COMPATIBILITY OF INSECTICIDES WITH NATURAL ENEMIES OF THE GLASSY-WINGED SHARPSHOOTER

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ABSTRACT
To enhance control of the glassy-winged sharpshooter (GWSS), relative toxicity of neonicotinoids, imidacloprid and thiamethoxam, and other conventional insecticides, chlorpyrifos, cyfluthrin and fenpropathrin, was examined to assess compatibility with parasitoids while being toxic to GWSS. Both imidacloprid and thiamethoxam when applied systemically through uptake in citrus leaves were found to be toxic to adult *Gonatocerus ashmeadi* and *Aphytis melinus*. However, systemic treatments of citrus and willow leaves infested with parasitized GWSS eggs did not impact the emergence of parasitoids significantly, suggesting they are relatively safe to the parasitoids while they are within the GWSS eggs. Fenpropathrin, a pyrethroid, was not toxic to *G. ashmeadi* for 3-4 days post-treatment while chlorpyrifos was quite toxic within 24 h to both *G. ashmeadi* and *A. melinus.* Future tests will measure the amounts of imidacloprid and thiamethoxam that are on the surface of citrus leaves affecting the survival of the parasitoids using ELISA. Based on these findings, our research will focus on understanding which chemicals are the most beneficial for maintaining a minimal impact on important parasitoids on citrus and grapes.

INTRODUCTION
The glassy-winged sharpshooter (GWSS) is an important agricultural pest in California because it is a vector of several strains of the bacterium *Xylella fastidiosa* (*Xf*). The bacterium *Xf* causing Pierce's disease (PD) is transmitted to a number of host plants including grapes, citrus, olive and liquid amber trees (Wong et al. 2003). Citrus plays a large role in producing large populations of GWSS during spring and summer in southern California and dispersal to summer ornamentals contributing to the spread of *Xf*-diseased plants. Regional control programs that have targeted GWSS in citrus have proven highly successful in reducing GWSS densities in various parts of California. It is therefore essential to address the issue of GWSS management in citrus by adopting approaches that will ensure sustainable control.

The degree of compatibility among various control measures being used against GWSS is an important consideration in the development of sustainable management programs. Both field (Akey et al. 2001) and toxicological studies in the laboratory (Toscano et al. 2001) have shown that GWSS are extremely susceptible to both conventional and the newer neonicotinoid insecticides and can thus be used effectively to suppress GWSS populations. However, there is little information available on the long-term impact that chemical control measures against GWSS are having on its natural enemies and other predators and parasitoids, as well as on other pest species that attack citrus. To date, biological control has been critical in citrus IPM in California for many years, but is now threatened by the advent of new pests and greater use of insecticides to regain control. It is therefore essential to attain greater understanding of the impact of insecticide use for GWSS control on its natural enemies in citrus and how best it can be integrated with existing, successful management programs. The overall objective of this research proposal will be to help determine compatible management tactics by focusing on chemical control that is being used against GWSS and evaluating its impact upon several important biological control agents.

Several new insecticides in the neonicotinoid class of pesticides that have become important in agriculture during the last 4-5 years potentially play an important role in the control of GWSS due to their selectivity. Use of these more effective and selective insecticides have made it possible to target pest populations selectively while conserving their natural enemies (Naranjo 2001, Naranjo et al. 2004). With the use of neonicotinoid group of insecticides coupled with increasing knowledge of the predators and parasitoids important in the control of GWSS, a new citrus IPM program can be established that will provide effective and sustainable control. Therefore, to benefit the most from these selective insecticides in their potential adoption into IPM program for GWSS, we proposed to test their action against both the pest and their natural enemies. Imidacloprid, a systemic insecticide in this group, has been presumed to be safe for many natural enemies based on its systemic action. However, our preliminary results have shown a limited but detrimental impact on the *Gonatocerus ashmeadi,* suggesting a need for further investigations. Formulation of an insecticide can influence the uptake and penetration in a natural enemy based on the distribution of pesticide residues in the pest. This can lead to the question of any secondary effects on natural enemies with systemic insecticides. Research conducted to answer these questions will aid in identifying the most suitable insecticides that are specifically suited to the development of a locally adapted management system for GWSS. In addition to neonicotinoids, the impact on both the pest and natural enemies of select conventional insecticides that are also utilized for control of GWSS and other pests on citrus and grapes should be investigated to utilize the most ideal chemicals in GWSS management practices. Often the use of conventional insecticides is considered to be extremely negative in IPM practices due to adverse direct and indirect effects against biological control agents. If toxicity assessment of conventional insecticides indicates preservation of natural enemies, it would allow a wider selection of products and responsible use patterns that may slow resistance development. The overall aim of this project is to evaluate the impact of a number of insecticides that are used in citrus and grapes on select natural enemies.

