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 from July 2017 to July 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).

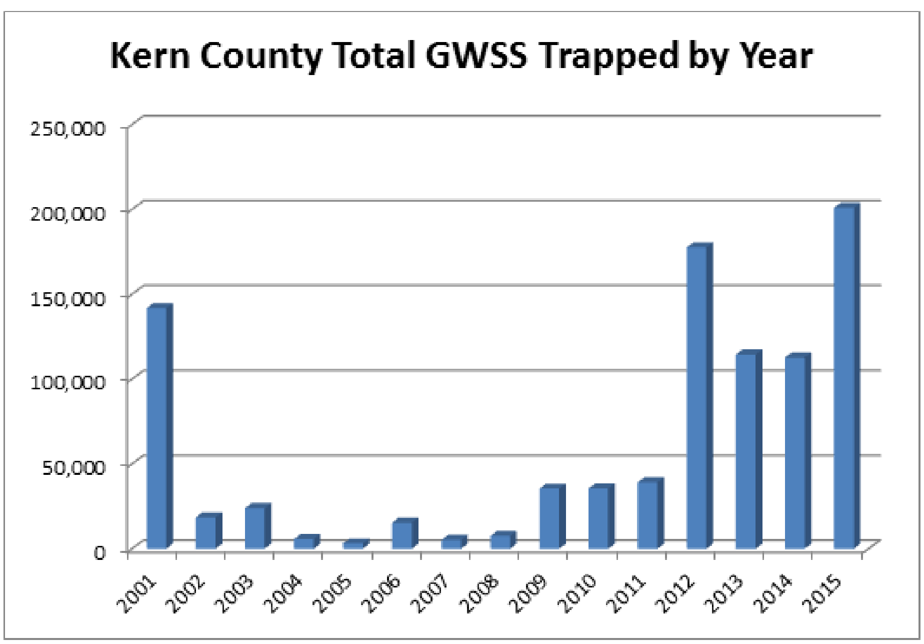

Figure 1. Total number of GWSS caught on CDFA traps in Kern Co. from 2001 – 2015. (From Haviland 2015)

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 neonicotinoids 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.

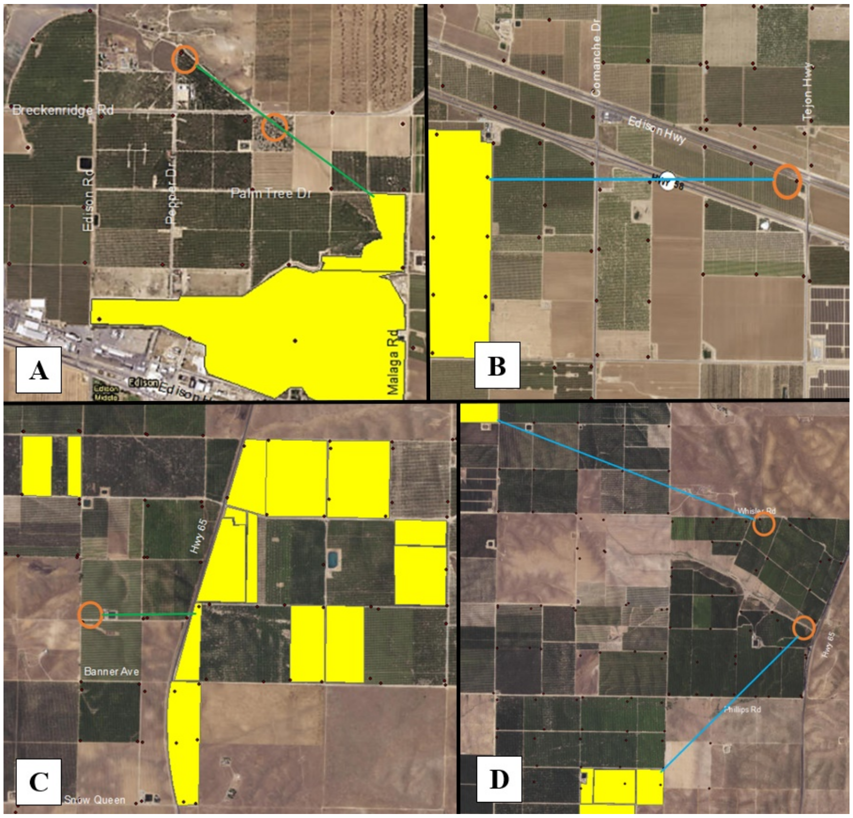

Figure 2. Four Kern County locations chosen for *H. vitripennis* collection and imidacloprid bioassays. (A) Treated Site 1 (T1), (B) Organic Site 1 (O1), (C) Treated Site 2 (T2), and (D) Organic Site (O2). Citrus or grapes treated with imidacloprid in 2017 are represented by the yellow areas. Orange 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.

