COMPATIBILITY OF SELECT INSECTICIDES WITH NATURAL ENEMIES OF THE GLASSY-WINGED SHARPSHOOTER AND OTHER PESTS

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ABSTRACT
The toxicity of two insect growth regulators (IGRs), buprofezin and pyriproxyfen; three neonicotinoids, acetamiprid, imidacloprid and thiamethoxam; and three conventional insecticides, bifenthrin, fenpropathrin, and chlorpyrifos; were tested in the laboratory for compatibility with egg parasitoids (*Gonatocerus ashmeadi*, *Gonatocerus triguttatus*) of glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* and against *Aphytis melinus* (*A. melinus*), an endoparasitoid of armored scale insects found on citrus in California. Most of the selected insecticides tested are used against GWSS and other pests on citrus and grapes. Survivorship of adult parasitoids on citrus leaves with residues of these insecticides (within Petri dishes) was determined after 24, 48 and 72h. Our results indicated that a number of insecticides tested are toxic to the egg parasitoids, *Gonatocerus* spp., as well as to *A. melinus* under laboratory conditions. Results from this study allow ranking of the eight insecticides based on their increasing toxicity as follows for *Gonatocerus* spp.: fenpropathrin > pyriproxyfen > buprofezin > imidacloprid > thiamethoxam > acetamiprid > bifenthrin > chlorpyrifos. All insecticides tested were highly toxic to *A. melinus*. In additional studies, higher concentrations of imidacloprid and thiamethoxam were found to be toxic over time to two predators, *Geocoris punctipes* and *Orius insidiosus*. Results from laboratory studies suggest that both systemics, imidacloprid and thiamethoxam, may not preserve these parasitoids as much as expected. To understand these bioassay results with the two systemics, further studies included the quantification of imidacloprid and thiamethoxam in both the parasitoids and the test citrus leaves by ELISA kits. Results indicated the presence of varying levels of these two compounds in the parasitoids as well as in citrus leaves suggesting that although parasitoids are non-plant feeders, they were exposed to the systemic chemicals. Further research is underway to determine how the parasitoids are exposed to the two systemics.

Relative numbers of GWSS and its natural enemies from Riverside were determined using two sampling methods, collection of fresh GWSS egg masses on two host plants (citrus and willow) and using yellow sticky cards. Relative numbers of the pest (GWSS) and beneficials (*Gonatocerus ashmeadii*, *Gonatocerus triguttatus* and *Ufens* spp.) varied based on the sampling method. Seasonal patterns were obvious with higher parasitoid numbers in summer compared to fall collections with a parasitism rate at >90% based on the egg collection method. Few predators were observed on the yellow sticky cards.

INTRODUCTION
Many insecticides that have been used to suppress GWSS populations appear to be quite effective (Akey et al. 2001, Bethke et al. 2001, Prabhaker et al. 2005). However, there is little information available on the long-term impact that different control measures are having on GWSS populations and its natural enemies on citrus and grapes. Although biological control has been the foundation of citrus IPM in California for many years, it is now threatened by the arrival of several new pests and greater use of non-selective insecticides to control these new pests. In particular, the recent registration of new insecticides for use on citrus is creating uncertainty over the longer-term impact they may have on established IPM programs (Grafton-Cardwell and Gu 2003). It is therefore essential to attain greater understanding of the various control options for GWSS in citrus and how they can be best integrated with existing, successful management programs. The overall objective of this research proposal is to help determine IPM compatible management tactics by focusing on chemical control being used against GWSS and by evaluating their impact upon several important biological control agents. To address this goal, the impact of various insecticides including those that are used against GWSS and other pests on citrus was assessed against GWSS egg parasitoids and *A. melinus* using two bioassay techniques, a Petri dish assay and systemic uptake bioassay (Prabhaker et al. 2005). The insecticides evaluated in this study included three conventional compounds, chlorpyrifos, bifenthrin, and fenpropathrin; two IGRs, pyriproxyfen and buprofezin; and three neonicotinoids, acetamiprid, imidacloprid, and thiamethoxam.

