Update on Bactericide use in HLB

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Historically Bactericides Have Been Used in Treating Greening

CITRUS GREENING DISEASE

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The Bactericides *streptomycin, tetracycline(s), & penicillin* have each been shown in studies since the 1970’s to *improve overall plant health* and fruit quality in HLB infected trees.

These studies included:

- Foliar sprays
- Budwood soak
- Injections (most historic work focuses on direct trunk injection)
The USDA-ARS & AgroSource CRADA Provides Lab-to-Market Tech Transfer

- The CRADA provides a merger of the scientific, regulatory, manufacturing and commercial acumen of each party.
  - ARS – provides scientific and intellectual property expertise
  - ASI – provides scientific, regulatory, manufacturing and commercial expertise to deliver technology to the grower

Lab → Greenhouse → Field → Market
Commercially Available Bactericides
Registered for Use in Agriculture

The CRADA is currently testing ASI FireWall50 and FireLine17 to manage HLB.

- FireWall 50WP (streptomycin), presently has Section 18 EPA approval for use in Florida Grapefruit for Canker management.
  - ASI does not endorse or support the off Label use of FireWall 50WP or FireLine 17WP. The use of these products (or others) off label could interfere with the registration of bactericides for use in control of HLB.
- The CRADA is also looking at novel chemical and biochemical agents that may provide solutions to HLB.
Components of a Successful Bactericidal Treatment Strategy

Active Bactericide

Successful Treatment

Extended Exposure

Delivery

Citrus Greening
What are the Best Active Bactericides for HLB? Penicillin? Streptomycin? Oxytetracycline?

**Penicillin:**
- Prior field work at the USDA-ARS indicated significant changes relative to controls in tree health in the spring following frequent treatment with Penicillin.

**Streptomycin:**
- Prior work with streptomycin alone, or streptomycin + penicillin had positive effects for treatment of HLB.

**Oxytetracycline:**
- HLB literature indicates that it and other tetracycline's are viable candidates for treatment.
**Penicillin or FireWall?**

**Extended Exposure (longevity) in Plant**

**FireWall Greenhouse and Field Studies:**

- CRADA able to achieve systemic movement of streptomycin throughout the tree
- Studies show biological activity against bacteria up to 22-weeks on treated plants

- Penicillin (Black solid line)
- Streptomycin (Red Dashed Line)

Note: Activity of Penicillin is gone by two weeks, while Streptomycin remains
Major Hurdles to Overcome for Penicillin Registration for Use in Agriculture in USA

Government Regulatory Concerns (USDA, CDC, FDA, EPA):

1. Penicillin's remain a major first line human use bactericide
   - EPA previously refused to register gentamycin
2. Not presently registered for agricultural crop use
   - No products are currently on the market
   - Registration for use could take up to 7 years (or longer).
3. Severe allergen in susceptible humans
   - Residue studies need to be done to ensure juice is penicillin free
   - Handler exposure would likely be an issue
4. All tools should be explored
   - It is expected that numerous tools will be required
5. Must be realistic about the costs, timelines and challenges
   - Prioritize projects accordingly
Selection of FireWall & FireLine:

- *Penicillin* at best is most likely a long term tool.
- *FireWall 50WP* and *FireLine 17WP*, each are presently formulated and registered for use in numerous agriculture crops.
  - *FireWall 50WP* is currently labeled for grapefruit use to combat canker, so we have a path to follow for an EPA registration for use against HLB in citrus.

Primary Objective of The Large (ARS/ASI) HLB Bactericide Study:

- **Near-term** apply *existing technology and products* to combat HLB
- **Mid-term** strategies to *develop optimal bactericidal therapies*
- **Long-term** develop novel treatment mechanisms for delivery to *most efficiently treat trees.*
Large Scale HLB Bactericidal Therapy Trials Overview

General Information:
- We are in commercial groves evaluating the performance of various bactericides under production processes throughout Florida.
- Evaluations are conducted with bactericides that show promise in published literature and in our greenhouse studies.
- We are evaluating various applications strategies.

Project Time-Line:
- This is a multi-year project, each strategy testing in parallel; however, year one’s primary goal is to provide a near-term solution ASAP.

