TOXICITY OF PESTICIDES USED IN CITRUS TO APROSTOCETUS VAQUITARUM (HYMENOPTERA: EULOPHIDAE), AN EGG PARASITOID OF DIAPREPES ABBREVIATUS (COLEOPTERA: CURCULIONIDAE)

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TOXICITY OF PESTICIDES USED IN CITRUS TO *APROSTOCETUS VAQUITARUM* (HYMENOPTERA: EULOPHIDAE), AN EGG PARASITOID OF *DIAPREPES ABBREVIATUS* (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

Twelve pesticides used in citrus were tested for their contact toxicity to *Aprostocetus vaquitarum* Wolcott (Hymenoptera: Eulophidae) a parasitoid of *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae). Sevin® 80 WSP, Malathion 5 EC, and Imidan® 70 WSB resulted in the most rapid death of *A. vaquitarum* adults. Admire® 2F, Danitol® 2.4EC, and Surround® WP were also very detrimental. Kocide® 101 WP, Citrus Soluble Oil, Micromite® 80 WGS, Acramite® 50 WS, Micromite® 80 WGS + Citrus Soluble Oil, Aliette WDG, and Agrimek® 0.15 EC + Citrus Soluble Oil were slightly to non-toxic to *A. vaquitarum*. The relative toxicity of the pesticides was consistent up to four weeks after application. Significantly fewer adult *A. vaquitarum* emerged from *D. abbreviatus* eggs laid on foliage treated in the field with Sevin® XLR and Imidan® 70 WSB than emerged from the water treated control. Field residues of Sevin® XLR remained toxic for seven days while the effects of Imidan® 70 WSB were no longer significant after one week. The number of *A. vaquitarum* adults emerging from host eggs laid on treated foliage was not significantly different among Micromite® 80 WGS, Acramite® 50 WS, and the control, but significantly fewer adults emerged from foliage treated with either Micromite® 80 WGS + Citrus Soluble Oil or Citrus Soluble Oil alone. There were no significant differences between oviposition or new generation adults when *A. vaquitarum* was exposed to Micromite® 80 WGS or a water control.

Key Words: insecticides, diflubenzuron, selectivity, toxicity, citrus IPM, biological control

RESUMEN

Se estudió la toxicidad de varios plaguicidas aplicados comúnmente en cítricos para *Aprostocetus vaquitarum* Wolcott (Hymenoptera: Eulophidae) un parasitoide de *Diaprepes abbreviatus* L. (Coleoptera: Curculionidae). Sevin® 80 WSP, Malathion 5 EC, e Imidan® 70 WSB fueron los que más rápidamente causaron la muerte de *A. vaquitarum*. Admire® 2F, Danitol® 2F, Danitol® 2.4 EC y Surround® WP fueron muy tóxicos para el parasitoide. Comparados con el testigo absoluto, Kocide® 101 WP, aceite soluble de cítricos, Micromite® 80 WGS, Acramite® 50 WS, Micromite® 80 WGS + aceite soluble de citricos, Aliette WDG y Agrimek® 0,15EC + aceite soluble de cítricos no resultaron significativamente tóxicos para el parasitoide. La toxicidad relativa de estos plaguicidas se mantuvo durante un período de 4 semanas. Emergieron significativamente menos adultos de *A. vaquitarum* de huevos de *D. abbreviatus* que habían sido depositados en hojas tratadas en el campo con Sevin® XLR e Imidan® 70 WSB en comparación con aquéllos que emergieron de huevos depositados en hojas tratadas con agua. Los efectos tóxicos de Sevin® XLR continuaron por 1 semana, mientras que los efectos de Imidan dejaron de ser significativos después de 1 semana. No hubo diferencias significativas entre el número de adultos *A. vaquitarum* emergidos de huevos de *D. abbreviatus* en follaje tratado con Micromite® 80 WGS y Acramite® 50 WS comparado con el testigo absoluto. Sin embargo, sí las hubo con Micromite® 80 WGS + aceite soluble de cítricos o con el aceite soluble de cítricos cuando este ultimo fue aplicado solo. No hubo diferencias significativas ni en la puesta ni en la emergencia de una nueva generación de parasitoides cuando se expuso las hembras a Micromite® 80WGS en comparación con aquéllas que se expuso al agua (testigo absoluto).

Translation provided by the authors.

Pesticides are a critical component of insect pest management in citrus production. Insect fauna affected by pesticide applications often encompasses a group extending beyond the pest species targeted. Of particular concern are beneficial insects which play a vital role in suppressing pest insect populations. Non-selective pesticide application can disrupt beneficial insect populations and may lead to outbreaks of pest insects (Barbosa & Schultz 1987). Much of Florida's citrus production is intended for juicing, which requires a less severe pesticide regime than the fresh fruit market (Michaud & Grant 2003). However, populations of specific pests, such as the root weevil, *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae), regularly necessitate the use of broad spectrum insecticides (Timmer et al. 2005).

