Title of Report
Interim Progress Report for CDFA Agreement Number 16-0511-SA

Title of Project
Insecticide Resistance in the Glassy-winged Sharpshooter: Using Historical Use Patterns to Inform Future Management Strategies

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Reporting Period
The results reported here are from work conducted July 2017 to March 2018

Introduction
This project, initiated in July 2016, is an extension of a pilot study that was conducted in 2014 and 2015 with support from the Consolidated Central Valley Table Grape Pest and Disease Control District and the CDFA Pierce's Disease Control Program. Despite continued efforts by CDFA in the Area-wide GWSS Management Program, numbers of sharpshooters had increased from 2012-2015, causing concern among the industry (Figure 1). At the same time, surveys of PD infected vines indicated an increase in disease incidence in the General Beale region of Kern County (Haviland 2015).

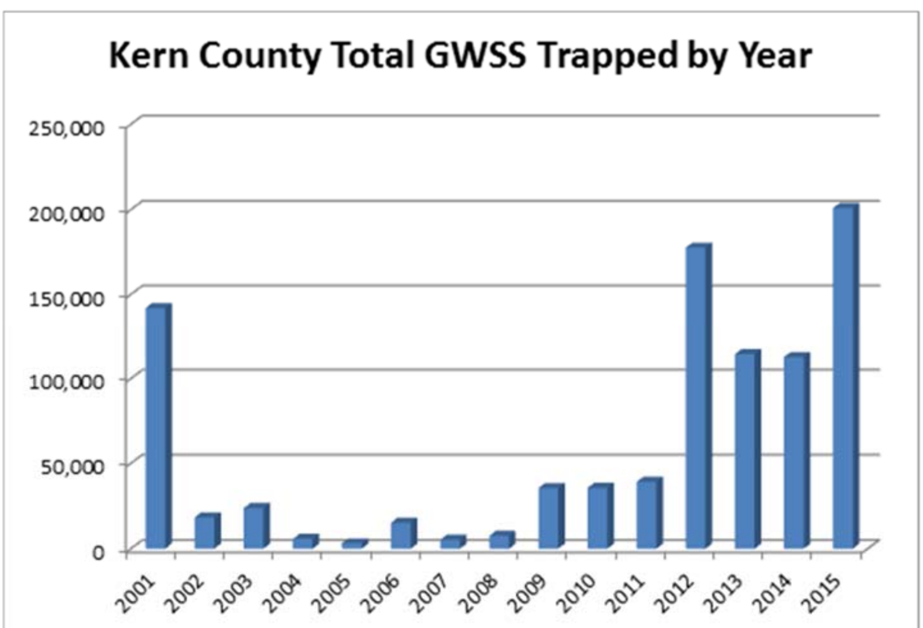

Figure 1. Total number of GWSS caught on CDFA traps in Kern Co. from 2001 – 2015. (From Haviland 2015)

In the 2015 study, we evaluated 8 commonly used compounds (Table 1), in both systemic uptake and foliar bioassays, collecting GWSS on 3 dates in July and August from an organic citrus grove in the Edison area, and 3 dates in September and October from the General Beale area. These studies showed that GWSS collected in 2015 were much less susceptible to the insecticides than they were in 2001 and 2002 (Prabhaker et al. 2006), when the Area-wide GWSS Management Program was initiated (Perring et al. 2015). For some insecticides, the studies showed LC_{50} values to be much higher in 2015, an indication of resistance in the populations. These results were similar to those obtained by Redak et al. (2015) in the same geographic region.

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

These high levels of resistance may explain the upsurge in GWSS number in the region. At the same time, we documented variation in the relative toxicities at different times and locations throughout the 2015 season (Perring et al. 2015). In particular, there was a 79-fold increase in the LC_{50} value for imidacloprid from the first bioassay of the season to the last, and there were differences in susceptibility of sharpshooters collected from different fields and geographic areas. This study suggested that toxicity was related to factors in the local context.

