Research and strategies for management of HLB in Brazil

Renato B. Bassanezi
Joseph M. Bové (in memoriam)
HLB CONTROL CHALLENGE

- No resistant commercial citrus varieties
- No feasible curative methods
- Rapid progress and severe damages
HLB MANAGEMENT: THREE-PRONGED SYSTEM

Prevents as many trees as possible from becoming infected

Healthy trees produced in insect-proof covered nurseries

Reduction of the sources of inoculum

Control of *Diaphorina citri*

“If citrus growers accomplish the TPS to manage HLB they will have a chance. If not, they will be out of citrus business”

“The HLB-management system (TPS) as described here is only a short-term solution to keep the citrus industry alive and to buy time for long-term solutions, probably based on engineered citrus genotypes, to come in, hopefully, within five to ten years. In the meantime, research on psyllid control and identification of infected but still symptomless trees might improve the system.”

J.M. Bové (2010)
ADVANTAGES OF SPS FOR HLB MANAGEMENT

Previous challenges constructed the basis for facing HLB

✓ Production of citrus healthy trees in insect-proof nurseries
✓ Grower experience to inspect groves for diseases
✓ Grower habit to reduce disease inoculum
✓ Grower experience to scout and control insect-vectors
✓ Partnership between Government Plant Protection Agency and Fundecitrus in disease eradication programs
✓ Open channel for knowledge and technology transfer from research institutions to growers
✓ Research institutions network
"The largest citrus industry in the world must have a research compatible with its size."

J.M. Bovê
FUNDECITRUS PARTNERSHIP WITH FOREIGN RESEARCH INSTITUTIONS
RESEARCH LINES ON HLB

Short and medium term researches to improve the TPS and make it more sustainable

Detection and Elimination of HLB-affected trees

✓ Strategies for early detection
  • Diagnosis methods (multiplex qPCR; biophotonics /LIFS)
  • Inspection methods (ground x platform, aerial images, glasses)
  • Sampling criteria for new areas (dissemination modeling)
  • Labor evaluation (safety, ergonometric, labor perception)

✓ Strategies to eliminate diseased tree
  • Methods for tree elimination

✓ Assessment of the impact of keeping diseased trees in the field
  • Epidemiological impact of low-frequency tree elimination
  • Crop loss assessments (scion/rootstocks, nutrition status, plant age)
  • Fruit quality assessments
  • Simulation of different management scenarios (benefit-cost analysis)
RESEARCH LINES ON HLB

Short and medium term researches to improve the TPS and make it more sustainable

Monitoring *Diaphorina citri*

- Favorable conditions for psyllid population increase
- Psyllid dispersal (distance, weather and phenological factors)
- Models to predict new vegetative flushes and its growth
- Scouting methods (visual, traps, D-vac, net,...)
- Sampling criteria for psyllid scouting (traps/ha, traps/meter)
- Positioning of yellow sticky cards in plant and in the grove
- Trap reading techniques
- Monitor grove or neighbor to predict psyllid migration
RESEARCH LINES ON HLB

Short and medium term researches to improve the TPS and make it more sustainable

Control of *Diaphorina citri*

- Contact and systemic chemical insecticides (selectivity, mode of application, rates, residual period of control, timing, frequency, and spray volume, efficacy in semi-dwarfing rootstocks)
- Natural insecticides (dillapiol from *Piper aduncum* formulation)
- Biological insecticides (*I. fumosorosea, B. bassiana*, formulations, rate, timing, compatibility)
- Biological control (*T. radiata* and *D. aligarhensis* rear and release methods, Bt isolates)
- Materials that interfere in the psyllid landing on citrus plants
- Optimization of regional management
- Benefits of external actions of HLB management
RESEARCH LINES ON HLB

Short and medium term researches to improve the TPS and make it more sustainable

Plan the planting and resetting / Intensive psyllid control at the edge

✓ Strategies to escape from HLB
  • Climate conditions favorable to ACP, Ca. Liberibacter titer, bacteria acquisition and transmission, and symptoms expression
  • Psyllid dispersion (distances, factors)
  • Influence of abandoned groves and backyard infected trees
  • Shape and size of groves and blocks ("edge effect")
RESEARCH LINES ON HLB

Short and medium term researches to improve the TPS and make it more sustainable

Plan the planting and resetting / Intensive psyllid control at the edge

- Strategies to facilitate intensive psyllid control at the edge and avoid its dispersal into the grove
  - Planting direction
  - Planting density
  - Scion/rootstock combination (flush pattern, semi-dwarfing)
  - Frequency of insecticide sprays at the edge
  - Eradication and reset at the edge
  - Reflective mulch
  - Trap crop with systemic insecticide at the edge

Solid high density planting with parallel rows to the grove perimeter

Continuos resetting at the edge

Citrus grove

Trap crop
RESEARCH LINES ON HLB

Short and medium term researches to improve the TPS and make it more sustainable

Speed up plant growth and production

✓ Strategies to escape from HLB most susceptible period and to mitigate crop loss
  • Nursery tree size for UHD system
  • Scion/rootstock combination (vigor, tolerance/resistance, productivity efficiency)
  • Planting density
  • Low-input Inter-cropping production system (economic alternatives)
  • Nutrients, hormones and SAR on root growth, symptoms suppression and yield maintenance
  • Irrigation
  • Plastic mulch
  • Screen-covered plants or caolim

