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

Project Leaders:

Nick. C. Toscano
Department of Entomology
University of California
Riverside, CA 92521
nick.toscano@ucr.edu

Joseph. G. Morse
Department of Entomology
University of California
Riverside, CA 92521

Cooperators:

N. Prabhaker
Department of Entomology
University of California
Riverside, CA 92521

Steven Castle
ALARC
USDA-ARS
Maricopa, AZ 85239

S. Naranjo
ALARC
USDA-ARS
Maricopa, AZ 85239

T. J. Henneberry
ALARC
USDA-ARS
Maricopa, AZ 85239

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ABSTRACT

To evaluate the compatibility of insecticides that have both a broad and limited spectrum of activity against biological control agents, laboratory studies were carried out to compare the relative susceptibilities of seven foliar and two systemic insecticides against four common species of beneficial insects: *Aphytis melinus* Debach, *Gonatocerus ashmeadi* Girault, *Eretmocerus eremicus* Rose & Zolnerowich, and *Encarsia formosa* Gahan. Evaluations with systemic insecticides also included two species of predators, *Geocoris punctipes* Say and *Orius insidiosus* Say. Foliar insecticides were evaluated by a petri dish technique across a range of concentrations to measure their effect on direct mortality of the parasitoids. A systemic uptake bioassay technique was used to determine the toxicity of systemics against the six species of beneficials. Insecticides tested are used against citrus and agricultural pests, and included acetamiprid, imidacloprid, thiamethoxam (all 3 are neonicotinoids); chlorpyrifos, (organophosphate); bifenthrin, cyfluthrin, fenpropathrin (all 3 are pyrethroids); and buprofezin and pyriproxyfen (two insect growth regulators = IGRs). Chlorpyrifos, a conventional organophosphate insecticide with broad-spectrum activity, was consistently the most toxic pesticide to all four species of beneficial insects tested. Among the pyrethroids, fenpropathrin demonstrated lower toxicity to parasitoids compared with bifenthrin or cyfluthrin. Acetamiprid, although efficacious against GWSS, exhibited fairly selective toxic characteristics to *G. ashmeadi* and *E. eremicus* until four days post-treatment while being toxic to *A. melinus* within 24 h after treatment. *Aphytis melinus* was the most susceptible hymenopterous parasitoid to all test insecticides. Buprofezin and pyriproxyfen, with a relatively narrow spectrum of activity, were less toxic to the parasitoids. Imidacloprid has been considered to be relatively selective, with limited impact on parasitoids because of its systemic activity. However, results from this laboratory study suggest that both systemics, imidacloprid and thiamethoxam, may not be as selective against parasitoids as was expected. To understand the bioassay results with the two systemics, quantification of imidacloprid and thiamethoxam in both the parasitoids and the test citrus leaves was evaluated using ELISA kits. Additional studies are underway in the laboratory to determine how the parasitoids are exposed to these two systemic materials through various routes of exposure. Selectivity of these two compounds to natural enemies is also being examined under field conditions. The results presented here will provide pest managers with specific information on the degree to which the tested insecticides are likely to be compatible with various natural enemies.

INTRODUCTION

The current management plan for glassy-winged sharpshooter, (GWSS), *Homalodisca vitripennis*, includes the use of a number of insecticides that are quite effective (Akey et al. 2001, Bethke et al. 2001, Prabhaker et al. 2006). However, if selected insecticides are effective against GWSS while showing minimal impacts on beneficial insects, biological control can be maximized. There has been 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 species. In particular, the recent registration of new insecticides for use on citrus is creating uncertainty over the long-term impact they may have on established IPM programs (Grafton-Cardwell and Gu 2003). Therefore, there is a need for accurate assessment of the impact of agrochemicals on both GWSS and nontarget insects, including parasitoids and predators. Such information is 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 project is to help determine IPM compatible management tactics by focusing on chemical controls being used against GWSS and evaluating their impact upon several important biological control agents. To address this goal, the impact of selected insecticides including those that are used against GWSS and other pests on citrus was assessed against a number of common beneficial parasitoids including *G. ashmeadi* (an egg parasitoid of GWSS), *A. melinus* (an endoparasitoid of armored scale insects on citrus), *E. eremicus* and *E. formosa* (two whitefly parasitoids), and two predators, *G. punctipes* and *O. insidiosus*. The relative selectivity of insecticides was determined in the laboratory using two bioassay techniques, a petri dish bioassay for foliar insecticides and a systemic uptake bioassay for systemic insecticides

(Prabhaker et al. 2006). The insecticides evaluated in this study were selected on their basis of utility and potential use, which included four conventional compounds, chlorpyrifos, bifenthrin, cyfluthrin and fenpropathrin; two IGRs, pyriproxyfen and buprofezin; and three neonicotinoids, acetamiprid, imidacloprid, and thiamethoxam.

