RNAi Systems for Reducing Psyllids and Leafhoppers in Grapevine and Citrus

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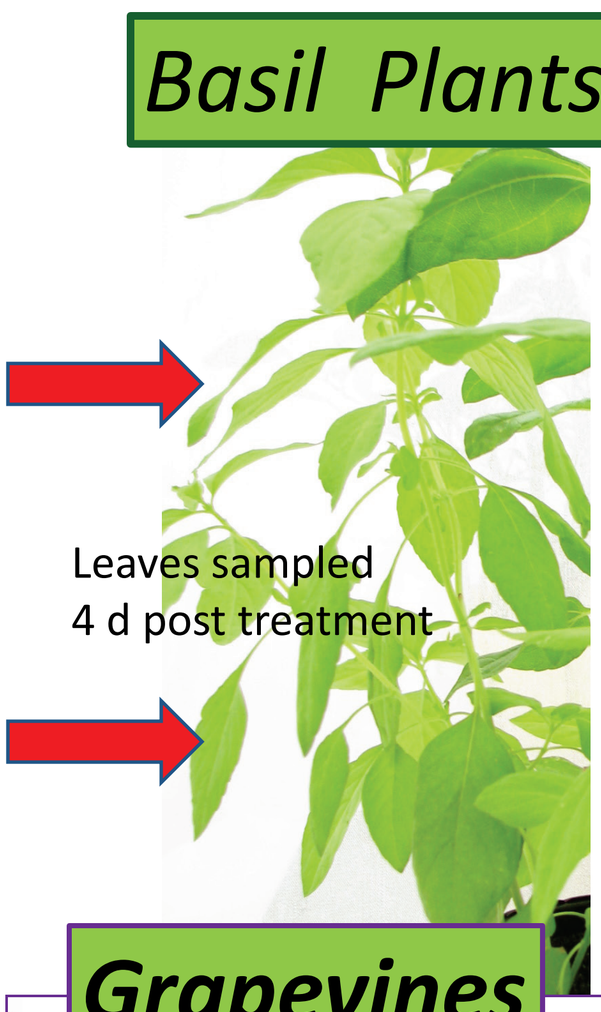
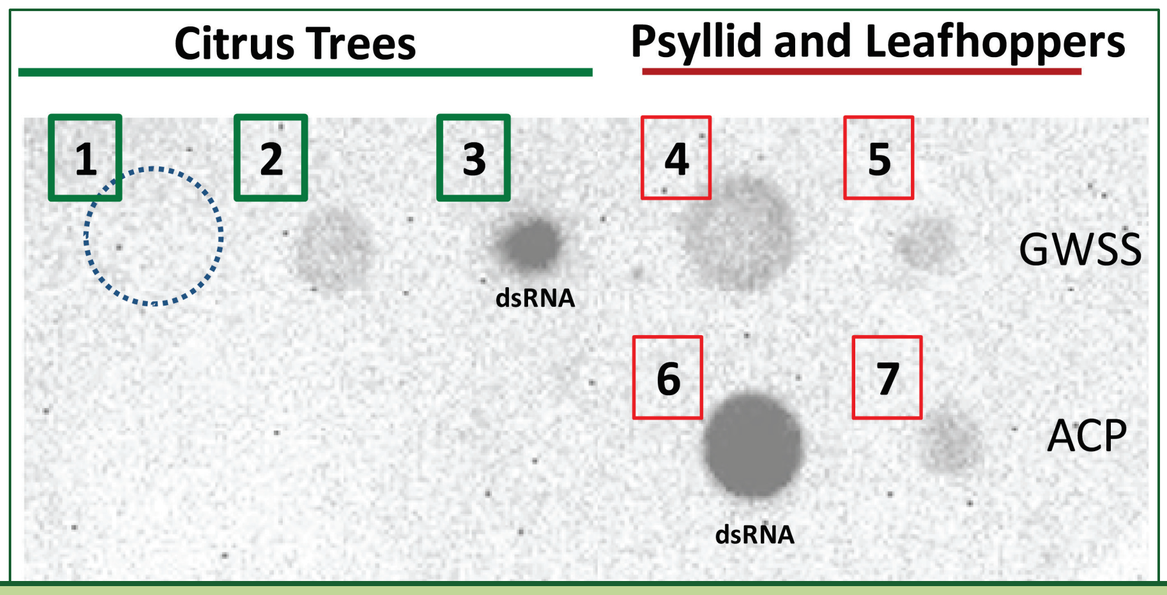
Abstract:
We demonstrated successful delivery of dsRNA constructs in whole-plant systems (herbaceous plants, woody grapevine and citrus seedlings and trees) which is currently being evaluated. Successful feeding of dsRNA in sucrose solutions to psyllids and leafhoppers was followed by using cut plant flush. Treatments of seedlings showed that dsRNA could be introduced into whole plant systems.

