Interim Progress Report for CDFA Agreement Number #14-0381-SA

MONITORING FOR INSECTICIDE RESISTANCE IN THE GLASSY-WINGED SHARPSHOOTER IN CALIFORNIA

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Introduction:
Chemical control has been a first line of defense against *H. vitripennis* populations ever since 2000 when the initial area-wide treatment program was conducted in the Temecula region of Riverside County. A similar program was initiated the following year in Kern County as high populations of *H. vitripennis* represented a critical threat to table grapes growing in proximity to citrus orchards. The systemic neonicotinoid insecticide imidacloprid was selected for use in both regions and has continued to be relied upon for suppressing *H. vitripennis* populations on an area-wide basis. Various attributes of imidacloprid including its systemic activity and long persistence within plants, its application versatility, and its semi-selective activity against sucking plant pests such as *H. vitripennis* have combined to make imidacloprid a leader in global insecticide sales. Applications of imidacloprid that could be made through the mini-sprinkler irrigation system of citrus orchards were viewed as a positive feature that promoted the use of imidacloprid early in the area-wide programs against *H. vitripennis* and have continued to reinforce its use. Although data on the frequency of imidacloprid use since 2000 has not been compiled for the area-wide program regions of Riverside and Kern counties, there is a general impression that imidacloprid has been used to a greater extent than other insecticides for control of *H. vitripennis*. In addition, citrus growers have used imidacloprid extensively for control of red scale and other citrus pests (Grafton-Cardwell et al. 2008) and grape growers have relied upon imidacloprid for vine mealybug control (Daane et al. 2006). The combined uses of imidacloprid across citrus and grape acreages over the past 15 years has likely elevated selection pressure for resistance to imidacloprid in *H. vitripennis* and other target pests.

Concerns about the potential for insecticide resistance developing in *H. vitripennis* populations have been heightened recently in areas of Kern Co. due to high population levels reminiscent of the early 2000s. In 2012, CDFA-monitored yellow-trap catches in the General Beale Road region east of Bakersfield matched historic levels and have again been at extreme levels in 2015. The long history of imidacloprid use in this region coupled with a resurgence in *H. vitripennis* populations raise important questions about possible factors contributing to the recent outbreaks. Resistance to imidacloprid has been documented for numerous insects including other sap-feeding insects (Liu et al. 2005, Nauen and Denholm 2005, Karunker et al. 2008). However, reports of resistance to insecticides by xylem feeding insects are rare, and to imidacloprid are unknown. In the arthropod pesticide resistance (APR) database (http://www.pesticideresistance.org/), only a single obscure record exists for a xylem feeder—a sugarcane feeding froghopper (spittlebug) reported in a book chapter (Fewkes 1968). Although fundamental arguments (Rosenheim et al. 1996, Gordon 1961) for why sap feeding insects might be less prone to resistance development compared to leaf-chewing insects are supported by the APR database, the possibility of pesticide resistance development remains in any organism subjected to a specific mortality treatment over time. There are few examples, if any, where a xylem-feeding insect has been subjected to the kind of intensive management program that has targeted *H. vitripennis* over the past 15 years in Kern Co. Because pesticides are an integral part of the high-yielding production agriculture in citrus and grapes, pest resistance to pesticides must be evaluated. This potential is magnified when overreliance on a few select products occurs, such as has been the case with the use of imidacloprid in the area-wide control programs and by growers and pest control advisors protecting their orchards and vineyards. The repeated use of the same product(s) for control of a pest population results in continual selection pressure, which ultimately may result in resistance development. The continued successful implementation of insecticides in management programs require that their efficacies be evaluated, especially under present circumstances where conspicuously large numbers of *H. vitripennis* are potentially initiating future epidemics of Pierce's disease in table grapes of Kern Co.

Objectives:

1. Conduct laboratory bioassays on field-collected *H. vitripennis* from Kern and Tulare Counties to determine susceptibility to neonicotinoid, pyrethroid, and organophosphate insecticides.
2. Investigate the geographic variation in susceptibility of *H. vitripennis* to determine if a pattern of resistance emerges associated with insecticide use patterns.