OBJECTIVES

1. Monitor citrus orchards in Riverside, Ventura County 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 *G. triguttatus* as well as other parasitoids in the system such as *Aphytis 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 *Aphytis melinus.*

RESULTS

Insects

Gonatocerus ashmeadi and *G. triguttatus* egg masses and adults were obtained from collections made in citrus and other hosts such as willow in Riverside. Insectary-reared shipments of *A. melinus* were obtained from Corona, California for tests conducted both in laboratory and field tests.

Bioassay Techniques

Petri-dish bioassay: For foliar treatment, the petri dish bioassay technique was used to determine toxicity to two pyrethroids and two neonicotinoids. Leaf discs from citrus trees were dipped in various concentrations of acetamiprid, fenpropathrin and cyfluthrin and after allowing them to dry were placed in petri dishes with agar beds for exposure to the parasitoids for various intervals. At least 10 parasitoids per replicate, and 6 replicates per concentration were tested. A minimum of 5 concentrations per test along with a water control was evaluated. Toxicity of GWSS was also tested using this technique to compare the responses of the pest and its parasitoids. Additionally, *A. melinus* nymphs and adults (50-100) per petri dish were placed in the dishes with a drop of honey. Mortality assessment was made after 24 and 48 h.

Field Collection of GWSS Egg-Infested Leaves

Willow and citrus leaves infested with GWSS eggs including parasitized eggs were also collected and subjected to the same treatment as above and placed in petri dishes to observe emergence and/or mortality. This test was conducted to examine toxicity of insecticides against parasitized eggs and the effect of insecticides on emergence or reduction due to mortality.

Leaf-Uptake Systemic Bioassay

The systemic toxicity of imidacloprid and thiamethoxam was assessed using excised citrus leaves to allow uptake through the petioles directly into the leaf. The excised leaves were placed in serial dilutions of each compound contained in aquapiks for 24 hours. After 24 hours uptake time, treated leaves were placed in aquapiks containing water only. Parasitoids were exposed to each compound by enclosing them in clip cages attached to the treated leaves. Mortality counts were made after 24 and 48 hours.

Objective 1.

Tests were initiated to determine the relative abundances of those natural enemies that are most active against GWSS in citrus orchards. Monitoring has not been initiated in Ventura County or Coachella Valley at the present time. Two methods were used to assess densities of the parasitoids *G. ashmeadi* and *G. triguttatus* as well as various predator species. Yellow sticky traps were posted at multiple locations within citrus orchards in Riverside for continuous monitoring of GWSS and natural enemies and changed once every week. Additionally, rates of parasitism by *G. ashmeadi* and *G. triguttatus* was evaluated by collecting citrus and willow tree leaves that were infested with GWSS egg masses and were placed in petri dishes with agar beds for incubation up to a week or longer. Numbers of GWSS nymphs and parasitoids emerging from each leaf were recorded throughout the season. Data collected from these traps shows that parasitoids were most abundant during mid-summer relative to GWSS activity. The parasitoids were also more abundant on willow leaves than citrus. In addition to *G. ashmeadi*, other species of parasites were also abundant in GWSS eggs on willow leaves. Predators were relatively few on the yellow traps and none were found using the petri dish technique. The numbers of *G. ashmeadi* decreased significantly from September in the leaf samples from both willow and citrus.

Objective 2.

