

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

Project Leaders:

Frank J. Byrne
Dept. of Entomology
University of California
Riverside, CA 92521

Nick C. Toscano
Dept. of Entomology
University of California
Riverside, CA 92521

Brian A. Federici
Dept. of Entomology
University of California
Riverside, CA 92521

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ABSTRACT

Geographically distinct populations of GWSS differ in their toxicological responses to pyrethroid insecticides. We have shown that these different responses are unlikely to be caused by an esterase-mediated mechanism. The distributions of esterase activity in insects tested from Riverside and Redlands citrus orchards remained unchanged after selection with an LD50 dose of esfenvalerate.

INTRODUCTION

We are using a multi-disciplinary approach to understand the biological and genetic mechanisms contributing to the toxicological differences between GWSS populations. This will allow us to determine whether the basis for decreased tolerance is due to target site changes or due to the selection of detoxification mechanisms. Whereas target-site modifications will only impact the pyrethroid class of insecticides, the selection of detoxification mechanisms are more critical due to their potential to confer cross-resistance to chemical classes that differ in their modes of action. In this first report, we describe selection experiments designed to test the potential involvement of esterases in conferring pyrethroid tolerance (Objective 2).

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

Bioassays

Topical application bioassays (Byrne et al., 2003) have been conducted on Riverside GWSS adults to determine an LD50 for esfenvalerate. The LD50 was determined to be 0.75ng esfenvalerate per insect.

Selections

For selection experiments, insects were collected from the UC Agricultural Operations orchard in Riverside. Adults were treated with 0.75ng esfenvalerate by topical application. Esterase activity was measured in a subsample of insects taken before the bioassay, and in the survivors (at 48 hours) from the bioassay (Figure 1). Although there were differences in activities between males and females, there were no differences in activities attributable to selection by esfenvalerate.

In additional selection experiments, insects from Redlands and Riverside orchards were treated with 0 (controls), 0.075ng (sub-lethal) and 0.75ng (LD50) esfenvalerate per insect. Control and survivors at each treatment were used to prepare target RNA for gene expression profiling studies.

Microarrays

PCR amplified inserts from 1,536 normalized library clones were spotted onto amino-silane coated glass slides. Each clone was spotted in side by side duplicate spots and the entire array was duplicated on each slide. Total RNA was isolated from two individual insects from each treatment for target preparation. Each total RNA was reverse transcribed and PCR amplified separately with Cy3- and Cy5-tagged dUTP. Slides were hybridized for 16 hours at 42°C on a Genomics Solutions GEN TAC[®] hybridization station and washed twice at medium stringency for 40 seconds. Each hybridization was repeated as a target dye swap. Slides were scanned on an Applied Precision Array Worn fluorescence scanner. Data is being evaluated using the Silicon Genetics GeneSpring program.

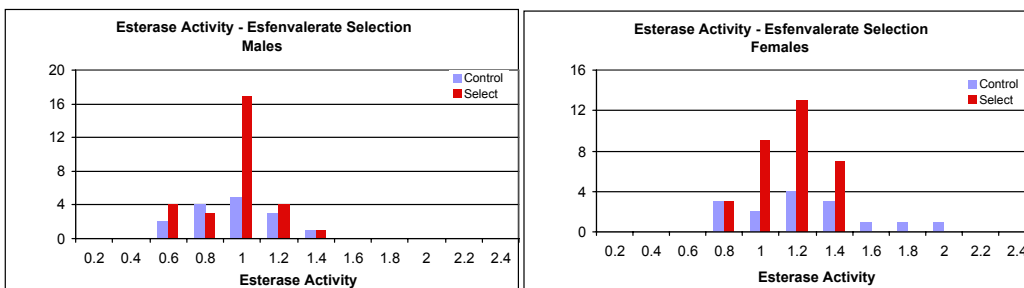


Figure 1. Distributions of esterase activity in adult male and female glassy-winged sharpshooters from a Riverside citrus orchard. Insects were treated topically with either acetone (Control) or 0.75ng esfenvalerate (Select), and esterase activity measured in survivors.

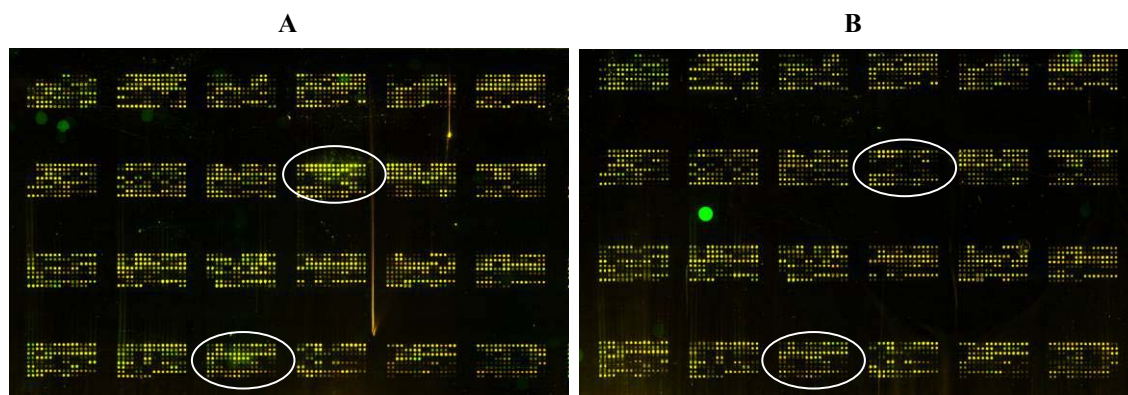


Figure 2. Scan data of microarrays hybridized to Cy3 labeled control target (green) and Cy5 labeled sub-lethal target (A) or LD50 target (B) (red). Circled results show obvious gene expression differences.

CONCLUSIONS

In this study, we tested populations of GWSS from Riverside citrus orchards with 0.75ng esfenvalerate. This dose of esfenvalerate is the LD50 for the Riverside population when topically applied to the insect abdomen. Distributions of esterase activity revealed that there were no differences between the untreated insects and the treated survivors. These results suggest that esterases do not contribute directly to the toxicological differences between these populations. In addition, many and different gene expression changes occur in GWSS in response to sub-lethal and LD50 doses of esfenvalerate.

REFERENCES

Byrne, F.J. and Toscano, N.C. 2003. Characterization of plant metabolites of imidacloprid in citrus trees and grapevines, and evaluation of their efficacy against the glassy-winged sharpshooter *Homalodisca coagulata*. Proceedings of the Pierce's Disease Research Symposium pp. 286-287, Coronado Island Marriott Resort, San Diego, California. Dec 8-11, 2003.

FUNDING AGENCIES

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