Description of Activities and Summary of Accomplishments and Results:

Objective 1: Conduct laboratory bioassays on field-collected *H. vitripennis* from Kern and Tulare Counties to determine susceptibility to neonicotinoid, pyrethroid, and organophosphate insecticides.

We have conducted bioassays of insects collected from Kern County and not Tulare County because this is where the GWSS were found in high enough numbers to support the studies. In addition, this reporting period focuses on objective 1, while objective 2 will be covered in the final report. In this study, we collected GWSS on three dates in July and August, 2015 in organic citrus groves in the Edison area, then shifted to the General Beale Road area for three more dates in September and October. Insects were subjected to a systemic uptake bioassay and a foliar insecticide bioassay adapted from Prabhaker et al. (2006), for the materials listed in Table 1. From these bioassays, LC_{50} (lethal concentration that kills 50% of the population) values were calculated and compared to LC_{50}s determined in 2001 and 2002 (Prabhaker et al. 2006).

Table 1. Insecticides tested in adult *H. vitripennis* bioassays in 2015.

Insecticide Class	Active Ingredient	Product	Application	Manufacturer
Neonicotinoid	Imidacloprid	Admire® Pro	soil	Bayer
	Thiamethoxam	Platinum® 75 SG	soil	Syngenta
	Acetamiprid	Assail® 70 WP	foliar	United Phosphorus
Butenolide	Flupyradifurone	Sivanto™ 200 SL	foliar	Bayer
Pyrethroid	Bifenthrin	Capture® 2 EC	foliar	FMC
	Fenpropathrin	Danitol® 2.4 EC	foliar	Valent
Organophosphorus	Chlorpyrifos	Lorsban® 4E	foliar	Dow
	Dimethoate	Dimethoate® 2.67 EC	foliar	Loveland

The data showed that GWSS tested in 2015 were less susceptible to the tested compounds than they were in 2001 and 2002. For the neonicotinoids, the LC_{50} values for thiamethoxam, imidacloprid, and acetamiprid were up to 1.78, 57.31, and 130 times, respectively, higher in 2015 (Table 2). Even larger differences existed for the pyrethroids bifenthrin (5066 times higher), and fenpropathrin (101 times higher) and the organophosphates chlorpyrifos (22190 times higher) and dimethoate (2150 times higher). We believe that the extraordinary differences in the pyrethroids and the organophosphates may be the result of different research protocols used in the 2001/2002 studies and the 2015 studies. In the earlier work, we used a petri dish assay which enclosed the treated leaves and insects, probably contributing to fumigation action and extremely the low LC_{50} values. In 2015, we used a screened clip cage which eliminated or greatly reduced the fumigation action of the insecticides. Even so, the data from all studies indicate that GWSS is less susceptible to most of the insecticides being used than it was 14 years ago. Similar results were obtained using topical bioassays for imidacloprid, bifenthrin, and fenpropathrin (Redak et al. 2015).

Table 2. LC_{50} values for 7 insecticides evaluated on GWSS in 2001, 2002 and 2015.