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.

<u>Objectives</u>

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.

<u>Activities and Accomplishments</u>

Objectives 1 and 2

The 2017 bioassays with imidacloprid were conducted on GWSS collected monthly from 4 different Kern Co. sites from July through October. Initially our bioassays were grouped and analyzed according to the 'organic' versus 'treated' site designations as reported in Perring et al. (2017). We have since analyzed each site individually to determine if susceptibility reduction over the season was related to the distance of the collection sites from field applications of imidacloprid. The previously named 'organic' sites included the E Edison (O1) and S Hwy 65 (O2) locations, and the 'treated sites' included the W Edison (T1) and N Hwy 65 (T2) locations.

We created a new map of our 4 sites which includes the timing of nearby imidacloprid applications (all formulations) applied to surrounding perennial hosts (grape, grapefruit, lemon, orange, pistachio, tangelo, and tangerine; listed in CDFA Plant Quarantine Manual, Section 454 <http://pi.cdfa.ca.gov/pqm/manual/pdf/454.pdf>) from January 1 through October 9, 2017 (Figure 3).

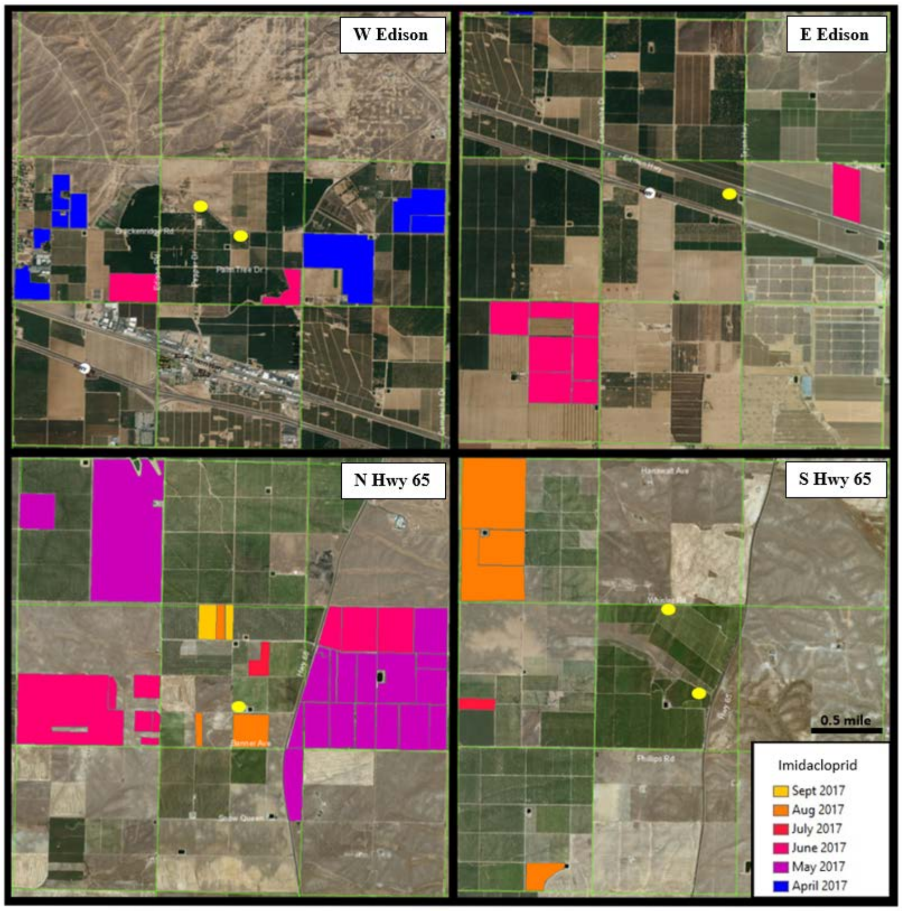

Figure 3. Locations of *H. vitripennis* collections in 2017. Yellow dots represent the exact collection sites. In the upper left quadrant is site W Edison; upper right is E Edison; lower left is N Hwy 65; and lower right is S Hwy 65. Each quadrant contains the approximately 3 mi^2 region surrounding each site. The legend indicates the months in which imidacloprid applications were made.

Each GWSS collection site had a unique situation of proximate imidacloprid applications and treatment timings. The two 'treated' sited, W Edison and N Hwy 65, had applications early in the growing season (April and May, respectively), whereas the previous 'organic' sites', E Edison and S Hwy 65, had the earliest applications in June and July, respectively. There also were more frequent applications within the 1.5 mi radius around the W Edison and N Hwy 65 collection sites as well as applications closer to these sites. Collections from citrus orchards and bioassays began in July and were repeated at each site in August (Table 2). Resulting 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, E and W Edison, could not be tested into late season as GWSS numbers were significantly lower in September. N and S Hwy 65 collections were bioassayed in mid-September, but then only S Hwy 65 could be tested in October. Analyzing these sites individually, we found that susceptibility of the GWSS collected at the N and S Hwy 65 sites decreased significantly from July to September and October, respectively (Table 2). At N Hwy 65, where imidacloprid was applied early and often, susceptibility dropped 29-fold. At S Hwy 65, with applications later and less frequent, susceptibility decreased 11-fold. These results suggest that seasonal reductions in susceptibility to imidacloprid 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.

