected to arcsine-square root transformation when needed to mitigate the skewness of the data and meet the requirements of normality. Analysis of variance was estimated using Proc ANOVA (SAS Institute 1996) to detect differences among treatments and treatment means were separated using Fisher's LSD.

Toxicity to nymphs was assessed using the same methods as for the eggs, except that 5 ovipositing females per tree were released into the cage for a period of 72 h. After 3 to 4 d, when waxy excretions from the first through third-instar nymphs were observed, the trees were sprayed as described above. The trees were left undisturbed for a period of 3-5 d and the number of unhatched eggs and dead or live nymphs on each shoot were counted under a dissecting microscope and recorded. The entire experiment was conducted twice. Statistical analyses were performed as for eggs.

Toxicity to adults was assessed by treating potted citrus trees after covering the exposed soil of the pot with a thin plastic film and then a double layer of paper coffee filters. The coffee filters were taped to the pot to prevent adults from becoming lost in the soil. The sprayed trees were pruned to fit into a plexiglass cylinder 50 cm tall with a 10-cm radius. Then, 20 adults that were approximately 2 weeks old were aspirated into a 50-mL plastic vial and introduced into the cylinder. The trees were placed in the greenhouse at the University of Florida, Department of Entomology and Nematology, Gainesville at 18-36°C, 35-80% RH under a 16L:8D photoperiod. The number of dead or live adults was estimated after 72 h. The entire experiment was repeated 5 times. Statistical analyses were performed as for eggs and nymphs.

#### Toxicity of Imidacloprid to Adult Psyllids on Detached Leaves vs. Attached Leaves

In order to evaluate whether our use of detached leaves from the field experiment was appropriate for evaluating residual toxicity of imidacloprid to adults of *D. citri*, 2 experiments were conducted simultaneously. Eight citrus trees were sprayed with imidacloprid at the LLR. Two of these trees were pruned on 4 dates (immediately after spray and 1, 2, and 4 weeks later) and covered with a plexiglass cylinder, as described above, and left at the greenhouse at 18-36°C, 35-80% RH under a 16L:8D photoperiod. Twenty adult psyllids were introduced into the cylinder and the number of dead or live adults was counted after 2 d to assess mortality on attached leaves. Two leaves were also removed from each tree and individually placed into a 50-mL vial and 2 adults were introduced into each vial as described for the residual toxicity tests. The vials were placed in a growth chamber at 23°C, and 70% RH under a 16L:8D photoperiod. The number of dead or live adults was estimated after 2 d. Water-treated trees and foliage from the same trees served as

controls. Mortality in each set of experiments across each time interval was compared with water-treated trees using Proc GLM (SAS Institute 1996) and differences across the treatments were evaluated using Fisher's LSD. The mortality achieved at each date in the 2 bioassay methods also was compared using the same procedure. Regression analyses were done using Proc REG (SAS Institute 1996) to compare the decline in adult mortality over time using the 2 assay methods.

## RESULTS

### Field Trial during Aug 2006 Demonstrating Efficacy of Silwet L-77 as an Insecticide

Pretreatment and immediate post-treatment counts of psyllid nymphs indicated no differences between water- and Silwet L-77-treated trees (Table 1). Counts on subsequent weeks revealed a lower mean number of nymphs on Silwet L-77-treated trees when compared to water-treated trees on weeks 1, 3, and 4 (Table 1). However, no differences were found between the 2 treatments on weeks 2 and 5. Very few nymphs were found during week 5, perhaps due to maturation of the tender shoots.

Pretreatment observations indicated no differences in shoot length between the treatments (Table 2). Immediate post-treatment observations indicated that shoots on water-treated trees were longer, perhaps due to an artifact of sampling. These differences, however, were not significant during subsequent samples and indicate that the Silwet L-77 application had no effect on shoot length. No symptoms of phytotoxicity on this cultivar were observed in the field.

### Laboratory Studies with Silwet L-77 and Imidacloprid Using Eggs, Nymphs, or Adults of *D. citri*

Silwet L-77 applied at the rate of 0.05% by itself killed all the nymphs tested using the shoot dipping protocol and hence no combinations with

TABLE 1. EFFECT OF SILWET L-77 (0.05% v:v) ON THE NYMPHS OF THE ASIAN CITRUS PSYLLID AT THE WATER CONSERVATION RESEARCH SITE IN FLORIDA DURING AUG 2006.

Sample interval	Mean $\pm$ SE number of psyllid nymphs/shoot		
	Water	Silwet L-77	P
Pretreatment	23.9 $\pm$ 3.7 a	24.0 $\pm$ 3.8 a	0.99
Immediate post treatment	9.6 $\pm$ 2.0 a	8.3 $\pm$ 2.4 a	0.67
Week 1	10.5 $\pm$ 2.1 a	2.3 $\pm$ 0.6 b	0.0001
Week 2	7.8 $\pm$ 2.9 a	3.8 $\pm$ 1.3 a	0.21
Week 3	3.1 $\pm$ 0.8 a	1.2 $\pm$ 0.4 b	0.03
Week 4	1.2 $\pm$ 0.4 a	0.1 $\pm$ 0.1 b	0.003
Week 5	0.1 $\pm$ 0.02 a	0.2 $\pm$ 0.03 a	0.45

\*Treatment means were analyzed using Proc ANOVA and treatment differences were analyzed using Fisher's LSD. Treatments with the same letter within a row are not significantly different from each other ( $P \leq 0.05$ ).

