CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)
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Project Number
S1998

Project Title
An Eco-friendly RNA Interference-based Insect Control for Management of Citrus Greening Disease Using a Model System

Abstract

Objectives/Goals
The goal of the project was to develop an RNA interference (RNAi)-based highly specific, bio-pesticide for the control of the psyllid vector of citrus greening disease. Citrus greening disease has killed over 50% of the trees in Florida, and is a threat to the 2-billion dollar California citrus industry. Because of quarantine regulations and ease of experimentation, a tomato model system was used. The objective was to demonstrate the efficiency of RNAi in effective control of the target psyllid and to develop a field delivery system using a viral vector.

Methods/Materials
Abnormal wing disc (awd) gene from tomato psyllid was partially sequenced. Using an awd clone, transcripts were synthesized in both directions using T7 RNA polymerase. Nymphs were treated with dsRNA and allowed to mature on tomato plants. Mortality and wing phenotypes were recorded, and awd gene expression levels were studied using real time PCR. The awd gene was cloned into a tobacco mosaic viral vector separately, in forward and reverse orientations, and viral transcripts were used to infect tomato seedlings. Nymphs were reared on these plants, mortality and wing phenotypes were recorded.

Results
A fragment of the awd gene from tomato psyllids was PCR amplified, cloned and sequenced. The treatment of psyllid nymphs with awd dsRNA preparations caused very high levels of mortality, as well as abnormal wing phenotypes in many surviving adults. Gene expression levels of awd, analyzed in 72 individuals, showed a much lower level of expression in dsRNA treated psyllids. Full length transcripts generated from an engineered viral vector (using a tobacco mosaic viral vector) with awd in forward and reverse orientations were used to infect tomato plants, and the effect on nymphal mortality and adult phenotypes are being analyzed.

Conclusions/Discussion
In absence of any other control strategies, the management of citrus greening disease is mostly dependent on the use of extensive insecticidal sprays. As many as 26 sprays/year have been reported from Brazil which may be harmful to beneficial insects as well as to human health and the environment. Using a tomato model system, a simple molecular method of gene silencing was shown to be an effective bio-pesticide targeted to the psyllid. No harmful effects are expected on other insects (eg. Honeybees) and humans. An effective way of field application is demonstrated with the use of a viral vector.

Summary Statement
An environmentally friendly and targeted bio-pesticide technology was developed using a tomato model system which can be applied to protect California#s citrus industry from an insect (Asian citrus psyllid) that transmits the deadly citrus

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