Toxicity of Seven Foliar Insecticides to Four Insect Parasitoids Attacking Citrus and Cotton Pests

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ABSTRACT Laboratory studies were carried out to compare the toxicity of seven foliar insecticides to four species of adult beneficial insects representing two families of Hymenoptera: Aphelinidae (*Aphytis melinus* Debach, *Eretmocerus eremicus* Rose & Zolnerowich, and *Encarsia formosa* Gahan) and Mymaridae (*Gonatocerus ashmeadi* Girault) that attack California red scale, *Aonidiella aurantii* (Maskell); sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (both *E. eremicus* and *E. formosa*); and glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar), respectively. Insecticides from four pesticide classes were evaluated using a petri dish bioassay technique across a range of concentrations to develop dosage–mortality regressions. Insecticides tested included acetamiprid (neonicotinoid); chlorpyrifos (organophosphate); bifenthrin, cyfluthrin, and fenpropathrin (pyrethroids); and buprofezin and pyriproxyfen (insect growth regulators [IGRs]). Chlorpyrifos was consistently the most toxic pesticide to all four species of beneficial insects tested based on LC_{50} values recorded 24 h posttreatment compared with 48-h LC_{50} values with the neonicotinoid and pyrethroids or 96 h with the IGRs. Among the three pyrethroids, fenpropathrin was usually less toxic (except similar toxicity to *A. melinus*) than was cyfluthrin, and it was normally less toxic (except similar toxicity with *E. formosa*) than was bifenthrin. Acetamiprid was generally less toxic than bifenthrin (except similar toxicity with *G. ashmeadi*). The IGRs buprofezin and pyriproxyfen were usually less toxic than the contact pesticides, but we did not test for possible impacts on female fecundity. For all seven pesticides tested, *A. melinus* was the most susceptible parasitoid of the four test species. The data presented here will provide pest managers with specific information on the compatibility of select insecticides with natural enemies attacking citrus and cotton, *Gossypium hirsutum* L., pests.

KEY WORDS *Homalodisca vitripennis*, *Homalodisca lacerta*, insecticide impacts, *Bemisia tabaci*, parasitoids

Major pests such as California red scale, *Aonidiella aurantii* (Maskell); citrus thrips, *Scirtothrips citri* (Moulton); and citricola scale, *Coccus pseudomagnoliarum* (Kuwana), have threatened citrus production in California for many years, at times causing heavy damage to various citrus groves (Flint et al. 1991, Morse and Luck 2003). More recently, the California citrus industry has gained an addition to its pest complex, the glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar). High populations of *H. vitripennis* are of minor direct concern on citrus, but they represent a threat to grape production in California, because this species is a prolific vector of the pathogen causing Pierce's disease (Hix et al. 2003, NRC 2004). Citrus is the major overwintering host of glassy-winged sharpshooter, and treatments applied on citrus to reduce its vector potential on grape threaten a biologically based citrus integrated management system that has operated successfully for many years (Grafton-Cardwell and Gu 2003, Morse and Luck 2003, Morse et al. 2007). Previous studies have reported on the successful control of major pests of citrus by using biological control agents (Luck 1981, Luck et al. 1986). For example, citrus growers have depended on parasitoids and predators such as *Aphytis* spp. and the vedalia beetle, *Rodolia cardinalis* (Mulsant), to control California red scale; yellow scale, *Aonidiella citrina* (Coquillett); purple scale, *Lepidosaphes beckii* (Newman); and cottony cushion scale, *Icerya purchasi* Maskell. Natural enemies do not eliminate pest populations, but they establish equilibria with pest insect populations that are generally below damage thresholds. When pest densities exceed these thresholds, an occasional insecticide treatment is needed. Thus, pesticide intervention is considered essential in some situations to control high infestations of specific pests, including *H. vitripennis* in citrus, so as to reduce economic damage and spread to other crops. Several insecticides that are widely used to suppress

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various pests can disrupt the effectiveness of these beneficial agents, but with other materials, it is less clear to what degree they are disruptive. Improved understanding of pest–natural enemy–insecticide interactions will assist in formulating more effective integrated pest management (IPM) strategies on citrus.

In the American Southwest, *Bemisia tabaci* (Gennadius) has been the principal pest of vegetable and field crops for many years. Devastating outbreaks in the early 1990s in California, Texas, and Arizona resulted in farm revenue losses in excess of hundreds of millions of dollars (Perring et al. 1993). For the past two decades, control of *B. tabaci* has been dependent on both conventional and selective insecticides such as neonicotinoids and insect growth regulators (IGRs). Pyriproxyfen, an IGR, is effective in the management of *B. tabaci* and *Trialeurodes vaporariorum* (Westwood) in agricultural crops and greenhouses (Horowitz et al. 1994, Ellsworth and Diehl 1996, Ellsworth and Naranjo 1999). Pyriproxyfen and buprofezin (also an IGR) have been reported to be highly compatible with certain parasitoids because of minimal developmental interference and lack of adverse effects on female foraging behavior (Hoddle et al. 2001, Naranjo 2001). Pyriproxyfen also has been shown to be benign to a variety of predatory arthropods (Naranjo et al. 2003). Although pyriproxyfen seems to be compatible with whitefly parasitoids, several reports indicate that outbreaks of cottony cushion scale have been observed due to its high toxicity to vedalia beetles if treatments are applied in the spring (e.g., Grafton-Cardwell and Gu 2003). Well-timed use of IGRs for whitefly control may serve to complement the activity of beneficial species in cotton and vegetables.