Diaprepes abbreviatus is native to the Caribbean and was presumably introduced from Puerto Rico. It was first reported in Florida in 1964 and is established across the citrus-producing regions of the state (Woodruff 1964). *Diaprepes abbreviatus* feeds on >270 species of plants from 59 families (Simpson et al. 1996). It is a significant pest for ornamental growers and is economically very important in the citrus industry where it is estimated to cost producers over 70 million dollars annually (Stanley 1996). Adult weevils feed along the edges of leaves leaving characteristic semi-circular notches but the most significant damage is done by larvae feeding on the root system which can weaken or kill the plant. Root feeding also may leave the plant more susceptible to root rot organisms such as *Phytophthora* spp. (Timmer et al. 2005). Though there is evidence that soil-applied pesticides may be effective against larvae (McCoy et al. 1995), pesticide regimes often target the adult stage with foliar applications, particularly during periods of new citrus growth.

Biological control is a vital component in an ongoing effort toward an integrated pest management system for *D. abbreviatus*. In the late 1990s, programs were initiated to introduce hymenopteran egg parasitoids from the Caribbean islands into Florida. In 2000 the ecto-parasitoid *Aprostocetus vaquitarum* Wolcott (Hymenoptera: Eulophidae) was introduced into Florida from the Dominican Republic (Jacas et al. 2005). *Aprostocetus vaquitarum* has been mass reared and released in several Florida counties since 2000 and is now considered to be established in parts of southern Florida (Jacas et al. 2005). *Aprostocetus vaquitartum* is one of the principal parasitoids of *D. abbreviatus* in its native range, and in areas where it has become established in south Florida, egg mortality rates of 70-90% have been observed (Peña et al. 2005).

Very little is known about the toxicity of insecticides used in citrus production to parasitoids of *D. abbreviatus*. Two products were tested against two other *D. abbreviatus* egg parasitoids (Amalin et al. 2004) but no information exists on the relative toxicities of commonly used pesticides to *A. vaquitarum*. Our study was initiated to determine the relative toxicities of several pesticides used in Florida citrus production to *A. vaquitarum*. Four pesticides registered for control of *D. abbreviatus* and three insecticides registered for other citrus insect pests were examined, as well as four mite control products and two fungicides. The contact toxicity of the pesticides was evaluated in the laboratory. Some products were also tested further in the field. Information on the relative potency of these crop protection products could be of use in the development of *D. abbreviatus* management strategies aimed to minimize adverse impacts on beneficial insects such as *A. vaquitarum*.

Materials and Methods

Pesticides

Twelve pesticides labeled for use against *D. abbreviatus* in Florida were tested for toxicity to *A. vaquitarum* (Table 1). The application rate for each pesticide was based on the 2005 Florida Citrus Pest management Guide (Timmer et al. 2005). All commercially formulated pesticides were diluted in water to an application rate of 935.4 liter/ha (100 gal/ac).

Pesticide Toxicity Trial

To test the toxicity of the 12 pesticides listed above, female *A. vaquitarum* (<72 h old) were exposed to the recommended field application rate of each commercial formulation. Filter paper (Fisherbrand® Filter Paper, P4 Medium-Fine porosity, slow flow rate, Cat. no.: 09-803-6G) was dipped in a mixed pesticide or a water control for 3 seconds and air dried for 2.5 h. The treated filter paper was then cut into 0.5 cm × 5.0 cm strips. Female *A. vaquitarum* were placed one individual per tube into 10-ml test tubes. A smear of honey was provided on the inner surface of each test tube as a food source and the open end of the test tube was covered with 2 ply of kimwipe (Kimberly-Clarke®, Kimwipes® EX-L) secured with rubber tubing to allow ventilation. The kimwipe was moistened with water daily. Pesticide-treated and control filter paper strips were placed in the test tubes and each parasitoid was examined for signs of life 8, 16, 24, 48, 72, and 96 h after exposure. Mortality was assessed under a 50× dissecting microscope. An insect was recorded as dead if it did not move or twitch in a 10-s period. The experiment was repeated 4 times with 10 to 14 individuals in each replicate for each treatment. Two of the four replicates also were monitored at 24-hour intervals until 100% mortality was achieved to assess longevity.

An experiment was carried out exactly as above with pesticide strips tested at intervals of 7 d, 14 d, and 21 d after pesticide application for treatments that resulted in higher mortality than the control when tested 0 d after application. Pesticide and control strips were weathered out of doors and protected from precipitation and direct

TABLE 1. LIST OF PESTICIDES TESTED, INCLUDING TRADE NAME, CLASS, ACTIVE INGREDIENT AND APPLICATION RATE.

Trade name	Class	Active ingredient	Application rate (product/1 litre water)	Manufacturer
Sevin 80 WSP[1]	Carbamate	Carbaryl (80%)	12.0 g	Bayer CropScience
Sevin XLR[1]	Carbamate	Carbaryl (44.1%)	10.0 ml	Bayer CropScience
Malathion 5 EC[1]	Organophosphate	Malathion (57%)	7.5 ml	Micro Flo Company
Imidan 70 WSB[1]	Organophosphate	Phosmet (70%)	2.4 g	Gowan Company
Admire 2 F[1]	Neonicotinoid	Imidacloprid (22%)	2.5 ml	Bayer CropScience
Danitol 2.4 EC[1]	Pyrethroid	Fenpropathrin (30.9%)	1.6 ml	Valent USA Corporation
Surround WP[1]	Kaolin clay	Kaolin clay (95%)	60.0 g	Engelhard Corporation
Kocide 101 WP[2]	Copper fungicide	Copper hydroxide (77%)	14.4 g	Griffin l.l.c
Alliete WDG[2]	Phosphonate	Aluminium tris (80%)	6.0 g	Bayer CropScience
AgriMek 0.15 EC'	Glycoside	Abmectin (2%)	0.5 ml	Syngenta
Citrus Soluble Oil[3]	Petroleum oil	Petroleum oil (99.3%)	10.0 ml	Platte Chemical Company
Micromite 80 WGS[3]	IGR	Diflubenzuron (80%)	0.47 g	Crompton Manufacturing Company, Inc.
Acramite 50 W5[3]	Unknown	Bifenazate (50%)	1.2 g	Crompton Manufacturing Company, Inc.