TABLE 2. EFFECT OF SILWET L-77 ON SHOOT LENGTH OF TREATED TREES AT THE WATER CONSERVATION RESEARCH SITE IN FLORIDA DURING AUG 2006.

Sample interval	Mean $\pm$ SE shoot length (cm)		
	Water	Silwet (0.05%)	P
Pretreatment	9.1 $\pm$ 0.5 a	10.1 $\pm$ 0.8 a	0.16
Immediate post treatment	10.3 $\pm$ 0.5 a	8.2 $\pm$ 0.4 b	0.002
Week 1	10.1 $\pm$ 0.5 a	10.5 $\pm$ 0.4 a	0.54
Week 2	13.5 $\pm$ 0.9 a	13.6 $\pm$ 0.8 a	0.95
Week 3	15.6 $\pm$ 0.6 a	14.7 $\pm$ 0.6 a	0.25
Week 4	18.1 $\pm$ 0.6 a	16.8 $\pm$ 0.5 a	0.10
Week 5	19.2 $\pm$ 0.7 a	18.8 $\pm$ 0.6 a	0.63
Week 6	19.1 $\pm$ 0.8 a	19.8 $\pm$ 0.7 a	0.47

\*Treatment means were analyzed using Proc ANOVA and treatment differences were analyzed using Fisher's LSD. Treatments with the same letter within a row are not significantly different from each other ( $P \leq 0.05$ ).

imidacloprid were tested (data not shown). At 50 ppm, Silwet L-77 killed more than 99% of the psyllid nymphs. The lethal concentrations and their fiducial limits are presented in ppm (Table 3). These rates are well below the 500 ppm (0.05%) rate recommended for field use.

A preliminary study using Silwet L-77 at 0.05% indicated that it was not toxic to eggs, because most eggs hatched and developed into healthy nymphs. As a result, a combination of Silwet L-77 (0.05%) + imidacloprid was tested (Table 4). Mortality of eggs included the eggs that did not hatch, as well as the first-instar nymphs that died soon after hatching. Silwet L-77 by itself caused the least mortality (0.3%) to eggs on shoots dipped into the solution among all treatments. The highest mortality was recorded on shoots treated with the LLR of imidacloprid (97.2%), while the combined treatment of Silwet L-77 + one-tenth the LLR of imidacloprid caused 71.6% mortality (Table 4). Imidacloprid at one-tenth the LLR resulted in only 50.4% mortality of eggs. Combining Silwet L-77 with one-tenth the LLR of imidacloprid clearly provided improved control of eggs compared to Silwet L-77 alone. Though not statistically significant, the combination also provided improved control of eggs compared to one-tenth the LLR of imidacloprid (Table 4).

Silwet L-77 applied topically at the rate of 0.05% killed 47% of adults, while imidacloprid at one-tenth the LLR killed 46.3% of adults (Table 4). A combination of Silwet L-77 and one-tenth the LLR of imidacloprid increased mortality of adults to 100%, which was equal to the LLR of imidacloprid (100%). All the water-treated adults survived, as expected (Table 4). These laboratory results suggest that control of eggs, nymphs, or adults could be achieved with a combination of one-tenth the LLR of imidacloprid plus Silwet L-77 (0.05%).

#### Field Trial during Oct and Nov 2006

Pre-treatment observations indicated that trees to be sprayed with imidacloprid at the LLR had fewer nymphs than the rest of the treatments (Table 5), but this could have been due to sampling error. There were significantly more living nymphs on the water-treated control trees immediately after treatment (Table 5), suggesting that Silwet L-77 and imidacloprid alone and in combination with Silwet L-77 killed most *D. citri* nymphs.

Over the total post-spray period, there were significantly more living psyllid nymphs/shoot on the water-treated trees (mean = 17.4) than on the trees treated with Silwet L-77 (1.8) or imidaclo-

TABLE 3. CONCENTRATION-MORTALITY RESPONSE OF ASIAN CITRUS PSYLLID NYMPHS TO SILWET L-77 UNDER LABORATORY CONDITIONS.

Lethal concentration	Concentration (ppm)	Fiducial limits (ppm)	Intercept	Slope
LC50	7.3	6.5-8.1	2.9484	-2.5485
LC90	19.9	18.8-21.1		
LC95	26.4	24.8-28.5		
LC99	45.0	40.6-50.9		

\*Mortality at each Silwet L-77 concentration was subjected to probit analysis using Proc Probit. Five shoots with at least 20 nymphs per shoot were tested for each concentration.

TABLE 4. TOXICITY OF SILWET L-77 ALONE OR IN COMBINATION WITH IMIDACLOPRID TO PSYLLID EGGS USING A SHOOT-DIP BIOASSAY AND TO ADULTS USING A TOPICAL APPLICATION METHOD AT 24°C, 77% RH UNDER A 16L:8D PHOTOPERIOD.

