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 2019

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. 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).

In the 2015 study, we evaluated 8 compounds in 4 insecticide classes: Neonicotinoids (Imidacloprid, Thiamethoxam and Acetamiprid), Butenolide (Flupyradifuron), Pyrethroids (Bifenthrin and Fenpropathrin), and Organophosphates (Chlopyrifos and Dimethoate) in both systemic uptake and foliar bioassays. 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. 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, evaluations of Imidacloprid, Thiamethoxam, Acetamiprid, Bifenthrin and Fenpropathrin showed similar levels of resistance to the 2015 bioassays (Perring et al. 2016, 2017). The data also showed declining susceptibility to the systemic neonicotinoids (imidacloprid and thiamethoxam) over the season, revealing a trend repeated from the 2015 bioassays.

Our 2017 bioassays were limited to imidacloprid because the numbers of GWSS available for collection were low. We selected 4 sites with unique patterns of nearby field applications of imidacloprid (Admire® Pro) for monthly testing to determine if seasonal reduced susceptibility occurred within different Kern Co. regions. Two of the sites were considered '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.

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

We reported on these objectives at the December 2018 meeting in San Diego (Perring et al. 2018) and have published the results of this research (Andreason et al. 2018). To summarize here, we found that, for imidacloprid, there was a 3.5-fold decrease in susceptibility from 2001/2002 (0.82 µg/ml) to the present (2.51 µg/ml – 3.43 µg/ml). However, this decrease is not significantly different. The thiamethoxam LC_{50} value determined in 2001/2002 could not be compared to the current value (1.03 µg/ml) because the compound was previously tested as a foliar insecticide. Therefore, this study establishes the baselines susceptibility level of GWSS to thiamethoxam applied systemically. For acetamprid, susceptibility decreased 7-fold from earlier studies (0.26 µg/ml in 2001/2002) until now (1.78 µg/ml). With no overlap in the 95% FL, this is a significant decrease. GWSS susceptibility to bifenthrin significantly decreased as well. There was a 152-fold decrease from 2001/2002 (0.0044 µg/ml) to now (0.67 µg/ml) with no overlap in the 95% FL. There was a 9.5-fold decrease in susceptibility to fenpropathrin (previously: 0.042 µg/ml) to current (0.40 µg/ml), but the slight overlap in the 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 has developed over the last 15 years. The 2017 bioassays with imidacloprid indicated that seasonal reductions in susceptibility to imidacloprid occurred and that differential proximity to field applications was a contributing factor to the degree of imidacloprid susceptibility.

Objectives 3 and 4

To explore the relationships between historical pesticide applications and GWSS resurgence in different areas, we need to work with 3 large data sets; the crop coverages, the pesticide use data, and the GWSS abundance data. For each of these data sets, we have obtained all the data that exists from 2001-2016 for CDFA Zones 1 (Hwy 65 Area, north of Bakersfield) and Zone 3 (General Beale/Edison Area east of Bakersfield).

<u>Crop Coverage:</u>

The foundation of the Geographic Information System (GIS) exists in the crop coverage data for each year. These data were obtained from the Kern County Department of Agriculture and Measurement Standards. Each field has a geographic location that has been placed on the map in the GIS, and also is identified by the Township, Range, Section (TRS) and Site ID. Using only the Site ID to define the parcel location proved inadequate because; 1) the Site ID is assigned to a land owner/lessor and the ownership could and did change over the years, and/or 2) multiple owners may use the same parcel notation (e.g., 1, 20, 13 or A, B, C). So we needed to keep track of the correct ID for that site as the years progressed. We used attribute layers for Townships and Sections provided by

ESRI (Redlands, CA), a major source of GIS data and software for the international market, to determine where the parcel was located within the section. We needed to gather data from adjacent parcels that fell into another section to insure that the parcel on which we wish to work is consist through the 16 years of the analysis.

Pesticide Use Data:

We have obtained the application records for imidacloprid, thiamethoxam, acetamiprid, flupyradifurone, fenpropathrin, bifenthrin, chlorpyrifos and dimethoate from the Kern County Agricultural Commissioner Pesticide Use database from 2001-2016. These 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. We received the records in Excel format and identified all formulations of the 8 materials because the pesticide use records contain trade names and we wanted to be sure to obtain all uses of each material for our analyses. The pesticide use database included Township, Range, and Section (TRS), allowing us to identify all applications of our eight compounds within a particular section. From this query, we could identify all applications to a particular parcel using the Site ID. At this point we identified each application and date of application for that parcel for each year. This was done for each parcel over the 16 years.

