International Research Conference on Huanglongbing VI

March 10-15, 2019
Riverside, California, USA
Research Updates

- Horticultural Practices- Dr. Tripti Vashisth
- Asian Citrus Psyllid- Dr. Lauren Diepenbrock
- HLB Pathology- Dr. Megan Dewdney
Horticulture/Cultural Practices Outline

- 30 oral presentation and 63 posters
- Rootstocks and Varieties - 4
- Cultural Control/Horticulture
  - Covered Production - 3
  - Soil amendments and Root health - 2
  - Nutrition - 1
- Effect of HLB on fruit - 1
- HLB Resistance and Tolerance - 12
- Regulatory and Others - 7
Rootstocks and Varieties
Bowman and Albrecht “Rootstock influences on tree health and growth in response to Candidatus Liberibacter asiaticus in grafted sweet orange trees”

- **Greenhouse Study**

- **Valencia on 11 commercial rootstock-healthy and HLB**

  1. Carrizo
  2. Cleopatra
  3. Sour orange
  4. Swingle
  5. Ridge sweet orange
  6. US-802
  7. US-812
  8. US-896
  9. US-897
  10. US-942
  11. US-1516
<table>
<thead>
<tr>
<th>Rootstock</th>
<th>No. of leaves on healthy tree</th>
<th>No. of leaves on Clas+ tree</th>
<th>HLB leaf number effect Clas+/healthy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>US-942</td>
<td>117</td>
<td>81</td>
<td>71 a</td>
</tr>
<tr>
<td>US-812</td>
<td>121</td>
<td>73</td>
<td>64 ab</td>
</tr>
<tr>
<td>US-802</td>
<td>115</td>
<td>57</td>
<td>48 a-c</td>
</tr>
<tr>
<td>Sour orange</td>
<td>139</td>
<td>50</td>
<td>39 bc</td>
</tr>
<tr>
<td>Carrizo</td>
<td>147</td>
<td>57</td>
<td>38 bc</td>
</tr>
<tr>
<td>US-1516</td>
<td>105</td>
<td>37</td>
<td>37 bc</td>
</tr>
<tr>
<td>US-896</td>
<td>145</td>
<td>45</td>
<td>33 bc</td>
</tr>
<tr>
<td>US-897</td>
<td>109</td>
<td>36</td>
<td>33 bc</td>
</tr>
<tr>
<td>Ridge sweet orange</td>
<td>150</td>
<td>40</td>
<td>23 c</td>
</tr>
<tr>
<td>Swingle</td>
<td>141</td>
<td>32</td>
<td>23 c</td>
</tr>
<tr>
<td>Cleopatra</td>
<td>159</td>
<td>31</td>
<td>20 c</td>
</tr>
</tbody>
</table>

- US-942 and US-812 maintained the most leaves in the canopy
- Ridge, Swingle, and Cleopatra maintained the fewest leaves in the canopy

Bowman et al. 07b-03
<table>
<thead>
<tr>
<th>Rootstock</th>
<th>Total leaf area on healthy tree (cm²)</th>
<th>Total leaf area on CLas+ tree (cm²)</th>
<th>Leaf area effect Clas+/healthy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>US-942</td>
<td>3803 a-c</td>
<td>1485</td>
<td>37 ab</td>
</tr>
<tr>
<td>US-812</td>
<td>3494 bc</td>
<td>1503</td>
<td>41 a</td>
</tr>
<tr>
<td>US-802</td>
<td>3396 bc</td>
<td>1216</td>
<td>35 ab</td>
</tr>
<tr>
<td>Sour orange</td>
<td>4105 ab</td>
<td>1015</td>
<td>25 a-c</td>
</tr>
<tr>
<td>Carrizo</td>
<td>3528 bc</td>
<td>1151</td>
<td>31 a-c</td>
</tr>
<tr>
<td>US-1516</td>
<td>3160 bc</td>
<td>817</td>
<td>25 a-c</td>
</tr>
<tr>
<td>US-896</td>
<td>3782 a-c</td>
<td>790</td>
<td>21 a-c</td>
</tr>
<tr>
<td>US-897</td>
<td>2943 c</td>
<td>563</td>
<td>19 bc</td>
</tr>
<tr>
<td>Ridge sweet orange</td>
<td>4478 a</td>
<td>868</td>
<td>17 bc</td>
</tr>
<tr>
<td>Swingle</td>
<td>3448 bc</td>
<td>598</td>
<td>17 bc</td>
</tr>
<tr>
<td>Cleopatra</td>
<td>4069 ab</td>
<td>513</td>
<td>12 c</td>
</tr>
<tr>
<td>P value</td>
<td>0.025</td>
<td>0.237</td>
<td>0.036</td>
</tr>
</tbody>
</table>