Treatment	Mean % mortality ± SE of eggs $P < 0.0001$	Mean % mortality ± SE of adults $P < 0.0001$
Silwet L-77 at 0.05%	0.3 ± 0.3 c	47.0 ± 4.5 b
Imidacloprid at one-tenth the LLR	50.3 ± 12.5 b	46.3 ± 10.6 b
Imidacloprid at the LLR	97.2 ± 1.3 a	100 a
Silwet + one-tenth the LLR of imidacloprid	71.6 ± 12.9 b	100 a
Water	—	0 c

\*Treatment means were analyzed using Proc ANOVA and treatment differences were analyzed using Fisher's LSD. Treatments with the same letter within a column are not significantly different from each other ( $P \leq 0.05$ ). Six shoots were sampled for each treatment to estimate egg mortality, each shoot had 3-113 eggs, number of adults sampled for each treatment and water check = 45-53.

prid at one-tenth the LLR (1.6) ( $P < 0.0001$ ) (Table 5). There were no significant differences among the Silwet L-77 and imidacloprid treatments over this total post-spray period, although there was a trend for trees treated with imidacloprid at the LLR and trees treated with Silwet L-77 + one-tenth the LLR to have lower psyllid densities (means = 0.3 and 0.9 per shoot, respectively, over the experiment). These data suggest that Silwet L-77 alone or in combination with one-tenth the LLR of imidacloprid provided control of nymphs equivalent to that of the LLR of imidacloprid.

When the data were analyzed by week there were always differences between the water-treated trees and the trees treated with Silwet L-77 and/or imidacloprid (Table 5). There were sometimes differences among the treatments, particularly during weeks 2 and 4.

Measurements of shoot length, recorded to determine whether Silwet L-77 or imidacloprid affected shoot elongation, did not exhibit any clear pattern among the treatments (data not shown). When imidacloprid was applied at one-tenth the LLR, shoots at the end of the experiment averaged 10.4 cm (S.E. = ±1.0), which was not different from shoot lengths of trees treated with the LLR of imidacloprid (9.6 ± 0.9) ( $P = 0.27$ ). Shoot lengths in both imidacloprid treatments (one-tenth the LLR and the LLR) were not different from the trees treated with Silwet L-77. Shoot length in the water-treated trees and the trees with the combined treatment of Silwet L-77 and imidacloprid were not different from each other. Weekly observations on shoot length indicated there were minor differences across treatments at weeks 1 and 5. However, because differences did not exhibit any clear pattern, we conclude that there was no effect of treatments on shoot length in this cultivar under these growing conditions. During the course of the trial, visual observations also indicated no phytotoxicity symptoms.

Shoot growth of tagged shoots, that were known to have been treated, over the 6 weeks of the experiment was not different across treat-

ments (Table 6). These results indicate that Silwet L-77 and imidacloprid, either alone or in combination, did not have any effect on shoot growth. The shoot length decreased during the last 2 weeks of sampling, probably due to frost injuries on shoot tips.

Residual toxicity of mature treated foliage to adults persisted for only 2 weeks in the foliage treated with the LLR of imidacloprid (Table 7). During the entire post-treatment period, the mortality of adults on treated citrus foliage was highest on leaves collected from trees sprayed with the LLR of imidacloprid (mean = 24%), followed by mortality on foliage sprayed with a combined treatment of Silwet L-77 and one-tenth the LLR of imidacloprid (11.3%), while 4.6% and 8.8% mortality of adults was observed on the water- and Silwet L-77-treated foliage, respectively (Table 7). The mortality on foliage treated with one-tenth the LLR of imidacloprid also averaged 4.6% over the post-treatment period. The highest rate of mortality (55%) occurred during the first week after treatment with the LLR of imidacloprid, indicating that adults are difficult to control. The increase in mortality of adults during the sixth week after treatment among all the treatments compared to weeks 3, 4, and 5, was due to unknown reasons, and was unlikely due to the effect of imidacloprid because mortality also increased in the 2 controls (water and Silwet L-77 treatments).

#### Concentration-Mortality Data for Silwet L-77 and Kinetic in Laboratory Bioassays

Mortality of psyllid nymphs caused by Silwet L-77 or Kinetic was not different from each other at the tested concentrations using a shoot-dip bioassay. At 50 ppm, both Silwet L-77 and Kinetic killed >95% of the psyllid nymphs. The lethal concentrations and their fiducial limits are presented in ppm (Table 8). These data reveal that both Silwet L-77 and Kinetic are equally effective in killing psyllid nymphs using the shoot dip assay in

TABLE 5. EFFECT OF SILWET L-77 ALONE OR IN COMBINATION WITH IMIDACLOPRID AT THE LOWEST LABEL RATE (LLR) ON SURVIVAL OF PSYLLID NYMPHS AT THE WATER CONSERVIR RESEARCH SITE IN FLORIDA BETWEEN 5 OCT AND 15 NOV 2006.

Treatment	Mean $\pm$ SE living nymphs / shoot							
	Pre spray <i>P</i> < 0.0001	Immediate post spray <i>P</i> < 0.0001	Week 1 <i>P</i> < 0.0001	Week 2 <i>P</i> < 0.0001	Week 3 <i>P</i> < 0.0001	Week 4 <i>P</i> = 0.05	Week 5 <i>P</i> = 0.30	Total post-spray period <i>P</i> < 0.0001
Water	33.9 $\pm$ 3.9 a	32.1 $\pm$ 3.9 a	29.3 $\pm$ 5.5 a	8.5 $\pm$ 2.0 a	10.3 $\pm$ 3.7 a	5.8 $\pm$ 3.2	1.8 $\pm$ 1.5 a	17.4 $\pm$ 5.3 a
Silwet L-77 (0.05%)	35.0 $\pm$ 3.0 a	1.2 $\pm$ 0.3 b	4.6 $\pm$ 1.0 b	3.5 $\pm$ 1.3 b	0.9 $\pm$ 0.4 b	0.1 $\pm$ 0.1 b	0.5 $\pm$ 0.4 a	1.8 $\pm$ 0.7 b
Imidacloprid at one-tenth the LLR	31.0 $\pm$ 2.9 a	2.1 $\pm$ 0.7 b	2.9 $\pm$ 0.8 b	1.0 $\pm$ 0.4 c	0.4 $\pm$ 0.3 b	3.1 $\pm$ 1.9 ab	0 a	1.6 $\pm$ 0.5 b
Imidacloprid at the LLR	23.1 $\pm$ 1.8 b	0.1 $\pm$ 0.1 b	1.0 $\pm$ 0.5 b	0.6 $\pm$ 0.3 c	0.2 $\pm$ 0.2 b	0.2 $\pm$ 0.1 b	0 a	0.3 $\pm$ 0.2 b
Silwet L-77 (0.05%) + one-tenth the LLR of imidacloprid	35.1 $\pm$ 3.5 a	0.9 $\pm$ 0.5 b	1.6 $\pm$ 0.5 b	1.0 $\pm$ 0.4 c	0.3 $\pm$ 0.3 b	2.1 $\pm$ 1.0 ab	0.1 $\pm$ 0.6 a	0.9 $\pm$ 0.3 b

