TITLE OF REPORT: Interim Progress Report for CDFA Agreement Number 14-0379-SA

TITLE OF PROJECT: Management of insecticide resistance in GWSS populations using toxicological, biochemical and genomic tools.

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TIME PERIOD: March 2019-July 2019

INTRODUCTION:

Systemic imidacloprid treatments have been the mainstay of GWSS management in citrus, grapes, and commercial nursery operations. The treatments in citrus groves are generally applied post-bloom to suppress the newly emerging spring populations. The use of winter or early spring foliar treatments of pyrethroid or carbamate treatments were introduced to the management program to suppress over-wintering adults and reduce the first early season cohort of egg-laying adults. The combination of early season foliar treatments combined with the more persistent systemic treatments has effectively managed GWSS populations in the Bakersfield area for many years.

In Kern County, GWSS populations have been monitored since the area-wide treatment program was instigated by the CDFA following an upsurge in GWSS numbers and an increase in the incidence of PD. The data show an interesting pattern of sustained suppression of GWSS populations, following the implementation of the area-wide treatment program, until 2009 when numbers began to increase again, culminating in a dramatic flare-up in numbers in 2012. In 2012, a single foliar treatment with either Lannate® (methomyl: carbamate insecticide class), Assail® (acetamiprid: neonicotinoid insecticide class) or Baythroid® (cyfluthrin: pyrethroid insecticide class) was applied in groves in late March while systemic treatments with imidacloprid (neonicotinoid insecticide class) were applied mid-March to early April. The application of systemic imidacloprid during 2012 mirrored the strategy used in 2001 when the imidacloprid treatments were highly effective in suppressing the GWSS populations. Despite the additional foliar treatments in 2012, the insecticide treatments failed to suppress the insect population at a level that had occurred previously. It is a worrying trend that in the 2 years prior to 2012, there was a steady increase in total GWSS numbers, an early indication that the predominant control strategy might be failing. Data collected after 2012 show that trap catches of GWSS numbers have remained high each year up until 2016 (when the most recent data were provided to us by David Haviland), despite more aggressive implementation of the area-wide treatment program that included a Winter 2015 pyrethroid application and a Spring 2016 foliar neonicotinoid in addition to the regular systemic imidacloprid treatment (Haviland and Stone-Smith, 2016). The consequence of the increase in GWSS populations has been a steady increase in the incidence of PD in the region. In the Temecula area, this worrisome increase in GWSS has not occurred, although trap counts for 2016 appeared earlier in the year and at higher levels than those recorded for most years since monitoring began (Daugherty, 2016). Since 2016, populations have remained low.

There is also significant concern for the development of insecticide resistance arising from the management of GWSS in commercial nursery production. The majority of commercial nurseries maintain an insect-sanitary environment primarily through the use of regular applications of soilapplied imidacloprid or other related systemic neonicotinoids. For nursery materials to be shipped outside of the Southern California glassy-winged sharpshooter quarantine area, additional insecticidal applications are required. Applications of fenpropathrin (pyrethroid insecticide class) or carbaryl (carbamate insecticide class) must be applied to all nursery stock shipped out of the quarantine area. As with citrus and vineyard production, the potential for the development of insecticidal resistance in nursery populations of GWSS to these three classes of insecticides (neonicotinoids, pyrethroids, and carbamates) is high.

The focus of this study is to investigate the role of insecticide resistance as a contributing factor to the increased numbers of GWSS that have been recorded since 2009 in commercial citrus and grapes in Kern County. In 2016, we confirmed the presence of resistance to imidacloprid and cross resistance to fenpropathrin in GWSS populations sampled from the General Beale Road area of Kern County. Resistance has since been confirmed by this project in GWSS sampled from additional sites in both Kern County and Tulare County.