Relative toxicity of select insecticides to *G. ashmeadi* and *A. melinus* was assessed for imidacloprid, thiamethoxam, chlorpyrifos, cyfluthrin and fenpropathrin using petri dish for foliar applications and systemic uptake method for imidacloprid and thiamethoxam as described above. Data indicates that imidacloprid and thiamethoxam were toxic to the parasitoids even though the insects were exposed systemically and not directly. These results suggest that the two neonicotinoids were toxic to GWSS and did not preserve its beneficials as expected. Similar test results were obtained for *A. melinus* and *Encarsia* spp.also. As expected, chlorpyrifos was quite toxic to the beneficials. The predator, *Chrysoperla*, was not as susceptible to the systemic insecticides immediately but over time became more susceptible after 3-4 d of exposure.

More tests are in progress to address the reason for toxicity of the parasitoids to imidacloprid and thiamethoxam when applied systemically. Using ELISA, tests will be conducted to determine at what levels the systemic chemicals (imidacloprid and thiamethoxam) can be detected on the leaves after exposure to the two insecticides. Multiple testing methods will be used to evaluate if a specific dose of the two insecticides makes contact with the insect while they move around on the surface of the treated leaves.

Objective 3.
Tests have been initiated to examine if *A. melinus* is exposed to systemic insecticides while feeding on honeydew or through host feeding by adult parasitoids on intoxicated hosts on citrus. These tests will determine if there are residues of imidacloprid and thiamethoxam in honeydew when they are applied systemically. Initial tests have shown that A. melinus is extremely susceptible when caged on leaves of citrus trees that had been treated with the two insecticides over a year ago. Further tests will be conducted to determine at what levels the systemic chemicals (imidacloprid and thiamethoxam) can be detected in the honeydew produced by homopterans using ELISA kits.

Objective 4.
The potential for mortality caused by systemic insecticides that are in the plant tissue to parasitoids of GWSS, *Gonotocerus* spp. was examined by systemically treating willow leaves infested with parasitized GWSS egg masses. The impact on emergence of *Gonotocerus* spp. was not extensive compared to the toxicity of imidacloprid against the adult parasitoids that were mobile on plant surfaces treated systemically. Further tests will be conducted to determine directly the titers of either imidacloprid or thiamethoxam within the leaf tissue as well as in GWSS eggs using ELISA methods.

CONCLUSIONS
Compatibility of select insecticides that are used for control of glassy-winged sharpshooter (GWSS) with representative parasitoids important in citrus was evaluated. Two systemic insecticides, imidacloprid and thiamethoxam were found to be toxic to the adult parasitoids of GWSS as well as to *A. melinus* in laboratory tests. However, systemic treatment with imidacloprid of willow leaves infested with parasitized GWSS eggs did not impact the emergence of parasitoids significantly suggesting that imidacloprid was relatively safe to *G. ashmeadi* and *G. triguttatus* during their development in the GWSS eggs. Results also indicated that chlorpyrifos was extremely toxic to the natural enemies while the pyrethroid, fenpropathrin, was not as toxic. Our results are expected to aid the development of pest management strategies based on the effective use of insecticides that selectively target pest species but are relatively harmless to GWSS parasitoids and other natural enemies present in citrus and grapes, thereby fostering enhanced biological control. Our research will focus on gaining an understanding of which chemicals are the most beneficial for maintaining a minimal impact on important parasitoids such as *G. ashmeadi* and *G. triguttatus*, as well as other parasitoids present on citrus and grapes. These data will also help to preserve IPM programs that have been established in different citrus-growing regions and help prevent pest flare-ups as a result of poor chemical control decision-making.

Toxicity of Select Insecticides to Parasitized GWSS Eggs

Chemical	Total # Egg S. T	#Eggs D	#Emerged (parasites)	#Egg S. Exit holes[a]
Admire (10 ppm)	42	18	78	14
Lorsban	63	40	41	35
Danitol	30	10	156	19
Knack	31	16	120	16
Platinum (10 ppm)	64	44	39	19

Age of egg sacs unknown at the time of treatment
[a]Parasitoid exit holes

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