\*Treatment means were analyzed using Proc ANOVA and means were separated using Fishers' LSD with *P* = 0.05; treatments with the same letter within a column are not significantly different from each other.

TABLE 6. EFFECT OF SILWET L-77 ALONE OR IN COMBINATION WITH IMIDACLOPRID AT THE LOWEST LABEL RATE (LLR) ON GROWTH OF SINGLE TAGGED SHOOTS (N = 10) AT THE WATER CONSERVIR RESEARCH SITE BETWEEN 5 OCT AND 15 NOV 2006.

Treatment	Mean shoot length (cm $\pm$ S.E.)						
	Week 1 <i>P</i> = 0.96	Week 2 <i>P</i> = 0.44	Week 3 <i>P</i> = 0.21	Week 4 <i>P</i> = 0.85	Week 5 <i>P</i> = 0.83	Week 6 <i>P</i> = 0.53	Entire post-spray period <i>P</i> = 0.25
Water	5.9 $\pm$ 0.7	9.6 $\pm$ 1.3	12.5 $\pm$ 1.2	14.0 $\pm$ 1.7	14.0 $\pm$ 1.7	12.6 $\pm$ 1.3	11.4 $\pm$ 1.4
Silwet L-77 (0.05%)	6.3 $\pm$ 0.6	8.9 $\pm$ 1.1	11.3 $\pm$ 1.5	12.5 $\pm$ 1.7	13.8 $\pm$ 1.5	11.9 $\pm$ 1.7	10.8 $\pm$ 1.2
Imidacloprid at one-tenth the LLR	6.4 $\pm$ 0.6	7.9 $\pm$ 0.7	9.3 $\pm$ 0.8	14.6 $\pm$ 2.7	12.5 $\pm$ 1.9	12.5 $\pm$ 2.3	10.5 $\pm$ 1.4
Imidacloprid at the LLR	6.2 $\pm$ 0.6	10.1 $\pm$ 0.8	13.0 $\pm$ 1.3	14.4 $\pm$ 1.5	14.3 $\pm$ 1.8	14.6 $\pm$ 2.0	12.1 $\pm$ 1.5
Silwet L-77 (0.05%) at one-tenth the LLR of imidacloprid	6.1 $\pm$ 0.4	9.4 $\pm$ 0.8	11.3 $\pm$ 1.7	12.6 $\pm$ 1.3	12.4 $\pm$ 1.0	11.0 $\pm$ 1.4	10.5 $\pm$ 1.1

\*Treatments means were analyzed using Proc ANOVA and means were separated using Fishers' LSD with *P* = 0.05.



TABLE 7. RESIDUAL TOXICITY OF SILWET L-77 ALONE OR IN COMBINATION WITH IMIDACLOPRID TO PSYLLID ADULTS HELD ON MATURE FIELD-TREATED FOLIAGE SAMPLED AT THE WATER CONSERVATION RESEARCH SITE DURING 5 OCT TO 15 NOV 2006.

Treatment	Mean percentage mortality of adults after foliage treatment						Entire post-treatment period <i>P</i> < 0.0001
	Week 1 <i>P</i> < 0.0001	Week 2 <i>P</i> < 0.0001	Week 3 <i>P</i> = 0.36	Week 4 <i>P</i> = 0.24	Week 5 <i>P</i> = 0.23	Week 6 <i>P</i> = 0.48	
Water	2.5 b	5.0 b	0	0	7.5	12.5	4.6 c
Silwet L-77 (0.05%)	5.0 b	5.0 b	0	7.5	1.8	17.5	8.8 bc
Imidacloprid at one-tenth the LLR	7.5 b	7.5 b	2.5	0	2.5	7.5	4.6 c
Imidacloprid at the LLR	55.0 a	37.5 a	7.5	1.0	12.5	22.5	24.1 a
Silwet L-77 (0.05%) + imidacloprid at one-tenth the LLR	12.5 b	12.5 b	5	12.5	5.0	20.0	11.3 b

\*Treatments means were analyzed using ProcANOVA and means are separated using Fishers' LSD with *P* = 0.05; treatments with the same letter within a column are not significantly different from each other. LLR indicates the lowest label rate. \*Number of adults in treatments and water check = 40.

the laboratory at concentrations far below the 500 ppm used in the field.