[1]Insecticide, [2]Fungicide, [3]Miticide.

sunlight in 1.5-liter wax paper dishes with perforated fitted plastic lids. All experiments were conducted in a walk-in growth chamber with a 16:8 (L:D) photoperiod, 23°C night:26°C day temperature regime, and 58-60% RH. Percent mortality was corrected with Abbott's Formula: 100 × (1 - % surviving on treatment/surviving on control) (Abbott 1925). Longevity of *A. vaquitarum* on the different treatments was compared by one-way ANOVA. Mean separations were performed with the Tukey HSD method (α = 0.05) (Statistix® 8 Analytical Software, 2003).

Effect of Residual Pesticide on Parasitism

To test the residual effects of specific pesticides on *A. vaquitarum*, products were applied out of doors to 1-1.5 m tall green buttonwood (*Conocarpus erectus* L.) host plants. In the first of two experiments Sevin® XLR, Imidan® 70 WSB, and a water control were tested in a randomized complete block design, and replicated 3 times with 3 green buttonwood plants per replicate. Treatments were applied with a hand-gun sprayer operating at 2413 kPa (350 psi) and delivering 935.4 liter/ha of finished spray (~3.79 liter/tree). Four hours after treatment, 15-cm long branches from each treatment (n = 30) were placed in 500-ml plastic containers with water and offered to 150 field collected *D. abbreviatus* inside 30 × 30 × 30-cm plexiglass cages. After being exposed to *D. abbreviatus* for 12 h, the branches with egg masses were removed and placed inside a clean plexiglass cage into which female *A. vaquitarum* (2 per egg mass) were released. The *A. vaquitarum* females were removed after 48 h. The branches remained in water for a further 72 h after which the egg masses were cut from the branches with scissors and placed into individual 10-ml test tubes until emergence. Host plant material from each treatment was collected 7 and 14 d after being sprayed and was exposed to *D. abbreviatus* and *A. vaquitarum* as above.

In a separate experiment Micromite® 80 WGS, Acramite® 50 WS, Citrus Soluble Oil, Micromite® 80 WGS + Citrus Soluble Oil, and a water control were tested with green buttonwood host plants exactly as described in the preceding paragraph. Data for both experiments were analyzed with a Kruskal-Wallis ANOVA (Statistix® 8 Analytical Software, 2003).

Micromite® 80 WGS Fecundity Trial

To test the effects of the insect growth regulator Micromite® 80 WGS on fecundity, female *A. vaquitarum* (<48 h old) were exposed to filter paper strips (1 cm × 5 cm) treated with Micromite® 80 WGS or a water control and placed in 9-cm diameter petri dishes. A smear of honey was placed on the inner surface of the lid as a food source, and a water soaked cotton disc (1 cm diameter, 0.3 cm height) was placed in the dish. A 3-cm diameter hole was cut in the lid and covered with fine mesh to provide ventilation. Females were exposed to each treatment for 70 h. After 70 h, one *D. abbreviatus* egg mass (<48 h old) was introduced into the dish. The two leaves which contained the egg mass were left intact and the petioles were inserted into a water soaked block of florist's foam covered in aluminum foil. The egg mass was exposed to a female for 24 h and then removed. A total of three egg masses were offered to each female at 24-h intervals. Fifty females were used for each treatment; a sub-sample of egg masses from 20 of the females on each treatment was dissected 40 h after being exposed. The egg masses from the other 30 females were reared to emergence. Egg masses opened after 48 h were examined under a dissecting microscope to determine the stage of *A. vaquitarum* development. The number of adult *A. vaquitarum* and the number of *D. abbreviatus* eggs available in each egg mass were calculated at the completion of the experiment with aid of a dissecting microscope. The experiment was conducted in a growth chamber, with conditions as above. Data were subjected to a Kruskal-Wallis ANOVA to analyze oviposition and adult emergence as well as detect significant differences between the means ($\alpha = 0.05$) (Statistix® 8 Analytical Software, 2003).

