CHEMICAL CONTROL OF GLASSY-WINGED SHARPSHOOTER: ESTABLISHMENT OF BASELINE TOXICITY AND DEVELOPMENT OF MONITORING TECHNIQUES FOR DETECTION OF EARLY RESISTANCE TO INSECTICIDES

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INTRODUCTION

Among the control practices used against the glassy-winged sharpshooter (GWSS) that spreads Pierce's disease, chemical control offers an immediate remedial option against this pest. Insecticides were not used extensively until recently to control this pest and therefore efficacy of insecticides should be evaluated and monitored. Our present challenge is to study the effectiveness of selected insecticides representing various chemistries against the GWSS and to establish baseline toxicity of various insecticides. Knowledge of baseline toxicities would facilitate the selection of the most effective products for use in GWSS management. The results of tests conducted in the laboratory under controlled conditions can provide practical guidance to individual growers without conducting expensive large-scale trials to determine the suitability of an insecticide. Conventional bioassays provide rapid and field-based information to establish baseline toxicity data.

In order to establish baseline susceptibility data to various insecticides against the GWSS, evaluation and development of simple and suitable bioassay techniques to detect toxicological responses of the insects to various insecticides is critical. Ideally, bioassay techniques should require less handling of insects and also be sensitive enough to allow early detection of changes in an insect's response to insecticides over time. Changes in the toxicological response of a population to an insecticide is usually the first indication of resistance development. Hence, the priority of our project was to evaluate and standardize 3 bioassay techniques at the present time with the possibility of developing 2 more techniques in the future.

The purpose of the report is to describe three techniques, a petri-dish and a leaf-dip bioassay technique used for testing the relative susceptibilities of GWSS to a number of contact insecticides, and a systemic-uptake bioassay method for systemic insecticides. The baseline data obtained so far to selected insecticides against the GWSS can be useful for future studies on resistance monitoring and management of this pest. The availability of these three techniques will allow us to also examine various strategies of management with chemicals to avoid or delay insecticide resistance. Testing various chemical application strategies to manage GWSS populations will be critical to understand the evolution of resistance in GWSS.

OBJECTIVES

1. Develop reliable bioassay technique(s) to evaluate baseline toxicity of insecticides from major classes of insecticides against all life stages of GWSS.
2. Monitor all life stages of the GWSS populations collected from insecticide-treated citrus orchards and vineyards in Riverside, Redlands, San Joaquin Valley and Temecula to determine baseline susceptibility to various insecticides.
3. Investigate the rate of development of resistance to a selected OP, pyrethroid and a neonicotinoid by artificial selection in the greenhouse.
4. Develop electrophoretic techniques to identify esterase profiles in individual GWSS of all life stages including eggs.

RESULTS AND CONCLUSIONS

Petri-dish Bioassay: Petri dishes of 60 mm were used for this assay. Agar beds were prepared in the petri dishes for maintenance of citrus leaves for up to a week. Excised citrus leaf discs of the same size as the petri dish were dipped for 30 sec in five concentrations of each insecticide and allowed to dry for an hour. The dried leaf discs were placed on the agar bed in each petri dish. Tests were conducted using 10 contact insecticides and one systemic: esfenvalerate, cyfluthrin, bifenthrin, and fenpropathrin (pyrethroids), chlorpyrifos and dimethoate (organophosphates), endosulfan (cyclodiene), acetamiprid, imidacloprid, thiamethoxam (neonicotinoids), and pymetrozine. Five GWSS were exposed to the treated leaves in each petri dish covered with a plastic top. Each test was replicated 6 times and included water-only dipped controls. Mortality was assessed after 24, 48 and 96 h. In the case of acetamiprid and thiamethoxam, mortality was assessed after only 4 and 16 h followed by 24 h because of their potency to the insects. No condensation was observed in the petri dishes while maintaining insects for exposure to the treated leaves even over a week.

Leaf-dip Bioassay: Leaf-dip bioassays of attached leaves on citrus plants were conducted in the greenhouse. Five serial dilutions of each of the 9 insecticides were used for dipping of the attached leaves and allowed to dry for an hour. Five adults or immatures were placed in clip cages and attached to the treated leaves. Mortality assessment was similar to that of the petri-dish bioassays.