OBJECTIVES

1. Monitor citrus orchards in Riverside Co., Ventura Co. and Coachella Valley to determine the relative abundance of select parasitoids and predators before and after treatment
2. Evaluate select foliar and systemic GWSS pesticides used on citrus and grapes for their impact on GWSS egg parasitoids such as *Gonatocerus ashmeadi* and *Gonatocerus triguttatus* as well as other parasitoids in the system such as *A. melinus*.
3. Determine if honeydew produced by homopteran insects on citrus can be contaminated with systemic insecticides such as imidacloprid and thiamethoxam.
4. Determine the impact of imidacloprid and thiamethoxam residues within plant or within plant-feeding intoxicated insects, on the survivorship of *Gonatocerus ashmeadi* (*G. ashmeadi*) , *Gonatocerus triguttatus* (*G. triguttatus*), and *A. melinus.*

RESULTS

Monitoring

Two sampling methods, collection of fresh GWSS egg masses and use of yellow sticky cards were used to survey the relative abundance of natural enemies including parasitoids and predators that are active against GWSS and other pests in citrus orchards in Riverside Co. The survey was initiated in July 2004 and continued through November 2004. Subsequent surveys were conducted from June 2005 through September 2005. Yellow sticky traps were posted at multiple locations within each orchard for continuous monitoring of GWSS and natural enemies and were changed once a week. Significant differences were found in the numbers of parasitoids collected from GWSS egg masses compared to the numbers collected on sticky traps. Data collected from the sticky traps showed a significantly lower number of parasitoids relative to GWSS (<10%). In contrast, direct observations of the numbers of parasitoids that emerged from egg masses were much higher than GWSS immatures, thus showing a higher rate of parasitism using this method. Numbers of parasitoids were much higher in summer than in early fall. This trend was observed by both monitoring techniques. The majority of the GWSS egg masses collected in September 2005 from Riverside Co. were parasitized by *Gonatocerus* spp. (mixed populations of *G. ashmeadi* and *G. triguttatus)*. These results provided a picture of relative GWSS activity within each orchard in addition to providing limited information on the activity and abundance of natural enemies. Our results indicate that both techniques were necessary to assess the activity of beneficials because of the significant differences obtained in relative numbers based on the monitoring technique. Predators were observed in lower numbers compared to parasitoids.

Toxicological responses of *Gonatocerus* spp.

Bioassay responses of *Gonatocerus* spp. under laboratory conditions to the two IGRs by the Petri dish method generated LC_{50}s that were higher than with the neonicotinoids (Table 1). Pyriproxyfen showed low toxicity to the parasitoids after 96h of exposure, when exposed to the insects as leaf residues in the Petri dish at higher concentrations. Similar results were observed with buprofezin. Toxicity was quite low for the first 96h but increased over time. The most toxic neonicotinoid to *Gonatocerus* spp. was acetamiprid as indicated by a low LC_{50} of 0.034 μg/mL, followed by thiamethoxam at 0.312 μg/mL, a 9-fold difference between the two compounds. A larger difference in toxicity was observed between the two systemics, imidacloprid and thiamethoxam. Both compounds were toxic to *Gonatocerus* spp. but thiamethoxam was more toxic after 24h exposure as indicated by the lower LC_{50} compared to imidacloprid which exhibited a higher LC_{50} of 11.06 μg/mL at 48 h observation, which decreased further after 72h exposure. Imidacloprid was not toxic to these insects at tested doses during the first 24h of exposure. A notable difference in responses of the parasitoids to fenpropathrin was observed using the Petri dish technique (Table 1). The LC_{50}s determined were the highest among all the insecticides tested and indicated that fenpropathrin was less toxic to *Gonatocerus* spp in contrast to the lower LC_{50} value observed with bifenthrin. This is a major difference in activity for the same class of chemistry. The most toxic compound among all classes of chemistry evaluated was chlorpyrifos as seen by the low LC_{50}.

Toxicological responses of *A. melinus*

Results with *A. melinus* were quite different from those obtained with *Gonatocerus* spp. (Table 2). The LC_{50} values were much lower to most of the compounds for *A. melinus* indicating that these insects were quite susceptible to these insecticides. In some of the bioassays, *A. melinus* were so susceptible that mortality averaged 90-95% for most of the tested concentrations. These results suggest that *A. melinus* is more susceptible than *Gonatocerus* spp. as measured by the Petri dish technique. In general, *A. melinus* mortality was high even at 24h compared to the GWSS egg parasitoids.

Toxicological responses of predators

Susceptibility of two predators, *Geocoris punctipes* and *Orius insidiosus*, to imidacloprid and thiamethoxam was also evaluated. Both systemic compounds were toxic to these predators (Table 3), although these results are not surprising because *Geocoris* spp. and *Orius* spp. are plant feeders at times. The LC_{50} values were low to both compounds but only after 72 or 96h exposure.

Quantification of imidacloprid and thiamethoxam

Quantitative estimates of imidacloprid and thiamethoxam were made using ELISA kits to determine if the mortality observed was correlated to levels of the compounds in leaves. Variable levels of imidacloprid were detected in the homogenates of both insects and bioassay leaves as well as in the surface leaf washes of citrus leaves. These results indicated that imidacloprid is present on treated leaves as well as in the insect. Similar results were observed with systemically applied thiamethoxam. These results will assist in interpretation of our bioassay results with systemic compounds.