In 2016, these studies were repeated. Despite a reduced number of sharpshooters compared to 2016, we evaluated two pyrethroids and 3 neonicotenoids on 2 dates from table grapes and 1 date from citrus. The data from 2016 showed similar resistance levels to those from 2015 for all 5 chemicals (Perring et al. 2016), demonstrating that resistance levels in 2015/2016 were higher than in 2001/2002, indicating declining susceptibility over the years. The data also showed declining susceptibility to the systemic neonicotinoids (imidacloprid and thiamethoxam) over the season, revealing a trend repeated from the 2015 bioassays.

With the limited numbers of GWSS available for collection in 2016 and because imidacloprid has been used extensively in citrus (Grafton-Cardwell et al. 2008) and grapes (Daane et al. 2006), our 2017 bioassays focused on imidacloprid testing. As previously mentioned, bioassays with imidacloprid in 2015 and 2016, demonstrated a trend of declining susceptibility from early season (June/July) bioassays to late season (September/October) bioassays. We followed up on this discovery by choosing 4 different sites with unique patterns of nearby field applications of imidacloprid (Admire® Pro) and similar mode of action compounds, acetamiprid (Assail® 70 WP) and thiamethoxam (Actara®) for monthly testing to determine if seasonal reduced susceptibility occurred within different Kern Co. regions (Figure 2). We called 2 sites 'organic' and 2 sites 'treated' based on distance, greater than 1 mile and less than 0.5 miles, respectively, from imidacloprid applications during the season. We found that seasonal reduced susceptibility did occur in both the 'organic' and 'treated' sites and that the degree of reduction was likely due to nearby field applications.

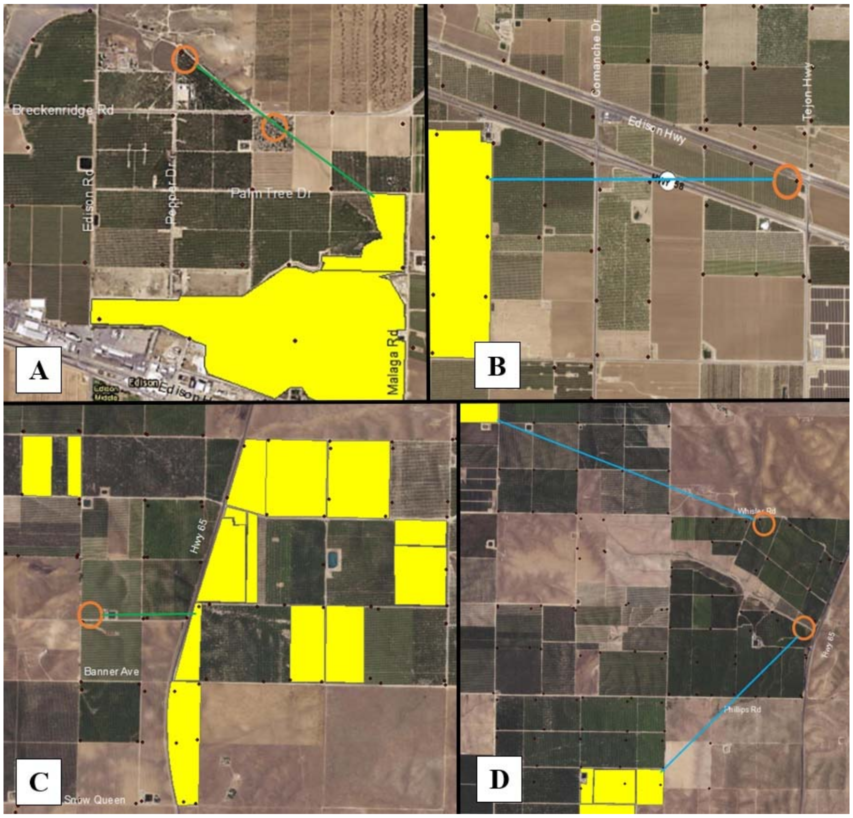

Figure 2. Four Kern County locations chosen for *H. vitripennis* collection and imidacloprid bioassays. (A) Treated Site 1 (T1), (B) Organic Site 1 (O1), (C) Treated Site 2 (T2), and (D) Organic Site (O2). Citrus or grapes treated with imidacloprid in 2017 are represented by the yellow areas. Orange circles indicate collection sites. Green lines represent distances between collection sites and treated areas that are less than 0.5 mile. Blue lines represent distances between collection sites and treated areas of over 1 mile (Perring et al. 2017).