Results

Pesticide Toxicity Trial

When the pesticides were tested on the day of application, Sevin® 80 WSP, Malathion 5 EC, and Imidan® 70 WSB resulted in more rapid death of *A. vaquitarum* than the other products or the control ($F_{13,322} = 27.5$, $P < 0.001$) (Table 2). Females survived only 15.3 ± 1.7 h on Sevin® 80 WSP. The pesticides Kocide® 101, Citrus Soluble Oil, Micromite® 80 WGS, Acramite® 50 WS, Micromite® 80 WGS + Citrus Soluble Oil, and Agrimek® 0.15 EC + Citrus Soluble Oil were not significantly different from the control on which *A. vaquitarum* survived 122 ± 10.1 h. Admire® 2 F, Danitol® 2.4 EC, Surround® WP, and Aliette were intermediate. Seven days after application, Sevin® 80 WSP, Malathion 5 EC, and Imidan® 70 WSB once again resulted in the most rapid death of *A. vaquitarum* which survived only 18.4 ± 1.4 h on Sevin® 80 WSP ($F_{9,270} = 40.5$, $P < 0.001$) (Table 2). Aliette, Kocide®101, and Agrimek® 0.15 EC + Citrus Soluble Oil were not significantly different from the control on which females survived a mean of 123.4 ± 9.3 h. Admire® 2 F, Danitol® 2.4 EC and Surround® WP resulted in intermediate survival times. Fourteen days after application Sevin® 80 WSP resulted in the most rapid death (16.4 ± 1.2 h) followed by Malathion 5 EC, Imidan® 70 WSB, Admire® 2F, Kocide®101, Danitol® 2.4 EC, and Surround® WP. Agrimek® 0.15 EC + Citrus Soluble Oil was not different from the control on which females survived for a mean of 124.8 ± 17.3 h ($F_{8,171} = 16.7$, $P < 0.001$) (Table 2). Twenty-one days after application Sevin® 80 WSP, Malathion 5 EC, and Imidan® 70 WSB again resulted in the most rapid death of *A. vaquitarum*, which survived 24.0 ± 2.5 h on Sevin® 80 WSP. Admire® 2 F, Danitol® 2.4 EC, and Surround® WP resulted in intermediate survival time, while Kocide®101 and Agrimek® 0.15 EC + Citrus Soluble Oil were not significantly different from the control on which females survived for a mean of 173.2 ± 21.2 h ($F_{8,198} = 25.5$, $P < 0.001$) (Table 2).

Sevin® 80 WSP was the most toxic pesticide when *A. vaquitarum* was exposed to the pesticides on the same day as pesticide application, resulting in 24% mortality after 8 h and 98% mortality after 24 h (Table 3). Malathion 5 EC and Imidan® 70 WSB were the only other products which resulted in mortality after 8 h, and following Sevin® 80 WSP were the most toxic of the products tested resulting in 100 and 98% mortality, respectively, after 48 h. All three products resulted in 100% mortality of *A. vaquitarum* by 72 h. Admire® 2 F and Danitol® 2.4 EC were the next most lethal products resulting in 96 and 85% mortality by the

Table 2. Mean time of death (h ± S.E.) after exposure to each pesticide treatment.

	Time after application			
Treatment	0 d	7 d	14 d	21 d
Sevin 80 WSP	15.3 (1.7) e	18.4 (1.4) c	16.4 (1.2) d	24.0 (2.5) d
Malathion 5 EC	26.0 (3.4) e	23.1 (2.4) c	33.2 (3.1) cd	25.4 (2.3) d
Imidan 70 WSB	23.0 (3.6) e	25.1 (2.4) c	45.6 (6.6) bcd	42.1 (4.6) d
Admire 2 F	62.0 (5.8) cd	58.3 (2.9) b	57.6 (3.2) bc	67.8 (5.4) bcd
Danitol 2.4 EC	95.0 (6.0) bc	59.1 (3.6) b	68.4 (6.1) bc	109.6 (7.8) b
Surround WP	91.0 (7.4) bc	66.9 (4.1) b	78.0 (8.7) b	93.9 (9.5) bc
Kocide 101 WP	108.0 (10.5) ab	103.7 (9.9) a	67.2 (4.1) bc	168.0 (20.2) a
Agrimek 0.15 EC & Oil	115.0 (9.4) ab	106.3 (8.0) a	126.0 (16.0) a	163.8 (15.1) a
Alliete WDG	93.0 (8.5) bc	132.9 (12.0) a		
Citrus Soluble Oil	104.7 (6.1) ab			
Micromite 80 WGS	115.0 (9.4) ab			
Acramite 50 ws	139.0 (9.1) a			
Micromite 80 WGS & Oil	135.0 (8.6) a			
Control (water)	122.0 (10.1) ab	123.4 (9.3) a	124.8 (17.3) a	173.2 (21.2) a

Means within each time of application followed by the same letter are not significantly different ($P = 0.05$).

TABLE 3. PERCENT MORTALITY OF *A. VAQUITARUM* FROM 8 TO 96 H AFTER EXPOSURE TO EACH PESTICIDE.

