EVALUATION OF RESISTANCE POTENTIAL IN THE GLASSY-WINGED SHARPSHOOTER USING TOXICOLOGICAL, BIOCHEMICAL, AND GENOMICS APPROACHES

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ABSTRACT
We used microarray expression profiling to identify genes that were differentially regulated in glassy-winged sharpshooters in response to treatment with the pyrethroid insecticide esfenvalerate. Targets were prepared from insects treated at both LD_{50} and sub-lethal doses. Of the 1,536 cDNAs in the array, only eight were differentially regulated in response to esfenvalerate. Of these, three aligned significantly with ferritin, lysozyme i-1, and polynucleotide phosphorylase. Additional pyrethroids have been included in our bioassay program. In topical application bioassays, LD_{50}s for bifenthrin and fenpropathrin were 1.2 and 3.6 ng/insect, respectively. Esfenvalerate, with an LD_{50} of 0.75 ng/insect, is the most toxic of the pyrethroids we have tested against the GWSS. Populations of insects have been selected with these insecticides at LD_{50} and sublethal doses for microarray analyses.

INTRODUCTION
Without an effective cure for PD, insecticides remain an important component of PD/GWSS management. There are several classes of insecticides available to growers for the management of the GWSS, providing them with options for control under various situations. Thus, the systemic attributes of the neonicotinoids have been exploited to provide long-term management of the insect on citrus and grapevines, whereas pyrethroid chemicals are more effective at dealing with incipient outbreaks, and for the disinfestation of nursery stock and fruit prior to shipment to non-GWSS areas of California.

Pyrethroids play an important part in GWSS management. They are quick-acting insecticides with modest persistence, making them ideal for pre-harvest cleaning of citrus trees. It is important to retain their efficacy for this purpose and, therefore, any evidence of regional variations in toxicological response should be evaluated in order to avoid resistance problems. This would involve assessment of tolerance levels and cross-resistance patterns, both within the pyrethroid class and across chemical classes, with the ultimate goal of eliminating all at-risk chemicals from the recommended treatment schedules.

By elucidating the resistance mechanism(s), we will have valuable information that can be used for better management of pyrethroid use. An analysis of target site resistance is especially important because of the potential for cross-resistance to other pyrethroids. If the target site mutation is not present in the populations showing increased tolerance, then this will lower the risk of substantial resistance problems. The presence of target-site resistance genes not only would present problems with pyrethroid use, but this type of resistance can provide a basis upon which other mechanisms can develop, particularly metabolic mechanisms. The latter mechanisms then have the opportunity to enhance the resistance problem within the pyrethroid group, but more significantly to extend the resistance problem to other insecticide classes. In this way, even modest use of one compound can have serious consequences for a more widely used product.

The genomics component of the study will identify genes that are differentially regulated as a consequence of insecticide exposure and, therefore, likely to contribute to resistance or tolerance of treated insects. DNA microarrays provide a format for the simultaneous measurement of the expression level of thousands of genes in a single hybridization assay. Hybridization intensities for each DNA sequence on the array are converted to a quantitative read-out of relative gene expression levels. The utility of this method lies in its ability to identify variation in expression patterns that correlate with events such as insecticide selection. It is an ideal technique for determining whether significant differences exist in the expression profiles of GWSS populations. By highlighting genes whose expression levels are affected, subsequent analyses can identify the gene function, allowing us to determine the relevance of that gene in resistance/tolerance to the selecting insecticide and the likelihood of that gene conferring cross resistance to chemically unrelated insecticides.

OBJECTIVES
1. Monitor toxicological responses of geographically distinct populations of GWSS to pyrethroid insecticides.
2. Measure biochemical activity of putative resistance-causing enzymes in these populations.
3. Clone and sequence the sodium-channel genes in GWSS populations differing in susceptibility to insecticides.
4. Perform microarray gene expression profiles in GWSS populations differing in susceptibility to insecticides to isolate novel genes involved in resistance.

RESULTS

Response of GWSS in Bioassays

Topical application bioassays have been conducted against the GWSS (adults only tested) with bifenthrin and fenpropathrin. The LD_{50}s for these compounds were 1.2 and 3.6 ng/insect, respectively. These compounds were less toxic to the adults than esfenvalerate, which has an LD_{50} of 0.75 ng/insect (Byrne et al., 2004). These three pyrethroid insecticides will now be used to determine the effect of insecticide dose (at LD50 and sublethal concentrations) on gene expression changes (both up-regulation and down-regulation) in GWSS populations.

Selections

Thus far, selections of GWSS populations from Riverside and Redlands locations have been completed for esfenvalerate, using 0.75 and 0.075 ng/insect as selecting concentrations. RNA extracted from the survivors of these selections is being prepared for subsequent hybridization to microarrays in our gene expression profiling studies.