Greenhouse Bioassays Using Potted Citrus Trees to Compare Silwet L-77 or Kinetic Alone and in Combination with Abamectin or Imidacloprid to Eggs, Nymphs, or Adults of *D. citri*

Silwet L-77 and Kinetic killed very few *D. citri* eggs when the potted citrus trees were sprayed (Table 9). The percentage of egg mortality on Silwet L-77- or Kinetic-treated trees (2.5%) was not different from the egg mortality observed on the water-treated (0.3%) or untreated (1.4%) trees (Table 9).

Egg mortality on the imidacloprid-treated trees tested at one-fourth, one-half, and at the LLR was not different (Table 9). The *D. citri* eggs experienced 99-100% mortality at all 3 rates of imidacloprid. When Silwet L-77 or Kinetic were combined with one-fourth or one-half the LLR of imidacloprid, egg mortality ranged from 95.2% to 100%, which was not different from mortality caused by imidacloprid alone at these rates. Thus, Silwet L-77 did not improve mortality caused by imidacloprid to *D. citri* eggs (Table 9).

Abamectin at one-fourth the LLR caused lower egg mortality (61.4%) than at one-half the LLR (96.5%) or at the LLR (92.9%) (Table 9). When Silwet L-77 was combined with abamectin at one-fourth and one-half the LLR, mortality increased to 98.8% and 97.8%, respectively, indicating that Silwet L-77 improved the toxicity of abamectin to *D. citri* eggs (Table 9). When Kinetic was combined with abamectin at one-fourth the LLR, mortality increased significantly from 61.4% to 86.3%. However, adding Kinetic did not improve the efficacy of one-half the LLR of abamectin, which was 83.5% with Kinetic and 96.5% without Kinetic (Table 9). These data suggest that Silwet L-77 is better at increasing the toxicity of abamectin to *D. citri* eggs than Kinetic.

Mortality of nymphs on untreated and water-treated trees averaged 0.4% and 1.7%, respectively, while 73.3% of nymphs on Silwet L-77-treated trees died (Table 9). Only 29.2% of the nymphs died on trees treated with Kinetic, suggesting that Silwet L-77 is more effective in suppressing *D. citri* nymphs (Table 9). Mortality of nymphs on trees sprayed with imidacloprid at one-fourth, one-half, and at the LLR ranged from 94.8 to 99.5% (Table 9). There was a slight increase in nymphal mortality when one-fourth and one-half the LLR of imidacloprid was applied in combination with Silwet L-77 (mortality was 99.4 and 99.7%, respectively), but the differences were not significant.

Kinetic applied with one-fourth or one-half the LLR of imidacloprid caused 92% and 100% mortality of nymphs, respectively, which was not significantly different from these rates of imidaclo-

TABLE 8. CONCENTRATION-MORTALITY RESPONSE OF ASIAN CITRUS PSYLLID NYMPHS TO SILWET L-77 IN COMPARISON WITH KINETIC UNDER LABORATORY CONDITIONS.

Lethal concentration of Silwet L-77	Concentration (ppm)	Fiducial limits (ppm)	Intercept	Slope
LC50	14.8	14.0-15.2	3.7546	-4.1267
LC90	28.0	26.8-29.3		
LC95	33.7	32.0-35.8		
LC99	52.3	47.8-57.9		
Lethal concentration of Kinetic				
LC50	12.6	11.8-13.3	4.5443	-5.2963
LC90	27.6	26.2-29.2		
LC95	34.5	32.3-37.0		
LC99	47.5	44.6-51.3		

\*Mortality at each Silwet L-77 and Kinetic concentration was subjected to probit analyses using Proc Probit. Five shoots with at least 20 nymphs per shoot were tested for each concentration.

prid alone, so adding Kinetic or Silwet L-77 to imidacloprid did not significantly increase mortality to nymphs (Table 9).

Abamectin at one-fourth the LLR killed fewer nymphs (42.0%) than one-half the LLR (87.8%) or the LLR (97.3%) (Table 9). However, when combined with Silwet L-77, mortality at one-fourth the LLR increased significantly from 42% to 96.8% (Table 9). Combining Silwet L-77 with one-half the LLR of abamectin resulted in 99% mortality compared to 87.7% with abamectin alone, but these were not significantly different. Nymphal mortality increased significantly from 42% to 85.6% when one-fourth the LLR of abamectin was combined with Kinetic. When one-half the LLR of abamectin was combined with Kinetic, mortality was 92.6% compared to 87.7% for abamectin alone, but this was not significantly different (Table 9).

Tests conducted by releasing adult psyllids into a plexiglass cylinder enclosing pruned and Silwet L-77- or Kinetic-sprayed trees resulted in mortality rates of 4.3% and 1.7%, respectively, which was not significantly different (Table 10). No mortality occurred when adults were exposed to water-treated or untreated trees. The mortality rate of adults released into cylinders containing trees sprayed with one-fourth the LLR of imidacloprid (49.9%) differed significantly from that of adults exposed to one-half the LLR (76.7%), which was in turn significantly lower than the mortality caused by the LLR (100%). When Silwet L-77 was combined with one-fourth the LLR of imidacloprid, mortality of adults increased significantly from 49.9% to 98.8%, which was not different from the LLR of imidacloprid. Likewise, mortality increased significantly from 76% to 96.9% when Silwet L-77 was combined with one-half the LLR of imidacloprid (Table 10). Mortality of adults exposed to foliage treated with one-half the LLR of imidacloprid plus Silwet L-77 was not signifi-

cantly different from the mortality of adults treated with the LLR of imidacloprid (Table 10).