Solid high density planting with irrigation and adequate nutrition

Conventional and semi-dwarfing rootstocks

Conventional and advanced nursery trees

Solid high density planting with plastic mulch
RESEARCH LINES ON HLB

Long term researches to improve the TPS and make it more sustainable

Strategies for *Diaphorina citri* control

- Light traps
- Automatic device for trap reading and data signaling
- Laser-based equipment for control
- Pheromone and attractants
- Repellent semiochemicals
- GM repellent citrus varieties and soybean (intercrop)
- Selection and evaluation of targets genes of ACP for RNAi
- Nanoparticles for RNAi formulation
- GM citrus and *Murraya* with RNAi against ACP
- Bt isolates and formulation for drench/contact application
- GM citrus with Bt gene against ACP
RESEARCH LINES ON HLB

Long term researches to improve the TPS and make it more sustainable

Strategies for *Candidatus* Liberibacter control

- Plant-bacteria and vector-bacteria interactions (screening for effector genes of Las)
- Analysis of genetic diversity of Las population
- Transcriptome of Las and Lam infection in citrus varieties (identification of genes associated with defense and stress response)
- Genetic mapping of tolerant citrandarins rootstocks
- Physiological changes in plants with HLB (starch accumulation and callose formation)
- Root transmission of Las
RESEARCH LINES ON HLB

Long term researches to improve the TPS and make it more sustainable

Strategies for *Candidatus Liberibacter* control

- Evaluation of resistance/tolerance of different citrus genotypes (gene expression, callose formation, starch accumulation, bacterial titer, disease severity and yield decline)
- Phloem-specific promoters for citrus transformation
- SPS strain CTV vector for screening potential genes against the bacteria
- GM citrus resistant to Las (AMP, genes from citrus transcriptome; prophages)
MAIN SOURCES FOR RESEARCH SUPPORT ON HLB
<table>
<thead>
<tr>
<th>INVESTMENTS ON HLB RESEARCH AFTER 2006</th>
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<tbody>
<tr>
<td><strong>US Dollar</strong></td>
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<tr>
<td>FUNDECITRUS</td>
</tr>
<tr>
<td>Embrapa</td>
</tr>
<tr>
<td>FAPESP</td>
</tr>
<tr>
<td>CNPq</td>
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<tr>
<td>CRDF</td>
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<tr>
<td><strong>Total</strong></td>
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</table>

Average rate
USD 1.00 = R$ 2.21
FUNDECITRUS ACTIONS ON HLB MANAGEMENT

Communication and Training

Eradication Program

Phytosanitary Alert – Psyllid -

*T. radiata* rearing and releasing
## INVESTMENTS ON HLB MANAGEMENT AFTER 2006

<table>
<thead>
<tr>
<th>Description</th>
<th>USD Value</th>
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</thead>
<tbody>
<tr>
<td>Eradication Program*</td>
<td>3,163,337.46</td>
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<tr>
<td>Communication and Training</td>
<td>2,050,777.34</td>
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<tr>
<td>Phytosanitary Alert – Psyllid</td>
<td>498,121.33</td>
</tr>
<tr>
<td>Tamarixia rearing and releasing</td>
<td>279,018.48</td>
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</tbody>
</table>

**Average rate**  
USD 1.00 = R$ 2.21  

**Total Investments**  
5,991,254.61

* Fundecitrus took part of official HLB eradication program from March/2005 to December/2009
INTEGRATED HLB MANAGEMENT

- Intensive Management at the Edges
- Speed up Plant Growth and Production
- Plant Healthy Nursery Trees
- Inspect Frequent for HLB-Affected Trees
- Plan the Planting and Resetting
- Monitor Psyllid Presence
- Control the Psyllid
- Eliminate Diseased Trees
- Partner up with Your Neighbors
- Take Part of Regional Management
GOOD RESULTS ON HLB MANAGEMENT IN SPS

Average annual disease incidence lower than 2%
INCIDENCE PROGRESS OF HLB IN SÃO PAULO STATE
**SEVERITY OF HLB IN SÃO PAULO STATE**

<table>
<thead>
<tr>
<th>Severity</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>No HLB symptoms</td>
<td>83.08%</td>
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<tr>
<td>≤ 25% canopy with symptoms</td>
<td>8.26%</td>
</tr>
<tr>
<td>26 – 50% canopy with symptoms</td>
<td>4.20%</td>
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<tr>
<td>51 – 75% canopy with symptoms</td>
<td>2.71%</td>
</tr>
<tr>
<td>&gt; 75 canopy with symptoms</td>
<td>1.74%</td>
</tr>
</tbody>
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INCIDENCE OF HLB BY REGIONS

- TMG - Triângulo Mineiro
- DUA - Duartina
- MAT - Malã
- LIM - Limeira
- VOT - Votuporanga
- BEB - Bebedouro
- BRO - Brotas
- AVA - Avaré
- SJQ - S. J. do Rio Preto
- ALT - Altinópolis
- PFE - Porto Ferreira
- ITG - Itapetinga

Incidence:
- 0.4%
- 0.9%
- 8.3%
- 5.9%
- 25.6%
- 48.3%
- 39.1%
- 25.9%
- 20.1%
- 8.2%
- 0.8%
The greatest challenge: Early infection of new groves
“The successful HLB management by the TPS must be continued in SPS not only during the next 5 to 15 years required to develop HLB-resistant, genetically modified citrus (GMC) trees, but also afterwards, when GMC trees will hopefully be available.

The farms with a high percentage of healthy trees represent the “hard-core-orchards” on which the future of the SPS citrus industry will be constructed. They offer appropriate conditions for hosting not only the GMC trees, but also for the uninterrupted production of regular, non-GMC trees in spite of HLB.”

J.M. Bové (2012)