Control of whiteflies by using biological agents alone has been attempted; however, in several cases, effective levels of control have not been attained (Oliveira et al. 2001), perhaps due to intensive use of pesticides that adversely affected natural enemies. Naranjo (2001) evaluated the impact of various pesticides on whitefly predators and parasitoids so that strategies might be developed to conserve natural enemies and encourage their use in established whitefly IPM systems. In addition to looking at the compatibility of several conventional insecticides with natural enemies of citrus pests, the current study examined the impact of pesticides on two parasitoids of *B. tabaci* (*Eretmocerus eremicus* Rose & Zolnerowich and *Encarsia formosa* Gahan), which are important in the biological control of *Bemisia* spp. These two parasitoids can adequately protect a crop with moderate infestations of whiteflies if they establish early in the season. However, in general, management of whiteflies on cotton, *Gossypium hirsutum* L., has been heavily dependent on insecticide use.

Several species of *Gonatocerus* egg parasitoids are present in large numbers during the summer on citrus, attacking *H. vitripennis* eggs. *Aphytis melinus* DeBach, which parasitizes California red scale and is widely used for augmentative field releases, also was selected for testing for compatibility with various foliar insecticides. Earlier studies have evaluated the toxicity of several insecticides used for control of citrus pests to *A. melinus* and several chemicals were found to be compatible with this parasitoid (Phillips et al. 1983; Morse and Bellows 1986; Bellows and Morse 1988, 1993; Bellows et al. 1993). However, studies focusing on determination of toxic values through direct exposure of natural enemies of citrus pests and whiteflies to insecticides are limited, and those studies that are reported do not include some of the newer pesticides that have been introduced over the past 10 yr. Therefore, more recent evaluation of foliar insecticide toxicity against select natural enemies seems timely. Toward this end, the present laboratory study was conducted with a focus on two main objectives: 1) to assess the relative numbers of egg parasitoids of *H. vitripennis* present in citrus and surrounding crops in Riverside, CA; and 2) to compare the relative toxicities of seven foliar insecticides across a range of concentrations to determine LC_{50} values for four parasitoids of citrus pests and whiteflies. Relatively few researchers have measured the direct dose–mortality effects of insecticides against parasitoids. In addition, we were interested in determining to what degree these four species varied in their response to a range of pesticides so as to suggest how such work might be extrapolated to other parasitoids.

Materials and Methods

Assessment of Relative Numbers of Egg Parasitoids Attacking *Homalodisca* spp. To assess the relative numbers of different species of egg parasitoids of *Homalodisca* spp., leaves infested with egg masses of two *Homalodisca* species were collected from citrus (orange, *Citrus sinensis* L. 'Valencia' and lemon, *Citrus limon* Burm. f., 'Lupe') and willow (*Salix goodingii* Ball.) trees. The collection site was in the vicinity of Fields five (willow) and seven (citrus) at Agricultural Operations at the University of California, Riverside, and the two fields are separated by only ≈150 m. Weekly collections of egg masses were made from July through November 2004 by turning over leaves of either citrus or willow until 100–130 egg masses from each type of tree were found. Egg masses collected from trees in this region are a mixture of two species of sharpshooter eggs, *H. vitripennis* and *Homalodisca lacerta* (Fowler), and they were not identified for this study, because *Gonatocerus* and *Ufens* spp. parasitize eggs of both species (Al-Wahaibi 2004). The portions of leaves containing the sharpshooter egg masses were excised from whole leaves to allow parasitoid emergence. Collection of emerging parasitoids was accomplished by placing the excised portions of leaves, with egg masses facing up, on 1.5% agar beds in 60-mm-diameter petri dishes, which were covered with lids to retain moisture. Four to six leaf sections per dish, each with an egg mass, were held for up to 3 wk to enable development of *H. vitripennis*, *H. lacerta*, or parasitoid embryos. As emergence occurred, inhabitants within each petri dish were removed and identified. Numbers of *Gonatocerus* spp. and *Ufens* spp. that emerged each day were recorded and transferred to screened cages to be

held for insecticide tests. At the time of this study, no attempt was made to differentiate whether more than one species of *Ufens* was present in this study region. However, a study published by Al-Wahaibi et al. (2005) subsequent to the initiation of this study, identified the presence of only two species of *Ufens* around the same study site, and named them *Ufens principalis* Owen and *Ufens ceratus* Owen (Hymenoptera: Trichogrammatidae). Therefore, results presented here as *Ufens* spp. include a mixture of these two species.

Counts of the number of emerging parasitoids were made daily for each petri dish, but they were ultimately summarized to produce one total for each dish. Total counts per dish were square root transformed to normalize variances and were subjected to a repeated measures multivariate analysis of variance (MANOVA) to determine the effects of tree species and time (sampling date) on densities of *Homalodisca* parasitoids. The majority of the *Gonatocerus* spp. that emerged and were collected were *G. ashmeadi*, with <1% of a second species, *Gonatocerus novifasciatus* Girault, present on any individual date. Therefore, attempts were not made to separate the two species for either toxicological tests or the parasitoid phenology analysis and the results reported in this study are reported as "*G. ashmeadi.*" A follow-up two-way analysis of variance (ANOVA) was used to determine whether significant tree species effects occurred for the two parasitoid genera studied.