	Percent mortality						Abbott's Corrected	
Treatment	8 h	16 h	24 h	48 h	72 h	96 h	72 h	96 h
0 d after application								
Sevin80 WSP	23.5	82.5	98.0	100	100	100	100	100
Malathion 5 EC	6.3	36.3	76.3	100	100	100	100	100
Imidan 70 WSB	6.3	47.8	86.8	98.0	100	100	100	100
Admire 2 F	0	4.5	26.3	59.5	89.5	95.8	87.3	91.6
Danitol 2.4 EC	0	2.3	2.3	22.0	55.5	85.0	46.1	70.0
Surround WP	0	0	0	21.3	36.5	67.0	23.0	34.0
Kocide 101 WP	0	0	0	13.0	39.0	72.8	26.1	45.6
AllieteWDG	0	0	2.0	10.5	40.8	80.5	28.2	61.0
Agri-Mek 0.15 EC & Oil	0	0	0	6.3	27.5	58.3	12.1	16.6
Citrus Soluble Oil	0	2.0	2.0	2.0	13.3	61.0	0	22.0
Micromite 80 WGS & Oil	0	0	0	2.0	8.8	36.8	0	0
Micromite 80 WGS	0	0	0	6.5	8.5	40.5	0	0
Acramite 50 WS	0	0	0	2.0	4.3	27.8	0	0
Control	0	0	0	8.8	17.5	50.0	x	x
7 d after application								
Sevin80 WSP	9.8	71.5	93.8	100	100	100	100	100
Malathion 5 EC	9.8	58.8	84.5	100	100	100	100	100
Imidan70 WSB	0	52.3	84.8	100	100	100	100	100
Admire 2 F	0	6.8	26.8	76.3	98.3	100	97.9	100
Danitol 2.4 EC	0	0	5.3	56.3	96.0	98.3	95.0	97.1
Surround WP	0	0	0	27.8	54.5	71.5	43.3	51.5
Kocide 101 WP	0	0	2.3	17.8	42.0	46.0	27.7	8.0
Agri-Mek 0.15 EC & Oil	0	0	0	8.0	34.8	48.8	18.7	12.8
Alliete WDG	0	0	0	0	19.3	36.8	0	0
Control	0	0	0	5.8	19.8	41.3	x	x
14 d after application								
Sevin80 WSP	18.5	64.5	95.8	100	100	100	100	100
Malathion5 EC	0	31.5	60.8	100	100	100	100	100
Imidan 70 WSB	0	24.3	55.3	92.5	95.0	95.0	92.6	88.2
Admire 2F	0	8.5	30.0	73.5	100	100	100	100
Danitol 2.4 EC	0	0	4.3	56.3	88.0	95.0	82.2	88.2
Surround WP	0	0	0	28.8	53.8	73.3	31.6	36.7
Kocide 101 WP	0	0	2.5	17.0	59.0	83.3	39.3	60.4
Agri-Mek 0.15 EC & Oil	0	0	4.5	11.3	30.0	43.8	0	0
Control	0	0	0	9.3	32.5	57.8	x	x
21 d after application								
Sevin80 WSP	4.3	64.8	87.0	100	100	100	100	100
Malathion 5 EC	4.8	33.3	69.8	100	100	100	100	100
Imidan 70 WSB	0	27.3	52.5	79.3	93.3	100	92.2	100
Admire 2 F	0	14.3	21.3	69.3	82.8	98.0	80.0	68.8
Danitol 2.4 EC	0	2.3	7.0	40.5	58.3	75.0	51.5	60.9
Surround WP	0	0	2.5	39.3	57.3	88.3	50.4	81.7
Kocide 101 WP	0	0	0	16.8	35.3	53.0	24.8	26.6
Agri-Mek 0.15 EC & Oil	0	0	0	9.5	21.5	44.0	8.7	12.5
Control	0	0	2.3	9.0	14.0	36.0	x	x

Treatments with survival equal to the control were not tested the following week.

completion of the 96-h experiment. Exposure to Surround® WP, Kocide® 101 WP, and Aliette resulted in substantially higher *A. vaquitarum* mortality than that observed on the water control, while mortality on Agrimek® 0.15 EC + Citrus Soluble Oil was marginally higher than that on the control. *Aprostocetus vaquitarum* exposed to Citrus Soluble Oil, Micromite® 80 WGS, Micromite® 80 WGS + Citrus Soluble Oil, and Acramite® 50 WS had mortality rates marginally lower than that observed on the control. These four treatments did not appear to have an effect on *A. vaquitarum* survival, thus these treatments were not subjected to testing two to four weeks after application. The results were similar when the pesticides were tested two weeks after pesticide application. Sevin® 80 WSP and Malathion 5 EC resulted in 10% mortality after 8 h and all *A. vaquitarum* were dead 48 h after exposure to Sevin® 80 WSP, Malathion 5 EC, and Imidan® 70 WSB. Admire® 2 F and Danitol® 2.4 EC resulted in 100 and 98% mortality, respectively, after 96 h. Substantial mortality (72%) was also observed on Surround® WP after 96 h. Females exposed to Kocide® 101 WP and Agrimek® 0.15 EC + Citrus Soluble Oil showed slightly higher mortality than those exposed to the water control while those exposed to Aliette had a mortality rate slightly less than the control. Testing three and four weeks after pesticide application yielded similar results to weeks one and two. Sevin® 80 WSP remained the most toxic followed by Malathion 5 EC, Imidan® 70 WSB, Admire® 2 F, Danitol® 2.4 EC, Surround® WP and Kocide® 101 WP (Table 3). The relative toxicity of the pesticides was strikingly consistent over the four week period. Mortality rates decreased slightly over the four weeks; however, the toxicity of each pesticide was generally preserved. Though the pesticide treated substrate was maintained out of doors, it was protected from sunlight and precipitation.

Effect of Residual Pesticide on Parasitism

Survival of *A. vaquitarum* was affected by Sevin® XLR and Imidan® 70 WSB applied to foliage in the field. Fewer adult *A. vaquitarum* emerged from *D. abbreviatus* eggs laid on foliage treated with Sevin® XLR (0.0) and Imidan® 70 WSB (2.3 ± 1.3) than emerged from the water treated control (17.9 ± 1.9) when host eggs were laid immediately following pesticide application ($F_{2,61}$ = 30.8, P < 0.001) (Table 4). The number of adults emerging from eggs laid one week after application was lower on the Sevin® XLR treated foliage than on foliage treated with either Imidan® 70 WSB or water ($F_{2,75}$ = 10.34, P < 0.001). The number of adult *A. vaquitarum* emerging from host eggs laid two weeks after pesticide application was not different among the treatments ($F_{2,76}$ = 0.54, P = 0.59). The number of *D. abbreviatus* larvae emerging from eggs laid immediately after pesticide application was higher on foliage treated with Imidan® 70 WSB than on either the Sevin® XLR or control treatment ($F_{2,61}$ = 23.9, P < 0.001) (Table 4). There were no differences in the number of *D. abbreviatus* larvae emerging among the treatments from eggs laid one week ($F_{2,75}$ = 1.11, P = 0.33) or two weeks after pesticide application ($F_{2,76}$ = 0.94, P = 0.39).