The purpose of this project was to determine if GWSS has become less susceptible to various insecticides over the last 15 years and if resistance development possibly contributed to the recent resurgence of GWSS in Kern Co. Additionally, we aimed to determine how patterns of GWSS resurgence (areas and timing) were related to historical insecticide applications. Increasing our understanding of the factors contributing to reduced resistance, both seasonal and over the years, may help growers in their selection of GWSS management materials and application timings in their particular areas.

Objectives

1. Conduct laboratory bioassays on field-collected *H. vitripennis* from Kern County to document the levels of resistance at the beginning of the 2016 and 2017 field seasons, and to document changes in susceptibility as each season progresses.
2. Document differences in insecticide susceptibility in GWSS collected from organic vs. non-organic vineyards (grapes) and/or orchards (citrus) and from different locations in Kern County.
3. Obtain and organize historic GWSS densities and treatment records (locations, chemicals used, and timing of applications) into a Geographic Information System for use in statistical analyses.
4. Determine the relationship between insecticide susceptibility of different GWSS populations and treatment history in the same geographic location and use relationships to inform future insecticide management strategies.

Activities and Accomplishments

Objectives 1 and 2

The 2017 bioassays with imidacloprid of GWSS collected monthly from 4 different Kern Co. sites starting in early July and concluded in October. Initially our bioassays were grouped and analyzed according to the ‘organic’ (O1 and O2; Figure 2) versus ‘treated’ (T1 and T2; Figure 2) site designations as reported in Perring et al. (2017). We have since analyzed each site individually to determine if seasonally-reduced susceptibility occurs regardless of distance from field applications.

Collections from citrus orchards and bioassays began in July and were repeated at each site in August (Table 2). LC_{50} values were similar to those determined at the beginning of 2016 and 2015 tests, indicating that the lowered susceptibility levels at the end of the previous year do not continue into the next year and that LC_{50} values revert back to previous years' early season levels. The LC_{50} values also were not significantly different among sites nor were they different from July to August. Unfortunately, 2 of the sites, T1 and O1, could not be tested into late season as GWSS numbers were significantly lower in September. T2 and O2 collections were bioassayed in mid-September, but then only O2 could be tested in October. Analyzing these sites individually, we found that susceptibility of the GWSS collected at the T2 and O2 sites decreased significantly from July to September and October, respectively. At T2, where a neonicotinoid application was made within 0.5 mi of the collection site, susceptibility dropped 29-fold. At O2, with the application greater than 1 mi away, susceptibility decreased 11-fold. These results suggest indicate that seasonal reductions in susceptibility to neonicotinoids occur and that differential proximity to field applications likely contributes to the degree of reduction.

Table 2. Toxicities of imidacloprid to *Homalodisca vitripennis* determined in uptake bioassays in multiple locations in Kern County, California, USA.

