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: October 2014-March 2015

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. The consequence of the increase in GWSS populations has been an increase in the incidence of PD. In the Temecula area, this worrisome increase in GWSS has not occurred (yet); however the selection pressure in this area remains high as similar management approaches are in use here as in Kern County.

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 application 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 materials (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. Although the primary focus of our research will be in Kern County, we propose broadening the scope of the project to include populations from agricultural, nursery and urban settings. This broader approach will enable us to provide a more comprehensive report on the overall resistance status of GWSS within southern California and develop more effective resistance management plans.

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:

Common to All Objectives:

Hired post-doctoral research associate and two laboratory technicians.

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.

We are waiting for GWSS summer populations to become readily apparent and collectable. Our main collections will be focused on the General Beale area where we expect to find a good source of GWSS. Although there was some GWSS activity at the end of February and early March (Beth Stone-Smith pers comm to FJB), growers had completed foliar treatments to citrus and numbers would have been difficult to gather for bioassays. With foliar treatments completed, conducting summer bioassays will be timely as putative resistant populations will have received an additional field selection and bioassays should demonstrate whether resistance is occurring in that region.

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).

Work accomplished here is the same as Objective 1. We are waiting for the development of field populations of GWSS. Once populations are available, we will commence with a determination of LC_{50} and LC_{95} doses of the proposed insecticides.

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

In order to identify markers that could be easily typed to determine insecticide resistance of field collected GWSS, it was first necessary to identify the genic targets of commonly used insecticides within the GWSS genome. Unfortunately, we found that the current genome assembly available through USDA is extremely fragmented and altogether lacks annotation of genes. To overcome this obstacle, we utilized gene sequences from housefly and mosquito to identify contigs (via BLAST) within the GWSS assembly that contained genic targets of insecticides. As an example of the fragmented nature of the genome, we identified nine different contigs that contained portions of the GWSS para sodium gated channel gene, which is the molecular target of pyrethroids (HVIT018256-PA, HVIT018257-PA, HVIT018258-PA, HVIT018259-PA, HVIT018260-PA, HVIT018261-PA, HVIT018262-PA, HVIT018263-PA, HVIT018265-PA). The exon/intron boundaries of the sodium channel gene were annotated using algorithms developed in house. After extensive bioinformatics work we believe we have reconstructed the entire sequence of the gene: 5925 nucleotides (1975 amino acids), exclusive of introns. To confirm this, and other, computational predictions we plan to extract RNA from field-collected insects, generate cDNA, and sequence clones corresponding to genes of interest.

Using similar strategies to those outlined above, we have identified two copies of acetylcholinesterase genes (target of organophosphates) within the GWSS genome. The first codes for 326 amino acids (HVIT023764-PA) and the second for 476 amino acids (HVIT012420-PA). Additionally, we have identified seven genes that appear to comprise the nicotinic acetylcholine receptor (the target of imidacloprid). The genes range from 181 to 532 amino acids in length and the relevant contigs are HVIT020383-PA, HVIT009815-PA, HVIT008519-PA, HVIT014300-PA, HVIT001761-PA, HVIT018233-PA, HVIT008084-PA.

To facilitate identification of specific genes in the future, we are now running an annotation program, AUGUSTUS, in order to more fully characterize the GWSS genome. The algorithm is computationally intense and was initiated in March. We expect the process to take at least four months to complete. However, this up-front time invested in improving the assembly will prove highly beneficial as we delve further into the molecular mechanisms underlying GWSS resistance.

Objective 4: Monitor populations for metabolic insecticide resistance, by testing enzymatic activity against organophosphates using the AChE biochemical assay

This objective will be addressed during the summer when GWSS populations emerge

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.

As with other objectives requiring substantial numbers of GWSS, we are waiting for GWSS summer populations to become readily apparent and collectable. These collections will be ongoing throughout the duration of the project and will provide insect material for both the molecular and biochemical components of the project. We are currently setting up collaborative arrangements with CDFA monitoring personnel to ensure adequate collections can be performed.

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

The main focus with this objective will be to develop an assay to quantify cytochrome P450 activity in GWSS. The cytochrome P450 enzyme system is the most important enzyme system used by insects for the detoxification of xenobiotics. We will evaluate multiple coumarin-based cytochrome P450 fluorogenic substrates with the goal of developing a microfluorometric assay similar to that used to quantify ethoxycoumarin-*O*-deethylase activity in individual insects. This work has yet to begin and is not planned to be initiated until late in the second year of the study. At that time, we should have a much-improved annotated version of the GWSS genome available that will enable us to coordinate the use of bioassay, biochemical and molecular techniques to better understand the contribution of this enzyme system to insecticide resistance.

PUBLICATIONS:

No publications to date.

RESEARCH RELEVANCE STATEMENT:

The bioassay techniques that were developed and the baseline toxicological data that were generated in early studies determining response to insecticides by GWSS can be used here as a reference point in our efforts to detect any shifts in susceptibility that have occurred as a consequence of the continued use of insecticides for the statewide control of GWSS. We intend to develop new assays that measure qualitative and quantitative changes in putative insecticide resistance-causing enzymes such that an accurate evaluation of the incidence of insecticide resistance in agricultural, nursery, and urban populations of GWSS can be made. Data derived from this project will enable growers and regulatory agencies to better manage and limit the spread of GWSS populations.

LAYPERSON SUMMARY OF PROJECT ACCOMPLISHMENTS:

Insecticide resistance is one of the major causes of pest control failures for growers. It 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 will address the recent upsurge in GWSS numbers in Kern County where reliance on a small number of insecticides may have selected for resistance. Associated with this work, we will also investigate whether heavy insecticide use has selected for resistance in the Western Riverside County (Temecula area) and in Orange County (commercial nursery industry). We will use diagnostic tools that 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 partial identification of the genes responsible for possible insecticide resistance in GWSS to neonicotinoid, pyrethroid and carbamate insecticides.

Our work is ongoing to determine if resistance is actually occurring in this insect in areas of the state in which these compounds are heavily utilized.

STATUS OF FUNDS:

$541,351 remain in the budget at this time.

SUMMARY AND STATUS OF INTELLECUAL PROPERTY:

Not relevant.

LITERATURE CITED:

Not applicable.