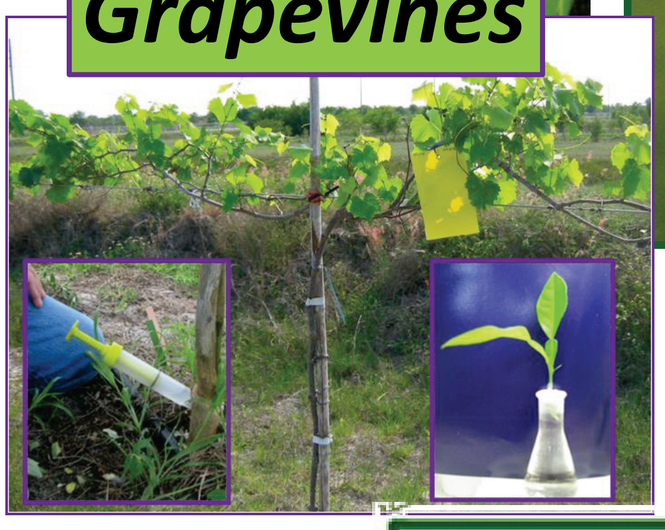
Citrus trees which are ~2.5 m tall, and grapevine (5 yr old) are currently being screened for dosage titers, uptake time, persistence within plant tissues. The citrus trees are 6 year old Mexican Limes. Analyses of fruits, and juice are also to be examined for presence and/or persistence of dsRNA constructs. RNA interference technology (RNAi) has been used successfully to silence endogenous insect genes both by injection and feeding, and produced by genetically improved plants. RNAi has been shown to work in many different types of insects leafhoppers, psyllids, beetles, bees, mosquitoes and other flies, lepidopteran butterflies and moths. Non-insects: ticks, nematodes, and other arthropods.