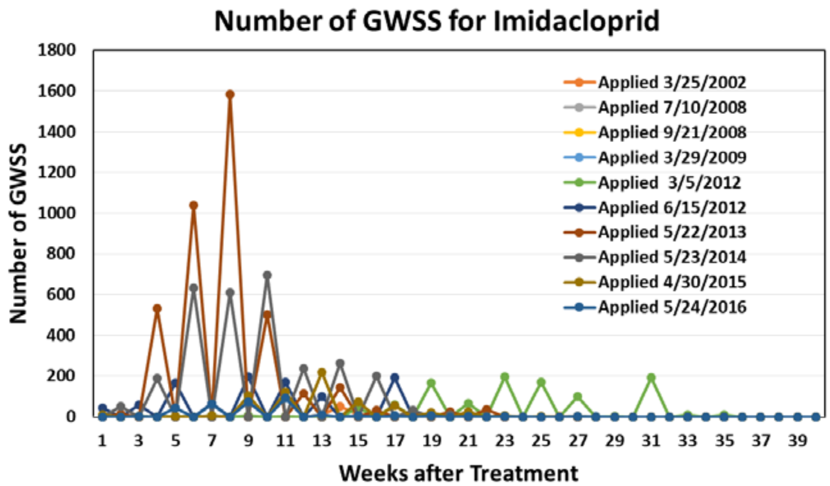
GWSS Abundance Data:

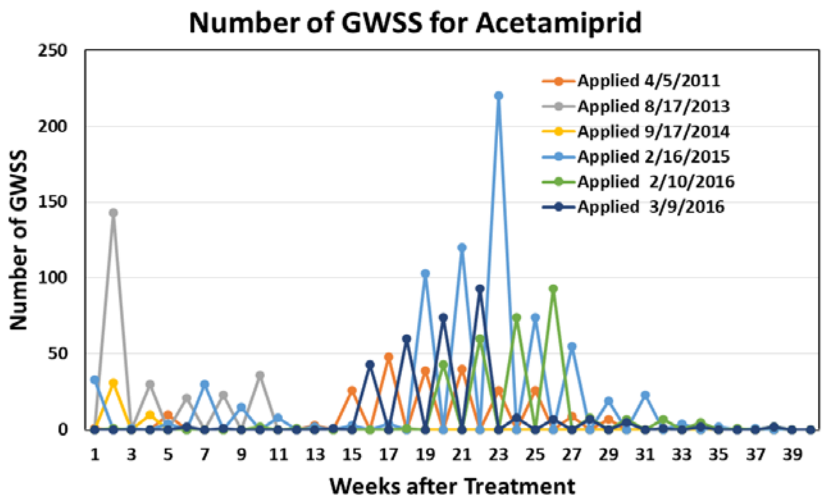
GWSS trap data have been collected by the CDFA GWSS Program and we obtained all trap counts from 2001-2016 for zones 1 and 3. We received these trap counts in GIS map format which shows the locations of each trap in the dataset. In the GIS we were able to determine which traps were located within one quarter mile of the parcel of interest. The number of traps per parcel varies depending on the shape of the parcel and the physical placement of traps. After identifying the traps by their unique ID, we were able to query the trap dataset by Trap ID, giving us access to Trap counts (adult GWSS), and the date of trap service, which are included in the trap data files. We have learned that not all traps were serviced with the same regularity (for example initially all traps were recorded weekly, but then it was changed to every 2 weeks on average; but this was not consistent across all trap sites. In addition, on some weeks the traps were not accessible, so there are "holes" in the dataset. For these dates, we needed to include a missing data point so that so count data could be aligned by date. Over the 16 years of the study, some traps were added and other traps were deleted. This requires us to query the traps annually to identify these changes. The trap data for each trap was transferred to another Excel file, organized horizontally by date, with a vertical listing of all traps within ¼ mile of the target field over the 16 years of trapping. The pesticide applications then could be added to the file on the appropriate date, allowing us to determine the average number of GWSS present from 1-40 weeks following an application.