Significantly more *A. vaquitarum* adults emerged from host eggs laid immediately after pesticide application on foliage treated with Micromite® 80 WGS or Acramite® 50 WS than from the control. Fewer *A. vaquitarum* emerged from foliage treated with Micromite® 80 WGS + Citrus Soluble Oil or Citrus Soluble Oil alone ($F_{4,133}$ = 29.3, P < 0.001) (Table 5). The number of *A. vaquitarum* emerging from host eggs laid on foliage treated one ($F_{4,145}$ = 13.9, P < 0.001) and two weeks ($F_{4,145}$ = 11.7, P < 0.001) earlier was not significantly different among Micromite® 80 WGS, Acramite® 50 WS, and the control, but significantly fewer adults emerged from foliage treated with either Micromite® 80 WGS + Citrus Soluble Oil or Citrus Soluble Oil alone. The number of neonate *D. abbreviatus* larvae emerging was lower on foliage treated with Citrus Soluble Oil than on

Table 4. Mean number (± S.E.) of adult *A. vaquitarum* and neonate *D. abbreviatus* emerging from *D. abbreviatus* egg masses laid on host plant material treated with Sevin XLR and Imidan 70 WSB 0, 7, and 14 d before oviposition.

Treatment	Mean number (± S.E.) Day 0	Day 7	Day 14
		Adult *A. vaquitarum*	
Control (water)	17.9 (1.9) a	12.0 (1.9) a	15.6 (2.1) a
Sevin XLR	0.0 (0.0) b	2.1 (1.4) b	12.6 (2.3) a
lmidan7OWSB	2.3 (1.3) b	13.6 (1.6) a	13.7 (1.7) a
		Neonate *D. abbreviatus*	
Control (water)	0.6 (0.4) a	3.4 (2.3) a	0.6 (0.6) a
Sevin XLR	0.8 (0.5) a	4.3 (2.1) a	1.2 (0.6) a
lmidan7OWSB	29.6 (5.4) b	0.7 (0.3) a	2.6 (1.5) a

Means followed by the same letter are not significantly different (P = 0.05).

TABLE 5. MEAN NUMBER (± S.E.) OF ADULT *A. VAQUITARUM* AND NEONATE *D. ABBREVIATUS* EMERGING FROM *D. ABBREVIATUS* EGG MASSES LAID ON HOST PLANT MATERIAL TREATED WITH MICROMITE 80 WGS, ACRAMITE 50 WS, CITRUS SOLUBLE OIL, AND MICROMITE + CITRUS SOLUBLE OIL 0, 7, AND 14 D BEFORE OVIPOSITION.

Treatment	Mean number (± S.E.)		
	Day 0	Day 7	Day 14
	Adult *A. vaquitarum*		
Micromite 80 WGS	22.6 (1.9) a	19.4 (2.9) a	12.9 (1.3) a
Acramite 50 WS	24.8 (3.3) a	20.8 (2.6) a	12.8 (1.8) a
Control (water)	9.3 (1.6) b	20.5 (2.7) a	14.2 (1.9) a
Micromite 80 WGS + Oil	0.8 (0.6) c	3.7 (1.0) b	3.3 (0.7) b
Citrus Soluble Oil	2.1 (1.1) c	1.4 (0.4) b	6.2 (1.8) b
	Neonate *D. abbreviatus*		
Micromite 80 WGS	0.0 (0.0) a	0.9 (0.9) a	0.3 (0.3) a
Acramite 50 WS	1.4 (0.7) a	0.3 (0.2) a	2.7 (1.4) a
Control (water)	2.6 (1.3)a	4.5 (2.2) a	2.7 (1.4) a
Micromite 80 WGS+ Oil	0.0 (0.0) a	0.5 (0.5) a	0.0 (0.0) a
Citrus Soluble Oil	23.6 (3.4) b	14.1 (2.5) b	12.8 (2.8) b

Means followed by the same letter are not significantly different ($P = 0.05$).

any of the other treatments when eggs were laid immediately ($F_{4,133} = 40.5$, $P < 0.001$), one ($F_{4,145} = 13.9$, $P < 0.001$), or two weeks ($F_{4,145} = 11.7$, $P < 0.001$) after pesticide application (Table 5).

Micromite® 80 WGS Fecundity Trial

There were no differences between oviposition by females exposed to Micromite® 80 WGS (20.3 ± 3.3) and those exposed to a water control (17.0 ± 2.8) ($F_{1,38} = 0.21$, $P = 0.65$) (Table 6). After 48 h, 45.8% of *A. vaquitarum* eggs laid by females exposed to Micromite® 80 WGS reached the first instar while 64.9% of eggs laid by females exposed to a water control reached the first instar after 48 h. There was no difference in the number of new generation *A. vaquitarum* adults emerging between Micromite® 80 WGS treated females (13.4 ± 2.5) and those females exposed to a water control (11.2 ± 1.0) ($F_{1,56} = 0.34$, $P = 0.56$). The number of host *D. abbreviatus* eggs was not different between the treatments for either the dissected egg masses ($F_{1,38} = 0.98$, $P = 0.33$) or those reared to emergence ($F_{1,56} = 0.29$, $P = 0.59$).

