RNA-interference for Reducing Insect Pests: Plant Infusion

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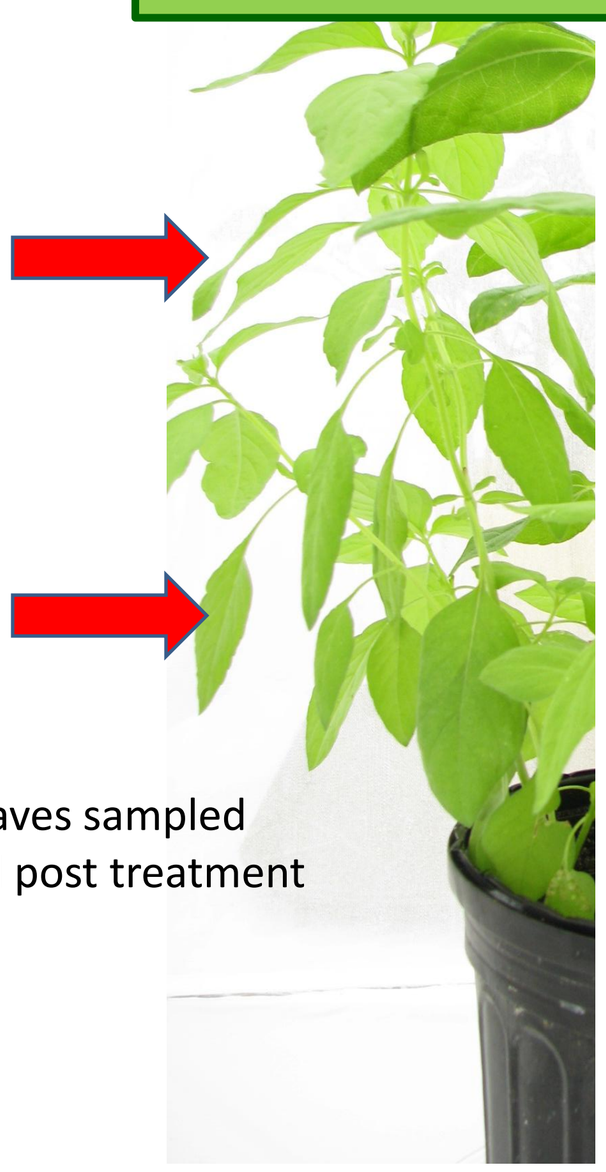
Abstract:
We developed a system for delivery of dsRNA constructs in whole-plant systems (herbaceous plants, woody grapevine and citrus seedlings and trees) which is currently being evaluated. Successful feeding of dsRNA in sucrose solutions to psyllids was followed by using cut plant flush. Treatments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

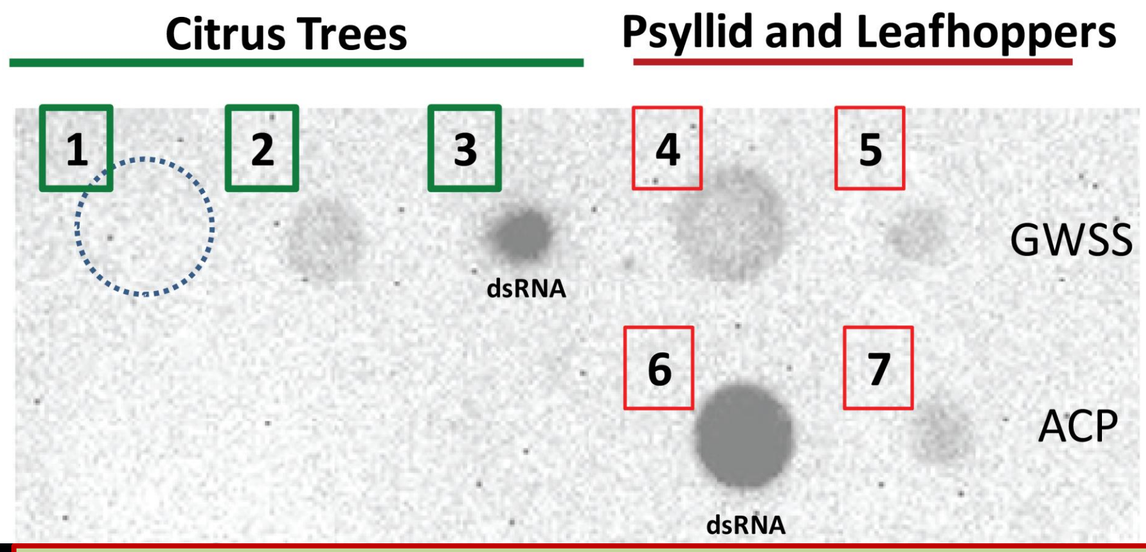
Citrus trees which are ~2.5 m tall are currently being screened for dosage titers, uptake time, persistence within plant tissues. These trees are 6 year old Mexican Limes, cv, which produce fruits which will also be examined for presence and/or persistence of dsRNA constructs. RNA interference technology (RNAi) has been used successfully to silence endogenous insect (including honey bee) genes both by injection and feeding, and in plants (genetically modified).

