

## Relative Susceptibility of *Haekeliana sperata* (Hymenoptera: Trichogrammatidae) to Pesticides Used in Citrus and Ornamental Systems in Florida

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J. Econ. Entomol. 102(3): 905–912 (2009)

**ABSTRACT** *Haekeliana sperata* Pinto is an egg-endoparasitoid of *Diaprepes abbreviatus* L. (Coleoptera: Curculionidae). We evaluated the relative susceptibility of *H. sperata* adults to commercially relevant pesticides used in citrus and ornamental production systems. Parasitoids were exposed to pesticide residues on leaf surfaces. Four concentrations of seven pesticides were tested; concentrations for each pesticide consisted of a dilution series using the label rate for field applications as the starting concentration. Lethal concentrations (LC<sub>50</sub>s and LC<sub>90</sub>s) were calculated 12 and 24 h after exposure to the pesticides. Lethal times (LT<sub>50</sub>s and LT<sub>90</sub>s) were estimated for each pesticide concentration. All tested pesticides had a negative effect on *Haekeliana*'s survivorship. However, some pesticides were significantly less harmful to this parasitoid. LC<sub>50</sub>s and LC<sub>90</sub>s of organophosphate, carbamate, and pyrethroid pesticides were less than one eighth of the label rates. LT<sub>50</sub>s and LT<sub>90</sub>s of these insecticides were <12 h even at the most diluted concentrations. Thus, applications of these pesticides might have a strong negative impact on the natural control of *D. abbreviatus* by *H. sperata*. Commercial formulations of imidacloprid, abamectin, petroleum oil, and a phosphonate fungicide allowed *H. sperata* to live longer compared with the previous pesticides, suggesting a certain degree of selectivity. Moreover, adults exposed to diluted concentrations of imidacloprid, abamectin, petroleum oil, and a phosphonate fungicide had a greater survival than those exposed to label concentrations. These findings suggest that the use of products that have less toxic effects on the introduced parasitoid will increase its chances to parasitize *D. abbreviatus* eggs.

**KEY WORDS** *Diaprepes* root weevil, biological control, egg parasitoid, conservation, pesticide residues

*Diaprepes abbreviatus* L. is a highly polyphagous root weevil that is native to the Lesser Antilles and was unintentionally introduced to Florida in the mid-1960s (Woodruff 1985). Since its first detection in Apopka (Orange County) in 1964, *Diaprepes* has spread throughout the central and southern part of the Florida peninsula. *Diaprepes* is now considered established in 23 counties, infesting >100,000 acres of citrus groves and many other agricultural, ornamental, and wild plants (Nguyen et al. 2003, Weissling et al. 2004). Estimates show that this pest has increased production costs >70 million dollars annually for the citrus industry in Florida (Stanley 1996, Muraro 2000). Moreover, the infestation of this weevil has spread to Texas and California (Grafton-Cardwell et al. 2004, CDFA 2006), resulting in drastic measures to restrict the introduction of ornamental plants from Florida (TDA

2001). Since 1997, there has been an effort toward achieving classical biological control of the *Diaprepes* root weevil resulting in the collection, introduction, rearing, and releasing of five parasitoid species. Two of these, *Aprostocetus vaquitarum* (Wolcott) (Hymenoptera: Eulophidae) and *Quadrastichus haitiensis* (Gahan) (Hymenoptera: Eulophidae), are established in southern Florida (Peña et al. 2004). Two additional species, *Fidiobia dominica* Evans and Peña (Hymenoptera: Platygasteridae) and *Haekeliana sperata* Pinto (Hymenoptera: Trichogrammatidae), are being released in other areas of Florida (Peña et al. 2006).

Two studies have addressed the toxicity of pesticides used in citrus to parasitoids of *D. abbreviatus*. Ulmer et al. (2006) evaluated the toxicity of pesticides used in citrus to *A. vaquitarum*. Their findings suggested that carbamate and organophosphate pesticides were the most toxic to *A. vaquitarum* adults, followed by neonicotinoid, pyrethroid, and kaolin clay pesticides. Copper and phosphonate fungicides, petroleum oil, abamectin, and insect growth regulators (IGRs) were slightly to nontoxic to *A. vaquitarum*. In another study, Amalin et al. (2004) tested the effect of an IGR, diflubenzuron, on *Ceratogramma etiennei* Del-

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Table 1. List of pesticides tested on *H. sperata*