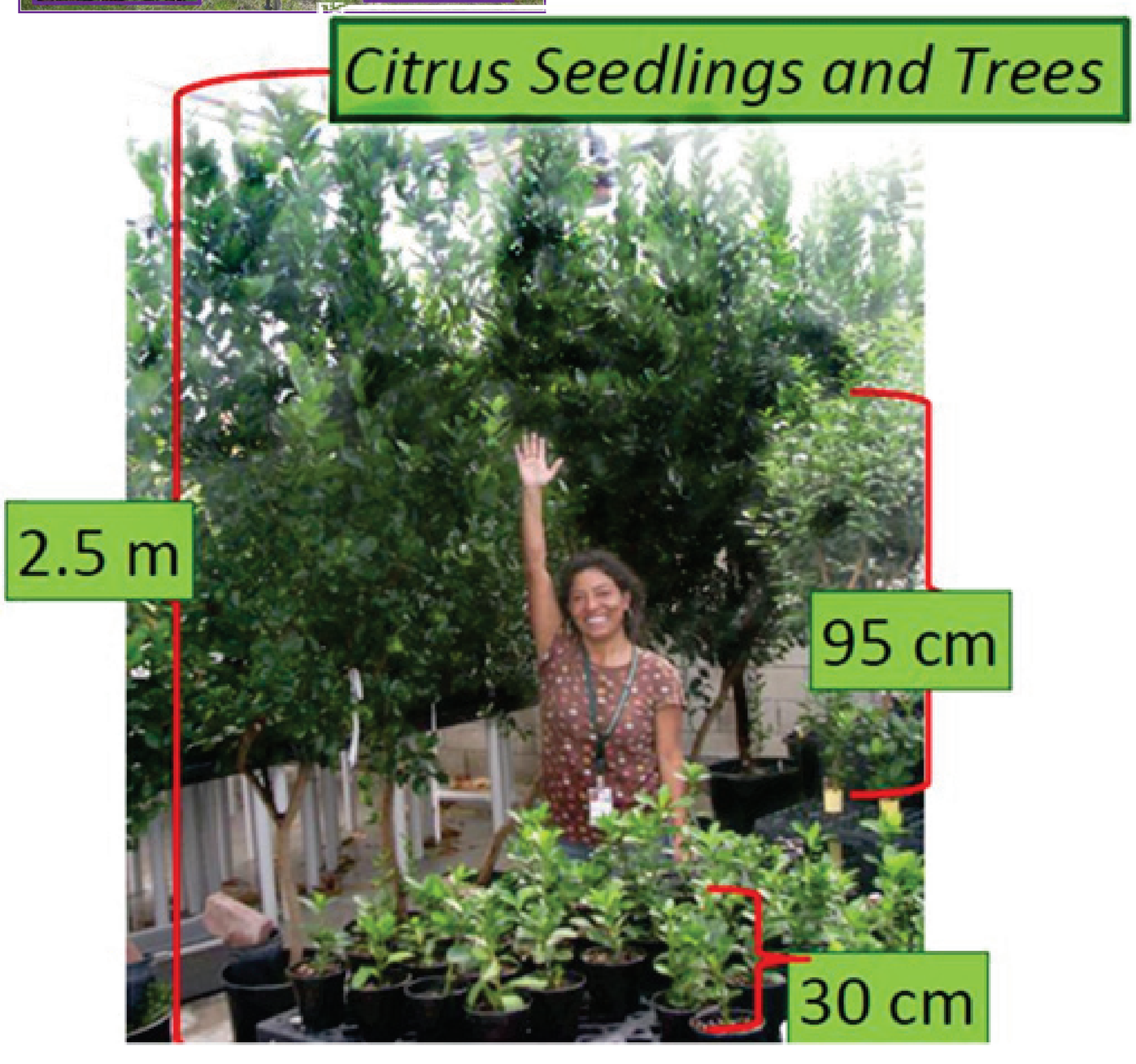
Previously, RNAi was used successfully to prevent bees from succumbing to infection from a viral disease, and treatment was shown not to persist in bee nor honey once treatment was stopped. We propose that in the case of citrus pests (ie. Psyllids) specific psyllid transcripts can be used to reduce and suppress psyllids across an Area-Wide program using RNAi strategies.





Spots: 1-Plant DNA control; 2-Citrus RNA control; 3-Citrus dsRNA treated;
4-GWSS-fed citrus dsRNA tree; 5-GWSS-fed citrus control tree;
6-ACP-fed Citrus dsRNA tree; 7- ACP-fed Citrus control tree.





Preliminary results: *Successfully show that we can get dsRNA uptake into trees* (Key Limes) as tall as 2.5 meters {~8 ft} within 4 days, at a dosage of 250 ml (10 mg/ml) dsRNA in 18.93 L {5 gal US} water, and in Grapevines and citrus trees by injection. Root mass rinsed to remove some of the soil, and then set into a plastic trash barrel, where they soaked in the solution. Insects were fed on control or treated trees for 3 days.

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 5 d in solution, water level roughly 1/3 up the container. Soil did not deter uptake nor detection of the dsRNA.

Future studies: Currently we have shown treated 4 trees (2.5 m) by watering, and a set of grapevine and citrus trees in the field which were injected, to determine uptake efficiency under field conditions.

Residue Inside Fruit. While our trees have some fruits, which are to be tested for dsRNA presence and longevity, we know from studies in bees that dsRNA does not remain very long in living systems and is broken down fairly rapidly (~30-60 days). Planned field tests on grapevines, and citrus trees will permit more stringent evaluation on juice and fruits from these treatments.

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 potato 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 5 day period.

CONCLUSIONS

RNAi was successful in increasing the mortality of Glassy-winged sharpshooters, GWSS, and Asian citrus psyllids, ACP. 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 after ingestion from plants*, containing dsRNA.

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