TABLE 6. MEAN NUMBER OF EGGS, LARVAE, HOST EGGS, AND TOTAL OVIPOSITION (± S.E.) BY *A. VAQUITARUM* AFTER 72 H EXPOSURE TO MICROMITE 80 WGS AND TOTAL MEAN OFFSPRING AND HOST EGGS AVAILABLE (± S.E.) FOR *A. VAQUITARUM* OVIPOSITION ON THREE CONSECUTIVE DAYS.

Treatment	Control	Micromite 80 WGS
Eggs	6.0 (1.2) a	11.0 (1.8) a
Larvae	11.0 (1.9) a	9.3 (2.4) a
Total Oviposition	17.0 (2.8) a	20.3 (3.3) a
Host eggs	185.5 (15.6) a	168.5 (11.8) a
Adult Offspring	11.2 (1.0) a	13.4 (2.5) a
Host Eggs	167.9 (6.4) a	171.4 (7.8) a

Means followed by the same letter are not significantly different ($P = 0.05$).

DISCUSSION

The impact of the 12 pesticides tested against *A. vaquitarum* ranged from harmless to highly toxic. The most acutely toxic products tested were Sevin® 80 WSP (carbamate), Malathion, and Imidan® 70 WSB (organophosphates). When tested immediately after application, mortality was more than twice as rapid as the next most toxic pesticides. Admire® 2 F (neonicotinoid) and Danitol® 2.4 EC (pyrethroid) were also toxic to *A. vaquitarum*. These and many other neurotoxic insecticides used in citrus are known to be extremely detrimental to a range of hymenopteran parasitoids and other beneficial insects (Easwaramoorthy et al. 1990; Villanueva-Jiménez & Hoy 1998; Jacas & García-Marí 2001; Wakgari & Giliomee 2003; Michaud & Grant 2003), including *Aprostocetus ceroplastae* (Girault) (Wakgari & Giliomee 2001). The regular use of these products will impede the establishment and productivity of *A. vaquitarum* and almost certainly have a negative impact on various other beneficial insects.

Surround® WP (kaolin clay) is touted as nontoxic and IPM-compatible. Though not as toxic as the neurotoxic insecticides, Surround® WP increased mortality and reduced the longevity of *A. vaquitarum* compared to the control and the other less harmful pesticides tested. The cause of death was not clear in the present study, but cadavers were often observed covered in kaolin particles. Application of Surround® WP to citrus has also been observed to increase scale insect infestations, most likely due to interference with parasitism (S. L. Lapointe, unpublished). The effect of Surround® WP in the present experiment may have been magnified by the exclusion of precipitation from the weathering process. Under these conditions, the Surround® WP residue would be expected to remain intact and not decline in potency.

Aprostocetus vaquitarum females exposed to Agrimek® 0.15 EC (avermectin), Kocide® 101 WP (copper hydroxide) and Aliette WDG showed a slight increase in mortality but longevity was not significantly different than that observed on the control. These products appear to be compatible with this parasitoid. However, at recommended rates Kocide® 101 WP and Agrimek® 0.15 EC were shown to be detrimental to *Ageniaspis citricola* (Logvinovskaya) (Hymenoptera: Encyrtidae), a parasitoid of the citrus leafminer (*Phyllocnistis citrella* (Stainton) (Lepidoptera: Gracillariidae) (Villanueva-Jiménez & Hoy 1998). Avermectin can affect citrus predatory mites negatively, including *Euseius stipulatus* (Athias-Henriot) (Jacas & García Marí 2001). Thus, further study of the effects on other beneficial insects in citrus is warranted.

Three of the products tested showed no contact toxicity to adult females, including Acramite® 50 WS, Micromite® 80 WGS, Citrus Soluble Oil, and Micromite® 80 WGS + Citrus Soluble Oil. Acramite® 50 WS (bifenazate) also has been shown to be compatible with the predatory mite *Phytoseiulus persimilis* (Kim & Yoo 2002) and only moderately harmful to ladybird beetles (James & Coyle 2001). Micromite® 80 WGS (diflubenzuron) is known to be toxic to several parasitoid species (Zaki & Gesraha 1987; Zijp & Blommers 2001; Amalin et al. 2004; Schneider et al. 2004), while it appears relatively harmless to others (Willrich & Boethel 2001; Amalin et al. 2004). Citrus Soluble Oil used alone is known to be compatible with other parasitoids and beneficial predators in citrus production (Amalin et al. 2000; Villanueva-Jiménez et al. 2000).