Application of Experimental Process

We recently completed our first attempts at putting the data together for correlation analyses. Four parcels were chosen: 2 adjoining parcels east of Bakersfield in the unincorporated area of Edison north of State Highway 50 in Area 3 of Kern County. We also selected 2 additional parcels on either side of State Highway 65 north of Bakersfield in Area 1. For each parcel, we created a new excel file in which the mean GWSS numbers were listed by "weeks after application" rather than by calendar date. This enabled visualization of the insect numbers as a function of time after application.

While we are still in the early stages of this analyses, we present data for one of the parcels for imidacloprid, acetamiprid and chlorpyrifos. Looking at imidacloprid, we determined that GWSS numbers were greatest between 3 and 11 weeks after application (Figure 1, top graph). For acetamiprid the greatest GWSS activity was 19 to 25 weeks after application (Figure 1, middle graph). For chlorpyrifos, there wasn't a strong relationship between timing after application and GWSS numbers, and overall numbers were low except for the 2015 data (Figure 1, bottom graph).





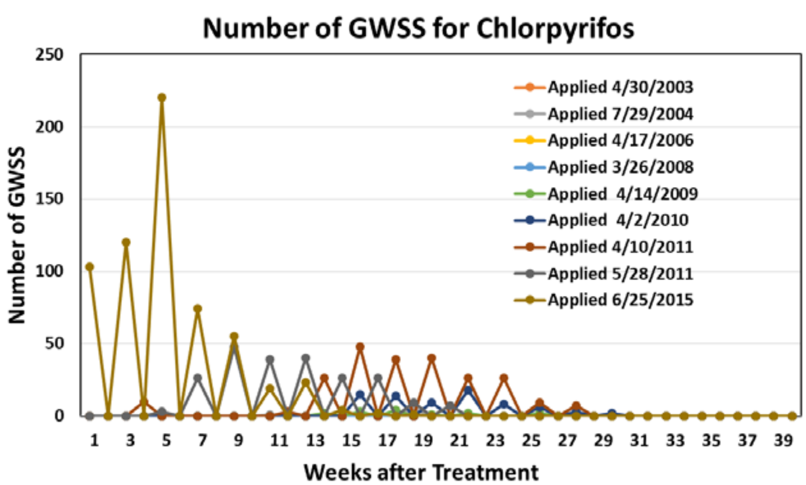


Figure 1. Average number of GWSS determined from 1-40 weeks after application of imidacloprid (top graph), acetamiprid (middle graph) and chlorpyrifos (lower graph) for insecticides applied from 2002-2016

At this point in time, we are not prepared to draw any conclusions from these graphs. However, we have developed a process for amalgamating the data from 3 very large data sets that are not cross referenced with each other. With these protocols, we are in a position to bring together data for additional parcels and additional insecticides and investigate analytical methods to determine if there is a relationship between treatments of particular materials and numbers of GWSS at certain times before and after treatment.

Publications and Presentations

Publications

Perring, T.M., N. Prabhaker, and S. Castle. 2015. Monitoring for insecticide resistance in the glassy-winged sharpshooter in California. Pp. 142-146 In. T. Esser and R, Randhawa (eds.) Research Progress Reports: Pierce's Disease and Other Designated Pests and Diseases of Winegrapes. December 2015. California Department of Food and Agriculture, Sacramento, CA.

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.

Andreason, S. A., Prabhaker, N., Castle, S. J., Ganjisaffar, F., Haviland, D. R., Stone-Smith, B., & Perring, T. M. 2018. Reduced Susceptibility of *Homalodisca vitripennis* (Hemiptera: Cicadellidae) to Commonly Applied Insecticides. Journal of Economic Entomology. 111: 2340-2348.

Perring, T.M., Prabhaker, N., Andreason, S., Castle, S., Haviland, D., Stone-Smith, B. 2017. Monitoring for insecticide resistance in the glassy-winged sharpshooter *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae) in California. Pp. 157-162 In T. 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.