Trade name	Class/active ingredient	Manufacturer	Application rates	Label concentration <sup>a</sup> (ml/liter)	Bioassay concentrations <sup>b</sup> (ml/liter)
Sevin XLR	Carbamate/carbaryl (44.1%)	Bayer CropScience	1.5 qt/acre	3.75	3.75, 1.87, 0.94, 0.47, 0
Lorsban 4E	Organophosphate/chlorpyrifos (44.9%)	Micro Flo Company	2-7 pt/acre	8.75-2.5	5.00, 2.50, 1.25, 0.63, 0
Provado 1.6F	Neonicotinoid/imidacloprid (22%)	Bayer CropScience	10-20 fl.oz/acre	1.56-0.78	1.0, 0.5, 0.25, 0.125, 0
Danitol 2.4 EC	Pyrethroid/fenprothrin (30.9%)	Valent USA Corporation	16-21.3 fl.oz/acre	1.66-1.28	1.50, 0.75, 0.38, 0.19, 0
Aliette WDG	Phosphonate/aluminium tris (80%)	Bayer CropScience	5.0 lb/acre	5.98 (g/liter)	6.0, 3.0, 1.5, 0.75, 0 (g/liter)
AgriMek 0.15 EC	Avermectin/abamectin (2%)	Syngenta	10-20 fl.oz./acre	1.56-0.78	1.0, 0.5, 0.25, 0.125, 0
Citrus soluble oil	Petroleum oil FC 435 (99.3%)	Platte Chemical Company	5 qt/acre	12.5	12.5, 6.25, 3.12, 1.56, 0

<sup>a</sup> Solutions were calculated using a standard volume of water of 100 gal/acre.

<sup>b</sup> Bioassay concentrations are a serial dilution using formulated pesticides at label rates as the primary solution.

vare (Hymenoptera: Trichogrammatidae) and *Q. haitiensis*. Their study concluded that this pesticide interferes with the development of *C. etiennei* but not the development of *Q. haitiensis*.

No prior studies have addressed the concentration dependent effects of commercially formulated pesticides on natural enemies of *D. abbreviatus*. Under field conditions, the deposition of the active ingredients on the target area is variable and dependent on several factors; among them, the water volume in the pesticide solution, the application equipment used, spray pressure, climatic conditions, tree size, shape and spatial arrangement, and age of the leaves (Ebert et al. 1999a, b; Hall 1991). Moreover, some pesticides are registered for controlling several pests in a single crop but with different rates depending on the target pest. Consequently, pest insects and natural enemies are often exposed to a range of pesticide concentrations that include the label concentration and also sublabel rates. There is increasing evidence that some pesticides have significantly variable effects on natural enemies at different doses (Villanueva-Jimenez and Hoy 1998, Delpuech et al. 1999); for this reason, it is important to understand the effects of pesticides on natural enemies at label rates but also at the lower rates which likely occur under field conditions.

Pesticides are a critical component of integrated pest management (IPM) in citrus and ornamental production. Thus, understanding their effects on *H. sperata* would provide critical background information to design tactics that could maximize the probability of establishment and utility of this parasitoid. Therefore, this study examines the relative susceptibility of adult *H. sperata* to a range of doses of commercially relevant pesticides used in citrus and ornamental production systems.

### Materials and Methods

**Stock Colonies.** *Haekeliana sperata* used in each of the experiments described below were collected in Dominica in 2003 and reared in the Tropical Research and Education Center (TREC) insectary (12-h pho-

toperiod, 26.5 ± 1°C, and 75% RH) for several generations. Parasitoids were reared on *D. abbreviatus* eggs from adult weevils that were collected from a pesticide-free commercial nursery in Homestead, FL. All the parasitoids used in the experiments were mated, fed, naïve with respect to hosts, and <1 d old.

**Bioassay.** The bioassay of Williams and Price (2004), developed for trichogrammatids and other minute Hymenoptera, was used in this study. Leaves from a pesticide-free lime grove were collected, and leaf disks (2.3 cm diameter) were excised with a cork borer. The leaf disks were dipped for 20 min in the different concentrations of the pesticides or in water for controls and air dried for 24 h. Four concentrations of seven pesticides were tested in the bioassays. Each pesticide was tested in a serial dilution where the starting solution was the recommended label rate for field applications, assuming a standard volume of water of 100 gal/acre. The tested pesticides (including trade name, class, active ingredient, application rate, label rate, and bioassay concentrations) are listed in Table 1.