Sources of Insects. Emerged *Gonatocerus* spp. from the above-mentioned field collections were the main source of test insects for the purpose of evaluating the toxicity of the tested insecticides. Citrus and willow in this area have no exposure to pesticides over the past 15 yr (except for herbicides used on weeds around the citrus). Insects that emerged each day in petri dishes were subsequently transferred to screened cages with citrus plants for maintenance for 3–4 d before toxicity tests were conducted. Honey drops were made available as a food source inside the cage and on the citrus leaves. Flying or actively feeding parasitoids were selected for each bioassay to minimize control mortality. Additionally, several field collections in citrus and willow trees were made in Riverside to obtain additional *Gonatocerus* spp. by using sweep net and bucket sampling devices (Castle et al. 2005). Collections were made at different times during summer as parasitoids became available. Insects collected were used in toxicological tests on the same day of collection.

Insectary reared *A. melinus* were obtained from a commercial insectary, Foothill Agricultural Research, Inc., Corona, CA, for laboratory tests. Insects were shipped as 2–3-d-old emerged adults, they were fed honey, and they were used in toxicological tests on the day of delivery. This insectary collects *A. melinus* from the citrus groves around their insectary each fall, rears those insects for at least three generations, and then mixes them with the previous year's colony in an attempt to maintain genetic variability (although genetic persistence of the field collected insects in the mixed population has not been studied). Citrus in this area receives little use of any of the tested insecticides with the exception of ground chlorpyrifos sprays for ant control. Because the trees in this area are skirt-pruned, we think *A. melinus* exposure to ant spray residues would be minimal. Note, however, that citrus in this area of southern California receives relatively little pesticide use in contrast, for example, to citrus in the San Joaquin Valley (Morse et al. 2007). As might be expected, past studies have shown that *A. melinus* collected from areas with differing pesticide use histories can have very different susceptibility to commonly used pesticides (Rosenheim and Hoy 1986). Thus, we consider the tested *A. melinus* to represent a relatively "pesticide-susceptible" population of this parasitoid.

Both species of whitefly parasitoids, *E. eremicus* and *E. formosa*, were supplied as pupae protected in their host whitefly pupae by Syngenta Bioline Inc. (Oxnard, CA). Insects supplied by Syngenta Bioline in California were in culture for ≈5 yr. These parasitoids were originally maintained and shipped from their facility in the United Kingdom to California. The original culture of the two parasitoid species was initiated from collections in and around glasshouses in the United Kingdom. No details of previous exposure of these parasitoids to pesticides were available. These parasitized insects were obtained loose in a bottle with bran flakes. Insects emerged ≈2–3 d after shipment. Freshly emerged insects were tested with pesticides within 1–2 d of emergence. As with *A. melinus* tests, results obtained for both whitefly parasitoids might not be representative of what might result when testing field populations of these parasitoids that received high past pesticide exposure resulting in selection for reduced susceptibility to commonly used materials. We did not conduct comparative bioassays between insectary reared and field-collected insects.

Insecticides. Insecticides used for toxicological tests against parasitoids were selected because they are applied for control of insect pests on various agricultural crops including citrus, cotton, and vegetables. The following commercial formulations of seven insecticides were evaluated: acetamiprid (Assail 70 WP [70% active ingredient [AI] wettable powder], provided by DuPont Agricultural Products (Wilmington, DE) at the time this study was initiated; this material is currently marketed by Cerexagri, King of Prussia, PA); chlorpyrifos (Lorsban 4E [0.479 kg [AI]/liter emulsifiable concentrate], Dow AgroSciences LLC, Indianapolis, IN); bifenthrin (Capture 2 EC [0.240 kg [AI]/liter emulsifiable concentrate], FMC Corp., Philadelphia, PA); cyfluthrin (Baythroid 2 EC [0.240 kg [AI]/liter], Bayer CropScience, Kansas City, MO); fenpropathrin (Danitol 2.4 EC [0.288 kg [AI]/liter], Valent USA Corp., Walnut Creek, CA); pyriproxyfen (Esteem 0.86 EC [0.103 kg [AI]/liter], Valent USA Corp.); and buprofezin (Applaud 70 WP, Nichino America, Inc., Wilmington, DE). All insecticides were diluted with deionized water on the day of testing to make a series of concentrations. At least five concentrations of each insecticide plus a water control were used to obtain dosage–mortality data for each parasitoid.

Bioassay Technique. Responses of *G. ashmeadi*, *A. melinus*, *E. eremicus*, and *E. formosa* to various concentrations of seven contact insecticides were determined