Year	**Date**	**Location**	***n***	**LC_{50} µg/ml (95% FL)**	**Slope ± SE**	**χ^2 (df)**
2017	July 24	E Edison	270	4.01 (0.63-11.31)	1.26 ± 0.23	3.15 (3)
		W Edison	140	*0.38 (0.02-12.49)	0.88 ± 0.13	9.12 (3)
		S Hwy 65	150	0.80 (0.13-2.07)	1.29 ± 0.36	2.46 (3)
		N Hwy 65	150	1.79 (0.54-3.98)	1.50 ± 0.37	1.73 (3)
	August 8	E Edison	238	1.27 (0.26-4.73)	0.95 ± 0.12	4.71 (3)
		W Edison	50	*1.12 (0.03-22.72)	0.90 ± 0.20	3.57 (3)
		S Hwy 65	237	0.56 (0.09-2.09)	1.11 ± 0.15	5.48 (3)
		N Hwy 65	59	*0.13 (0.08-0.18)	1.37 ± 0.58	0.09 (3)
	September 12	S Hwy 65	150	*8.99 (1.00-47.78$^+$)	1.15 ± 0.25	6.48 (3)
		N Hwy 65	150	51.53 (21.33-204.99)	1.02 ± 0.27	2.50 (3)
	October 9	S Hwy 65	504	8.71 (2.93-27.28)	0.89 ± 0.09	5.62 (3)

* LC_{50} determined by probit analysis using PoloSuite because of high variability in dose responses.
$^+$ 90% FL reported in place of indeterminable 95% FL.

With three years of bioassays complete, comparisons of annual LC_{50} estimates were made. For each compound tested, annual LC_{50} estimates, regression line slopes, and χ^2 values were determined by combining all bioassay results per compound per year and running probit analysis (Table 3). We did not include data for some previously tested compounds (flupyradifurone, chlorpyrifos, and dimethoate) because there were very few GWSS in 2016 and we had high variation in the responses of the few tests we were able to conduct in that year. For each of the neonicotinoid and pyrethroid compounds, the annual LC_{50} values were not significantly different from 2015 to 2016. For this reason, we also combined all bioassays to determine an overall LC_{50} estimate for each of these compounds (Table 3).

Table 3. Toxicities of various insecticides to *Homalodisca vitripennis* collected from multiple locations in Kern County, California, USA from 2015 through 2017 as determined by uptake and leaf dip bioassays.

Compound	Year	*n*	LC_{50} µg/ml (95% FL)	Slope ± SE	χ^2 (df)
Imidacloprid	2015	1,171	2.51 (0.98-5.29)	0.77 ± 0.06	53.68 (13)
	2016	575	3.43 (0.61-17.76)	0.74 ± 0.07	10.02 (3)
	2017	2,098	2.90 (1.05-6.45)	0.88 ± 0.05	11.59 (3)
	Overall	3,844	2.91 (1.93-4.21)	0.82 ± 0.04	47.27 (15)
Thiamethoxam	2015	775	0.74 (0.35-1.50)	0.93 ± 0.07	15.53 (6)
	2016	563	1.48 (0.35-4.94)	1.02 ± 0.08	11.33 (3)
	Overall	1,338	1.03 (0.54-1.87)	0.97 ± 0.05	20.67 (6)
Acetamiprid	2015	450	2.88 (1.06-8.13)	0.77 ± 0.07	4.41 (3)
	2016	450	0.94 (0.15-3.59)	0.59 ± 0.07	4.23 (3)
	Overall	900	1.78 (1.11-2.75)	0.67 ± 0.05	2.36 (3)
Bifenthrin	2015	746	0.54 (0.21-1.15)	0.74 ± 0.06	3.15 (3)
	2016	302	1.03 (0.29-3.72)	1.09 ± 0.11	6.73 (3)
	Overall	1,048	0.67 (0.30-1.29)	0.82 ± 0.06	4.00 (3)
Fenpropathrin	2015	735	0.33 (0.19-0.54)	0.60 ± 0.05	3.46 (4)
	2016	150	0.80 (0.32-1.70)	1.13 ± 0.20	1.13 (3)
	Overall	885	0.40 (0.19-0.77)	0.66 ± 0.05	4.45 (4)

For each year, the total mortality responses at each exposure concentration was calculated and plotted for comparison of regression line slopes for each of the above compounds (Figures 3-7). Slopes of the regression lines for the neonicotinoids had less variation among test years ($m \leq 0.18$) than did the pyrethroids ($m \geq 0.35$). The slope is an indication of the sensitivity of GWSS to the test concentration and the correlation between the test concentration and the mortality response (Georghiou and Metcalf 1961, Prabhaker et al. 2006). Higher slopes indicate a greater rate of increase in mortality responses per increased exposure concentration. Interestingly, the annual slopes for bifenthrin (Figure 6) and fenpropathrin (Figure 7) increased from 2015 to 2016. However, we have not yet determined if this difference in response indicates any shifts in exposure response in the field.