OBJECTIVES

1. Monitor citrus orchards in Riverside, 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 *G. ashmeadi* and *G. 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 *G. ashmeadi*, *G. triguttatus*, and *A. melinus.*

RESULTS

Objective 1. Assessment of relative numbers of egg parasitoids of *H.vitripennis*.

Relative numbers of different species of egg parasitoids of *Homalodisca* spp. were assessed through collection of leaf samples infested with egg masses. The estimation process was repeated on a weekly basis at the same sites and by collecting the same number of egg-infested leaves from two hosts, citrus and willow, for a period of five months. Results showed emergence of four species of egg parasitoids, *G. ashmeadi*, *G. novifasciatus, Ufens principalis*, and *U. ceratus*. The majority of the parasitoids of *Gonatocerus* spp. were *G. ashmeadi* whereas less than 1% of total collections were *G. novifasciatus*. Large number of parasitoids emerged during the summer months compared to the fall. 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.

In addition to the above-mentioned method, a survey for estimation of the relative abundance of natural enemies, including parasitoids and predators that are active against GWSS and other pests in citrus orchards in Riverside Co., was done using yellow sticky traps. 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. Large differences were found in the numbers of parasitoids collected on sticky traps compared to the numbers collected from GWSS egg masses in petri dishes. Data collected from the sticky traps showed a significantly lower number of parasitoids relative to GWSS (<10%). These results are in contrast to those obtained through direct observations of the numbers of parasitoids that emerged from egg masses in petri dishes, which were much higher than were GWSS immatures.

Objective 2. Toxicological responses of four species of parasitoids.

Our study has focused on non-target effects of commonly used insecticides for control of agricultural pests against beneficial insects as measured by direct toxicity. We compared the relative toxicities of seven contact insecticides and two systemics to a number of beneficial insects that are important in biological control of both citrus pests and whiteflies. Variation in susceptibility to different insecticides was observed among the different species of natural enemies. *A. melinus* appears to have a generally lower susceptibility to many insecticides tested compared to *G. ashmeadi* or *E. eremicus* (Table 1). Compared to *A. melinus*, responses of *G. ashmeadi* and *E. eremicus* were similar to the test chemicals as exhibited by lower sensitivities in general. However, some similarities in trends were also observed among the four species of parasitoids. For example, sensitivity to chlorpyrifos was highest among the seven contact insecticides for all beneficial species. Bioassay responses of *G. ashmeadi* 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). Although, differences in responses to certain pyrethroids were observed with respect to toxicity among the various species of natural enemies, fenpropathrin appeared to be less harmful to most of the beneficials compared to bifenthrin or cyfluthrin. A larger difference in toxicity was observed between the two systemics, imidacloprid and thiamethoxam against the parasitoids. Both compounds were toxic to *G. ashmeadi* but thiamethoxam was more toxic after 24 h exposure compared to imidacloprid which was not toxic to these insects at tested doses during the first 24 h of exposure. A follow-up study involving detection of imidacloprid using ELISA revealed variable amounts of this compound in insects even though they do not feed on plant tissue.

Evaluation of the susceptibility of two predators, *G. punctipes* and *O. insidiosus,* to imidacloprid and thiamethoxam revealed that both systemic compounds were toxic to these predators (Table 2). The LC_{50} values were low but only after 96 h exposure. These results are not surprising because *Geocoris* spp and *Orius* spp. will feed on plants.

Objectives 3 & 4. Impact of systemic compounds on parasitoid survival.

