
AGRICULTURAL RESEARCH SERVICE
U.S. HORTICULTURAL RESEARCH LABORATORY
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Microbiological Media
PLANT
PATHOLOGY
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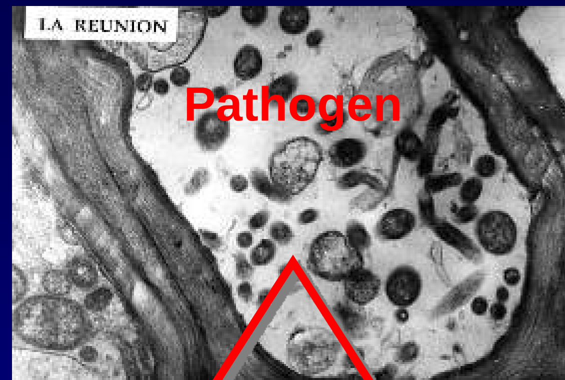
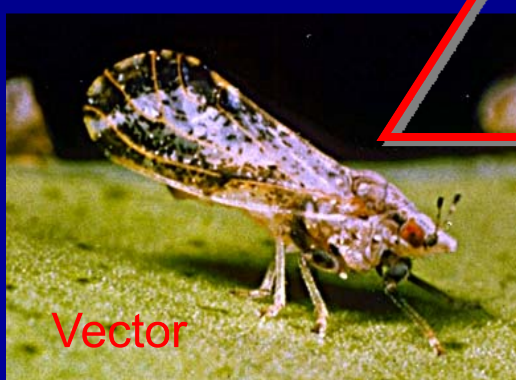
USDA-ARS Citrus Greening Disease Research



Subtropical Plant Pathology
Research Unit

Subtropical Insects
Research Unit



Horticulture and Breeding
Research Unit

Vector-Oriented Project Components

A. Diagnostics and Detection
Phenology of *Liberibacter* in Field Populations of Adult *Diaphorina citri*

B. Molecular Characterization
HLB-Psyllid interactions- Genetics Approach

C. Culturing and Collection of Isolates
HLB-Psyllid Cell Culture
Artificial Systems for *Diaphorina citri*

D. Pathogen Transmission
Latency Period of *Liberibacter* in Adult *Diaphorina citri*: Detection and Transmission

E. Resistance
Evaluation of citrus relatives for resistance to psyllid
Development of phloem infusion assay and testing disease control compounds

F. Epidemiology/Vector Control
New Biological Control Agents
Microbial control of Asian citrus psyllid
Asian citrus psyllid management and Murraya paniculata
Asian citrus psyllid reproductive biology and behavior
Sampling Asian citrus psyllid populations
Asian citrus psyllid migrations
Population genetics and phylogeny of Asian citrus psyllid
Quantitative ecology of Asian citrus psyllid

Hall, Hentz

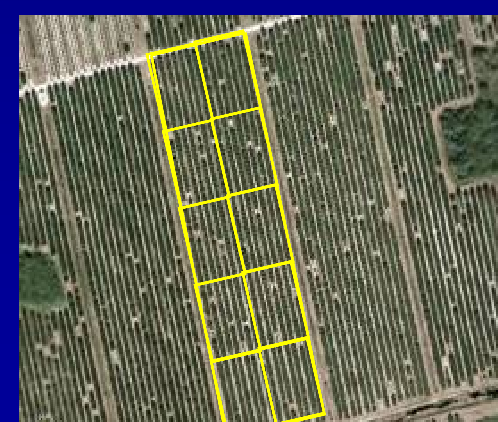
Sampling Asian citrus psyllid populations

- Methods of detecting, monitoring and estimating infestation levels of psyllids are needed by growers, researchers and regulatory personnel

- Research will be conducted to determine protocols for:

Trapping adults
Detecting adults with traps or stem tap sampling
Sampling to detect eggs and nymphs
Hierarchical methods of estimating population levels based on dispersion parameters

How and where to sample



Asian citrus psyllid migrations

Information on the migratory habits of adult psyllids is lacking

Research will be conducted to characterize:

Movement of adults between trees in a block

Research will be conducted to determine:

If migrations occur and if they are seasonal

If infestation densities promote migration

The research will clarify if defined migrations occur and, if so, the seasonality of these migrations.




Hall et al.

New Biological Control Agents

- Determine if there is genetic evidence for different biotypes of *Tamarixia radiata* among populations in Florida, Texas, Puerto Rico, and other locations.
- Biotype differences may explain why *T. radiata* is more effective in some geographical areas than others. It might behoove the industry to import new strains of the parasitoid.
- Import a bi-parental biotype of *Diaphorencyrtus aligarhensis* from Vietnam and release into Florida citrus.
- We anticipate that the Vietnamese biotype of *D. aligarhensis* would establish in Florida and boost biological control of the psyllid (ornamental settings and commercial citrus).

Microbial control of Asian citrus psyllid

Identify pathogens that attack Asian citrus psyllid in Florida.