OBJECTIVES:

1. For commonly used pyrethroid, carbamate, and neonicotinoid insecticides, determine LC_{50} data for current GWSS populations and compare the response to baseline susceptibility levels generated in our previous studies.
2. Define diagnostic concentrations of insecticides that can be used to identify increased tolerance to insecticides in insects sampled from other locations (where numbers are relatively low).
3. Monitor populations for known molecular markers of resistance to pyrethroids
4. Monitor populations for target-site insecticide resistance, by testing enzymatic activity against carbamates using the AChE biochemical assay
5. Monitor populations for broad-spectrum metabolic resistance, by comparing esterase levels in current populations of GWSS to baseline susceptibility levels we previously recorded.
6. Develop assays for additional resistance mechanisms not previously characterized in GWSS.

ACTIVITIES:

Objective 1: For commonly used pyrethroid, carbamate, and neonicotinoid insecticides, determine LC_{50} data for current GWSS populations and compare the response to baseline susceptibility levels generated in our previous studies.

AND

Objective 2: Define diagnostic concentrations of insecticides that can be used to identify increased tolerance to insecticides in insects sampled from other locations (where numbers are not so high).

<u>Neonicotinoid</u>

Each year, we plan our monitoring work of Central Valley GWSS populations based upon the CDFA areawide trapping data. The numbers have been dramatically lower this year, and the normal surge in populations that occurs in mid-June was not evident this year. Several reasons can account for the lower numbers, including the cooler winter/spring temperatures, and more aggressive control of populations in both conventional and organic groves (Beth Stone-Smith, personal communication, July 2019). Additional treatments for ACP control will also have had an effect on GWSS populations, particularly the post petal fall treatments applied during May. Despite the lower numbers, sharpshooters are now starting to resurge, and we have begun our testing for the 2019 season. We collected sufficient numbers at our HWY65 collection site to conduct a full dose response bioassay, that yielded an LD_{50} of 293 ng imidacloprid/insect. We also collected insects from the General Beale Rd area, and from urban locations in Bakersfield (1 site) and Riverside (2 sites). At the Bakersfield urban site, we conducted a full dose response bioassay (LD50 = 14 ng imidacloprid/insect), while at the other sites we were limited to bioassays at a single discriminating dose of 50 ng imidacloprid/insect, a dose that should provide 100% mortality of a susceptible population. While one of the Riverside sites was fully susceptible, there was only 50% mortality recorded at the other. Numbers were low at the latter site, but we will attempt a further collection in order to run an additional discriminating dose bioassay. Data for 2019 are summarized in **Figure 1**.