using a petri dish bioassay technique that was adapted from previous studies with glassy-winged sharpshooter (Prabhaker et al. 2006). Agar beds were layered in the base of each petri dish for maintenance of test leaves of citrus or cotton for up to 7 d. Freshly cut leaf discs of citrus (for *G. ashmeadi* and *A. melinus*) and cotton (for the two whitefly parasitoids), sized to fit in the base of each petri dish, were dipped in each concentration of each insecticide for 30 s. Citrus leaves were chosen for bioassay tests with *G. ashmeadi* and *A. melinus*, and cotton leaves were chosen for whitefly parasitoids to mimic the host plants they are most commonly found on in agricultural settings. In this way, we hoped to reduce potential variability in insect responses to insecticides had they been placed on an unnatural host substrate. Treated leaf discs were allowed to dry for 1 h, and were then placed on the agar beds. Each bioassay included at least five replications of each concentration for each insecticide. For exposure to treated leaves, 10 *G. ashmeadi*, 20 *E. eremicus* (except on one of the three test dates, only 15 wasps were used per petri dish), and 20 *E. formosa* per replicate were placed in each petri dish by using an aspirator. Approximately 25–50 adults of *A. melinus* were aspirated gently and released through an opening in the top of the petri dish onto treated leaves held in the dish. With all four parasitoids, a thin strip of honey was smeared on the underside of each petri dish lid to provide food for the insects. Each bioassay was replicated at least three times on each of three dates. Mortality of insects was recorded at 24 h for chlorpyrifos due to higher toxicity with all four species and at 48-h intervals for acetamiprid and the pyrethroids. Observations with the two IGRs (buprofezin and pyriproxyfen) were taken at 96 h posttreatment for all species due to the concern that mortality impacts might be delayed. The ambient temperature in the laboratory during all experiments ranged between 24 and 27°C.

Statistical Analysis. The LC_{50}, 95% fiducial limits (FL), and slopes of the regression lines were estimated by probit analysis using POLO (Russell et al. 1977, LeOra 1987). Differences in LC_{50} values were considered significant between pesticides and insect species if there was no overlap of 95% fiducial limits. The POLO probit analysis model generates a "g" factor to indicate the level of fit for analyzed data. With almost all good sets of data, g will be substantially smaller than 1.0 and seldom >0.4 (Russell et al. 1977).

Results

Assessment of Relative Numbers of Egg Parasitoids of *Homalodisca* spp. The emergence of *G. ashmeadi* and *Ufens* spp. from *Homalodisca* spp. egg masses was recorded from weekly samples of citrus and willow leaves collected in early July through mid-October 2004. Some parasitoid emergence was observed from every egg mass sample period, but peak emergence for both parasitoid genera and on both host plants occurred during July (Fig. 1). In citrus, as expected given two summer generations of sharpshooters, a bimodal emergence pattern was observed for *G. ashmeadi* with a second peak of emergence in late August before levels tapered off by mid-September. This second peak was not seen with *G. ashmeadi* on willow or with *Ufens* spp. on either host plant. The MANOVA results revealed highly significant effects of collection date ($F_{11,576} = 14.2$; $P < 0.0001$), tree species ($F_{1,576} = 219.6$; $P < 0.0001$), and an interaction between tree and date ($F_{11,576} = 18.4$; $P < 0.0001$) for both parasitoid genera. The profile of *G. ashmeadi* emergence was similar on both citrus and willow (Fig. 1), although significantly higher numbers were recovered from citrus ($F_{1,576} = 165$; $P < 0.0001$). For *Ufens* spp., significantly higher numbers emerged from egg masses on willow leaves compared with citrus leaves ($F_{1,576} = 80.3$; $P < 0.0001$), because relatively few *Ufen* spp. were collected on citrus season-long (Fig. 1).

Pesticide Toxicity across the Four Parasitoid Species. Control mortality was always below 10% with all four species of parasitoids, and the g factor for all data sets was ≤0.38 (Tables 1–4). *A. melinus* (range, 0.5–0.8 mm; mean length ± SD, 0.6 ± 0.10 mm; $n = 20$) and *E. formosa* (0.6–1.0 mm; 0.7 ± 0.13 mm; $n = 40$) were the smallest parasitoids tested followed by *E. eremicus* (0.8–1.1 mm; 0.95 ± 0.12 mm; $n = 40$), and then the relatively larger *G. ashmeadi* (1.1–1.8 mm; 1.5 ± 0.22 mm; $n = 40$). Consistently, with all seven pesticides, *A. melinus* was the most susceptible parasitoid of the four species tested (Tables 1–4). The other three parasitoids responded similarly to the tested organophosphate (chlorpyrifos) and all three pyrethroids (bifenthrin, cyfluthrin, and fenpropathrin) based on overlap of 95% fiducial limits. With the remaining three pesticides (acetamiprid, buprofezin, and pyriproxyfen), the order of parasitoid species susceptibility varied with pesticide. *G. ashmeadi* was most susceptible to acetamiprid followed by *E. eremicus* and then *E. formosa*. *E. formosa* and *E. eremicus* were more susceptible to buprofezin than was *G. ashmeadi*, but *G. ashmeadi* and *E. eremicus* were more susceptible to pyriproxyfen than was *E. formosa*. With the two IGRs buprofezin and pyriproxyfen and across all four parasitoid species, no changes in morphological appearance were noticed among either live or dead parasitoids, suggesting that the IGRs exhibited only acute toxicity. Note, however, that we did not evaluate possible sublethal impacts on parasitoid fecundity.