The bioassay chambers consisted of one piece of transparent PVC tube (2.54 cm ID by 3.5 cm long) with organdy-covered ventilation holes, two vial scintillation caps (cat. no. 74521-22400; Kimble Glass, Vineland, NJ) each containing 3 ml of agar and a treated leaf disk (each pair treated with the same concentration and placed on top of the agar), a piece of dialysis membrane, and a feeding tube (Williams and Price 2004). The chambers were assembled by sliding one cap into each end of the tube so that the edge of leaf disk was aligned with the edge of the ventilation holes. The upper surface of the leaf disk formed the floor and the under surface of the leaf disk formed the ceiling of the chamber. A strip of dialysis membrane was used to seal the chambers. A piece of borosilicate glass capillary (5 cm long by 1.5 mm diameter) that was previously flamed was used to make a small hole in one of the ventilation holes. Through this hole, 10 parasitoids (presumed mated and 1:1 ♂:♀) were introduced to each chamber using an aspirator constructed with a capillary of the same type

**Table 2.** Lethal time (50 and 90%) of *H. sperata* exposed to four concentrations of selected pesticides

Pesticide	Concentration	LT <sub>50</sub> (h)	LT <sub>50</sub> FL (h)	LT <sub>90</sub> (h)	LT <sub>90</sub> FL (h)	$\chi^2$	Slope
Aliette WDC	0 g/liter	184.61a	154.1–234.8	978.69a	660.8–1665	15.35	1.76
	0.75 g/liter	39.74b	38.7–40.7	62.11b	60.23–64.24	19.10	6.6
	1.5 g/liter	33.03c	30.71–35.48	58.52b	52.39–68.16	71.25	5.15
	3 g/liter	30.95cd	28.29–33.77	58.79b	51.47–71.08	88.63	4.59
	6 g/liter	21.67d	17.00–26.77	57.84b	43.10–100.57	198.41	3
Provado 1.6F	0 ml/liter	184.61a	154.1–234.8	978.69a	660.8–1665	15.35	1.76
	0.12 ml/liter	29.03b	25.20–32.83	104.07b	86.00–134.82	147.48	2.31
	0.25 ml/liter	26.56b	22.88–30.18	99.02b	81.59–128.88	135.96	2.24
	0.50 ml/liter	13.84c	11.80–15.81	65.03c	56.21–77.77	54.67	1.9
	1.00 ml/liter	7.66d	6.46–8.83	52.85c	46.67–61.18	26.76	1.52
AgriMek 0.15 EC	0 ml/liter	184.61a	154.1–234.8	978.69a	660.8–1665	15.35	1.76
	0.12 ml/liter	25.37b	22.07–28.63	77.58b	66.32–95.24	157.19	2.64
	0.25 ml/liter	20.58b	17.84–23.23	74.61b	63.75–91.25	92.57	2.29
	0.50 ml/liter	15.24c	14.07–16.38	55.03c	51.06–59.88	17.58	2.29
	1.00 ml/liter	9.42d	8.54–10.27	30.69d	28.29–33.59	13.57	2.5
Citrus soluble oil	0 ml/liter	179.33a	150.5–226.1	908.12a	621.6–1515	15.99	1.81
	1.56 ml/liter	26.42b	24.45–28.40	162.84b	141.7–191.7	34.06	1.62
	3.12 ml/liter	20.53c	18.09–22.93	136.27b	114.3–169.5	47.36	1.55
	6.25 ml/liter	13.49d	10.55–16.31	62.27c	51.56–79.43	146.85	1.92
	12.5 ml/liter	12.16d	10.15–14.11	50.5c	44.15–59.18	85.27	2.07
Sevin XLR	0 ml/liter	179.33a	150.5–226.1	908.12a	621.6–1515	15.99	1.81
	0.45 ml/liter	8.10b	6.38–9.74	23.28b	18.24–34.68	19.13	2.79
	0.94 ml/liter	4.89b	3.09–6.41	13.59bc	10.40–21.19	29.48	2.88
	1.87 ml/liter	3.86bc	2.65–4.91	12.3c	10.02–16.34	12.88	2.54
	3.75 ml/liter	2.41c	1.78–2.97	7.91d	6.92–9.21	2.56	2.48
Lorsban 4E	0 ml/liter	179.33a	150.5–226.1	908.12a	621.6–1515	15.99	1.81
	0.63 ml/liter	2.88b		3.3b			22.06
	1.25 ml/liter	2.73b		3.14b			20.93
	2.50 ml/liter	2.73b		3.14b			20.93
	5.00 ml/liter	2.4b		2.82b			18.38
Danitol 2.4 EC	0 ml/liter	179.33a	150.5–226.1	908.12a	621.6–1515	15.99	1.81
	0.19 ml/liter	3.43b	2.92–3.89	8.31b	7.41–9.59	4.26	3.34
	0.38 ml/liter	2.75b	2.33–3.09	5.2c	4.67–6.00	2.33	4.63
	0.75 ml/liter	2.6b	2.14–2.96	5.1c	4.56–5.91	1.97	4.37
	1.50 ml/liter	1.16		3.76		2.91	2.51