- US-942 and US-812 maintained the most total leaf area
- Ridge, Swingle, and Cleopatra maintained the least total leaf area

Bowman et al. 07b-03
<table>
<thead>
<tr>
<th>Rootstock</th>
<th>Root titer of CLas @ 50 wai (genomes per 1 mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>US-942</td>
<td>157</td>
</tr>
<tr>
<td>US-802</td>
<td>263</td>
</tr>
<tr>
<td>US-896</td>
<td>738</td>
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<tr>
<td>Swingle</td>
<td>837</td>
</tr>
<tr>
<td>Carrizo</td>
<td>880</td>
</tr>
<tr>
<td>US-812</td>
<td>885</td>
</tr>
<tr>
<td>Cleopatra</td>
<td>983</td>
</tr>
<tr>
<td>US-1516</td>
<td>1778</td>
</tr>
<tr>
<td>US-897</td>
<td>2019</td>
</tr>
<tr>
<td>Sour orange</td>
<td>6193</td>
</tr>
<tr>
<td>Ridge sweet orange</td>
<td><strong>8171</strong></td>
</tr>
<tr>
<td>P value</td>
<td><strong>0.48</strong></td>
</tr>
</tbody>
</table>

Bowman et al. 07b-03
<table>
<thead>
<tr>
<th>Rootstock</th>
<th>Leaf titer of CLas @ 50 wai (genomes per 1 mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swingle</td>
<td>18,930 c</td>
</tr>
<tr>
<td>US-1516</td>
<td>21,642 c</td>
</tr>
<tr>
<td>Carrizo</td>
<td>26,945 c</td>
</tr>
<tr>
<td>Sour orange</td>
<td>42,892 c</td>
</tr>
<tr>
<td>Ridge sweet orange</td>
<td>56,887 bc</td>
</tr>
<tr>
<td>US-896</td>
<td>57,143 bc</td>
</tr>
<tr>
<td>US-802</td>
<td>89,518 bc</td>
</tr>
<tr>
<td>Cleopatra</td>
<td>102,382 a-c</td>
</tr>
<tr>
<td>US-812</td>
<td>104,292 a-c</td>
</tr>
<tr>
<td>US-942</td>
<td>167,593 ab</td>
</tr>
<tr>
<td>US-897</td>
<td>215,410 a</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td><strong>0.007</strong></td>
</tr>
</tbody>
</table>

Bowman et al. 07b-03
Why don’t rootstocks that make healthier canopies have lower CLas titer in scion?

- Perhaps because healthier leaves support larger bacterial titer than sick leaves

- The rootstocks US-942 and US-812 tended to have the most favorable effects on the scion
- The rootstocks Cleopatra, Swingle, and Ridge tended to have the least favorable effects
- The relationship of CLas titer to observed canopy health was unclear

Bowman et al. 07b-03
Stover et al., “USDA Efforts to Develop Resistance and Tolerance to Huanglongbing in Citrus Scions”

- First released scion for fruit use containing Poncirus (1/8 Poncirus): US SunDragon
- Strong tolerance to HLB at many locations
SunDragon progeny selections-2018

Not only interesting as a cultivar but as a parent as well

USDA/ARS
Ft. Pierce, FL

Stover et al. 05b-06
Summary Stover et al., USDA

- Tolerance to HLB has been shown to be present in some cultivated citrus and greater resistance may be drawn from more distant members of the gene pool.

- USDA has identified tolerant cultivars and advanced selections which are in replicated trials including plantings with growers.