Compound	2001	2002	2015
Acetamiprid	0.44 (0.18 – 0.56)	0.08 (0.02 – 0.14)	0.74 (0.31 – 1.76) – July 9 3.97 (1.40 – 9.97) – Oct 7 10.41 (5.18 – 22.64) – Oct 21
Thiamethoxam	1.87 (1.18 – 2.06)	2.12 (1.49 – 3.28)	0.13 (0.08 – 0.23) – July 9 0.35 (0.19 – 0.67) – July 22 1.74 (0.71 – 4.25) – Sept 16 3.34 (1.25 – 7.70) – Oct 7 1.19 (0.55 – 2.60) – Oct 21
Imidacloprid	1.27 (0.68 – 2.54)	0.36 (0.09 – 0.52)	0.26 (0.15 – 0.46) – July 9 1.92 (0.74 – 6.01) – July 22 8.56 (4.47 – 14.56) – Aug 3 7.03 (2.73 – 23.30) – Sept 16 8.63 (2.63 – 29.92) – Oct 7 20.63 (8.27 – 47.71) – Oct 21
Compound	**2001**	**2002**	**2015**
Bifenthrin	0.0006 (0.0002 – 0.004)	0.013 (0.008 – 0.035)	0.53 (0.15 – 1.29) – July 7 0.13 (0.05 – 0.30) – July 22 0.78 (0.25 – 2.49) – Sept 16 0.14 (0.04 – 0.40) – Oct 7 3.04 (1.27 – 8.23) – Oct 21
Fenpropathrin	0.063 (0.045 – 0.204)	0.020 (0.006 – 0.059)	0.19 (0.08 – 0.41) – July 7 0.04 (0.02 – 0.09) – July 22 2.02 (0.86 – 5.09) – Sept 16 0.21 (0.01 – 1.42) – Oct 7 0.49 (0.17 – 1.22) – Oct 21
Compound	**2001**	**2002**	**2015**
Chlorpyrifos	0.002 (0.001 – 0.003)	0.001 (0.0007 – 0.005)	0.28 (0.06 – 1.76) – July 7 4.55 (1.54 – 18.04) – July 22 22.19 (6.93 – 96.61) – Oct 21
Dimethoate	0.046 (0.029 – 0.081)	0.031 (0.024 – 0.044)	10.99 (2.68 – 118.43) – Aug 3 66.67 (11.76 – 4177) – Sept 16 5.66 (0.93 – 30.11) – Oct 7

Taken in total, our work has shown that GWSS is less susceptible to commonly used insecticides than it was in 2001-2002. Furthermore, we are evaluating the levels of susceptibility in different geographic areas.

Publications/Presentations:

Perring, T.M., Prabhaker, N., Castle, S. 2014. Monitoring for insecticide resistance in the glassy-winged sharpshooter in California. Pierce's Disease Control Program, CDFA. p.31-35. Sacramento, CA.

Perring, T.M., Prabhaker, N., Castle, S.J. 2015. Monitoring for insecticide resistance in the glassy-winged sharpshooter *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae) in California. Annual Report. Central Valley Consolidated Table Grape District. 6p.

Perring, T.M. Reduced susceptibility in imidacloprid in General Beale Road populations of GWSS. Kern/Tulare table grape/PCA meeting. Bakersfield, CA 12/2015

Research Relevance Statement:
The variation in toxicity that was demonstrated in the 2015 season suggest that GWSS has become resistant to commonly used insecticide. Further studies on location and seasonality suggest that factors like insecticide usage in a local context may be important determinants for how effective certain insecticides are in certain areas. Understanding these dynamics will lead to more informed selection of materials in the future.

Layperson Summary:
Insecticides have played a key role in suppressing *H. vitripennis* populations ever since 2000 when area-wide control programs were first implemented in Riverside County. Reliance on insecticides has been particularly heavy in parts of Kern County where a mixed culture of citrus and table grapes supports development of *H. vitripennis* populations and promotes the spread of Pierce's disease. Spikes in *H. vitripennis* populations in recent years have raised concerns that insecticide management programs are no longer as effective as before due to the development of insecticide resistance. The study presented here examined responses of *H. vitripennis* to seven insecticides in a series of laboratory bioassays that evaluated mortality in relation to insecticide concentration. Studies showed that GWSS tested in 2015 were less susceptible to the tested compounds than they were in 2001 and 2002. Further analyses on location of GWSS collection and timing of bioassay may shed light on the use of insecticides in the local context and provide information useful for selecting insecticides in the future.

Status of Funds:
This 2-year project is approaching its last quarter, and funding levels are sufficient to finish the project.

Summary and Status of Intellectual Property:
The aim of UC Riverside's policies on the protection of intellectual property rights is to make available research to others for the public benefit, while providing recognition to individual researchers and inventors and encouraging the prompt and open dissemination of research results. UC's Research Policy Analysis and Coordination office provides intellectual property guidance on the technology transfer efforts of the 10 UC campuses and the Lawrence Berkeley National Laboratory. For more information, please go to: http://www.ucop.edu/research-policy-analysis-coordination/policies-guidance/intellectual-property-ex/index.html.

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