LT<sub>50</sub>s and LT<sub>90</sub>s of each pesticide followed by the same letter are not significantly different because of fiducial limits overlap.

used for making the holes. The hole was covered with one pipette tip 1.5 cm long (5–300  $\mu$ l; Finntip, Thermo Fisher Scientific Inc., Waltham, MA) filled with honey-water solution (1:1) that served as a feeding tube. Once assembled, the chambers were placed in an environmentally controlled room maintained at 26.5  $\pm$  1°C, 12:12 L:D, and 75% RH.

Mortality was scored under a stereoscope every 3 h after the starting of the bioassays. Mortality was defined by immobility and a complete lack of movement by mouthparts, wings, and legs. Five replicates per insecticide concentration were evaluated.

**Data Analysis.** Lethal time 50 (LT<sub>50</sub>) and lethal time 90 (LT<sub>90</sub>) were estimated for each insecticide concentration using the SAS-PROBIT procedure (SAS Institute 1999). Significant differences between lethal times were indicated when the 95% fiducial limits of one concentration did not overlap with the fiducial limits of the other concentrations. Lethal concentrations at LC<sub>50</sub>s and LC<sub>90</sub>s were calculated 12, 24, and 48 h after parasitoids were exposed to the pesticides using the SAS-PROBIT procedure. Abbott's transformation was used to correct for control mortality (Abbott 1925), which was usually  $\leq$ 10%.

## Results

Carbamate, organophosphate, and pyrethroid insecticides used in the bioassays were highly toxic to *H.*

*sperata*. Contact with any of these pesticides at any of the tested concentrations resulted in death of all *Haemaphysalis* wasps within a few hours. LT<sub>50</sub>s of *H. sperata* exposed to residues of these pesticides ranged from 2.4 to 8.1 (Sevin), 2.4 to 2.8 (Lorsban), and 1.1–3.43 h (Danitol) (Table 2). In some cases, because of rapid onset of mortality, the analyses did not produce lethal concentration values or fiducial limits. This was caused by the high mortality registered in the first evaluations; 42, 82, and 61% of the total number of wasps were dead 3 h after being exposed to Sevin, Lorsban, and Danitol, respectively. No differences on the LT<sub>50</sub>s of the four tested concentrations were observed for Lorsban and Danitol, suggesting that the lower concentrations (dilutions) are as toxic as the high concentrations (label rate concentrations). In contrast, lower concentrations of Sevin had a significantly ( $P < 0.01$ ) reduced toxic effect on *H. sperata* than the higher concentrations (Fig. 1). The estimated lethal concentrations (LC<sub>50</sub>s) were out of the range of tested concentrations. Considering that all the tested concentrations of these insecticides produced rapid and high mortality on *H. sperata*, the LC<sub>50</sub>s of these pesticides will necessarily be lower than the ones used in these bioassays.

Contact with Agrimek and Provado also caused high mortality to *H. sperata*, but it occurred later than for those exposed to Sevin, Danitol, and Lorsban. LT<sub>50</sub>s for wasps exposed to Agrimek and Provado ranged from 9.42 to 25.37 and 7.66 to 29.03 h, respectively. In