Impact of residues of insecticides in honeydew

Results of this objective have not been completed. When tests were conducted to expose *A. melinus* by contact with surface residues of citrus leaves from trees that were treated with imidacloprid two years prior in a Riverside Co. location, results showed high mortality (92%) of *A. melinus*. This test was conducted to assess toxicity in general to *A. melinus* because these parasitoids will feed on available honeydew in citrus and not just on armored scales. If there are residues of treated compounds such as imidacloprid and/or thiamethoxam in honeydew, ELISA tests can determine if the systemic chemicals can be detected in honeydew produced by homopterans. Results were not conclusive because some of the insects showed mortality as high as 45% when confined on leaves from untreated trees. Future research plans will include more replications of this test.

Work in Progress

Work is in progress evaluating the impact of imidacloprid and thiamethoxam within plants on the survivorship of *Gonatocerus* spp. and *A. melinus*. Our preliminary results have shown that systemics have an impact on GWSS egg parasitoids and *A. melinus*. The potential for mortality caused by systemic insecticides in non-plant feeding insects such as parasitoids of GWSS needs to be evaluated further. The lethal effects on *Gonatocerus* spp. and *A. melinus* that occurred when exposed to systemically treated plant surfaces will be measured further by quantifying the titers of either imidacloprid or thiamethoxam within the leaf tissue as well as in GWSS eggs or scale nymphs in which parasitoids develop**.** In future tests, we will attempt to relate survivorship of parasitoids to the titers of either material within the treated leaf tissue or within GWSS eggs. The effect of imidacloprid and thiamethoxam treatments on *Gonatocerus* spp. will be studied during the second year of this research project.

CONCLUSIONS

Differences were observed in estimates of the relative numbers of natural enemies of GWSS using two monitoring methods. Numbers of parasitoids and rates of parasitism were much higher using the egg mass collection method versus the yellow sticky card technique. Both techniques showed seasonal differences in numbers of natural enemies, with higher levels in summer than in fall. This study helped fill the gap in knowledge regarding the effect of selected insecticides against natural enemies of GWSS. The work reported here investigated the toxicological effects of three neonicotinoids, imidacloprid (Admire), acetamiprid (Assail) and thiamethoxam (Platinum); two IGRs, buprofezin (Applaud) and pyriproxyfen (Esteem); two pyrethroids, bifenthrin (Capture) and fenpropathrin (Danitol); and an organophosphate, chlorpyrifos (Lorsban); against parasitoids of GWSS and *A. melinus*. Contrary to widespread assumption that systemic insecticides may not be toxic to natural enemies, our data showed that systemically applied imidacloprid and thiamethoxam were toxic to parasitoids that do not feed on plant tissue. Additionally, naturally occurring honeydew on citrus leaves may be toxic to *A. melinus*. These data will help determine the relative compatibility of particular insecticides to foraging natural enemies.

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Table 1. Toxicity of various insecticides to *Gonatocerus* spp.

Compound	Bioassay Technique	Exposure Time	# Tested	LC50 (µg/mL)
Buprofezin	Petri dish	72	232	115.21
Pyriproxyfen		24		102.78
Acetamiprid	Petri dish	24	267	0.062
Imidacloprid	Uptake	24	253	166.64
		48		11.06
Thiamethoxam	Uptake	24	295	0.312
Bifenthrin	Petri dish	24	198	0.034
		48	252	0.007
Fenpropathrin	Petri dish	24	248	323.30
		48		278.05
Chlorpyrifos	Petri dish	24	208	0.002

Table 2. Toxicity of various insecticides to *A. melinus*

Compound	Bioassay Technique	Exposure Time	# Tested	LC50 (µg/mL)
Buprofezin	Petri dish	24	812	0.215
Pyriproxyfen		24		0.436
Acetamiprid	Petri dish	24	823	0.017
Imidacloprid	Uptake	24	905	2.147
		48		0.416
Imidacloprid + honey		24	1564	0.0008
Thiamethoxam	Uptake	24	1695	0.044
Bifenthrin	Petri dish	24	738	0.001
Fenpropathrin	Petri dish	24	854	0.064
		48		0.008
Chlorpyrifos	Petri dish	24	578	0.0007

Table 3. Toxicity of two neonicotinoids to two predators using an uptake bioassay technique

Compound	Exposure time	# Tested	LC_{50} (µg/mL)
Orius insidiosus			
Imidacloprid	24	294	1.38
	72		0.018
Thiamethoxam	24	268	0.307
	72		0.007
Geocoris punctipes			
Imidacloprid	96	240	1.94
Thiamethoxam	96	250	5.39

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