- Some mandarins, trifoliate, and citron are the most convincingly documented sources of resistance.
Grosser et al., “Progress in breeding rootstocks to prevent or mitigate HLB in commercial trees”

- **NEW STRATEGY: Breeding Somatic Hybrid Rootstocks at the Tetraploid Level**
  - Products can have direct rootstock potential including adequate polylembryony, ability to control tree size due to polyploidy, and improved disease resistance
Valquarius on Orange #15 tetrazyg rootstock – just < 5 years at St. Helena, Dundee FL – released as UFR-3

Candidate for ACPS

Grosser et al. 05b-05
UFR-17 Emerging as good HLB-tolerant rootstock for higher-density plantings

- UFR-17 is [Nova+HBPummelo x sour orange+Carrizo]
- HLB+ over 4 years, grown
- 2 psyllid sprays per year;
- Picked 2.13 boxes/tree in 2018 season.

February 2018

Same trees, July 2018

Same trees, February 2019

Grosser et al. 05b-05
## Top Performing Gauntlet Rootstock Status

<table>
<thead>
<tr>
<th>Rootstock</th>
<th>Seed Tree</th>
<th>Producing Seed</th>
<th>PTP Status</th>
<th>TC</th>
<th>MAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>A+VolkxOrange19-11-8</td>
<td>Yes</td>
<td>Yes</td>
<td>Available</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Milam+HBPxOrange14-09-10</td>
<td>Yes*</td>
<td>Yes*</td>
<td>In process</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>A+VolkxOrange19-11-31</td>
<td>Yes</td>
<td>No</td>
<td>Available</td>
<td>Initiated</td>
<td>No</td>
</tr>
<tr>
<td>A+HBJL1-OP-09-36 (sour type)</td>
<td>No</td>
<td>No</td>
<td>Soon</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Milam+HBPxOrange14-09-9</td>
<td>No</td>
<td>No</td>
<td>Soon</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Milam+HBPxOrange14-09-14</td>
<td>No</td>
<td>No</td>
<td>Available</td>
<td>Initiated</td>
<td>No</td>
</tr>
<tr>
<td>A+HBPxOrange19-08-2</td>
<td>Yes</td>
<td>Zygotic</td>
<td>Available</td>
<td>Initiated</td>
<td>No</td>
</tr>
</tbody>
</table>

### Flying Dragon Hybrids (for ACPS)

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Seed Tree</th>
<th>Producing Seed</th>
<th>PTP Status</th>
<th>TC</th>
<th>MAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>B21-R1-T2-11-2</td>
<td>No</td>
<td>No</td>
<td>Available</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>B21-R1-T25-11-10</td>
<td>No</td>
<td>No</td>
<td>Available</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>B21-R1-T25-11-6</td>
<td>No</td>
<td>No</td>
<td>Available</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>A+FDxOrange19-11-10</td>
<td>Yes</td>
<td>Yes</td>
<td>Soon</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

R&D Tissue Culture Collaborators: Phillip Rucks Nursery TC Lab and Agromillora FL
Combination of good scion genetics, good rootstocks genetics and evolving nutrition (McKenna nitrate program): OLL-8 sweet orange/UFR-4 rootstock, 4 year old trees - Working!

Grosser et al. 05b-05
Rodrigues et al. “HLB differential response of ‘Valencia’ sweet orange grafted on several citrus rootstocks in a endemic area”

- Poster

- Out of 16 commercial rootstocks, Flying Dragon, the rootstock with smallest canopy, least flushing shoots per canopy, showed the least incidence of HLB over a period of 3 years
  
  - Possibly, fewer flushing shoots resulted in limited infestation by psyllid

Rodrigues et al. 05b-05
Cultural Management
Li et al., “Flush phenology manipulation by NAA and GA application in sweet orange”

- Poster
- Greenhouse and Field Experiment-1-year old trees
- NAA can effectively delay flushing when applied through soil or foliar
- GA induced vegetative flush, depends on previous flush

- NAA and GA can manipulate flowering, however timing of application is critical for desired effect; further research is needed
Schumann et al., “Hydroponically-grown grapefruit maximize HLB-free fresh fruit production in CUPS”

Grapefruit grown in CUPS, 871 trees/acre, hydroponics

100% pack-out

2,100 boxes/acre (200 tonnes/ha) in years 1-4

HLB-free citrus with the legendary Fresh From Florida taste!
Ferrarezi et al., “Citrus Under Protected Screen for grapefruit HLB management in Florida’s East Coast”