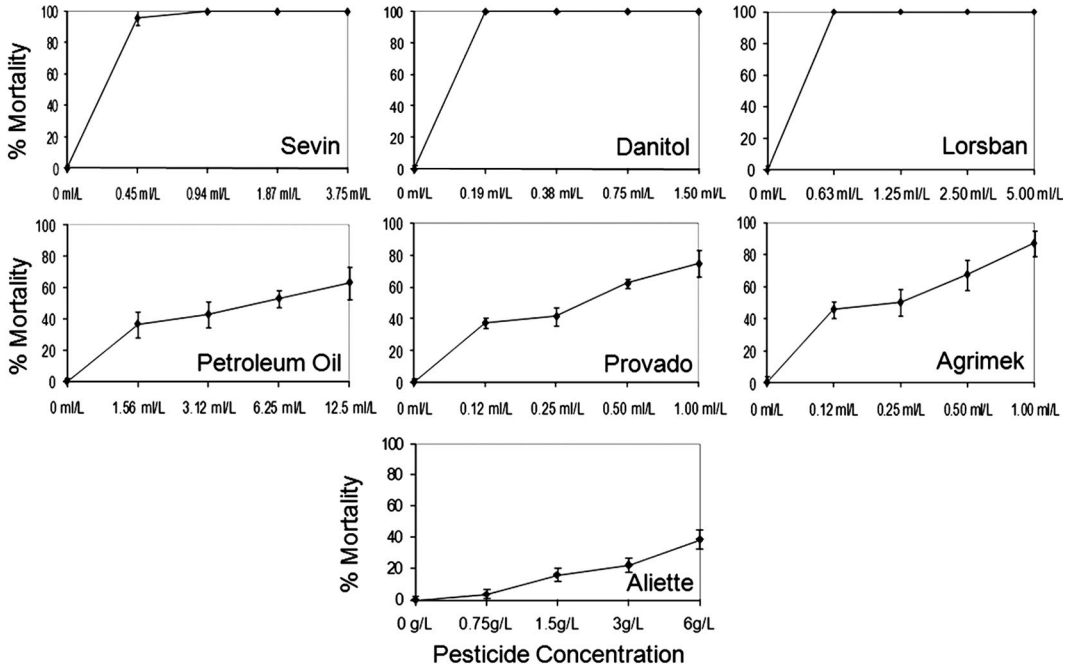


Fig. 1. Percent mortality of *H. sperata* 24 h after exposure to four different concentrations of pesticide residues on lime leaves. Pesticide commercial names are shown. Class and active ingredient of each material are listed in Table 1.

both cases, an effect of the concentration on the time of death was observed. The two lower concentrations (0.125 and 0.25 ml/liter) had a significantly longer  $LT_{50}$  than the two higher concentrations (0.5 and 1.0 ml/liter; Table 2). Accordingly,  $LT_{90}$ s for wasps exposed to the lower concentrations were significantly longer than those exposed to higher concentrations (Table 2). In both cases, the  $LC_{50}$ s calculated 12 h

after exposure to the insecticides were inside the range of the recommended field rates (Tables 1 and 3), which are the concentrations delivered directly from the applicator nozzle. Nonetheless, an effect of the concentration on the mortality was observed (Fig. 1), which suggests that sub label rates found under field conditions could cause less harm to *H. sperata*. Results of the bioassays indicate that these two pesticides

Table 3. Lethal concentrations (50 and 90) at 12, 24, and 48 h after *H. sperata* adults were exposed to leaf disks treated with seven pesticides at four different concentrations

Pesticide	Time after exposure to insecticide	$LC_{50}$ (ml/liter)	$LC_{50}$ FL (ml/liter)	$LC_{90}$ (ml/liter)	$LC_{90}$ FL (ml/liter)	$\chi^2$	Slope
Aliette WDG	12	19.92	11.27-77.24	111.59	38.50-1543	1.38	1.71
	24	9.34	6.46-18.17	73.18	31.68-371.66	1.84	1.43
	48	0.26	0.07-0.47	3.01	2.19-5.24	4.3	1.22
Provado 1.6F	12	0.64	0.48-1.02	10.62	4.20-74.84	0.57	1.05
	24	0.28	0.20-0.36	3.56	1.92-11.68	2.04	1.16
	48	0.17	0.12-0.21	1.21	0.86-2.12	3.06	1.52
AgriMek 0.15 EC	12	0.81	0.56-1.68	22.27	6.34-487	2.64	0.89
	24	0.19	0.13-0.24	1.77	1.14-3.90	4.46	1.32
	48	0.12	0.07-0.15	0.82	0.61-1.35	1.34	1.53
Citrus soluble oil	12	11.86	6.73-87.29	2091	179.65-2,209	0.69	0.57
	24	4.78	3.17-7.56	231.56	66.90-10,572	0.19	0.76
	48	0.8	0.30-1.29	9.62	6.70-19.04	4.56	1.19
Sevin XLR	12	0.21		1.55		5.68	1.5
	24	0.36		0.42		0	18.3
	48	a		a			
Lorsban 4E	12	a		a			0
	24	a		a			
	48	a		a			
Danitol 2.4 EC	12	0.14		0.17			17.63
	24	a		a			0
	48	a		a			0

<sup>a</sup> PROBIT analysis did not produce LC values because of rapid onset of mortality at the tested concentrations.

are less toxic to *H. sperata* than Sevin, Lorsban, and Danitol.