Work is on-going for objectives 3 and 4, evaluating the impact of imidacloprid and thiamethoxam within plants on the survivorship of *G. ashmeadi* 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 is being evaluated under field conditions. The lethal effects on *G. ashmeadi* and *A. melinus* that occur when exposed to systemically treated plant surfaces will be measured by determining the titers of both compounds 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. The effect of imidacloprid and thiamethoxam treatments on *G. ashmeadi* within GWSS eggs will be studied.

Tests are in progress to expose *A. melinus* to cottony cushion scale honeydew after exposure of the scale to systemic treatments of imidacloprid. Preliminary tests assessing dose-mortality responses of cottony cushion scale to three rates of imidacloprid applied to potted citrus have been initiated. The mortality of cottony cushion scale was determined at seven, 14 and 21 d post-treatment. Preliminary tests also included quantifying titres of imidacloprid in cottony cushion scale, leaf and stem extracts and honeydew using commercially available ELISA kits. Imidacloprid was detected variably in the insect, leaf and stem extracts and honeydew based on the rate applied. Higher levels were found in the leaf extracts within one week of treatment with lower titres detected in the insect and honeydew. Future tests will determine the impact if any, of concentrations of imidacloprid present in the honeydew on *A. melinus* and *H. covergens*. These tests will assess toxicity in general to parasitoids because these parasitoids will feed on available honeydew on citrus produced by cottony cushion scale. If there are residues of imidacloprid in honeydew, ELISA tests can detect the presence of systemic chemicals.

CONCLUSIONS

This study showed differences in the relative number of natural enemies of GWSS using two monitoring methods. Numbers of parasitoids and rates of parasitism were higher using the egg mass collection method versus the yellow sticky trap 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); three pyrethroids, bifenthrin (Capture), cyfluthrin (Baythroid), and fenpropathrin (Danitol); and an organophosphate, chlorpyrifos (Lorsban) against four parasitoids. 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. However, results presented here are presently limited to laboratory observations. Field confirmation is needed and is underway.

Table 1.: Toxicity of various insecticides to *G. ashmeadi* and *A. melinus.*

Compound	**Bioassay Technique**	**Exposure Time**	***A. melinus* No. Tested**	***A. melinus* LC_{50}µg(AI)/ml**	***G. ashmeadi* # Tested**	***G. ashmeadi* LC_{50} (µg(AI)/ml)**
Chlorpyrifos	Petri dish	24	4148	0.0008	2106	0.006
Bifenthrin	Petri dish	48	4117	0.001	1006	0.010
Cyfluthrin	Petri dish	48	3683	0.007	1215	0.067
Fenpropathrin	Petri dish	48	4140	0.010	1554	166.88
Acetamiprid	Petri dish	48	3257	0.005	1744	0.134
Buprofezin	Petri dish	96	4531	0.764	1804	315.52
Pyriproxyfen	Petri dish	96	3767	0.421	1794	132.53
Imidacloprid	Uptake	48	2248	2.14	1278	11.06
Thiamethoxam	Uptake	48	2156	0.044	1209	0.312

Table 2.: Toxicity of two neonicotinoids to two predators using a systemic uptake bioassay.

Compound	**Exposure time**	**# Tested**	**LC_{50} (µg(AI)/ml)**
Orius insidiosus			
Imidacloprid	24	352	1.63
	96		0.013
Thiamethoxam	24	341	0.297
	96		0.005
Geocoris punctipes			
Imidacloprid	96	334	2.01
Thiamethoxam	96	311	4.83

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IDENTIFY THE SPECIES OF MYMARIDAE REARED IN ARGENTINA AND MEXICO FOR POTENTIAL INTRODUCTION TO CALIFORNIA AGAINST THE GLASSY-WINGED SHARPSHOOTER AND PREPARE AND SUBMIT FOR PUBLICATION A PICTORIAL, ANNOTATED KEY TO THE *ATER*-GROUP SPECIES OF *GONATOCERUS* – EGG PARASITOIDS OF THE PROCONIINE SHARPSHOOTERS (HEMIPTERA: CICADELLIDAE: PROCONIINI) IN THE NEOTROPICAL REGION

Project Leader:
Serguei V. Triapitsyn
Department of Entomology
University of California
Riverside, CA 92521

Cooperators:
Guillermo A. Logarzo
USDA, ARS South American
Biological Control Laboratory
Hurlingham, Buenos Aires, Argentina