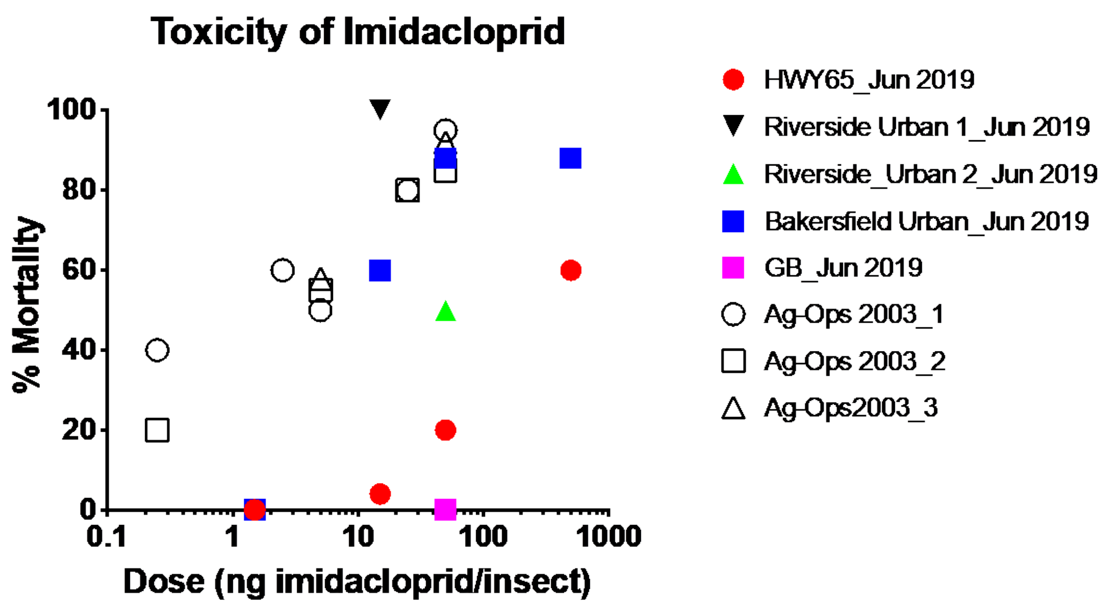

Figure 1. Dose-response data for 2019 GWSS populations. Data for some populations are limited to a single point due to the availability of low numbers. Data for Ag-Ops (3 separate bioassays) are included as a reference.

Over the next few months, our priority will be to evaluate the cross-resistance potential between imidacloprid and other insecticides. We have already shown that there is cross resistance to acetamiprid, so this compound should be used sparingly in the region as an alternative to imidacloprid. We will also evaluate flupyradifurone (Sivanto®), which is in IRAC Group 4, and is being used increasingly in the Central Valley. Although in a different subgroup within Group 4, flupyradifurone also targets the nACh receptor. While target site resistance is not suspected at this time, based on our sequence analysis to date, cross-resistance could occur if there was a common metabolic detoxification mechanism.

Objective 3: Monitor populations for known molecular markers of resistance to pyrethroids.

AND

Objective 6: Develop assays for additional resistance mechanisms not previously characterized in GWSS.

We have collected insects from nursery, urban, and field locations, for later use in analyses for the presence of resistance markers. We have already identified several cytochrome P450, glutathione S-transferase and ABC transporter genes based on the genome database of GWSS. In order to facilitate a more comprehensive analysis of their potential involvement in conferring resistance to imidacloprid and fenpropathrin, we are conducting RNA-seq analysis to compare individuals sampled from the Riverside, Tulare and Kern County locations where differences in toxicological response to the insecticides occur. We have completed the first round of RNA-seq experiments on bioassay survivors and the data are currently being analyzed (see below). In these experiments, we compare survivors from the topical application bioassays, as these individuals are more likely to express resistance-causing genes. Metabolism by cytochrome P450 (Cyt P450) enzymes is of particular interest because these enzymes are known to confer resistance to imidacloprid in several insect species, and our preliminary bioassays with the Cyt P450 inhibitor piperonyl butoxide show these enzymes increase susceptibility in resistant GWSS populations.

In preliminary evaluations using sample treatment as the variable in the analysis, the populations cluster by RNAseq similarity according to their resistance status (R populations cluster separately from the S and SR populations). The analysis on the first round of data is ongoing, with additional work required to compare genetic differences between individual insects.

Objective 4: Monitor populations for target-site insecticide resistance, by testing enzymatic activity against carbamates using the AChE biochemical assay.

AND

Objective 5: Monitor populations for broad-spectrum metabolic resistance, by comparing esterase levels in current populations of GWSS to baseline susceptibility levels we previously recorded.

No new data have been generated for this objective, since these objectives were largely addressed during the 2016 monitoring season (Redak et al, 2016), during which an assay was developed for GWSS that enabled the measurement of both the total esterase activity and the sensitivity of the AChE to paraoxon in an individual insect. In populations sampled from the Central Valley (GBR, HWY65, Tulare 2016) and Southern California (TEM2016), all insects were sensitive to the diagnostic concentration of 30 µM paraoxon. Insects were also tested from a nursery location in Orange County, and these insects were also sensitive to the OP.

Esterase activity was measured in GWSS collected from the Kern, Riverside, and Tulare County populations in 2016, and compared with data from our studies in 2003 (Riverside County) and 2015 (Kern County). We found no significant differences in esterase levels between the 5 populations, including the 2003 Ag-Ops population, and concluded that elevated levels of esterase activity cannot be used as a marker for pyrethroid resistance, as no causal link was established.