Mortality of adult *D. citri* exposed to foliage from trees treated with abamectin at one-fourth or one-half the LLR was 4.2% and 12.8%, respectively (Table 10). However, abamectin applied at the LLR killed 73.6% of the adults, which was significantly lower than the mortality caused by the LLR of imidacloprid (100%). Combining Silwet L-77 with either one-fourth or one-half the LLR of abamectin did not increase the toxicity of abamectin to adults, with mortality rates of 15.2% and 1.8%, respectively, compared to 4.2 and 12.8% mortality, respectively, for abamectin alone at one-fourth and one-half the LLR.

The combination of Kinetic with abamectin at one-fourth the LLR also did not increase toxicity. At one-half the LLR of abamectin, adding Kinetic resulted in 23.4% mortality compared to 12.8% without Kinetic. These data indicate that abamectin is not as effective in suppressing adults of *D. citri* as imidacloprid and that combining Kinetic with abamectin did not result in increased mortality of adults.

#### Greenhouse Bioassays Using Potted Citrus Trees to Examine the Residual Toxicity of Imidacloprid to Adult Psyllids Using Attached or Detached Leaves

As expected, mortality of adults on the water-treated attached leaves was always lower than on the imidacloprid-treated leaves, ranging from 0% to 15% over the 4 weeks after treatment (Table 11). Mortality on the imidacloprid-treated attached leaves immediately after treatment was 100%, and was 100% 1 week after treatment, but declined to 71% during the second, to 47% during the third, and was 58% during the fourth week after treatment. Regression analyses performed on the data indicated a good correlation between decline in mortality and time ( $R^2 = 0.7834$ , slope =

TABLE 9. TOXICITY OF SILWET L-77 AND KINETIC IN COMBINATION WITH IMIDACLOPRID OR ABAMECTIN TO EGGS OR NYMPHS OF THE ASIAN CITRUS PSYLLID AT 18-36°C, 35-80% RH UNDER A 16L:8D PHOTOPERIOD USING SPRAYED POTTED TREES.

Treatment	Mean % mortality of eggs $\pm$ SE	Mean % mortality of nymphs $\pm$ SE
	$P < 0.001$	$P < 0.0001$
Untreated	1.4 $\pm$ 0.5 e	0.4 $\pm$ 0.2 g
Water	0.3 $\pm$ 0.1 e	1.7 $\pm$ 0.1 g
Silwet L-77 (0.05%)	2.5 $\pm$ 0.5 e	73.3 $\pm$ 4.6 e
Kinetic (0.05%)	2.5 $\pm$ 2.5 e	29.2 $\pm$ 4.9 f
Imidacloprid at one-fourth the LLR	99.2 $\pm$ 0.1 ab	97.0 $\pm$ 3.0 abcd
Imidacloprid at one-half the LLR	100 a	99.5 $\pm$ 0.3 ab
Imidacloprid at the LLR	99.8 $\pm$ 0.2 a	94.8 $\pm$ 3.0 abcd
Abamectin at one-fourth the LLR	61.4 $\pm$ 17.2 d	42.0 $\pm$ 2.1 f
Abamectin at one-half the LLR	96.5 $\pm$ 3.5 ab	87.8 $\pm$ 9.7 cde
Abamectin at the LLR	92.9 $\pm$ 4.0 bc	97.3 $\pm$ 2.2 abcd
Silwet L-77 + imidacloprid at one-fourth the LLR	95.2 $\pm$ 4.8 ab	99.4 $\pm$ 0.4 abc
Silwet L-77 + imidacloprid at one-half the LLR	100 a	99.7 $\pm$ 0.3 ab
Silwet L-77 + abamectin at one-fourth the LLR	98.8 $\pm$ 0.5 ab	96.8 $\pm$ 0.3 abcd
Silwet L-77 + abamectin at one-half the LLR	97.8 $\pm$ 0.1 ab	99.0 $\pm$ 1.1 abc
Kinetic + imidacloprid at one-fourth the LLR	99.5 $\pm$ 0.1 a	92.0 $\pm$ 7.4 bcde
Kinetic + imidacloprid at one-half the LLR	99.1 $\pm$ 0.8 ab	100 a
Kinetic + abamectin at one-fourth the LLR	86.3 $\pm$ 0.2 c	85.6 $\pm$ 10.8 de
Kinetic + abamectin at one-half the LLR	83.5 $\pm$ 11.5 c	92.6 $\pm$ 7.4 abcd

\*Treatments means were analyzed using Proc GLM and treatment differences were analyzed using Fisher's LSD. Treatments with the same letter within a column are not significantly different from each other. LLR indicates the lowest label rate. Twelve shoots in the treatments and untreated check were sampled for eggs and nymphs, each shoot had 10-425 eggs and 6-335 nymphs, respectively.

-0.0573, intercept = 6.3111). The decline in mortality could be attributed to a decline in the residual activity of imidacloprid.

Also as expected, mortality of adults on water-treated detached leaves was zero except for week 2, when it was 25% (Table 11). Mortality of adults immediately after the spray was 100% on the detached leaves, and remained 100% for weeks 1 and 2, declining to 75% for week 3 and was 100% for week 4. Regression analyses on the detached leaves, however, indicated a weak correlation between decline in mortality and time ( $R^2 = 0.0556$ , slope = -0.0222, intercept = 4.1111), indicating that imidacloprid remained toxic to the adult psyllids using this detached leaf assay method. The reason(s) for the different results obtained using these 2 assay methods is unclear, because the detached leaves were from the same trees as the attached leaves.