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.

Basil Plants

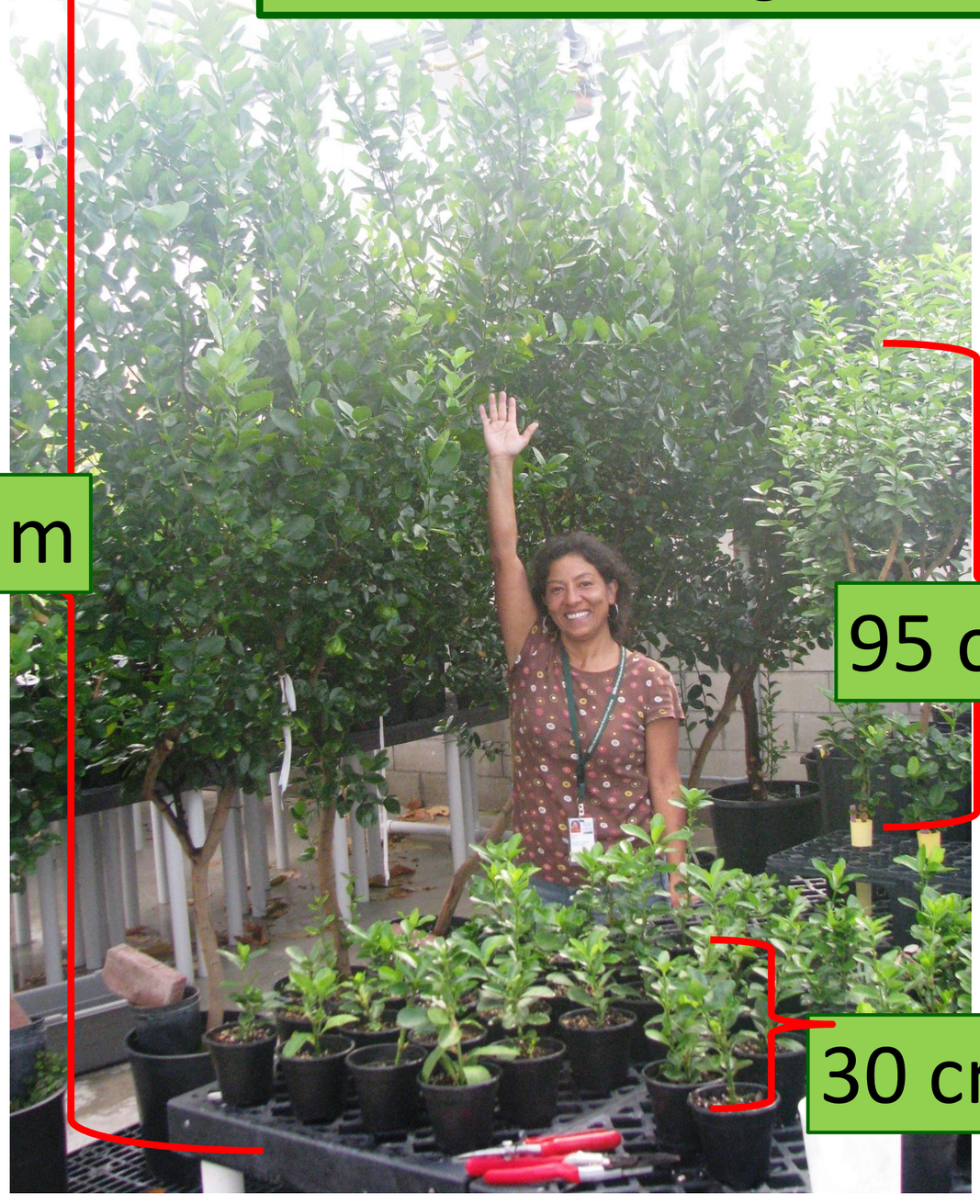


Leaves sampled
4 d post treatment



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.

Citrus Seedlings and Trees



Preliminary results: *Successfully show that we can get dsRNA uptake in 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. Trees had 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 treated 4 trees (2.5 m) by watering from the surface, but water still drains down inside of pots, thus a field trial using a full sized mature citrus tree is planned to use surface watering to determine uptake efficiency under field conditions.

Residue Inside Fruit. While our tall 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. Planned field tests on a larger number of fruits and juice will establish the data for regulatory requirements.

Industry Partner: Beeologics, LLC, Miami, FL, USA.

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