- Psyllids were detected @ IRREC CUPS even with intensive pest management program
  - Other insects such as thrips and mites still need to be managed
- CUPS is able to maintain HLB-free grapefruit trees up-to-date
  - 2018/19 in-ground trees on US-897 had higher yield than potted
- In-ground trees are vigorous with more vegetative growth and larger canopy volume than potted trees
- Potted trees have better fruit quality
Alferez et al., “Individual Protective Covers (IPCs) for young citrus trees”

IPC performance, one year after planting

![Graph showing IPC performance](image)

- All plants tested negative for HLB:
  - IPC: 27% negative
  - no IPC: 13% negative (Full dosage)
Graham and Morgan, “Root damage on HLB-affected trees: Consequences and mitigation with soil acidification”

High soil pH exacerbates HLB symptoms and fruit drop
6 yr old Valencia/Swingle trees

Soil pH 6.4: Fruit drop minimal

Soil pH 7.2: Fruit drop resulted in early harvest
Reduction in soil pH increased yield & fruit soluble solids

<table>
<thead>
<tr>
<th>Acidification treatment</th>
<th>Fruit weight (40 fruit, lbs)</th>
<th>Fruit yield (Boxes/ tree)</th>
<th>Soluble solids (lbs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (soil pH 7.3)</td>
<td>19.5 D</td>
<td>1.57</td>
<td>5.23 BC</td>
</tr>
<tr>
<td>Control w/ Sulfur</td>
<td>20.9 C</td>
<td>2.03</td>
<td>5.06 C</td>
</tr>
<tr>
<td>pH 6.0 w/o Sulfur</td>
<td>20.9 C</td>
<td>2.79</td>
<td>4.60 D</td>
</tr>
<tr>
<td>pH 6.0 w/ Sulfur</td>
<td>21.9 B</td>
<td>2.19</td>
<td>5.14 C</td>
</tr>
<tr>
<td>pH 5.0 w/o Sulfur</td>
<td>20.7 C</td>
<td>1.96</td>
<td>5.05 C</td>
</tr>
<tr>
<td>pH 5.0 w/ Sulfur</td>
<td>21.5 BC</td>
<td>1.80</td>
<td>5.34 ABC</td>
</tr>
<tr>
<td>pH 4.0 w/o Sulfur</td>
<td>21.6 BC</td>
<td>1.94</td>
<td>5.45 AB</td>
</tr>
<tr>
<td>pH 4.0 w/ Sulfur</td>
<td>23.2 A</td>
<td>2.55</td>
<td>5.53 A</td>
</tr>
</tbody>
</table>
Ghimire et al. “Effect of irrigation water pH on HLB-affected sweet orange”

- Poster
- Greenhouse study with healthy and HLB plants
- pH 5.8, 7.0, and 8.0 for 60 days

Both HLB and HLY plants showed a tendency of bringing soil pH close to 7 in course of experiment.
Ghimire et al. P2-11

Bar graph showing the percentage of death rate and leaf drop rate at different pH levels:

- **Death Rate**
  - pH 5.8: 0%
  - pH 7: 12.5%
  - pH 8: 37.5%

- **Leaf Drop Rate**
  - pH 5.8: 5.8%
  - pH 7: 7%
  - pH 8: 8%

The graph shows a positive correlation between pH and leaf drop rate, with an R² value of 0.90.
Summary

- There is a HLB-pH interaction
- HLB plants seem to perform better at pH range close to 5.8
- Soil and plants have pH buffering capacity therefore, pH adjustment is needed to be continuous process
- Poor performance of HLB plant at high cannot be solely attributed to nutrient availability
Strauss et al., “Benefits of biochar on HLB-affected citrus trees in sandy soil”

- Poster
- Greenhouse study on 2-year old Hamlin
- Compost and biochar influenced the microbiome
- Compost on HLB-affected trees altered the microbiome diversity; the diversity was similar to that of healthy trees
- Compost improved the nutrient availability to the tree
McCollum et al., “Anaerobic soil disinfestation impacts the soil microbiome and growth of citrus trees infected with CLas”

- Poster
- Field with 3 rootstocks
- ASD controls soilborne pathogen and nematodes

- In CLas positive trees:
  - ASD treated trees had significantly greater canopy area
  - Affected the soil microbiome
  - Did not prevent or reduce HLB incidence or CLas titer
Pitino et al. “Physiological effects of oak bioactive compounds on HLB-affected trees”

- Poster
- Greenhouse study-Preliminary work
- Oak extract treatment on HLB positive trees
  - Reduced starch accumulation
  - Improved chlorophyll content
  - Improved root and shoot growth
  - Slight reduction in CLas titer
Bassanezi et al., “Can additional supply of Ca and/or Mg reduce the progress of HLB incidence, severity and crop loss?"