Results of the field studies were consistent with the laboratory tests. Sevin® XLR sprayed on host plant material was extremely toxic, resulting in no parasitoid reproduction when *D. abbreviatus* eggs were laid immediately after spraying, and continued to significantly reduce *A. vaquitarum* populations one week after application. It was not until 14 d after treatment that Sevin® XLR no longer had a significant impact on *A. vaquitarum*. Imidan® 70 WSB significantly reduced *A. vaquitarum* reproduction but lost efficacy more quickly than Sevin® XLR and no longer reduced *A. vaquitarum* reproduction after one week in the field. Very low numbers of neonate *D. abbreviatus* eclosed on the control, due to high levels of parasitism, or on foliage treated with Sevin® XLR, due to its toxicity to both the parasitoid and the pest. However, treatment with Imidan® 70 WSB resulted in high mortality of the beneficial but not *D. abbreviatus*. Though Imidan® 70 WSB broke down relatively quickly in the field, its negative impact on *A. vaquitarum* and minimal effect on *D. abbreviatus* make it a very poor candidate for an IPM program.

Insect growth regulators such as Micromite® 80 WGS (diflubenzuron) have generally been considered environmentally safer alternatives to broad-spectrum insecticides. Diflubenzuron has been shown to have a minimal impact on some hymenopteran parasitoids (Villanueva-Jiménez & Hoy 1998; Willrich & Boethel 2001; Amalin et al. 2004). However, it is known to have devastating effects on other parasitoid species (Zaki & Gesraha 1987; Zijp & Blommers 2001; Amalin et al. 2004; Schneider et al. 2004). Many pesticide toxicity tests are carried out on only one developmental stage of the parasitoid, usually the adults, but Schneider et al. (2003a, 2003b) showed that diflubenzuron can have detrimental effects on developmental processes while appearing relatively harmless to adult parasitoids. In the present study, Micromite® 80 WGS was not toxic to adult *A. vaquitarum* but was further tested under field conditions and in the laboratory to assess oviposition and development from egg to adult. Consistent with the contact toxicity test with adult females, Micromite® 80 WGS had no negative affect on *A. vaquitarum* when host eggs were laid on field treated host plants and tested 0 to 14 d after application. Oviposition by females exposed to Micromite® 80 WGS in the laboratory was not significantly different from the control, though there was an indication that the time from oviposition to first-instar eclosure may be extended for eggs laid by Micromite® 80 WGS exposed females. If detrimental, diflubenzuron, a chitin synthesis inhibitor, often inhibits egg hatch and disrupts the molting process (Marx 1977; Weiland et al. 2002). In the present study there were no apparent differences in development from egg to adult between the Micromite® 80 WGS treatment and the water control.

Micromite® 80 WGS (diflubenzuron) is effective in reducing *D. abbreviatus* populations (Schroeder 1996) and had no affect on *A. vaquitarum* in the present study. Micromite® 80 WGS use appears to be compatible with both *A. vaquitarum* and *Quadrastichus haitiensis* (Gahan) (Amalin et al. 2004), two of the primary parasitoids of *D. abbreviatus* now established in south Florida. Diflubenzuron was also considered harmless for citrus predatory mites (Jacas & García Marí 2001). However, diflubenzuron was shown to adversely affect *Ceratogramma eteinnei* (Delvare) (Amalin et al. 2004), an endoparasitoid of *D. abbreviatus* which was released and failed to establish in south Florida, as well as several other hymenopteran parasitoids (Zaki & Gesraha 1987; Zijp & Blommers 2001; Amalin et al. 2004; Schneider et al. 2004). Its impact on the agroecosystem merits further study.

Though Micromite® 80 WGS alone had no negative impact on *A. vaquitarum*, Micromite® 80 WGS + Citrus Soluble Oil and Citrus Soluble Oil alone did significantly reduce *A. vaquitarum* reproduction. It appears that the *D. abbreviatus* egg masses laid between leaves treated with Citrus Soluble Oil do not remain closed and become exposed to the elements. *Aprostocetus vaquitarum* will not oviposit into an exposed egg mass and if oviposition occurs before the egg mass opens, parasitoid eggs and larvae desiccate and die when the egg mass opens (J. E. Peña, unpublished). Though Citrus Soluble Oil does not appear to be toxic to *A. vaquitarum*, it does indirectly affect this parasitoid by reducing the efficiency of the adhesive *D. abbreviatus* uses to secure and protect its eggs between two leaves. Citrus Soluble Oil treatments had a negative affect on *C. eteinnei* (Amalin et al. 2004). *Aprostocetus vaquitarum*, an ecto-parasitoid, is extremely vulnerable to the environment and possibly even more severely impacted when the host egg mass is opened and exposed.

The aim of this study was to evaluate the relative toxicity of citrus pesticides in an effort to promote the use of compounds with low toxicity levels to *A. vaquitarum* and other beneficial insects. At recommended rates, Sevin® 80 WSP, Malathion 5 EC, and Imidan® 70 WSB, were extremely toxic to *A. vaquitarum* and will discourage the establishment of this insect. Admire® 2F, Danitol® 2.4 EC, and Surround® WP were also moderately toxic to *A. vaquitarum* and regular use would be detrimental to a control program aimed at establishing and maintaining this parasitoid. Kocide® 101 WP, Aliette WDG, and Agrimek® 0.15 EC + Citrus Soluble Oil were relatively non-toxic. Micromite® 80 WGS, Acramite® 50 WS, and Citrus Soluble Oil were non-toxic to *A. vaquitarum* adults and these products appear to be very suitable for an IPM program. Micromite® 80 WGS was also shown not to disrupt development of *A. vaquitarum* while Citrus Soluble Oil, though not toxic, did reduce the success of *A. vaquitarum* by causing host egg masses to become exposed. Given the restricted field evaluations conducted in the present study, further research should focus on the impact of these pesticides under field conditions.

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