Toxicity Tests with *A. melinus*. *A. melinus* was most sensitive to leaf residues of chlorpyrifos, bifenthrin, and acetamiprid. The LC_{50} values across the seven pesticides ranged from 0.0008 μg (AI)/ml (chloryprifos) to 0.764 μg (AI)/ml (buprofezin), a 955-fold range, compared with a much larger range in LC_{50} values for the other three parasitoids (see below). The seven pesticides can be ranked from high-to-low toxicity to *A. melinus* (based on LC_{50} values) as (chlorpyrifos, [bifenthrin), (acetamiprid], cyfluthrin, fenpropathrin) > pyriproxyfen > buprofezin where () or [] indicate overlap of 95% fiducial limits. Given that they are IGRs, it is not surprising that buprofezin and pyriproxyfen were the least toxic pesticides tested against *A. melinus* despite observations being recorded at 96 h versus 24 or 48 h with the other pesticides.

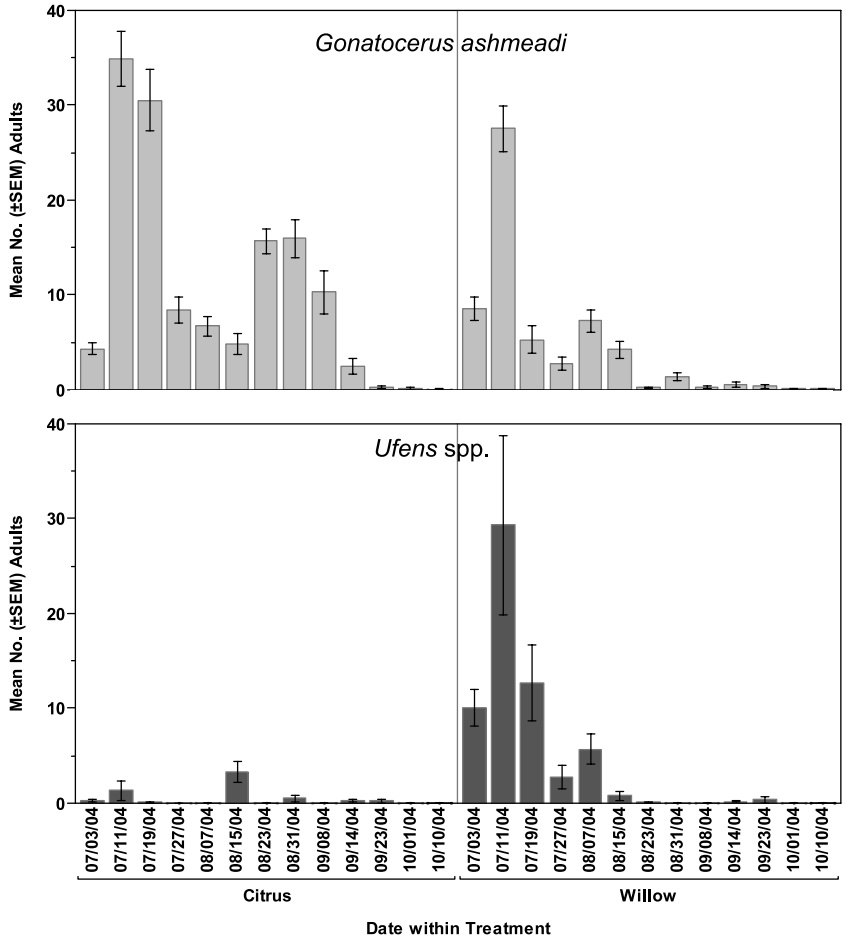

Fig. 1. Seasonal occurrence of *G. ashmeadi* and *Ufens* spp. in cultivated blocks of citrus and willow based on emergence from *Homalodisca* spp. egg masses. One outlier sample (not depicted) of 203 *Ufens* spp. occurred on 11 July 2005 in willow.

Toxicity Tests with *G. ashmeadi*. The large range of toxicity to *G. ashmeadi* across all tested pesticides (buprofezin LC_{50}/chlorpyrifos LC_{50} = 52,587) and in particular, within the pyrethroid chemistry (fenpropathrin LC_{50}/bifenthrin LC_{50} = 16,688), was remarkable, although the ranking of the seven tested pesticides from high-to-low toxicity was similar to that observed with *A. melinus*. With *G. ashmeadi*, this order was chlorpyrifos, bifenthrin > cyfluthrin, acetamiprid > (fenpropathrin, [pyriproxyfen), buprofezin]. Among the pyrethroids, fenpropathrin was much less toxic than either of the other two pyrethroids for three of the parasitoids (range of toxicity was 8,032 with *E. formosa*, 10,129 with *E. eremicus*, and 16,688 with *G. ashmeadi*) but not with *A. melinus* (range in pyrethroid LC_{50} values was only 10-fold). The tested

Table 1. Toxicity of various insecticides to adult *A. melinus* by using a petri dish bioassay

Insecticide class	Compound	Exposure time (h)	*n*	Slope ± SE	LC_{50} (μg [AI]/ml) (95% FL)[a]	χ^2 (df)	g (0.95)
Organophosphate	Chlorpyrifos	24	4,148	1.4 ± 0.05	0.0008 (0.0006–0.001) a [a]	11.06 (4)	0.02
Pyrethroid	Bifenthrin	48	4,117	1.8 ± 0.08	0.001 (0.0009–0.003) ab [a]	6.9 (4)	0.05
	Cyfluthrin	48	3,683	1.6 ± 0.07	0.007 (0.004–0.010) c [a]	14.4 (4)	0.12
	Fenpropathrin	48	4,140	1.2 ± 0.04	0.010 (0.004–0.019) c [a]	12.4 (4)	0.09
Neonicotinoid	Acetamiprid	48	3,257	1.9 ± 0.21	0.005 (0.002–0.011) bc [a]	5.6 (4)	0.11
IGR	Buprofezin	96	4,531	3.0 ± 0.09	0.764 (0.575–0.968) e [a]	7.29 (4)	0.09
	Pyriproxyfen	96	3,767	2.9 ± 0.11	0.421 (0.330–0.510) d [a]	13.9 (4)	0.06

[a] LC_{50} values for *A. melinus* followed by the same letter are not significantly different based on overlap of 95% FL across pesticides. LC_{50} values followed by the same letter within brackets are not significantly different for a particular pesticide, across the four tested parasitoid species (i.e., across Tables 1–4).