The petroleum oil showed results similar to those of Provado and Agrimek (Fig. 1).  $LT_{50}$ s for wasps exposed to petroleum oil ranged from 12.16 to 26.42 h. The two lower concentrations (1.56 and 3.12 ml/liter) had a significantly longer  $LT_{50}$  than the two higher concentrations (6.25 and 12.5 ml/liter; Table 2).  $LT_{90}$ s of wasps exposed to residues of petroleum oil at the two lower concentrations were larger than those of any of the other tested pesticides (Table 2).  $LC_{50}$ s at 12, 24, and 48 h after exposure to petroleum oil were equal or lower than the label rates, which suggests that contact with fresh residues of petroleum oil at label concentrations will cause the death of one half of the *Haeckeliania* wasps in <12 h.

The fungicide evaluated in this study (Aliette WDG) showed the least effect on *H. sperata*.  $LT_{50}$ s for wasps exposed to Aliette ranged from 21.67 to 39.74 h, being significantly longer at the lowest test concentration (0.75 g/liter; Table 2).  $LT_{90}$ s of wasps exposed to Aliette were very similar to the other pesticides such as Provado and Agrimek but still lower than those showed by the petroleum oil. This was the only pesticide on which the  $LC_{50}$  and  $LC_{90}$  at 12 and 24 h after exposure were significantly higher than the label concentrations (Tables 1 and 3). However,  $LC_{50}$  and  $LC_{90}$  48 h after exposure to the pesticide were lower than the label concentration. These results suggest that contact with fresh residues of Aliette at label concentrations will cause the death of one half of the *Haeckeliania* wasps in <48 h. An effect of the concentration on the mortality of *H. sperata* was observed, suggesting that a reduction in the fungicide concentration favors the longevity of *H. sperata* (Fig. 1). These results support that Aliette has a lower acute toxicity than the other tested pesticides (Fig. 1); the long-term effects of Aliette are relatively similar to the other pesticides used in the bioassays.

## Discussion

*Haeckeliania* adults search for weevil egg masses while walking on the surface of leaves of their host's plant, where they also mate and rest. In contrast, the immature stages develop inside the weevil eggs, which in turn are enclosed between two sealed leaves. These aspects of the biology and behavior of *H. sperata* suggest that adults are more likely to come into contact with pesticides that could result in short-term mortality or long-term sublethal effects.

In our bioassays, organophosphate, carbamate, and pyrethroid pesticides showed a rapid and strong toxic effect on *H. sperata* adults. No reduction in mortality was caused by the dilutions of the pesticide solution, which were as low as one eighth the recommended label rates. These findings indicate that these three insecticides have a high acute toxicity to *H. sperata*. These results are similar to those found on *A. vaquitarum*, where Ulmer et al. (2006) reported that label rates of organophosphate and carbamate insecticides were more toxic to *A. vaquitarum* adults than other

insecticides, whereas pyrethroids were less toxic to *A. vaquitarum*. In contrast, *H. sperata* was affected similarly by carbamate, organophosphate, and pyrethroid insecticides. These three groups of insecticides are neurotoxins that interfere with the transmission of impulses in the nervous system of the insect (Scharf 2003). Insecticides targeting the nervous system are considered broad spectrum pesticides. It is not surprising that neurotoxic insecticides could have negative effects on natural enemies, as reported in other studies (Villanueva-Jimenez and Hoy 1998; Wakgari and Giliomee 2001, 2003).

Carbamate, organophosphate, and pyrethroid insecticides used in our experiments are registered to control several citrus pests with varying label rates depending on the target pest. Sevin XLR is recommended for the control of various citrus pests at rates ranging from 1.5 to 3 qt/acre ( $\approx 7.5$ – $33.7$  ml/liter solution assuming a volume of 100 gal/acre) (Browning et al. 2007, Stansly et al. 2007). For root weevils, the rate is much higher (1–2 gal/acre  $\approx 10$ – $20$  ml/liter), and application is recommended in mixture with petroleum oil (+1 gal/acre  $\approx 10$  ml/liter of petroleum oil) (McCoy et al. 2007). Lorsban 4 EC is registered for the control of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), at a rate of 5 pt/acre ( $\approx 6.25$  ml/liter) (Rogers and Stansly 2007). Danitol is recommended for control of several pests including *D. citri*, flower thrips (*Frankliniella* spp.), and orchid thrips (*Chaetanaphothrips* spp.) at a rate of 1 pt/acre ( $\approx 1.25$  ml/liter) and for citrus root weevils at a rate of 16–21 fl. oz./acre ( $\approx 1.25$ – $1.63$  ml/liter) (Rogers and Stansly 2007, Stansly et al. 2007). Results of this study suggest that applications with any of these pesticides at any of the recommended rates will be extremely harmful to *H. sperata*. We conclude that these insecticides are nonselective to *H. sperata*.