When these laboratory and greenhouse results are compared to those obtained in the field trial conducted at the Water ConservII Research Site during Oct and Nov 2006 (Table 7), the laboratory and greenhouse results indicate that imidacloprid remains toxic to adults longer than when the spray is applied in the field. In the field test, mortality declined from 55% to 37% and then to 7.5%, respectively, during the first 3 weeks post spray (Table 7). By contrast, mortality of adults using

the detached leaves from the potted trees was 100%, 100%, 100%, 75%, and 100%, respectively, over the 5 weeks of this laboratory assay (Table 9).

## DISCUSSION

The potential use of Silwet L-77 as an insecticide to suppress *D. citri* was initially discovered when it was included as a control in a field trial to evaluate an entomopathogenic fungus (Hoy et al., unpubl.). Silwet L-77 has been shown to increase the efficacy of *Bacillus thuringiensis* as a control agent of the citrus leafminer, *Phyllocnistis citrella* Stainton, by increasing penetration into leaf mines (Shapiro et al. 1998). Others have shown that Silwet L-77 by itself causes direct toxicity to insects. Imai et al. (1994) found that Silwet L-77 at 0.10% caused 100% mortality of green peach aphid, *Myzus persicae* Sulzer. Silwet L-77 was found to be toxic to at least 3 species of tephritids (Purcell & Schroeder 1996). Silwet L-77 also is toxic to mealybugs, thrips, spider mites, and whiteflies (Chandler 1995; Skinner 1977; Smitley & Davis 1997; Wood & Tedders 1997; Liu & Stansly 2000; Cowles et al. 2000; Tipping et al. 2003). In our laboratory studies, although the mechanisms are unclear, Silwet L-77 seems to exhibit direct toxicity primarily to nymphs of *D. citri*. Our laboratory, greenhouse, and field trials

TABLE 10. RESIDUAL TOXICITY OF SILWET L-77 OR KINETIC ALONE OR IN COMBINATION WITH IMIDACLOPRID OR ABAMECTIN TO ADULTS OF THE ASIAN CITRUS PSYLLID AT 18-36°C, 35-80% RH UNDER A 16L:8D PHOTOPERIOD USING SPRAYED POTTED TREES.

	Mean % mortality ± SE
Treatment	$P < 0.001$
Untreated	0 g
Water	0 g
Silwet L-77 (0.05%)	4.3 ± 3.0 egf
Kinetic (0.05%)	1.7 ± 1.7 gf
Imidacloprid at one-fourth the LLR	49.9 ± 8.9 c
Imidacloprid at one-half the LLR	76.7 ± 7.8 b
Imidacloprid at the LLR	100 a
Abamectin at one-fourth the LLR	4.2 ± 2.7 egf
Abamectin at one-half the LLR	12.8 ± 4.3 ed
Abamectin at the LLR	73.6 ± 12.2 b
Silwet L-77 + imidacloprid at one-fourth the LLR	98.8 ± 1.2 a
Silwet L-77 + imidacloprid at one-half the LLR	96.9 ± 2.0 a
Silwet L-77 + abamectin at one-fourth the LLR	15.2 ± 6.8 def
Silwet L-77 + abamectin at one-half the LLR	1.8 ± 1.8 gf
Kinetic + imidacloprid at one-fourth the LLR	98.6 ± 2.0 a
Kinetic + imidacloprid at one-half the LLR	93.0 ± 2.1 ab
Kinetic + abamectin at one-fourth the LLR	8.5 ± 2.0 def
Kinetic + abamectin at one-half the LLR	23.4 ± 6.7 d

\*Treatments means were analyzed using Proc ANOVA and treatment differences were analyzed using Fisher's LSD. Treatments with the same letter are not significantly different from each other. LLR indicates lowest label rate. Number of adults in treatments and untreated check = 100.

indicate that Silwet L-77 can suppress psyllid nymphs when applied at rates of 0.05% (Tables 1, 3, 5, 9). When Silwet L-77 is combined with lower-than-label rates of imidacloprid or abamectin, mortality of eggs is significantly increased (Tables 4, 9). Silwet L-77 in combination with one-fourth or one-half the LLR of imidacloprid increased mortality of *D. citri* adults significantly (Table 10). However, under field conditions, the LLR of imidacloprid was significantly more effective in controlling adults than one-tenth the LLR combined with Silwet L-77 (Table 7). There were no significant differences in densities of psyllid nymphs in the field trial conducted during Oct-Nov 2006 among the treatments (Silwet L-77 alone, one-tenth the LLR + Silwet L-77, and the LLR of imidacloprid without Silwet) (Table 5). These results suggest that Silwet L-77 could become an important tool in managing Asian citrus psyllid because it is inexpensive (estimated to cost approximately \$2.00/acre) and could reduce the use of chemical pesticides, thus reducing production costs for citrus growers. However, large-scale field trials are needed to confirm this under the diverse growing conditions and cultural practices employed in Florida.

