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 2018-July 2018

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 shows 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).

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 soil-applied 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 fenpropathrin in GWSS populations sampled from the General Beale Road area of Kern 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>Neonicotinoids</u>

During the 2016 and 2017 seasons, we completed a comprehensive series of bioassays on insects collected from several sites within the Central Valley (Kern and Tulare Counties), and Temecula Valley (Riverside County). In the Tulare County population that was collected from organic citrus, there was a *ca*. 4-fold increase in tolerance to imidacloprid relative to Ag-Ops_2003. In contrast, insects from conventionally managed citrus in the General Beale Road area exhibited high levels of resistance (>3,000-fold), with minimal survival of insects when treated with doses as high as 5,000 ng imidacloprid per insect. The response of the HWY65 population was intermediate between the Tulare and GBR populations, a consequence of intermingling of insects from adjacent organic and conventionally managed groves. At a new smpling site near Edison, resistance levels were high, but not at the levels measured in General Beale Road. However, in all populations that were tested, we measured cross resistance to the foliar neonicotioid acetamiprid.

Each year, we plan our monitoring work based around the CDFA areawide trapping data. We will begin our monitoring work for 2018 during the week of July 23 with a sampling visit to two sites in the Bakersfield area – General Beale Road and Edison. The most recent CDFA areawide trap data (July 1-July 14, 2018) shows a dramatic increase in GWSS numbers throughout the Central Valley, particularly in the Edison area where we confirmed the presence of resistance in 2017, and near Muller/Comanche. Our primary goal is to evaluate the cross-resistance levels between imidacloprid and acetamiprid in populations from these two sites, as our data set for 2017 was incomplete due to a fall off in numbers late in the season. During the next few months, we will continue to establish current resistance trends by revisiting sites we monitored during 2016 and 2017. The most recent trap catches in Temecula also indicate that GWSS numbers are on the rise, so we will conduct monitoring in those groves also.

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 are currently collecting insects from nursery, urban, and field locations, for 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 were detected in 2016. We have completed the first round of RNA-seq experiments on bioassay survivors and the data are currently being analyzed. 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.

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. Further experiments will be conducted during 2018 to provide additional replication for the RNAseq analyses.

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

Organophosphate (OP) and carbamate insecticides target the neurotransmitter acetylcholinesterase (AChE). Target-site resistance arises as a consequence of mutations in the enzyme that affect the binding efficiency of the insecticide. 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.

We will test insects from the General Beale Road, HWY65, Temecula and Tulare populations during the next several months, using the diagnostic concentration of 30 µM paraoxon. We will also test insects from nurseries and urban locations as they become available.

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 has been generated for this objective, but will be during the 2018 season.

PUBLICATIONS:

Other than the CDFA Proceedings, we have no publications to date.

[Office1]

RESEARCH RELEVANCE STATEMENT:

Bioassay techniques used in this project have identified high levels of resistance to imidacloprid, and moderate levels of resistance to the pyrethroid fenpropathrin. The data generated in 2016 and 2017 confirm a major shift in toxicological response of sharpshooters to insecticides that are routinely 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 2018 season, we will continue to monitor for resistance to imidacloprid and fenpropathrin in GWSS populations, 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 (2016 and 2017) 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. Thus far, there does not appear to be a major shift in resistance to organophosphate and carbamate insecticides.

STATUS OF FUNDS:

$164,927 (direct) $16,493 (indirect) remain in the budget at this time.

SUMMARY AND STATUS OF INTELLECUAL PROPERTY:

Not relevant.

LITERATURE CITED:

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Daugherty, M. 2016. The Riverside County glassy-winged sharpshooter program in the Temecula Valley. In: Esser, T. (Ed.), Proceedings of the Pierce's Disease Research Symposium, 12-14 December 2016, San Diego, California, pp. 195-198.

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