Further analysis of our bioassay results using a generalized linear mixed model (GLMM) corroborated the significance of the observed seasonal decreases. With all sites combined, there was was a significant decrease from an average 50.5% mortality in July to 23.7% and 29.6% in September and October, respectively (Table 3). When the sites were analyzed separately, mortalities at S Hwy 65 significantly decreased from 61.3% to 29.6%, while mortalities at N Hwy 65 significantly decreased from 53.3% to 20.0%.

Table 3. Imidacloprid-induced mortality of *Homalodisca vitripennis* collected in 2017 at different locations in Kern County, CA analyzed by a GLMM.

Year	Date	Combined Mortality (%)	S Hwy 65 Mortality (%)	N Hwy 65 Mortality (%)
2017	July 24	50.5 (147) a	61.3 (30) a	53.3 (30) a
	August 8	46.4 (120) b	47.5 (48) b	62.1 (12) a
	September 12	23.7 (60) c	27.3 (30) c	20.0 (30) b
	October 9	29.6 (101) c	29.6 (101) c	--------

Values within the same column followed by the same letter are not significantly different, Tukey's test ($P < 0.05$). The number of replicates (clip cages containing five insects) on each date are given in parentheses.

Comparing this study's results to the baseline susceptibility levels determined in 2001/2002 (Prabhaker et al. 2006), all bioassays conducted on imidacloprid, thiamethoxam, acetamiprid, bifenthrin, and fenpropathrin were combined to determine annual LC_{50} values for each compound per tested year (Table 4). We did not include data from some previously tested compounds (flupyradifurone, chlorpyrifos, and dimethoate) because of a lack of adequate bioassays resulting from fewer GWSS in 2016 and because of high variation in the responses of the few tests we were able to conduct. For each of the neonicotinoid and pyrethroid compounds, the annual LC_{50} values were not significantly different from 2015 to 2016.

Table 4. 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 imidacloprid, we found that there was a 3.5-fold decrease in susceptibility from 2001/2002 (average LC_{50} = 0.82 µg/ml; 2001 95% FL: 0.68 – 2.54 µg/ml and 2002 95% FL: 0.09 – 0.51 µg/ml) to now. However, with overlapping 95% FL, this decrease was not significant. The thiamethoxam LC_{50} value determined previously could not be compared to the current value because the compound was previously tested as a foliar insecticide. Thus, this study establishes the baselines susceptibility level of GWSS to thiamethoxam applied systemically. For acetamiprid, susceptibility decreased 7-fold from the previous assays (average LC_{50} = 0.26 µg/ml; 2001 95% FL: 0.18 – 0.56 and 2002 95% FL: 0.02 – 0.14) until now. With no overlap in 95% FL, this was a significant decrease. GWSS susceptibility to bifenthrin significantly decreased, as well. There was a 152-fold decrease from 2001/2002 (average LC_{50} = 0.0044 µg/ml) to the present with no overlap in 95% FL. There was a 9.5-fold decrease in susceptibility to fenpropathrin (previously: average LC_{50} = 0.042 µg/ml; 95% FL: 0.007 – 0.205), but the slight overlap in 95% FL indicates that the decrease is not significant. Overall, of the 5 compounds repeatedly tested, acetamiprid and bifenthrin were determined to be significantly less toxic to GWSS, indicating that resistance to these compounds as likely developed over the last 15 years.

Objectives 3 and 4

After the recent publication of our findings related to Objectives 1 and 2 (Andreason et al. 2018), we have shifted our focus to Objectives 3 and 4. To begin exploring the relationships between historical pesticide applications and GWSS resurgence in different areas, we have obtained the Kern Co. pesticide application records and identified all applications of the 8 compounds of interest from 2001 through 2017. These filtered records include applications of every formulation of each compound to all reported hosts of GWSS, both annual and perennial, within Kern Co. zones 1 and 3 over the last 17 years. We are focusing on zones 1 and 3 because they have historically and recently been the areas of Kern Co. with the highest GWSS populations. These data are maintained in an excel spreadsheet for input into 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 are currently determining the best methods of analysis of these data and working to input the GWSS trap data from the past 17 years.

Publications and Presentations

Publications

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.

