**RNA INTERFERENCE TO REDUCE SHARPSHOOTERS, THE GLASSY-WINGED SHARPSHOOTER, AND THE ASIAN CITRUS PSYLLID**

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**ABSTRACT**
Short interfering RNAs which can be used to reduce gene expression, or 'silence' this expression in a sequence specific manner is call RNA interference, RNAi. In the case of hemipteran pests, RNAi has only recently been shown to disrupt their biology. We propose that RNAi has the potential to be applied in an area wide management strategy, thereby suppressing the pest populations to reduce disease spread. Previously we generated leafhopper genetic datasets from three species of known vectors of *Xylella fastidiosa (Xf)*, the plant infecting bacterium which causes Pierce's disease (PD) of grapevine. Using genomic analyses we identified a subset of potential genetic targets which may be used to suppress these sharpshooter leafhoppers. The primary disease vector, the glassy-winged sharpshooter (GWSS; *Homalodisca vitripennis*) vectors the *Xf* xylem-limited bacteria that causes PD in grapevine as well as 'Scorch-like' pathogens of other woody agricultural fruit, nut, and ornamental crops. But many other species of sharpshooters are also capable of transmitting *Xf.* Therefore, we chose to produce two constructs of dsRNA to Arginine kinase, one from the GWSS leafhopper, and one for the Asian citrus psyllid. Both insects feed on citrus and transmit different bacterial pathogens. We treated several host plants (Grapevine, Citrus, Okra, and Chrysanthemum), using cut flush, to seedlings, to mature grapevines and citrus trees for this study. The dsRNA treated plants caused an increase in mortality for both the GWSS and ACP. However, when each insect fed upon the dsRNA specific to the other insect no increase in mortality was observed. RNAi due to this specificity may be an excellent treatment to reduce insect pests while protecting the non-target beneficial organisms.

**LAYPERSON SUMMARY**
We demonstrate that a naturally occurring molecule, double-stranded RNA, dsRNA, can induce an RNA interference, RNAi, response in leafhoppers and psyllids. This means that insect specific genes can be down regulated such that the insects' fitness is reduced (ie. lays fewer eggs or dies). The specificity of the dsRNA's showed that each was only functional within the designed species, so the leafhopper –dsRNA worked only in leafhoppers but not in the psyllid, and *vice versa*. RNAi has become one of the most studied systems and is being developed for human health, as well as to solve problems in agriculture. The RNAi strategy uses the natural systems already in place within animals, insects and plants. We propose that RNAi strategies should be pursued for area wide insect pests suppression programs, and may further provide new management approaches to other pest species.

**INTRODUCTION**
RNA interference, RNAi, refers to the process of sequence-specific post-transcriptional gene silencing in animals mediated by short interfering RNAs (siRNAs) (Fire *et al*., 1998) and is a phenomenon that may be useful in many avenues of disease and pest management, including , we hypothesize, management of hemipteran pests. These approaches, such as the implementation of RNAi in insect pest management are rapidly being developed on many fronts, such as to improve the health of beneficial insects (Hunter et al, 2010). RNAi applications have been proposed and evaluated for their use in many biological problems from human health to agricultural pests. Thus, we propose that RNAi has the potential to be applied in an area wide management strategy, thereby suppressing leafhopper and psyllid populations to reduce disease spread.

To identify the mRNA's, which would be targeted by RNAi, we previously generated expression libraries from sharpshooter, based on expressed sequence tags, EST's, from three species of known vectors of *Xylella fastidiosa (Xf)*, which causes Pierce's disease (PD) of grapevine (Hunter et al,2003-2010, NCBI). Using genomic analyses we identified a subset of potential genetic targets which may be used to suppress these sharpshooter leafhoppers.

One of the major breakthroughs which launched RNAi approaches into the realm of practical applications has been the development of the ability to produce kilogram quantities of dsRNA (www.beeologics.com). An example of this is the power of an RNAi product which has been shown to increase the health of honey bees in the presence of bee viruses,

demonstrating the first study of its kind (Hunter et al, 2010). With the development of similar RNAi products it is predicted that soon these products will be approved and commercially available for agricultural use. Once there is a pathway for the evaluation and approval of RNAi products it is possible to envision many such solutions being developed to address PD and other insect transmitted diseases.