John T. Huber
Canadian Forest Service
c/o Canad. Natl. Collection of Insects
Ottawa, Ontario, K1A OC6, Canada

Jesse H. de León
USDA, ARS
Beneficial Insects Research Unit
Weslaco, TX 78596

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ABSTRACT
At least sixteen species of *Gonatocerus* were reared in Argentina and Neotropical Mexico from eggs of the proconiine sharpshooters in the course of classical and neoclassical biological control projects against the glassy-winged sharpshooter (GWSS) *Homalodisca vitripennis* (Germar) in California. The objectives of this project are to identify them taxonomically and to prepare a pictorial, easy-to-use, annotated key to more than 60 Neotropical species of the *ater* group of *Gonatocerus* – mymarid egg parasitoids of the proconiine sharpshooters including *Homalodisca*. Results obtained during the first three months of this new project are being reported.

INTRODUCTION
In North America, eggs of proconiine sharpshooters, which are known vectors of *Xylella fastidiosa* (*Xf*), are parasitized by various Mymaridae and Trichogrammatidae. An illustrated, annotated key to the genera and species of proconiine-parasitizing Trichogrammatidae is available (Triapitsyn 2003), and a key to such North American Mymaridae was published recently (Triapitsyn 2006).

Recommendation 3.12 (NRC 2004) calls for support for the classical biological control (over augmentative approach) against the glassy-winged sharpshooter (GWSS). Recently (during 2000-2006), major efforts have been undertaken to survey for egg parasitoids of GWSS and the related proconiine sharpshooters in Mexico (Hoddle & Triapitsyn, 2004, 2005; Morgan et al., 2000; Pilkington et al., 2005; Triapitsyn et al. 2002; Triapitsyn & Hoddle 2001, 2002; Triapitsyn et al. 2006) as well as in Argentina (Jones 2001; Jones et al. 2005; Logarzo et al. 2004; Pilkington et al. 2005; Virla et al. 2005). As the result, 12 species of *Gonatocerus* (Mymaridae) were reared in Argentina from eggs of the proconiine sharpshooter genera related to *Homalodisca* (G. Logarzo, unpublished USDA-ARS South American Biocontrol Lab. reports for 2004 and 2005). During 2002-2004, some of these species were imported under permits into the Universiey of California, Riverside (UCR) and USDA-APHIS, Mission, Texas quarantine facilities and their colonies were established on GWSS eggs. Several species are still being maintained and evaluated (Jones, Logarzo, Triapitsyn et al. 2005; Jones, Logarzo, Virla et al. 2005; Hoddle & Triapitsyn 2005). However, importation and quarantine evaluation of other available species from Argentina have not been initiated because their identification is not possible without a careful comparison with more than 60 already described Neotropical species of *Gonatocerus*. Several other species of *Gonatocerus* were also reared from eggs of proconiine sharpshooters in Mexico (Triapitsyn et al. 2002; Hoddle & Triapitsyn 2005), Chile (Logarzo et al. 2006), and Peru (Logarzo et al. 2004). The major problem, however, is taxonomic identification of these species, which has been impossible, except for a few of them. Thus, further introductions of these unnamed species (including applications for their release) are hampered because no positive identifications could be made before this project was initiated.

The *ater* species group of the genus *Gonatocerus* is mostly associated with Proconiini in the New World (Triapitsyn 2002; Triapitsyn et al. 2002). It is extremely speciose in the Neotropical region, with at least 60 described species, mainly from Argentina by A. A. Ogloblin, and probably with at least 100-150 undescribed species. Some of them have wide distributions from Mexico to Argentina (Triapitsyn et al. 2006). Unfortunately, almost 50 species described from Argentina and Ecuador by A. A. Ogloblin cannot be positively identified at present because there are no taxonomic keys for their separation, no adequate illustrations that accompany their descriptions in Spanish, and because the type specimens of almost half of these species were not available. Some of these were not marked by A. A. Ogloblin as types; they were located among the miscellaneous slides of *Gonatocerus* in his collection deposited in La Plata Museum in La Plata, Argentina, and needed to be identified. Therefore, to make identification of any specimen of *Gonatocerus* reared from eggs of proconiine sharpshooters anywhere from Mexico to Argentina, it needs to be compared with about 60 already described Neotropical species from the