For both the petri-dish and the leaf-dip technique, toxicity was determined based on the effects of exposure time and location effect (Table 1). Initial tests with GWSS were limited to populations collected from citrus orchards in Riverside. Tests on populations from citrus orchards in Redlands were initiated during late summer and compared with the Riverside populations. Considerable variation in susceptibility to insecticides was observed with both techniques. The petri-dish technique provided stable LC_{50}s with no or low control mortality in 24 h. Mortality increased in the controls over time. Monitoring data for chlorpyrifos indicated a difference of 13- and 15-fold between the two techniques in 24 and 48 h respectively. Toxicity of chlorpyrifos appears to be similar to both Riverside and Redlands populations. No significant differences in LC_{50}s were observed to fenpropathrin using the petri-dish technique between the two locations (LC_{50} = 0.019 to 0.042 ppm). However, a significant difference in LC_{50} was observed to esfenvalerate with the petri-dish test. Esfenvalerate was more potent to GWSS from Redlands (LC_{50} = 0.00003 ppm) compared to the insects from Riverside (LC_{50} = 0.002 ppm) showing a significant difference of 90-fold. Similarly, acetamiprid was also quite toxic to GWSS from Redlands with an LC_{50} of 0.003 ppm compared to 0.01 ppm for the Riverside insects using the petri-dish technique. Also the GWSS from Redlands were more susceptible to cyfluthrin (LC_{50}=0.004 ppm) indicating a 10-fold difference compared to Riverside insects (LC_{50}=0.038 ppm). In all tests, mortality increased with time, higher at 48 h compared to 24 h.

Systemic Bioassay: To assess the toxicity of imidacloprid action systemically, the leaf-dip method was modified to accommodate a system that allows excised leaves to take up imidacloprid through the petioles. The uptake and systemic translocation of imidacloprid through the leaf closely approximates the exposure pattern of GWSSs in an agricultural setting. The excised leaves were placed in serial dilutions of imidacloprid in aquapiks for 24 h. After 24 h uptake of imidacloprid, exposed leaves were placed in aquapiks containing water only. Exposure time of the insects to imidacloprid is similar to that of the other insecticides. Mortality was checked after 24, 48 and 96 h. Results as shown by LC_{50}s indicate that susceptibility to imidacloprid was not significantly different in 24 h between the Riverside and Redlands insects. However, with longer exposure time, a significant difference was observed between the responses of GWSS from the two locations (LC_{50} =0.0008 ppm for Redlands insects vs. 0.015 ppm for Riverside insects).

In general, GWSS are quite susceptible to all insecticides tested so far. However, insects from Redlands appear to be more susceptible to most compounds than the Riverside populations. Techniques were sensitive enough to detect even small differences in LC_{50}s between locations and exposure time. Monitoring results of GWSS also demonstrated seasonal variations in their responses to various insecticides.

Table 1. Susceptibility of glassy-winged sharpshooter from 2 locations to selected insecticides using 2 techniques.

			LC_{50} (ppm)	
Insecticide Class	**Insecticide**	**Field Location**	**Petri Dish**	**Leaf Dip**
Neonicotinoid	Acetamiprid	Riverside	0.01	0.09
		Redlands	0.003	0.008
	Imidacloprid	Riverside	1.646	—
		Redlands	0.612	—
	Thiamethoxam	Riverside	0.0037	—
		Redlands	0.0004	—
Pyrethroid	Cyfluthrin	Riverside	0.038	—
		Redlands	0.004	—
	Esfenvalerate	Riverside	0.0027	0.022
		Redlands	0.00003	0.00004
	Fenpropathrin	Riverside	0.042	0.168
		Redlands	0.019	0.012
Organophosphate	Chlorpyrifos	Riverside	0.001	0.013
		Redlands	0.001	0.015
Cyclodiene	Endosulfan	Riverside	0.006	—
		Redlands	0.003	—