Table 2. Comparison of the toxicity of insecticides to adult *G. ashmeadi* by using a petri dish bioassay

Insecticide class	Compound	Exposure time (h)	*n*	Slope ± SE	LC_{50} (μg [AI]/ml) (95% FL)[a]	χ^2 (df)	g (0.95)
Organophosphate	Chlorpyrifos	24	2,106	1.3 ± 0.06	0.006 (0.004–0.010) a [b]	9.5 (4)	0.09
Pyrethroid	Bifenthrin	48	1,006	1.1 ± 0.07	0.010 (0.004–0.019) a [b]	5.2 (4)	0.23
	Cyfluthrin	48	1,215	1.6 ± 0.11	0.067 (0.034–0.121) b [b]	12.1 (4)	0.22
	Fenpropathrin	48	1,554	2.6 ± 0.33	166.88 (126.45–497.60) cd [b]	8.7 (4)	0.38
Neonicotinoid	Acetamiprid	48	1,744	1.0 ± 0.04	0.134 (0.026—0.489) b [b]	5.0 (4)	0.23
IGR	Buprofezin	96	1,804	2.7 ± 0.13	315.52 (229.28–406.67) d [c]	13.04 (4)	0.11
	Pyriproxyfen	96	1,794	2.3 ± 0.12	132.53 (70.19–209.97) c [c]	13.8 (4)	0.05

[a] LC_{50} values for *G. ashmeadi* followed by the same letter are not significantly different based on overlap of 95% FL across pesticides. LC_{50} values followed by the same letter within brackets are not significantly different for a particular pesticide, across the four tested parasitoid species.

neonicotinoid (acetamiprid) seemed harmless during the first 24-h exposure period to all four parasitoids (data not shown); however, with a longer exposure of 48 h, the four parasitoids varied greatly in their susceptibility to this material (compare Tables 1–4). Based on LC_{50} values, *G. ashmeadi* was 413 and 315 times less sensitive to buprofezin and pyriproxyfen, respectively, compared with *A. melinus.*

Toxicity Tests with *E. eremicus* and *E. formosa.* Ranking of pesticide toxicity from high to low with the two whitefly parasitoids followed the same general pattern as with *G. ashmeadi* (Tables 3 and 4) (*E. eremicus:* chlorpyrifos, bifenthrin > cyfluthrin > pyriproxyfen, acetamiprid, fenpropathrin, buprofezin; *E. formosa:* chlorpyrifos, bifenthrin > cyfluthrin > acetamiprid > pyriproxyfen > fenpropathrin, buprofezin). One key difference, however, was the relatively low toxicity of acetamiprid to *E. formosa* and in particular, *E. eremicus.* Whereas acetamiprid was much more toxic to *G. ashmeadi* than were the two IGRs (buprofezin: 2,355-fold; pyriproxyfen: 989-fold), this range in toxicity was only 0.9 (*E. eremicus:* pyriproxyfen LC_{50}/acetamiprid LC_{50}) to 8.2-fold (*E. formosa:* buprofezin LC_{50}/acetamiprid LC_{50}) with the two whitefly parasitoids.

Discussion

The method that was used for estimating the relative numbers of egg parasitoids of *H. vitripennis* yielded numbers of parasitoids that emerged in petri dishes from citrus and willow collections, but it did not correct for differential mortality before emergence across plant or parasitoid species. Thus, it is uncertain why emergence levels of *Ufens* spp. differed dramatically between the two plant species compared with *Gonatocerus* spp. Similar levels of *Gonatocerus* spp. emergence on citrus and willow indicate that *Homalodisca* spp. eggs were present on both hosts, but *Ufens* spp. apparently preferentially oviposited on willow based on their limited emergence from egg masses collected from citrus. These results were similar to those reported by Al-Wahaibi (2004).

Emergence data showed that four species of egg parasitoids, *G. ashmeadi*, *G. novifasciatus*, *U. ceratus*, and *U. principalis* were recovered from the *Homalodisca* egg masses held within the petri dishes. The majority of the parasitoids of *Gonatocerus* spp. were *G. ashmeadi* with <1% *G. novifasciatus.* No attempt was made to differentiate the two species of *Ufens* because at the time of this work they were unnamed. Large numbers of parasitoids emerged during the summer compared with the fall. These parasitoids are important in suppressing populations of glassy-winged sharpshooters (Al-Wahaibi 2004); however, studies on the impact that insecticides have on these parasitoids are limited.

