

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 2017-July 2017

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 overwintering 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 LC₅₀ 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 LC₅₀ 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).

Neonicotinoids

During the 2016 season, we completed a comprehensive series of bioassays on insects collected from several sites within the Central Valley (Kern and Tulare Counties), and from one site in 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.

We have begun our monitoring work for 2017. In March 2017, we tested insects from two of our key sampling locations in the Central Valley – the Tulare organic site and the HWY65 organic/conventional site. Typical of bioassays conducted on GWSS at that time of year, the control mortality was high, likely due to the age of the insects. Despite that, increased imidacloprid tolerance in the HWY65 population

was again evident with 71% mortality at the 500 ng diagnostic dose. In contrast, the Tulare population exhibited over 90% mortality when treated with a 150 ng dose, indicating a typical response for a susceptible population.

The most recent CDFA maps showed a dramatic increase in GWSS numbers throughout the Central Valley. During the next few months, we will focus on establishing current resistance trends by revisiting the same sites we monitored during 2016. In addition, we will sample from other sites (both citrus and grapes) within the region. Imidacloprid and fenpropathrin bioassays will remain part of our standard bioassay program. In 2017, we plan to evaluate cross resistance patterns to other insecticides, including acetamiprid (which is used as a foliar treatment in the region) and bifenthrin. GWSS numbers in Temecula have also increased during the past month, and we generated fenpropathrin bioassay data for insects sampled from two distinct locations. Resistance was not detected in either population.

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.

These two objectives will be explored more thoroughly during the forthcoming months. We are currently collecting insects from nursery and urban locations, which will be used in later 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 will use RNA-seq analyses to 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. We have identified several Cyt P450 genes in our initial RNA-seq experiments, but it is too early to establish a causal link between any specific gene family and imidacloprid resistance without further analysis of the data. With the recent upsurge in numbers in the Temecula area, we will be able to include these insects in our RNA-seq analysis.

We have identified 49,040 unique genes from the GWSS transcriptome *de novo* assembly. These unique genes have been annotated using different annotation procedures, and then separated into three functional groups based on biological process, cellular component and molecular function. The combined use of the various databases identified 13,338 unique genes having the same annotation. Our objective is to produce a consensus annotation for GWSS genes that we can ultimately use to analyze the effects of insecticide selection (using bioassays) on gene expression.

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 μ M 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 2017 season.

PUBLICATIONS:

Other than the CDFA Proceedings, we have no publications to date. The resistance data from the 2016 monitoring effort were presented at two meetings.

Pacific Branch ESA, Portland, OR (April 2-5, 2017)

Title: Insecticide resistance in California populations of the glassy-winged sharpshooter *Homalodisca vitripennis*. Frank Byrne, Ming Li, Rick Redak, Bradley White.

2017 CAPCA Spring Summit, Pechanga Resort, Temecula, California (May 23-24, 2017)

Title: Update on GWSS area-wide monitoring and insecticide resistance. Frank Byrne, Matt Daugherty, Rick Redak.

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 confirms 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 2017 season, we will continue to monitor for resistance to imidacloprid and fenpropathrin in GWSS populations. We will also assess cross-resistance patterns within these two classes of insecticide, which 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 led to an increased incidence in 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. 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:

\$245,889.60 (direct) and \$24,588.99 (indirect) remain in the budget at this time.

SUMMARY AND STATUS OF INTELLECUAL PROPERTY:

Not relevant.

LITERATURE CITED:

- Byrne, F.J., Toscano, N.C., 2005. Characterization of neonicotinoids and their plant metabolites in citrus trees and grapevines, and evaluation of their efficacy against the glassy-winged sharpshooter and the egg parasitoid *Gonatocerus ashmeadi*. In: Tariq, M.A., Blincoe, P., Mochel, M., Oswalt, S., Esser, T. (Eds.), Proceedings of the Pierce's Disease Research Symposium, 5-7 December 2005, San Diego, California, pp. 287-289.
- Byrne, F.J. and N.C. Toscano. 2006. Detection of *Gonatocerus ashmeadi* (Hymenoptera: Mymaridae) parasitism of *Homalodisca coagulata* (Homoptera: Cicadellidae) eggs by polyacrylamide gel electrophoresis of esterases. *Biol. Control* 36: 197-202.
- 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.
- Haviland, D. and B. Stone-Smith. 2016. Monitoring glassy-winged sharpshooter and Pierce's Disease in Kern County, California. In: Esser, T. (Ed.), Proceedings of the Pierce's Disease Research Symposium, 12-14 December 2016, San Diego, California, pp. 75-80.
- Nauen, R., Hungenberg, H., Tollo, B., Tietjen, K., and A. Elbert. 1998. Antifeedant-effect, biological efficacy and high affinity binding of imidacloprid to acetylcholine receptors in tobacco associated *Myzus persicae* (Sulzer) and *Myzus nicotianae* Blackman (Homoptera: Aphididae). *Pestic. Sci.* 53:133-140.