Assessments:
- Assessments of tree health, size, vigor, fruit drop, qPCR of CLas infection, and bactericidal residue are being performed to gauge treatment effects.
## Large Scale HLB Bactericidal Therapy Trial

### Locations and Citrus Diversity Overview

Cooperator sites, trees, and general HLB status of trees included in the study

<table>
<thead>
<tr>
<th>Cooperator Grower</th>
<th>Site</th>
<th>Location</th>
<th>Age/Tree/~Size</th>
<th>Quantity</th>
<th>General HLB status†</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>S1</td>
<td>Indian River</td>
<td>3yr/Hamlin/5-6 ft</td>
<td>144</td>
<td>Early HLB</td>
</tr>
<tr>
<td></td>
<td>S2</td>
<td>Indian River</td>
<td>6yr/Valencia/7-8 ft</td>
<td>30</td>
<td>Overt HLB</td>
</tr>
<tr>
<td>C2</td>
<td>S3</td>
<td>Indian River</td>
<td>3yr/Hamlin/4-5 ft</td>
<td>120</td>
<td>Overt HLB</td>
</tr>
<tr>
<td></td>
<td>S4</td>
<td>Indian River</td>
<td>3yr/Ray Ruby/5-6 ft</td>
<td>120</td>
<td>Overt HLB/some Canker</td>
</tr>
<tr>
<td></td>
<td>S5</td>
<td>Indian River</td>
<td>8yr/Ray Ruby/8-10 ft</td>
<td>25</td>
<td>Overt HLB/some Canker</td>
</tr>
<tr>
<td>C3</td>
<td>S6</td>
<td>Ridge</td>
<td>4yr/Valencia/6-7 ft</td>
<td>120</td>
<td>Overt HLB</td>
</tr>
<tr>
<td></td>
<td>S7</td>
<td>Ridge</td>
<td>8yr/Valencia/8-10</td>
<td>120</td>
<td>Overt HLB</td>
</tr>
<tr>
<td></td>
<td>S8</td>
<td>Ridge</td>
<td>5yr/Hamlin/6-8 ft</td>
<td>30</td>
<td>~50% clear HLB</td>
</tr>
<tr>
<td>C4</td>
<td>S9</td>
<td>Peace River</td>
<td>6yr*/Hamlin/7-10 ft</td>
<td>120</td>
<td>Severe HLB, heavy ‘healthy’ fruit load</td>
</tr>
<tr>
<td></td>
<td>S10</td>
<td>Peace River</td>
<td>6yr/Valencia/8-10 ft</td>
<td>25</td>
<td>Overt HLB</td>
</tr>
</tbody>
</table>

†Designates that all trees in the study are qPCR positive for *Clas*
*Designates block contains resets at 3 years of age
Progress & Early Findings:
- Treatments began mid-to-late summer; all treatments were completed using a 30 day treatment schedule.
- Across all the sites and citrus varieties in the study, we have no evidence of Phytotoxicity on trees treated with label rates of FireWall or FireLine.
- Early findings appear to indicate that treated trees are showing signs of increased canopy density relative to non-treated controls.
- CLas status is being processed and is not yet reportable.

2015 Plans:
- Given our current understanding, we have refined our plans for the next season and are preparing these now to be performed early, mid, and late season.
- We plan to continue to treat certain trees from 2014 year (to see a cumulative result year-to-year).
- We plan to begin new trees for treatments at typical application timings.
Summary Conclusions

- The CRADA between USDA-ARS and ASI allows for:
  
  \[ \text{Lab} \rightarrow \text{Greenhouse} \rightarrow \text{Field} \rightarrow \text{Market} \]

- Components of a viable treatment regimen include:
  - Active HLB Bactericide (Penicillin, **Firewall 50WP**, **FireLine 17WP**)
  - Develop improved/optimized delivery methods
  - Extended Exposure (long term residual to kill over time)
    - Penicillin is short lived, and has significant hurdles to cross to get registered, no crop uses registered by EPA.
    - FireWall 50WP is bioactive for far longer than penicillin treatment
    - Studies indicate that after 60-days **no residue** is found in grapefruit juice, already registered by EPA for tree fruits.
    - FireLine 17WP studies are in process and are not currently reportable, already registered by EPA for tree fruits.
  
- Applications of bactericides have been applied for a large scale HLB study in Florida and results are now beginning to be processed.
Thanks to All Involved In This Work

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