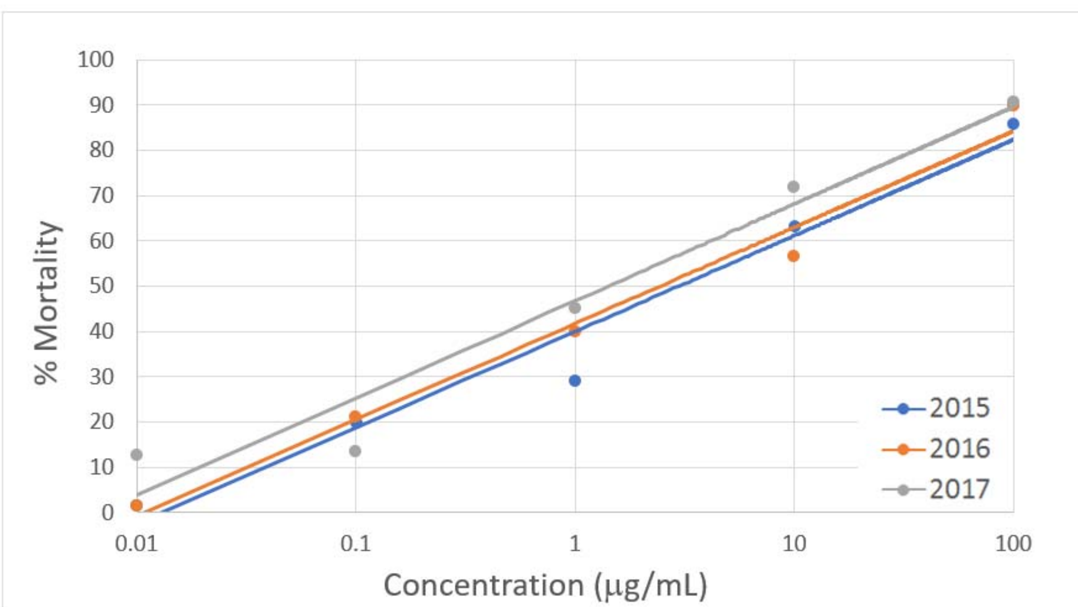

Figure 3. Annual mortality responses of *H. vitripennis* to imidacloprid. Corresponding regression line slopes reported in Table 2.