Microarrays

The LD_{50} concentration for esfenvalerate for the Riverside population of GWSS was determined to be 0.75ng per insect. This concentration and a sub lethal concentration of 0.075 ng per insect were used to generate samples for microarray target preparations. To identify a few genes differentially regulated by pesticide treatment, cDNA microarray hybridizations were performed using a subset of 1,536 clones of the 10,848 cones isolated from the GWSS cDNA library. Overall, the expression of most genes was not significantly altered. Figure 1 shows the complete dendrogram of the 1,274 genes, which are clustered on the basis of expression profiles in response to pesticide treatment at different doses. The expression data were consistent between duplicate spots and between the dye swap experiments and were, therefore, averaged for each clone. They are displayed in red (overexpressed) and green (under-expressed), relative to the control. Only eight genes were shown to be differentially regulated in response to esfenvalerate (Figure 1). Of these genes, only three showed significant homology to genes of other organisms. These genes aligned significantly with ferritin, polynucleotide phosphorylase, and lysozyme i in the NCBI databases. Ferritin was up-regulated in response to both treatment levels, while polynucleotide phosphorylase and lysozyme i were down-regulated in response to the LD_{50} dose only. Of the remaining gene clones, which showed significant changes in expression, one was down-regulated in response to the sub-lethal dose. Four genes were up-regulated in response to the LD_{50} dose of esfenvalerate, and one was up- regulated in response to both concentrations of the pesticide.

CONCLUSIONS

Management of sharpshooter populations is key to minimizing the spread of PD. This project will benefit the PD program by characterizing the pattern of resistance observed in GWSS populations, and by identifying the mechanisms involved. The potential for cross-resistance will also be evaluated. The cDNA microarray hybridization experiment utilizing a subset of the GWSS library provided the first insight into broad genome responses of GWSS to esfenvalerate, and identified a few important genes that are differentially regulated. For example, the increase in ferritin RNA levels observed in GWSS treated with esfenvalerate is indicative of a generalized stress response, while the observed down-regulation of lysozyme in our studies suggests that the pesticide has a direct effect on the immunity of the GWSS. Thus far, we have not detected any significant up-regulation of genes that are known, from studies on other insect pests, to confer metabolic resistance to pyrethroids. These include esterases, cytochrome P_{450}s, and glutathione transferases. Although the activity of these enzymes can be detected biochemically, our current data suggests that none of these genes is over-expressed following selection with esfenvalerate. The lack of detection of significant up-regulation of esterase genes concurs with our biochemical data, which shows no difference in esterase levels in the survivors of esfenvalerate selections relative to untreated controls (Byrne et al., 2004). We have a GWSS carboxylesterase clone in hand that will serve as a control when our microarray experiments are expanded to include all 10,848 library clones.

REFERENCES

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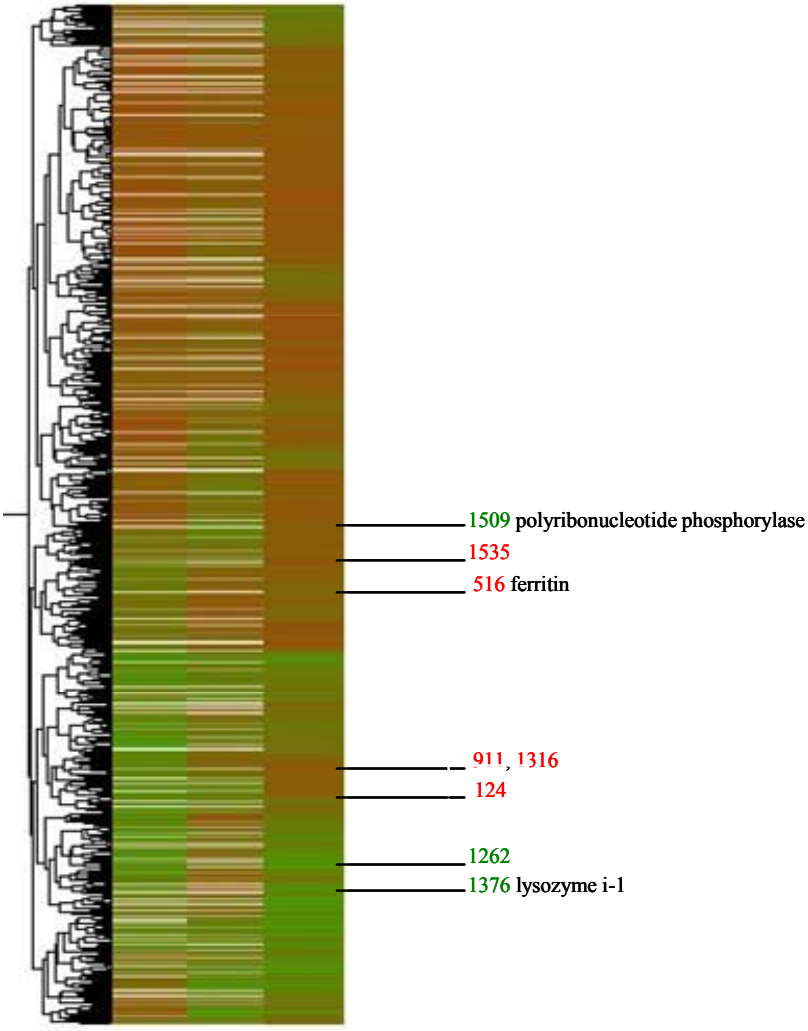

Figure. 1. Microarray analysis of esfenvalerate dosage responses. Total RNA from insects treated with no pesticide, 0.075 ng, and 0.75 ng of esfenvalerate were used to generate fluorescent targets which were hybridized in dye swap experiments to 6,144 cDNA microarrays representing 1,536 cDNAs spotted in quadruplicate. Data was filtered on fold change with two fold considered to be significant. Gene clones labeled were significantly up-regulated (red) or down-regulated (green) in reciprocal dye experiments.