The period of parasitoid emergence observed between July and October coincides with the period of peak *H. vitripennis* activity in southern California. Adult emergence from the spring generation of *H. vitripennis* nymphs in citrus begins in mid-June and peaks by mid-July (Castle et al. 2005). Egg laying by the new generation of adults begins soon after emergence and, in the current study, eggs were conspicuously present on both citrus and willow. Although

Table 3. Comparison of toxicity of insecticides to adult *E. eremicus* by using a Petri dish bioassay

Insecticide class	Compound	Exposure time (h)	*n*	Slope ± SE	LC_{50} (μg [AI]/ml) (95% FL)[a]	χ^2 (df)	g (0.95)
Organophosphate	Chlorpyrifos	24	1,782	1.6 ± 0.08	0.012 (0.008–0.018) a [b]	14.9 (4)	0.08
Pyrethroid	Bifenthrin	48	1,012	1.2 ± 0.09	0.011 (0.002–0.030) a [b]	5.5 (4)	0.32
	Cyfluthrin	48	1,219	1.5 ± 0.11	0.096 (0.040–0.222) b [b]	7.7 (4)	0.31
	Fenpropathrin	48	1,533	1.5 ± 0.15	111.42 (66.81–195.58) c [b]	10.3 (4)	0.31
Neonicotinoid	Acetamiprid	48	1,475	1.1 ± 0.11	108.27 (43.12–221.93) c [d]	11.2 (4)	0.36
IGR	Buprofezin	96	1,801	1.0 ± 0.10	120.41 (90.72–205.11) c [b]	12.7 (4)	0.25
	Pyriproxyfen	96	1,819	2.8 ± 0.14	95.56 (66.06–126.28) c [c]	16.31 (4)	0.14

[a] LC_{50} values for *E. eremicus* followed by the same letter are not significantly different based on overlap of 95% FL across pesticides. LC_{50} values followed by the same letter within brackets are not significantly different for a particular pesticide, across the four tested parasitoid species.

Table 4. Comparison of the toxicity of seven insecticides to adult *E. formosa* by using a petri dish bioassay

Insecticide class	Compound	Exposure time (h)	*n*	Slope ± SE	LC_{50} (μg [AI]/ml) (95% FL)[a]	χ^2 (df)	g (0.95)
Organophosphate	Chlorpyrifos	24	1,807	1.2 ± 0.06	0.017 (0.009–0.029) a [b]	8.9 (4)	0.08
Pyrethroid	Bifenthrin	48	1,185	1.4 ± 0.14	0.015 (0.005–0.025) a [b]	7.5 (4)	0.24
	Cyfluthrin	48	1,324	1.6 ± 0.10	0.063 (0.035–0.104) b [b]	16.8 (5)	0.12
	Fenpropathrin	48	1,997	1.0 ± 0.06	120.48 (74.01–233.59) e [b]	7.4 (4)	0.06
Neonicotinoid	Acetamiprid	48	1,522	2.8 ± 0.17	12.02 (9.93–14.52) c [c]	3.8 (4)	0.04
IGR	Buprofezin	96	1,817	1.0 ± 0.12	98.15 (68.72–152.07) e [b]	12.9 (4)	0.08
	Pyriproxyfen	96	1,815	2.7 ± 0.16	60.51 (54.90–65.99) d [b]	12.4 (4)	0.01

[a] LC_{50} values for *E. formosa* followed by the same letter are not significantly different based on overlap of 95% FL across pesticides. LC_{50} values followed by the same letter within brackets are not significantly different for a particular pesticide, across the four tested parasitoid species.

percentage of parasitism was not recorded for the egg masses collected from citrus and willow, overall parasitism during this period is known to be consistently high (Triapitsyn et al. 1998, Hoddle 2005).

Of the four parasitoids studied, all but *A. melinus* are endoparasitoids and even the ectoparasitic *A. melinus* is protected from direct pesticide exposure during the immature stages because eggs are laid under the armored scale cover where both larvae and pupae also develop. Several factors affect the degree to which adult parasitoids are exposed to a pesticide treatment. These include whether they are present in the field during the pesticide application and are directly exposed to the spray, the concentration of pesticide that is applied, the type of spray coverage that is used (e.g., on large citrus trees, outside coverage can be much different from thorough coverage targeted to wet scale insects on interior wood), how persistent residues of a particular pesticide are, and to what degree and where the parasitoids search over plant material containing residues, thus affecting the amount of pesticide they come in contact with. Our study focused on the nontarget effects of commonly used foliar insecticides on four parasitoids as measured by direct toxicity of surface pesticide residues after the spray had dried. Thus, we did not consider how persistent field residues of a particular pesticide might be in affecting parasitoid mortality.

Another factor that affected our results but was not quantified in our study was the inherent susceptibility of the parasitoid strains we tested with respect to their past exposure to some or all of the pesticides they were exposed to. Rosenheim and Hoy (1986) showed that *A. melinus* collected from areas with differing pesticide use histories had differing susceptibility to five commonly used pesticides. With *G. ashmeadi* and *A. melinus*, we intentionally chose parasitoid strains for evaluation with limited past exposure to pesticides. Results might have been different for some of our tested pesticides (e.g., chlorpyrifos, which has been in use on citrus in California for >30 yr) if we had chosen strains for evaluation with heavy past exposure to insecticides. By contrast, the two whitefly parasitoids, *E. eremicus* and *E. formosa*, were in culture for ≈5 yr with no fresh infusion of field-collected insects into the colonies. Results presented here for the whitefly parasitoids might have varied if the insects had recent exposure to any of the insecticides tested in this study.