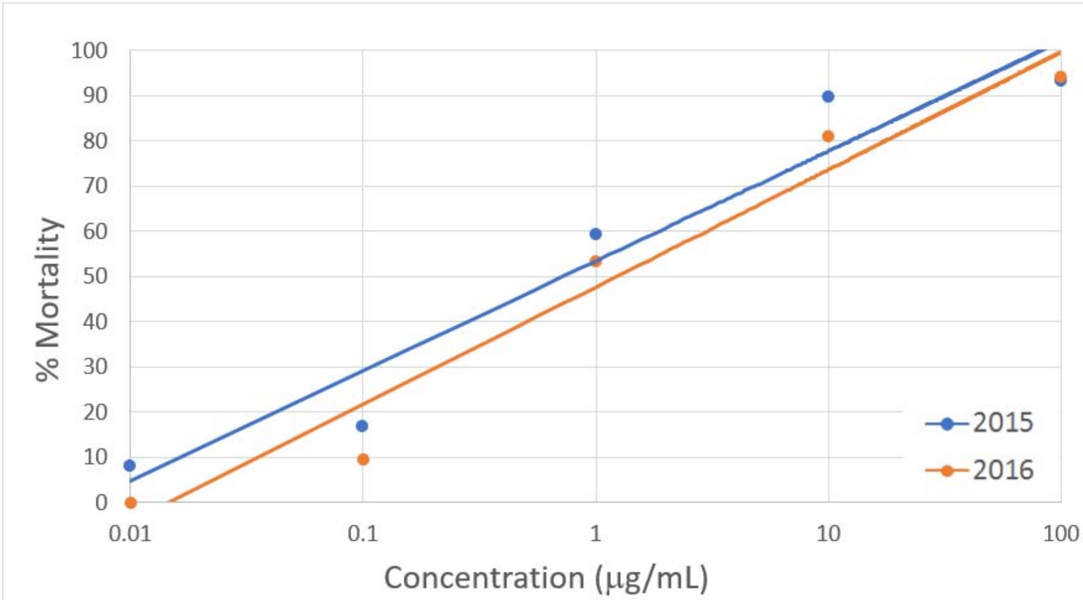

Figure 4. Annual mortality responses of *H. vitripennis* to thiamethoxam. Corresponding regression line slopes reported in Table 2.

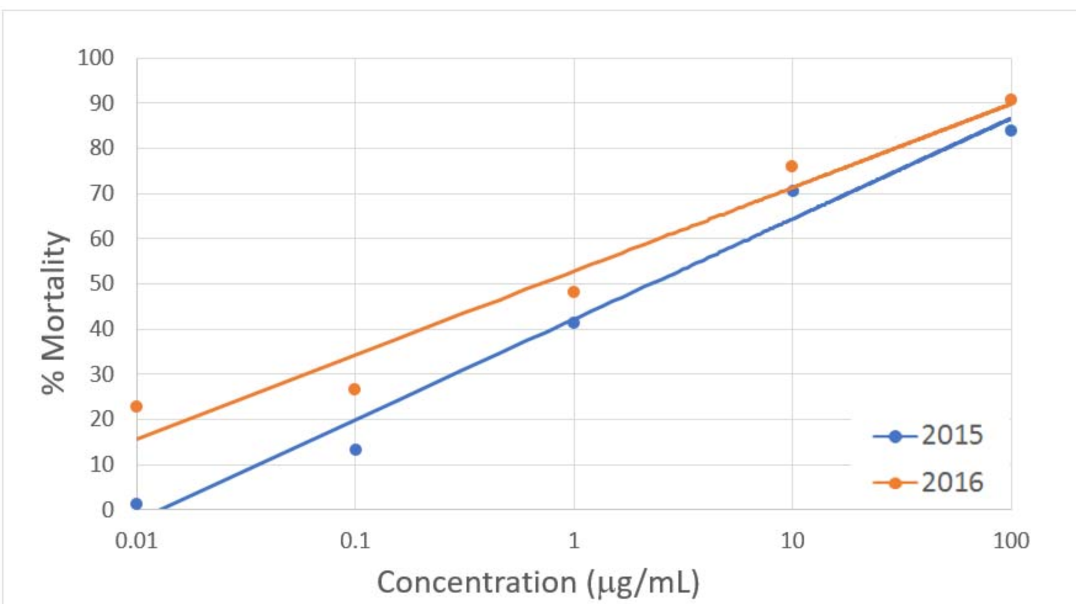

Figure 5. Annual mortality responses of *H. vitripennis* to acetamiprid. Corresponding regression line slopes reported in Table 2.