- 10 year old Hamlin on Rangpur lime
- Foliar treatments:
  - T1 = NPK with N as NH4NO3
  - T2 = NPK with N as CaNO3
  - T3 = T1 + Mg (as MgSO4.7H2O)
  - T4 = T2 + Mg (as MgSO4.7H2O)
- 6 year long study
Treatments did not prevent HLB incidence

Bassenezi et al. 01b-06
Plot yield did not increase with treatment

Bassenezi et al. 01b-06
Mattos et al., “Can negative effects of HLB be mitigated by calcium and magnesium fertilizer in citrus trees?”

- Poster
- Regardless of treatment, HLB positive trees showed poor growth parameters
- Rate of photosynthesis increased in Ca treatment
- Carbohydrate (starch) accumulation increased in roots with Ca treatment
- Mg improved plant response to stress
Kadyampakeni, “Citrus nutrient uptake, plant growth as influenced by citrus greening and regulated deficit irrigation”

- Poster
- Greenhouse study
- HLB-affected trees uptake limited water, leaving root zone wet
- For young tree, full irrigation is recommended, deficit irrigation not recommended
- Use of compost improved water use/uptake

[Image showing biomass accumulation at the end of a greenhouse for healthy (two trees on the left) and HLB-affected (two trees on the right).]
Grosser et al., “Ground applied overdoses of Mn show a therapeutic effect against HLB in established grove”

- Poster
- ‘Vernia’ on rough lemon
- Soil application of nutrients is beneficial
- With use Mn, a reduction in Ct was observed with improvement in yield
- Mn-B interaction is not clear; need further research

Nonetheless, good nutrition program has potential
Creste et al., “Fruit quality of orange trees with different nutritional treatment”

- Poster
- Effect of Calcium and Potassium on fruit quality
- Foliar Calcium and Potassium nitrate
- Calcium and potassium significantly improved Brix and ratio
- However, if 40% or more of the tree canopy showed HLB symptoms, there was no improvement
Tang et al., “HLB-associated fruit drop in sweet orange”

- Poster
- Valencia-18-year old

Mild; PAR: 150
Moderate; PAR: 310
Severe; PAR: 415
Severely symptomatic trees drop more fruit!

Fruit Drop Rate (%) vs. PAR value

- Severe tree
- Moderate tree
- Low tree

R² = 0.71
p-value = 0.0006

Tang et al. P2-12
Low carbohydrate availability may not be the main cause of pre-harvest fruit drop

- Loose fruit from severe trees had the greatest concentrations of fructose, glucose, and inositol among all groups.
- Loose fruit did not have lower concentrations of sucrose, fructose, glucose, or inositol in juice than tight fruit.

Tang et al. P2-12
Severely symptomatic trees have smaller fruit. The fruit that drops is of smaller size.

For loose fruit and severely symptomatic trees, higher sugar concentrations were likely due to more concentrated juice (smaller fruit), suggesting lower water uptake by the tree.

Fruit type: $P = 0.0007$
Symptom level: $P = 0.0037$
Take home message

- Psyllid exclusion in CUPS and IPC is promising
- Better performing rootstocks are available
- Flush phenology can be influenced for better ACP control via PGRs or appropriate rootstock
- Adding organic matter to soil can be beneficial
- Soil pH adjustment to below 6.5 is beneficial for HLB-affected plants
Take home message contd...

- Good nutrition program is helpful for HLB-affected trees
  - Focus should be on constant and balanced fertilizer program
  - Maintaining leaf nutrient in optimum to high levels
  - Regular leaf and soil nutrient analysis is the key
  - DO not rely solely on foliar programs as they have limited efficacy

- Small dose and frequent irrigation

- Good tree maintenance can improve tree health therefore, can positively influence fruit quality including fruit size