Our data show that the methods used in the laboratory bioassays affect our ability to predict whether the laboratory rates tested of imidacloprid (one-tenth, one-fourth, or one-half the LLR) are likely to be as effective under grove conditions

in Florida. For example, when imidacloprid at one-tenth the LLR was applied in combination with Silwet L-77 to adults using a topical treatment method in the laboratory, high rates (71%) of mortality were observed (Table 4), but only 12.5% mortality was observed in the field trial bioassay using treated foliage (Table 7), perhaps principally due to UV-related photodegradation (Wamhoff & Schneider 1999). Lower insecticide residual activity in the field was suspected to be due to the use of detached leaves in the assay, but the bioassays that compared detached and intact leaves showed differences in residual toxicity of imidacloprid to adults only during week 4 when mortality on attached leaves was 57.9%, but was 100% on detached leaves (Table 11). Clearly, it is difficult to predict the toxicity of a product precisely from laboratory bioassays due to differences in treatment and environmental conditions.

The laboratory data indicate that Kinetic is less effective than Silwet L-77 in causing direct mortality to nymphs (29% vs. 73% for Kinetic and Silwet, respectively) (Table 9). When Kinetic was combined with different rates of imidacloprid and applied to eggs, it increased egg mortality equivalent to that observed when eggs were treated with Silwet L-77 and the same rates of imidacloprid (Table 9). Kinetic and Silwet L-77 alone were equivalent in causing low toxicity to adults (Table 10), but when each was combined with one-fourth or one-half the LLR of imidacloprid equivalent

TABLE 11. RESIDUAL TOXICITY OF IMIDACLOPRID AT THE LOWEST LABEL RATE TO PSYLLID ADULTS ON ATTACHED OR DETACHED CITRUS FOLIAGE AT 18-36°C, 35-80% RH UNDER A 16L:8D PHOTOPERIOD AND AT 24°C, 77% RH UNDER A 16L:8D PHOTOPERIOD.

Sampling interval	Treatment	% Mortality ± SE on attached leaves	% Mortality ± SE on detached leaves
Immediate post spray	Water	15 ± 5.0 aB	0 aA
	Imidacloprid	100 bA	100 bA
Week 1	Water	0 aA	0 aA
	Imidacloprid	100 bA	100 bA
Week 2	Water	7.6 ± 2.9 aA	25 ± 14.4 aA
	Imidacloprid	71.2 ± 6.5 aA	100 bA
Week 3	Water	2.5 ± 2.5 aA	0 aA
	Imidacloprid	47.4 bA	75.0 ± 14.4 bA
Week 4	Water	5.0 ± 5.0 aA	0 aA
	Imidacloprid	57.9 ± 5.3 bA	100 bB

\*Treatments means were analyzed using Proc ANOVA and treatment differences were analyzed using Fisher's LSD for each time interval. Treatments with the same letter within a row (upper case) or column (lower case) are not significantly different from each other. Number of adults in treatments and water check for each time interval: attached leaves = 40, detached leaves = 8.

and high mortality occurred (93-98.8%) (Table 10). Kinetic, however, did not increase the effectiveness of abamectin over that of abamectin alone (Table 10). Dr. R. Buker (Helena Chemical Company, personal communication) indicated that Silwet L-77 is less stable than Kinetic in water with a high pH and impurities. Because our tests were conducted with clean water at a moderate pH, field trials to compare Kinetic and Silwet L-77 in different regions should be conducted to resolve whether water quality affects efficacy of these products. It should also be noted that Kinetic is a proprietary mixture of organosilicone compounds and other surfactants. Hence Kinetic has a lower concentration of organosilicone surfactants than Silwet L-77, and might therefore have poorer wetting properties than Silwet L-77.

Greenhouse bioassays, involving sprayed trees to which adults were introduced shortly after the spray droplets had dried, suggest that one-fourth and one-half the LLR of imidacloprid in combination with Silwet L-77 may be sufficient to suppress adults of *D. citri* under grove conditions (Table 10). These results need to be confirmed under grove conditions in different geographic regions of Florida.

Silwet L-77 at 0.05% has not been reported to exhibit phytotoxicity or to affect the growth of plants on which it was tested, and we observed no phytotoxicity or negative effects on shoot growth in Silwet L-77-treated trees when compared to water-treated trees in either of the 2 field trials. Additional field trials in citrus in Florida using different citrus cultivars under different growing conditions will confirm the lack of phytotoxicity of Silwet and Kinetic.

Natural enemies (such as lady beetles, lacewings, spiders (Michaud 2004) and a specialist parasitoid, *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae), which was released in a classical biological control program (Hoy et al. 1999; Hoy & Nguyen 2000; Skelley & Hoy 2004) were considered sufficient to suppress *D. citri* in Florida's citrus groves prior to the confirmation that greening disease is widely distributed in Florida. Because greening disease is such a serious threat to Florida's citrus industry, other control options need to be considered. One issue that was not resolved in this study is the effect of Silwet L-77 alone or in combination with lower-than-label rates of imidacloprid or abamectin on these important natural enemies. Multiple applications of pesticides will likely disrupt the natural enemies of psyllids, as well as of other pests, and could lead to pest outbreaks.

Current study suggests that Silwet L-77 or Kinetic, by exhibiting direct toxicity, could efficiently manage *D. citri* nymphs alone but not eggs or adults. However, Silwet L-77 or Kinetic, in conjunction with insecticides, appear to be effective in managing all life stages of *D. citri*. Neither Silwet L-77 nor Kinetic is currently registered as a pesticide and can only be applied as a spray adjuvant. The lower efficacy of Silwet or Kinetic against eggs and adults of *D. citri* suggests that Silwet L-77 or Kinetic may not be a potential candidate for registration as an insecticide by itself and the better solution may be to apply the organosilicone adjuvants in conjunction with low rates of insecticides. Such an approach would be ecologically less harmful and delay the development of resistance.

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