Perring, T.M., Prabhaker, N., Andreason, S., Castle, S., Haviland, D., Stone-Smith, B. 2018. Insecticide resistance in the glassy-winged sharpshooter: Using historical use patterns to inform future management strategies. Pp. 153-160 In T. Esser (Ed.) Proceedings of the 2018 Pierce's Disease Research Symposium. California Department of Food and Agriculture, Sacramento, CA.

Presentations

Perring, T.M. 2016. Insecticide resistance in the GWSS: Using historical use patterns to inform future management strategies. 2016 Pierces Disease Research Symposium, San Diego, CA. December 2016

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.

Perring, T.M. 2018. Insecticide resistance in the GWSS: Using historical use patterns to inform future management strategies. 2018 Pierces Disease Research Symposium, San Diego, CA. December 2018

Research Relevance Statement

Previous work has shown that GWSS are less susceptible to insecticides commonly used in management programs than they were in 2001 and 2002. Of five compounds studied, we found significant reductions in GWSS susceptibility to acetamiprid and bifenthrin over the last 16 years. In addition, we have determined that susceptibility to imidacloprid decreases over each field season, and that differential proximity to field where imidacloprid has been applied was a contributing factor to the degree of imidacloprid susceptibility. Having shown this relationship, we have turned our attention to exploring the relationships between historical pesticide applications and GWSS resurgence in different areas. This requires manipulation of 3 very large data sets (the crop coverages, the pesticide use data, and the GWSS abundance data), and here we report our progress in this process. Our goal is to understand if there is a link between site-specific pesticide usage and GWSS abundance following treatment. 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 resistance 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 analyzing historical records of insecticide applications alongside area-wide GWSS counts from 2001-2016 to determine if pesticide use has resulted in GWSS resurgence following application. This may indicate pesticide resistance and may be used to advise the future selection of insecticides for GWSS control.

Status of Funds

This was originally a two year project initiated in July 2016. In June of 2018, we were granted a no-cost extension to continue working on the last 2 objectives of this project. Funds have now been expended and we are in the final quarter of this funded project.

Summary and Status of Intellectual Property

Aside from the published papers and the presentations at various conferences, no intellectual property was produced as a result of this research project.

Literature Cited

Andreason, S. A., Prabhaker, N., Castle, S. J., Ganjisaffar, F., Haviland, D. R., Stone-Smith, B., & Perring, T. M. 2018. Reduced Susceptibility of *Homalodisca vitripennis* (Hemiptera: Cicadellidae) to Commonly Applied Insecticides. Journal of Economic Entomology. 111: 2340-2348.

Haviland, D. 2015. Monitoring the resurgence of Pierce's Disease in Kern County vineyards. Pp. 1-2 In Consolidated Central Valley Table Grape Pest and Disease Control District Newsletter: Fall 2015.

Perring, T.M., N. Prabhaker, and S. Castle. 2015. Monitoring for insecticide resistance in the glassy-winged sharpshooter in California. Pp. 142-146 In. T. Esser and R, Randhawa (eds.) Research Progress Reports: Pierce's Disease and Other Designated Pests and Diseases of Winegrapes. December 2015. California Department of Food and Agriculture, Sacramento, CA.

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 *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae) in California. Pp. 157-162 In T. 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.

Perring, T.M., Prabhaker, N., Andreason, S., Castle, S., Haviland, D., Stone-Smith, B. 2018. Insecticide resistance in the glassy-winged sharpshooter: Using historical use patterns to inform future management strategies. Pp. 153-160 In T. Esser (Ed.) Proceedings of the 2018 Pierce's Disease Research Symposium. California Department of Food and Agriculture, Sacramento, CA.

Prabhaker, N., S.J. Castle, F.J. Byrne, N.C. Toscano, and T.J. Henneberry. 2006. Establishment of baseline susceptibility to various insecticides for glassy-winged sharpshooter, *Homalodisca coagulata*, by comparative bioassays. J. Econ. Entomol. 99: 141-154.

Redak, R., B. White, and F. Byrne. 2015. Management of insecticide resistance in glassy-winged sharpshooter populations using toxicological, biochemical, and genomic tools. Pp. 157-163 In T. Esser and R, Randhawa (eds.) Research Progress Reports: Pierce's Disease and Other Designated Pests and Diseases of Winegrapes. December 2015. California Department of Food and Agriculture, Sacramento, CA.

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