**OBJECTIVES**

1. Get dsRNA into grapevines and citrus trees for delivery of RNAi to leafhoppers and psyllids.
2. Demonstrate functionality of RNAi in leafhoppers and psyllids.
3. Ultimately develop RNAi for leafhopper and psyllid area wide suppression and disease management.

**RESULTS AND DISCUSSION**

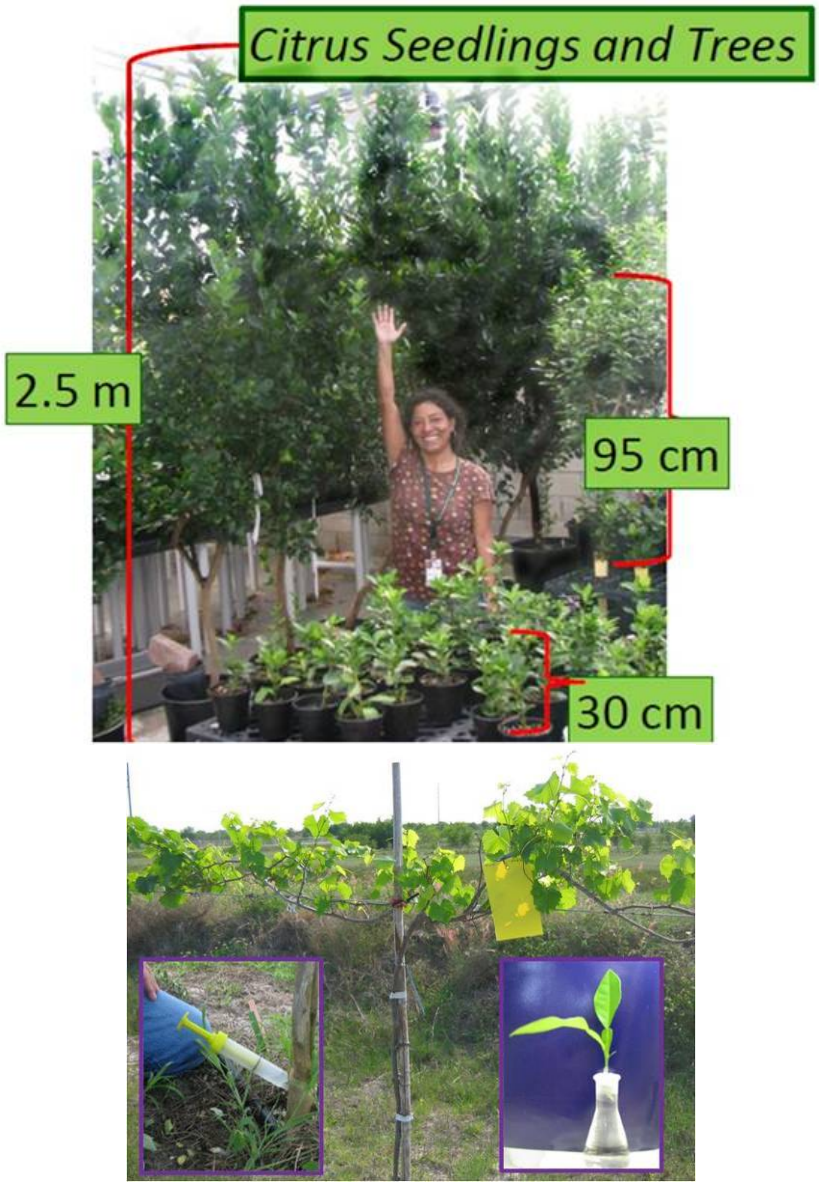


**Figure 1**. Plants from seedlings to mature citrus trees and grapevine were successfully treated with dsRNA. When the glassy-winged sharpshooter (GWSS; *Homalodisca vitripennis)*, and the Asian citrus psyllid were fed on these treated plants they were both shown to have ingested the dsRNA. This is an important finding as the leafhoppers are feeding from the xylem and the psyllids are feeding from the phloem, thus the dsRNA moved throughout the trees vascular system.

