Control and Management Strategy for Citrus Huanglongbing in USA

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Discovery of HLB in Florida

$9.3 \text{ billion annual economic impact on the state}$

Palmetto Bay *Citrus* sp.

First find locations in August, 2005

Florida City *Citrus maxima*

Photos: Xiaoan Sun and Susan Halbert
Strategies for Citrus HLB control

- Integrated Pest Control
- Cultural Control
- R gene and GM plant
- Chemical control of HLB bacterium
Cultural Control

- Remove all infected plants quickly
  - Pruning (not very helpful)
- Isolation distance (0.9 miles)

![Image of cultural control measures]
Clean budwood and nurseries

- Clean budwood! Citrus Health Program in Florida
- Prevent movement of citrus and citrus relatives
- Eliminate citrus and citrus relatives in the vicinity of commercial production
Chemical control of HLB bacterium

- In vitro systems to screen the effective molecules
- Evaluation of the screened molecules
- Applications of the effective molecules
CITRUS GREENING DISEASE

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1. Injections of tetracycline and penicillin improve overall plant health and fruit quality – Studies since the early 1970’s show this.
2. Antibiotic residues are short lived (~15 days or less).
3. Only injections were used
4. Improvement lasts for 1 year (often with two injections)
5. Only method that was successful was injection:
   1. Costly
   2. Problems with phytotoxicity using this method.
Regeneration protocol of Las-infected periwinkle cuttings

1. Las-infected periwinkle
2. Cuttings from the plants
3. Treated with CA for 4 hr.
4. Regenerated plants transplanted into big pots after two months
5. Regenerated plants from the treated cuttings
6. Cuttings planted in the pots for 2 months
Graft-based protocol for testing candidate antimicrobials:

- Monitor Las in treated scion
- Monitor ability of antimicrobials to effect migration of Las into seedling
Using the above two optimized screening system, more than 100 molecules were evaluated against HLB bacterium

1. Antibiotics: 31
2. Biocides: 25
3. Peptides: 5
4. Fungicide: 6
5. SAR substances: 6
6. Others: 29
Efficiency of the tested compound were divided into 4 groups based on the Ct value in the inoculated plants, scion infected percentage and Las transmission percentage. Highly effective: \( Ct \geq 36.0 \); Effective: \( 36.0 > Ct \geq 32.0 \); Partly effective: \( 32.0 > Ct \geq 28.0 \); Non-effective: \( Ct < 28.0 \).
Scions survival rate and rootstock infection rate (A) of rootstock and their grafted HLB-affected scions treated with different chemical compounds (PS: 1.0g/L penicillin G and 100 mg/L streptomycin; Ksg: 1.0 g/L Kasugamycin; Oxy: 1.0 g/L Oxytetracycline; Met: 100 mg/L Metronidazole; DBNPA: 200 μl/liter of 20% solution of the biocide agent 2,2-dibromo-3-nitrilopropionamide; CK: Tap water was used as a control).
Las bacterial titers (B) of rootstock and their grafted HLB-affected scions treated with different chemical compounds (PS: 1.0g/L penicillin G and 100 mg/L streptomycin; Ksg: 1.0 g/L Kasugamycin; Oxy: 1.0 g/L Oxytetracycline; Met: 100 mg/L Metronidazole; DBNPA: 200 μl/liter of 20% solution of the biocide agent 2,2-dibromo-3-nitrilopropionamide; CK: Tap water was used as a control).
HLB-affected citrus in field treated with antimicrobial compounds

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Effective compounds evaluated by graft-based screening method

<table>
<thead>
<tr>
<th>Chemical compounds</th>
<th>Ct value</th>
<th>Phytotoxicity(Y/N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicilin</td>
<td>39.7±0.1</td>
<td>N</td>
</tr>
<tr>
<td><strong>Actidione (cycloheximide)</strong></td>
<td><strong>39.2±0.8</strong></td>
<td><strong>Y</strong></td>
</tr>
<tr>
<td>80WG</td>
<td>35.8±1.1</td>
<td>N</td>
</tr>
<tr>
<td><strong>SAR</strong></td>
<td><strong>37.8±0.1</strong></td>
<td>N</td>
</tr>
<tr>
<td>Sulfadimethoxine sodium</td>
<td>36.8±1.7</td>
<td>N</td>
</tr>
<tr>
<td><strong>Nicotine</strong></td>
<td><strong>36.3±0.4</strong></td>
<td><strong>Y</strong></td>
</tr>
<tr>
<td>Ciprofloxacin hydrochloride</td>
<td>35.7±3.5</td>
<td>N</td>
</tr>
<tr>
<td>Sulfathiazole sodium</td>
<td>35.7±1.5</td>
<td>N</td>
</tr>
<tr>
<td>DL-buthionine-sulfoximine</td>
<td>35.6±0.3</td>
<td>N</td>
</tr>
<tr>
<td>Silver colloidal</td>
<td>35.0±1.8</td>
<td>N</td>
</tr>
<tr>
<td>Poly-l-arginine</td>
<td>34.8±2.1</td>
<td>N</td>
</tr>
<tr>
<td>Surfactin from bacillus subtilis</td>
<td>34.8±0.7</td>
<td>N</td>
</tr>
<tr>
<td>Poly-D-lysine</td>
<td>34.5±1.3</td>
<td>N</td>
</tr>
<tr>
<td><strong>P-cymene</strong></td>
<td><strong>34.0±1.1</strong></td>
<td>N</td>
</tr>
<tr>
<td>Carvacrol</td>
<td>33.9±3.1</td>
<td>N</td>
</tr>
<tr>
<td><strong>Zhongshengmycin</strong></td>
<td><strong>33.7±1.0</strong></td>
<td>N</td>
</tr>
<tr>
<td>Silver nitrate</td>
<td>32.9±1.6</td>
<td>N</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>32.6±2.5</td>
<td>N</td>
</tr>
<tr>
<td>CK</td>
<td>25.3±0.8</td>
<td>N</td>
</tr>
</tbody>
</table>
* Bark application delivered penicillin more efficiently than foliar spray.
Duration of Penicillin in Plant

![Graph showing the log2 of average radius (mm) of Penicillin over days after treatment.](image)

- **A avg**
- **B avg**
- **C avg**

Days after treatment:
- 0
- 2
- 4
- 6
- 8
- 10
- 12
- 14
- 16
SECOND QUESTION: Can Basal Bark Application Treatment Reduce Clas Abundance in Infected Citrus? (Monthly Applications over ~ 1 year of symptomatic potted trees ~ 1 meter)
FUTURE RESEARCH

- Develop more efficient delivery systems to enable chemicals to quickly become systemic in the citrus.

- Develop a control-released formula of the screened compounds coupled with thermotherapy.

- Discover most potent actives to be registered for use in citrus.