Significant variation in susceptibility to tested insecticides was observed among the natural enemies examined in this study. *A. melinus* was significantly more susceptible to all seven tested pesticides in comparison with the other three parasitoid species. Body size ranged from 0.5 to 0.8, 0.6 to 1.0, 0.8 to 1.1, and 1.1 to 1.8 mm for *A. melinus*, *E. formosa*, *E. eremicus*, and *G. ashmeadi*, respectively. The lower susceptibility of *A. melinus* might be expected due to its somewhat smaller size, but this pattern did not hold for the other three parasitoids. The three parasitoids exclusive of *A. melinus* showed similar LC_{50} values with chlorpyrifos and the three pyrethroids, and only with buprofezin did the largest species (*G. ashmeadi*) show a significantly higher LC_{50}.

Similar trends in toxicity among the seven pesticides were observed with the four species of parasitoids. Based on LC_{50} values, all four parasitoids were most sensitive to chlorpyrifos followed by bifenthrin and generally the third most toxic material was cyfluthrin (all species except *A. melinus* where it was fourth). All four parasitoids were least sensitive to buprofezin, which was usually followed (three of four cases) by fenpropathrin. Pyriproxyfen also generally ranked low in toxicity among the seven pesticides.

There were several differences in how the four parasitoids responded to the tested pesticides. As mentioned, *A. melinus* was statistically more susceptible to all seven pesticides. What was also notable, however, was its relatively low susceptibility to the two IGRs, buprofezin and pyriproxyfen, compared with any of the other three parasitoid species (the second most susceptible species was *E. formosa* with 128.5 and 143.7-fold higher LC_{50} values, respectively). Both IGRs are active primarily against the immature stages of insects, causing an inhibition of chitin synthesis (pyriproxyfen) and interruption of the physiological processes involved during a molt (buprofezin) (Uchida et al. 1985, Ishaaya et al. 1988, Ishaaya and Horowitz 1992, Miyamoto et al. 1993, Toscano et al. 2001). Several studies have reported good selectivity of IGRs toward various natural enemies (Hoddle et al. 2001; Naranjo 2001; Naranjo et al. 2003, 2004). Results from other studies indicate that residues of buprofezin had negligible impacts on foraging adult *Eretmocerus* spp. (Jones et al. 1995). Hoddle et al. (2001) reported on the compatibility of buprofezin with *E. eremicus* in relation to their ability to control

B. argentifolii. Similarly, pyriproxyfen leaf residues did not affect survivorship of *Encarsia* adults (Liu and Stansly 1997). However, in some cases, IGRs may not be as selective as expected. For example, sublethal effects including reduced longevity, reduced fecundity, and deformed wings manifested as a result of buprofezin and pyriproxyfen treatments in surviving aphelinid parasitoids (Liu and Stansly 1997, Jones et al. 1998). Based on our results, the relatively low susceptibility of adult *A. melinus* to the two IGRs warrants further study.

A second major difference in pesticide toxicity among the four parasitoids was the large range in acetamiprid LC_{50} values (0.005, 0.134, 12.02, and 108.27 with *A. melinus*, *G. ashmeadi*, *E. formosa*, and *E. eremicus*, respectively). Although our work was with parasitoids, acetamiprid has been shown to be nondetrimental to certain predators (Fitzgerald 2004), whereas it was toxic to others (Ruberson et al. 2004, Naranjo and Akey 2005). Also notable in our study with the three pyrethroids was the much lower toxicity of fenpropathrin to three of the four parasitoids (all except *A. melinus*). We have no good explanation for why bifenthrin and cyfluthrin were relatively toxic to *G. ashmeadi*, *E. eremicus*, and *E. formosa* but fenpropathrin was not. This finding also warrants further research.

With relevant information, the adverse impact of insecticides on natural enemies can be estimated and if there is a choice between several products, one can select the material that is likely to have the least impact. For example, cyfluthrin, fenpropathrin, and acetamiprid are the three common foliar sprays used for suppression of glassy-winged sharpshooter on citrus in California (N.C.T., unpublished data), and the lowest recommended use rate for each material is 15.0, 239.6, and 44.6 μg (AI)/ml, respectively (Grafton-Cardwell et al. 2005). If one compares the LC_{50} with *A. melinus* as a percentage of these use rates, one obtains 0.047, 0.004, and 0.011%, respectively. All three of these pesticides result in persistent residues on citrus (>3 wk; J.G.M., unpublished data), and this persistence, coupled with the low ratio of LC_{50} values to use rates, is consistent with the observation that glassy-winged sharpshooter treatments are incompatible with *A. melinus* augmentative releases (Morse and Luck 2003, Morse et al. 2007).

If, however, one has a citrus grove in which *G. ashmeadi* plays a large role in reducing glassy-winged sharpshooter levels via egg parasitism, but a glassy-winged sharpshooter treatment is needed, then our data clearly show that fenpropathrin is the best control option. Comparing *G. ashmeadi* LC_{50} values as a percentage of the lowest recommended use rate, one obtains 0.45, 69.64, and 0.30% with cyfluthrin, fenpropathrin, and acetamiprid, respectively. Knowledge of common pesticide use rates for particular pest species could be used with the data presented herein to conduct similar analyses in other situations where conservation of one or more of the four parasitoids that we tested is considered important.

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