Provado (imidacloprid) is a plant systemic pesticide that was also highly toxic to *H. sperata*. Systemic pesticides have been shown to be more selective to natural enemies because they can be applied as a soil drench, causing little effects on free-living natural enemies (Hull and Beers 1985). However, Provado is a formulation of imidacloprid designed for foliar sprays. Imidacloprid is another neurotoxic molecule that causes insect death because of prolonged neuroexcitation through stimulation of acetylcholine receptor (Scharf 2003). Foliar residues of imidacloprid were found to be highly toxic to parasitoids and predators but less toxic to predatory mites (Mizell and Sconyers 1992). Williams and Price (2004), using the same methods found, that residues of imidacloprid on leaves were highly toxic to *Anopheles iole* Girault (Hymenoptera: Mymaridae). In addition, Villanueva-Jimenez and Hoy (1998) reported that foliar sprays of imidacloprid were highly toxic to the parasitoid *Ageniaspis citricola* Loginovskaya (Hymenoptera: Encyrtidae) but only slightly affected its host, the citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae). In contrast, drenched imidacloprid had a moderate effect on the parasitoid while controlling the pest. Provado was highly toxic to *H. sperata*, but it



allowed the parasitoids to live longer than the carbamate, organophosphate, and pyrethroid insecticides. Moreover, *Haeckeliana* wasps exposed to low concentrations of imidacloprid lived significantly longer than those exposed to higher concentrations. These results suggest that, even though detrimental, Provado had a lower acute toxicity on *H. sperata* than other neurotoxic pesticides and could allow the parasitoids to live longer at sublabel rates found under field conditions. These results agree with those reported by Ulmer et al. (2006), which stated that Admire (the drench version of imidacloprid) is detrimental to *A. vaquitarum* but it does not act as fast as carbamate and organophosphates. Provado is registered for control of the Asian citrus psyllid and several species of aphids at a rate of 10–20 fl. oz/acre ( $\approx 0.78$ – $1.56$  ml/liter) (Browning et al. 2007, Rogers and Stansly 2007). At this rate, Provado had a negative effect on *H. sperata*. For insecticides used in the bioassays, Provado was the one that allowed the wasps to live longer, which could suggest that this insecticide is more compatible than the other evaluated products.

Agrimek had similar effects to those caused by Provado. Agrimek is an avermectin that targets the nervous system of the insect. In contrast to the former pesticides, it targets the  $\text{Cl}^-$  channel and causes neuron inhibition (Scharf 2003). Agrimek was also highly toxic to *H. sperata*, but it allowed the parasitoids to live longer than the tested carbamate, organophosphate, and pyrethroid insecticides. In our bioassay, *Haeckeliana* wasps exposed to low concentrations of Agrimek lived longer than those exposed to the label concentrations. This suggests that under field conditions, parasitoids encountering sublabel rates of this pesticide could live longer than those exposed to the label rates. Similar to what was observed on Provado, this pesticide was detrimental to *H. sperata* but showed a lower acute toxicity than the carbamate, organophosphate, and pyrethroid. These results are different to those reported by Ulmer et al. (2006), who found that Agrimek caused a slight increase in mortality of *A. vaquitarum* without affecting its longevity, but partially agree with those reported by Villanueva-Jimenez and Hoy (1998), who found that this pesticide was highly toxic to *A. citricola* and inappropriate for IPM programs.

Agrimek is recommended in a mixture with petroleum oil for the control of citrus leafminer, rust mites [*Aculops pelekassi* Keifer and *Phyllocoptruta oleivora* Ashmead (Acari: Eriophyidae)], and broad mites [*Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae)]. The recommended rate for controlling the citrus leafminer (5 fl. oz/acre of Agrimek  $\approx 0.39$  ml/liter + 1 gal/acre of petroleum oil  $\approx 10.0$  ml/liter) and mites (10 fl. oz/acre of Agrimek  $\approx 0.78$  ml/liter + 3 gal/acre of petroleum oil  $\approx 30.0$  ml/liter) are different (Childers et al. 2007, Rogers and Stansly 2007). Based on results obtained with the concentrations used in our bioassays, we could expect that applications of Agrimek with petroleum oil targeting the citrus leafminer should have less impact on *H. sperata* than those made targeting mites.