*Preliminary results*: Successfully show that we can get dsRNA uptake in trees (Key Limes) as tall as 2.5 meters {~8 ft} within four days, at a dosage of 250 ml (10 mg/ml) dsRNA in 18.93 L {5 gal US} water. Injection trials were also used on citrus as well as into grapevines. Grapevines and/or citrus trees had root mass rinsed to remove some of the soil, and then set into a plastic barrel where they soaked in the solution. Insects were placed on either controls treated with water or dsRNA treated grapevines or citrus trees for three days post treatments.

*Soil interaction study:* Citrus seedlings (Valencia, 95 cm {~3 ft}) tall in octagon pots, either rinsed of soil from roots, or left fully intact in pots were also permitted to soak for five d in solution, water level roughly 1/3 up the container. Soil did not deter uptake nor detection of the dsRNA.

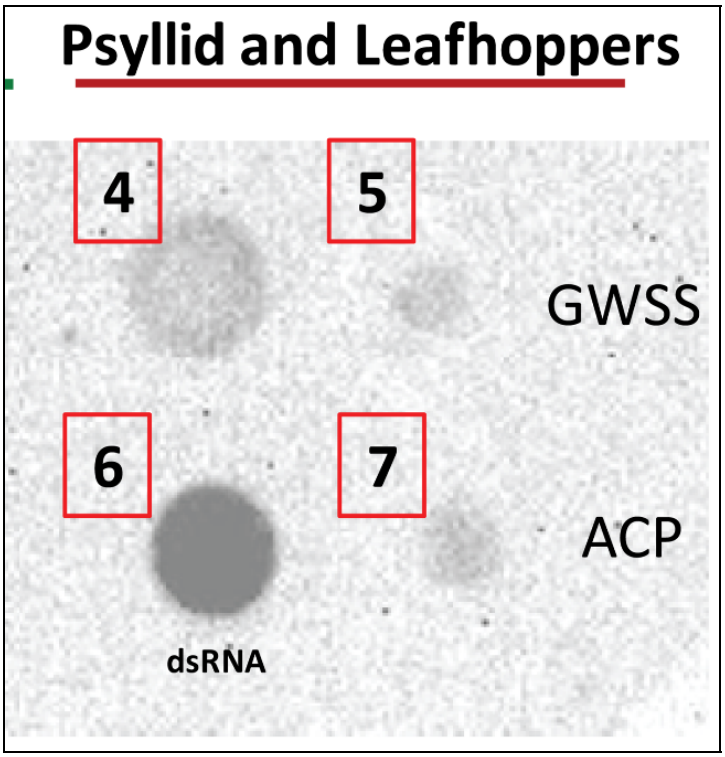


**Figure 2**. Detection of *ingested dsRNA* in both glassy-winged sharpshooter (GWSS), and the Asian Citrus Psyllid after feeding for 3 days on either grapevine or citrus treated with dsRNA.

***RNAi increases GWSS mortality:*** In two separate studies using the same dsRNA for Arginine kinase (dsRNA-AK) [Hunter, ARS, FL using citrus, and grapevine] and [Bextine and Hail, using Chrysanthemum stems], both determined that when GWSS fed upon plant seedlings or flush which had absorbed the dsRNA-AK, the sharpshooters died earlier and at a higher rate than the controls within a five day period.

*Future studies*: Currently in Florida we have eight citrus trees (sweet orange) (>2.5 m) and 4 grapevine (Noble) being treated by injection to determine dsRNA movement and persistence under field conditions.

**CONCLUSIONS**

RNAi was successful in increasing the mortality of GWSS and the Asian citrus psyllids. Sharpshooters and Psyllids were shown to ingest dsRNA from cuttings, seedlings, mature grapevine and/or citrus trees. This is the first demonstration of an RNAi effect for these insects, and the ability to induce RNAi in these insects from feeding on plants containing dsRNA. The results support the proposal that a RNAi host delivery strategy may be an efficient area wide approach to population suppression against these hemipteran insects {ie. The GWSS and the Asian citrus psyllid}.

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