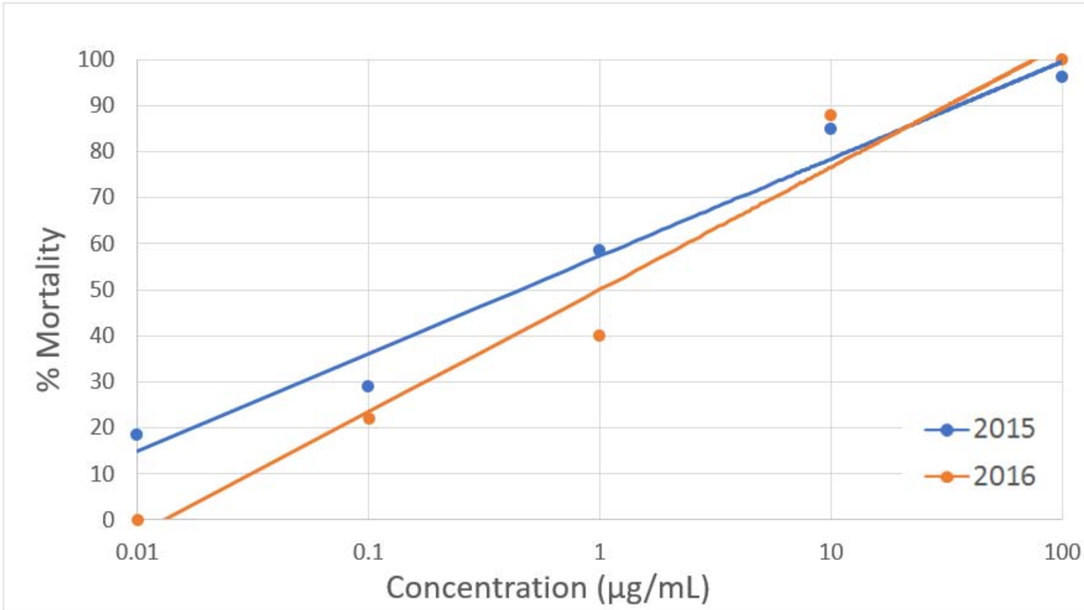

Figure 6. Annual mortality responses of *H. vitripennis* to bifenthrin. Corresponding regression line slopes reported in Table 2.

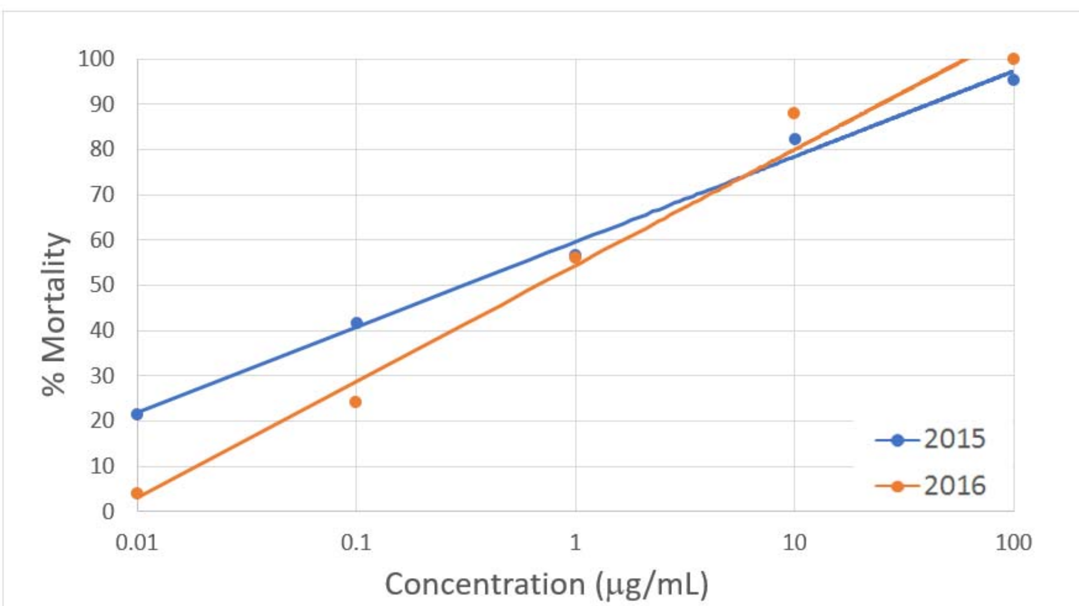

Figure 7. Annual mortality responses of *H. vitripennis* to fenpropathrin. Corresponding regression line slopes reported in Table 2.