Andreason, S.A., N. Prabhaker, S.J. Castle, F. Ganjisaffar, D.R. Haviland, B. Stone-Smith, and T.M. Perring. 2018. Reduced susceptibility of *Homalodisca vitripennis* (Hemiptera: Cicadellidae) to commonly applied insecticides. *Journal of Economic Entomology* <https://doi.org/10.1093/jee/toy192>

Presentations

Perring, T.M., N. Prabhaker, S. Castle, S. Andreason, D. Haviland, B. Stone-Smith. 2017. Insecticide resistance in the glassy-winged sharpshooter, a vector of *Xylella fastidiosa*. Entomological Society of America Annual Meeting, Denver, Co. 7 November 2017.

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

Bioassays have been conducted over the past 3 years on imidacloprid, thiamethoxam, acetamiprid, bifenthrin, and fenpropathrin. Comparing these results with bioassays conducted in 2001 and 2002 showed that of these 5 compounds, acetamiprid and bifenthrin were significantly less toxic to GWSS, indicating that resistance to these compounds has developed over the last 15 years. Further studies showed that susceptibility to imidacloprid and thiamethoxam decreased as the season progressed. In 2017, we found that GWSS collected near fields that had been treated with imidacloprid earlier in the season and more frequently had lower susceptibility as the season progressed than GWSS collected near fields with later season, infrequent applications. This suggests that continued applications of imidacloprid contributes to lower susceptibility of GWSS in the nearby vicinity. As we continue in this project, we will be investigating the relationships between site-specific resistance levels and recent usage of insecticides in the surrounding areas.

Layperson Summary

Insecticides remain the most frequently used tool for the management of GWSS and Pierce's Disease. Our interest in this project was due to the high GWSS numbers from 2012-2015, despite continued monitoring and treatments. This suggested that the populations may be changing with respect to their susceptibility to commonly used products. Our studies in 2015, 2016, and 2017 showed varying levels of resistance to insecticides in Kern County populations of GWSS. Compared to similar studies conducted in 2001 and 2002, we find resistance for the chemicals acetamiprid and bifenthrin. We also found that as the season progressed, the insects were less susceptible to one of the most widely used materials, imidacloprid. In 2017, we documented that insects collected near fields that had been treated often and early with imidacloprid were less susceptible later in the season than insects collected near fields that had not been treated early and often. This suggests that timing and frequency of imidacloprid impacts the season-long susceptibility of GWSS to this material.

Status of Funds

In the course of our studies, we encountered a very low abundance of GWSS in Kern County, so our scheduled bioassays had to be reduced. We were unable to conduct bioassays with the proposed number of materials and we were restricted to fewer sites than we had originally planned. The 2016 season had fewer GWSS than 2015, and 2017 was even more restrictive. Therefore, we focused our attention in 2017 solely on imidacloprid. The fewer number of GWSS resulted in more time analyzing trap data looking for potential collection sites, more trips to the field, and more bioassays with fewer numbers of GWSS in each bioassay. While we were able to complete the field work for Objectives 1 and 2, we were unable to devote the necessary time and resources to Objectives 3 and 4. We have been approved for a no-cost extension to continue work on these objectives. To date, we have obtained and organized historic GWSS densities and treatment records (locations, chemicals used, and timing of applications) into a Geographic Information System for use in statistical analyses. We will analyze historical spray records and GWSS counts to see if treatments in specific fields can be tied to subsequent GWSS numbers in traps adjacent to the treated fields. We will investigate both the distance from treated fields and the GWSS densities at different times after the applications. By showing relationships between sprays with particular insecticides and subsequent GWSS in the areas of the sprays, we hope to shed some light on how various pesticides have impacted GWSS densities in Kern County over the years. Success in this part of our project will be informative to growers and the GWSS control program as materials are being considered for use against GWSS in the future

Summary and Status of Intellectual Property

Aside from published manuscripts and presentations, no intellectual property was produced as a result of this research project.

Literature Cited

Andreason, S.A., N. Prabhaker, S.J. Castle, F. Ganjisaffar, D.R. Haviland, B. Stone-Smith, and T.M. Perring. 2018. Reduced susceptibility of *Homalodisca vitripennis* (Hemiptera: Cicadellidae) to commonly applied insecticides. *Journal of Economic Entomology* <https://doi.org/10.1093/jee/toy192>

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Acknowledgements

We thank the outstanding support of Stephanie Rill and the staff in David Haviland's lab. They have been instrumental in helping us collect GWSS for our bioassays. Dr. Fatemeh Ganjisaffar provided support in collecting GWSS, assisting in bioassays, and conducting statistical analyses. We also thank Tim Lewis for his contribution of GIS expertise and the Perring lab team of Nancy Power, Shayla Hampel, Seanathan Chin, and Shylla Taqi to collect GWSS and conduct bioassays.