Petroleum oil caused an acute mortality similar to that of Provado and Agrimek but allowed the parasitoids to live longer. Observations during the bioassays suggest that the effect of petroleum oil on *H. sperata* is mechanical. The oil apparently coats the wasps and immobilizes them. However, this effect was only conspicuous during the first evaluations. Our results contrast with those reported by Ulmer et al. (2006) and Villanueva-Jimenez and Hoy (1998); the former reported that petroleum oil showed no contact toxicity to *A. vaquitarum*, whereas the later considered it an IPM compatible product. The smaller size of *H. sperata* may make it more susceptible than other parasitoids to mechanical effects of petroleum oil. Our experiments did not include the effects of petroleum oil mixed with other insecticides that could be more favorable for *H. sperata*. Petroleum oil alone is recommended for control of various citrus arthropod pests at a rate of 5 gal/acre ( $\approx 50.0$  ml/liter). It is also recommended for control of greasy spot, *Mycosphaerella citri* Whiteside (Dothideales: Dothideaceae), at a rate of 5–10 gal/acre ( $\approx 50.0$ – $100.0$  ml/liter) (Timmer and Chung 2007). All these concentrations are much higher than those used in our bioassays and could have an acute toxic effect on *H. sperata*. We conclude that this product caused a high acute mortality similar to that caused by Provado and Agrimek but lower than that caused by carbamate, organophosphate, and pyrethroid insecticides.

The fungicide Aliette WDG was the pesticide that showed the least impact on *H. sperata*. Ulmer et al. (2006) found somewhat coinciding results that showed that Aliette was not toxic to *A. vaquitarum*. Aliette is a protectant, curative, and systemic fungicide recommended for the control of *Phytophthora* spp., foot rot, and brown rot of fruit at a rate of 5 lb/acre ( $\approx 6$  g/liter) (Graham and Timmer 2007). At this rate, Aliette was toxic to *H. sperata*. Nonetheless, parasitoids survived longer at sublabel rates than could be found under field conditions.

We conclude that all the pesticides that were included in our experiments had a negative effect on *Haeckeliana*'s survivorship. Based on this study, we could not say that these products are selective to *H. sperata*. In other words, we did not find that any of the tested pesticides preserves the ability of *H. sperata* to control *D. abbreviatus*. However, within the registered insecticides that we tested, there are some that cause significantly less harm to this parasitoid. Results presented here and those reported by Ulmer et al. (2006) suggest that the organophosphate, carbamate, and pyrethroid pesticides are not good candidates to preserve the natural control of *D. abbreviatus* by any introduced egg parasitoids. Our results showed that Provado, Agrimek, petroleum oil, and Aliette allowed *H. sperata* to live longer than Lorsban, Sevin, and Danitol, which suggests a certain degree of selectivity. Moreover, our results show that *Haeckeliana* adults exposed to lower concentrations than the recommended rates of Provado, Agrimek, petroleum oil, and Aliette have more chances of surviving than those exposed to the label concentrations. It is unclear if

these parasitoids that can live for a certain period of time after being exposed to a pesticide remain reproductively active and continue parasitizing hosts. There is evidence that the behavior of parasitoids could be affected by the exposure of sublethal doses of some pesticides, as seen in *Trichogramma brassicae* (Bezdenko) (Hymenoptera: Trichogrammatidae). Delpuech et al. (1999) reported that exposure of *T. brassicae* to sublethal doses of deltamethrin, a pyrethroid, modified its sex pheromonal communication, which reduced mating and therefore the fitness of this biological control agent. As seen in many other beneficial arthropod species (Johnson and Tabashnik 1999, Desneux et al. 2007), sublethal effects of pesticides could also be present in *H. sperata*.

Results of this study show that the applications of pesticides are likely to have a negative impact on the success of biological control programs targeting *Diaprepes*. Unfortunately, since the initial finding of Citrus Greening (Huanglongbing) disease in Florida in 2005, the application of pesticides targeting the Asian citrus psyllid (vector of the disease) has increased. Citrus growers are now making five to six applications of broad-spectrum insecticides (including products such as fenpropathrin, zeta-cypermethrin, chlorpyrifos, carbaryl, and dimethoate) per year, which will obviously have a significant impact on biological control in citrus (M.E.R., unpublished results). We propose the use of products that have less toxic effects on the introduced parasitoid. This will increase the chances of *H. sperata* to control *D. abbreviatus* and might reduce the application frequency of pesticides targeting the weevil.

### Acknowledgments

This study is partial fulfillment of requirements for the MSc degree, University of Florida, by D.C. We thank M. Scharf (University of Florida, Gainesville, FL) for helpful suggestions to improve this manuscript. We thank D. Long, R. Duncan, Z. Alegria, and J. Alegria for assistance. This work was supported in part by a CSREES grant to J.E.P.

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Received 16 August 2008; accepted 11 January 2009.