Objectives 3 and 4
To begin elucidating the relationships between historical applications and GWSS resurgence in different areas, we have identified the locations of all applications of the 8 compounds, including every applied formulation of each compound, on all reported hosts of GWSS, both annual and perennial, within Kern Co. zones 1 and 3 from 2001 through 2017. Zones 1 and 3, historically and recently, have been the areas of Kern Co. with the highest GWSS populations. These data are maintained in an excel spreadsheet for input into the GIS. To date, we have added the 2015, 2016, and 2017 neonicotinoid data to the GIS, and we continue to work on previous years. We currently are determining the best methods of analysis of these data and working to input the GWSS trap data from the past 16 years into the GIS. This will enable us to determine if pesticide usage impacted the levels of GWSS at subsequent sampling times.

Publications and Presentations

Publications

Perring, T.M., N. Prabhaker, S. Castle, D. Haviland, B. Stone-Smith. 2016. Monitoring for insecticide resistance in the glassy-winged sharpshooter *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae) in California. Pp. 221-229 In T. Esser (Ed.) Proceedings, 2016 Pierce's Disease Research Symposium. California Department of Food and Agriculture, Sacramento, CA.

Perring, T.M., Prabhaker, N., Andreason, S., Castle, S., Haviland, D., Stone-Smith, B. 2017. Monitoring for insecticide resistance in the glassy-winged sharpshooter in California. Pp. 157-162 in Esser (ed.) Research Progress Reports: Pierce's Disease and Other Designated Pests and Diseases of Winegrapes. December 2017. California Department of Food and Agriculture, Sacramento, CA.

Presentations

Andreason, S., Perring, T.M., Prabhaker, N., Castle, S., Ganjisaffar, F., Haviland, D., Stone-Smith, B. 2018. Insecticide resistance in glassy-winged sharpshooters in Kern County, California. Current Wine and Winegrape Research. Davis, CA. 21 February 2018.

Research Relevance Statement

Studies conducted in 2015 showed that GWSS were less susceptible to insecticides commonly used in management programs than they were in 2001 and 2002. Of particular concern was that the LC_{50} values for the neonicotinoids, imidacloprid, thiamethoxam, and acetamiprid, increased as the 2015 growing season progressed. Subsequent bioassays conducted in 2016 found GWSS susceptibility to each tested compound was within the 2015 range, and the trend of seasonal susceptibility reduction was again observed for imidacloprid and thiamethoxam. In 2017, this trend was explored by focusing on imidacloprid susceptibilities at 4 different sites over the course of the testing season, which revealed that seasonal susceptibility reductions occur at different sites with different patterns of nearby neonicotinoid treatments. These nearby treatments may have an affect on the degree of susceptibility reduction, with close treatments likely resulting in higher reductions. Additionally, annual and current overall LC_{50} values were determined for each compound. Our goal is to understand if there is a link between site-specific resistance levels and recent usage of insecticides of interest in the surrounding areas. If so, this may inform the selection of chemicals in the future.

Layperson Summary

Insecticides are key to the management of Pierce's Disease, through their reducing impact on GWSS numbers. High insect numbers from 2012-2015, despite continued monitoring and treatment suggested a change in the susceptibility to commonly used products. Our studies in 2015, 2016, and 2017 showed varying levels of susceptibility to insecticides in Kern County populations of GWSS, with declining susceptibility as the season progressed. Fortunately, there was no further reduction in susceptibility from the initiation of our tests in 2015 to our final tests in 2017, but levels of susceptibility to two tested insecticides were still much lower than in 2001 when the area-wide GWSS program was initiated. We are now digging into historical records of insecticide applications alongside area-wide GWSS counts from 2001-2017 to reveal any factors leading to GWSS population spikes. Should any strong relationships be shown, the information may be used to advise the future selection of insecticides for GWSS control.

Status of Funds

This is a two year project that was initiated in July 2016. Funding expenditures are appropriate for the current place in the grant cycle.

Summary and Status of Intellectual Property

Aside from the published proceedings and the presentation at the CDFA PD conference, no intellectual property was produced as a result of this research project.

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