Promising HLB Research

U.S. Horticultural Research Laboratory

Ft. Pierce, FL

C.E. Arnold, Lab Director

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COMPONENTS OF A SUCCESSFUL ANTIMICROBIAL TREATMENT OF CITRUS TO KILL CITRUS GREENING BACTERIUM

Active Antimicrobial
Extended Exposure
Penetrant (We Need Systemic)
Successful Treatment

Citrus Greening (HLB)
We have screened over 80 chemicals, about a dozen of them are being tested in the field.
Identifying Antimicrobials that affect C. Las Spread

C. Las Detection in Citrus leaves post monthly basal bark application by Real-time PCR

- Untreated
- Antibiotic Treated

Graph showing the detection of bacterial cells per plant cell over time for untreated and antibiotic-treated samples.
Basal Bark Application Concept

- **Trunk Applications:**
  - Allow Minimum Surface Area (reduced chemical cost)
  - Potential for reduced environmental exposure
MEASURING ANTIMICROBIAL MOVEMENT IN CITRUS
(We have done this on up to 3-year old trees)

About 2 g of tissue is required for 100 µl of clarified leaf extract.
Spray-on Antibiotic Bark Application

- Studies of antimicrobial bark application formulations are currently underway on large citrus trees in private groves and smaller trees in the USDA-ARS Ft. Pierce greenhouse.
Systemic Movement of Different Tetracycline Derivatives-Bark Applications

Tetracycline requires combination with penetrant

Without Penetrants

With Penetrant Aid

Tet-d1    Tet-d2

Tet-d1    Tet-d2
Heat Treatment Eliminates ‘\textit{Candidatus Liberibacter asiaticus}’ from Infected Citrus Trees Under Controlled Conditions

Michele T. Hoffman, Melissa S. Doud, Lisa Williams, Mu-Qing Zhang, Fang Ding, Ed Stover, David Hall, Shouan Zhang, Lisa Jones, Mark Gooch, Laura Fleites, Wayne Dixon, Dean Gabriel, and Yong-Ping Duan

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time (days)</th>
<th>Initial Ct value</th>
<th>30 DAT\textsuperscript{a} Ct value</th>
<th>270 DAT Ct value</th>
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<td>21.57</td>
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HLB-affected citrus (A) Before heat treatment and (B) 6 months after exposure to heat
Picos Farm Heat Therapy Summer 2012

• Valencia on Carrizo rootstock
• 4 rows with approximately 55 trees
• Half of each row is exposed to high heat using tents
Commercial Grove

Before-May 2012

8 months later – Jan 2013
ACP reproduces and develops only on the young leaves of Citrus and related plants.

Therefore, we are developing scent formulations that mimic the aromas of young Citrus leaves.
Collecting aroma compounds from citrus foliage

Aroma compounds pulled towards vacuum line

Aroma compounds collected on filter in vacuum line

Analysis by gas chromatography-mass spectrometry

Stem with young leaves inside flask
The aromas of young citrus leaves are complex and dynamic. They consist of fragrances called *terpenes* that vary from species to species.
Scent lures developed at USHRL are being tested by CDFA to determine if they improve ACP catches on sticky card traps.
ACP Pest Management:
Inhibition of the Stylet Sheath Formation Process
Other Important Insects That Require Stylet Sheaths For Successful Feeding: Sharpshooter, Whitefly, and Aphid
ACP Stylet Sheath Structure Appears to Contain Proteins and Polysaccharide Chains (not just protein as previously thought)

- Polysaccharide appears to be the major component of ACP stylet sheaths
- Proteins appear to be present but may be variably distributed (e.g. flange vs. trunk)
- Proteins appear to play a fundamental role in sheath solidification
- We speculate that ACP stylet sheaths may act like a ‘cloaking’ system to protect psyllids from plant defenses during feeding

ACP stylet sheaths could be compared to a wooden barrel, the wood planks being the polysaccharide beta-linked poly-glucoses and the metal bands the protein components.
Discovered Method to Degrade Salivary Sheaths

Two Sheaths Being Degraded Over Time
Kill Psyllids using RNAi Strategy
(Inhibiting a gene necessary for insect survival)

*The sequence of the dsRNA fed to the insect is an exact match to a specific psyllid gene that, when prevented from functioning, results in psyllid death.
Water solution drenches with dsRNA designed to kill psyllids were absorbed into citrus trees.

The dsRNA treatments in citrus lasted for 2.5 months. Total degradation after 3 months (no longer detected).
With RNAi we want to target the *pest* *NOT* the beneficial insects.
Reverse dsRNA feeding did not cause RNAi effects in the other insect. But feeding each species their own designed dsRNA, did cause RNAi effects and increased mortality within each insect. Demonstrates species specificity and safety.
**About RNAi** - RNA interference is a *natural immune response* found in insects, worms, cows, pigs, chickens (and all other animals), and plants (e.g. citrus, grapes, fruits, nuts, vegetables).

--Uses natural gene regulatory systems already in place.

--RNAi can respond in a highly specific manner to biological pathways or organisms.

--Uses information derived from psyllid genome data.

--RNAi approaches are NON-transgenic, thus do NOT genetically alter crops.

--RNAi for gene disruption and regulation is fast becoming the focus of human medicine, and will become just as important in agriculture.