The conclusions from experiments conducted for these objectives are that AChE insensitivity and elevated esterases are not contributing to resistance in GWSS. The data also suggest that pyrethroid resistance in Central Valley populations is a result of cross resistance to imidacloprid.

Objective 6: Develop assays for additional resistance mechanisms not previously characterized in GWSS.

With the departure of Dr Brad White from UCR, Dr Jason Stajich of the Dept of Microbiology and Plant Pathology at UCR is collaborating with us on the RNAseq analysis. He is analyzing sequencing data generated for GWSS populations expressing different toxicological phenotypes, and is assisting with the design and execution of further molecular experiments that will be required to identify and validate resistance markers. Now that the GWSS populations are increasing in the Central Valley, we will be able to proceed with carrying out experiments to achieve our goal of developing a marker for imidacloprid resistance.

PUBLICATIONS:

No publications since the March 2019 report.

RESEARCH RELEVANCE STATEMENT:

Bioassay techniques used in this project have identified high levels of resistance to imidacloprid, cross resistance to the related insecticide acetamiprid, and moderate levels of resistance to the pyrethroid fenpropathrin. Our data confirm a major shift in toxicological response of sharpshooters to one of the most important insecticides used for their control. The consequence of using ineffective insecticides, or insecticides whose efficacy has been compromised by resistance, is that insects will survive treatments and then have the potential to act as vectors of Pierce's Disease. We have made good progress in developing assays that measure qualitative and quantitative changes in putative insecticide resistance-causing enzymes. These assays will allow us to evaluate the incidence of insecticide resistance in agricultural, nursery, and urban populations of GWSS. Data derived from this project will enable growers, pest managers and regulatory agencies to better manage and limit the spread of GWSS populations. During the 2019 season, we will continue to monitor for resistance to neonicotinoids and pyrethroids (including those used in organic production), and assess cross-resistance patterns within these two classes of insecticide. The latter work will enable us to make recommendations to growers on how to overcome problems with resistance and improve GWSS management.

LAYPERSON SUMMARY OF PROJECT ACCOMPLISHMENTS:

Failure to control GWSS has resulted in an increased incidence of PD in the Central Valley. Insecticide resistance is one of the major causes of pest control failures for growers, and is most likely to occur where there is reliance on one insecticide. In many cases, the selection for resistance to the principal insecticide used for pest management within a system may also confer cross-resistance to other insecticides. Our project addresses the recent upsurge in GWSS numbers in Kern County where reliance on a small number of insecticides (most notably imidacloprid) has selected for resistance. In addition to our work in the Central Valley, we are investigating whether heavy insecticide use has selected for resistance in Western Riverside County (Temecula area) and in Orange County (commercial nursery industry). Data generated thus far show no evidence of resistance in Temecula. We are using diagnostic tools to detect resistance, and the information generated will enable pest managers to refine existing control strategies and minimize the impact that resistance has on future management efforts.

Accomplishments of this project to date include the confirmation of imidacloprid and pyrethroid resistance in Central Valley populations of the GWSS, particularly in the Bakersfield area. We have also confirmed that imidacloprid resistance confirms cross-resistance to acetamiprid, a worrying trend given the usage of this chemical in citrus in the region. Resistance has not been detected in the Riverside County area. We have been able to show that there is a direct link between the levels of imidacloprid resistance and the degree to which insects have been exposed. Our data suggest that the high levels of imidacloprid resistance are responsible for conferring cross-resistance to the pyrethroid, and it is therefore not inconceivable that cross-resistance to other non-neonicotinoid insecticide classes could also arise. We are also concerned that any increase in the use of natural pyrethrins in organic production could intensify the resistance problem for nurseries and vineyards adjacent to organic groves. Thus far, there does not appear to be a major shift in resistance to organophosphate and carbamate insecticides.

STATUS OF FUNDS:

$104,708.39 (direct) and $10,471.10 (indirect) remain in the budget at this time.

SUMMARY AND STATUS OF INTELLECUAL PROPERTY:

Not relevant.

LITERATURE CITED:

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