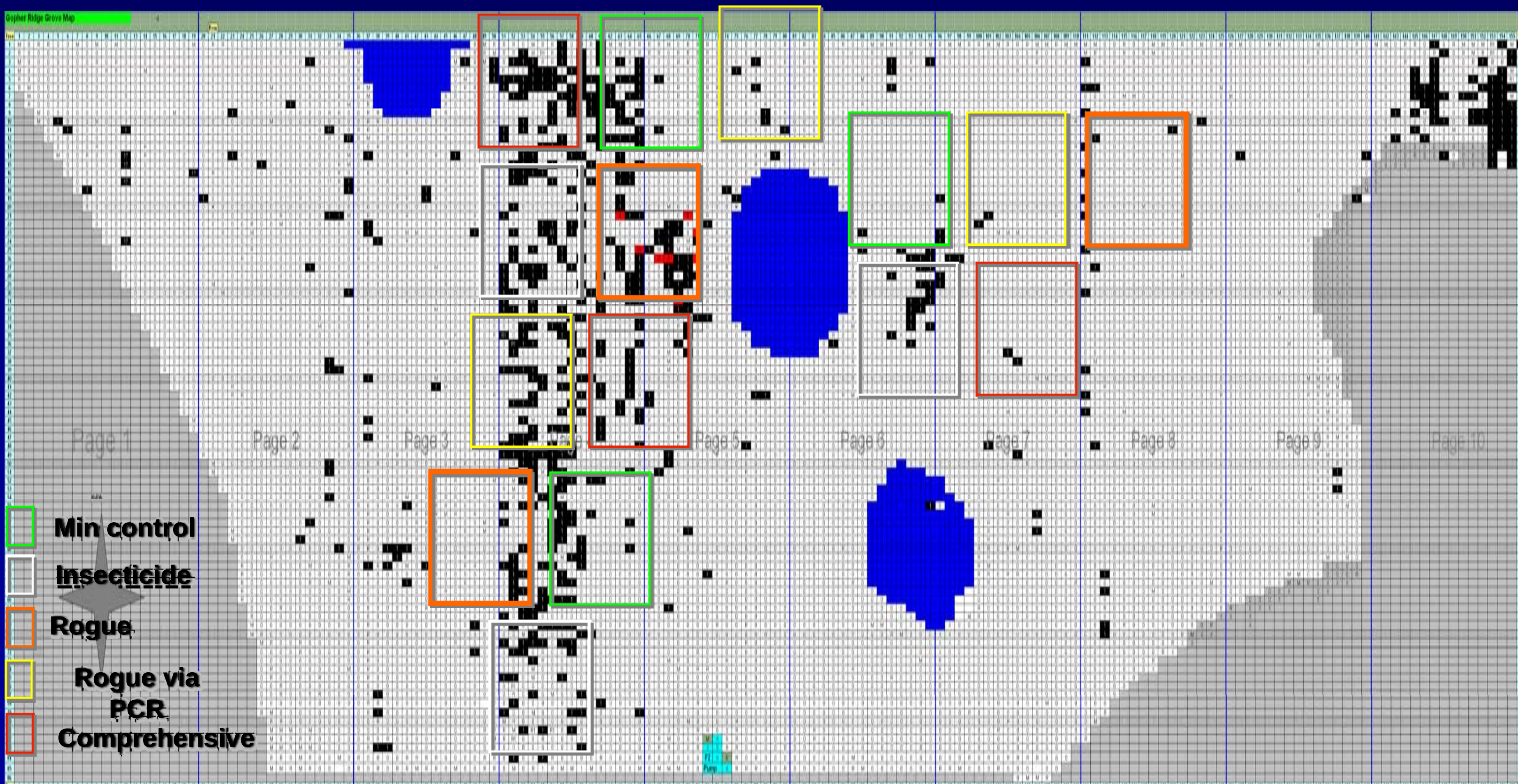
Determine which life stages these pathogens attack and seasonality of their infections.

An new fungal species of the genus *Hirsutella* that attacks adult psyllids will be investigated.

Screen candidate pathogens (fungi, bacteria, and viruses) available at ARS repositories.

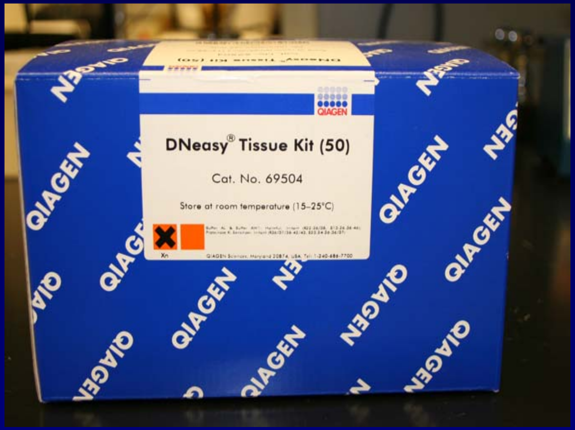
Epidemiology of HLB Disease Control Study - Gopher Ridge, Consolidated Citrus

- At time of first survey, HLB-positive trees were not showing good symptoms - symptoms had receded throughout the planting.
- Map shows variability attributed to various surveyors
- Resurvey needs to occur in Fall
- Plots were each 10 rows by 15 trees per row = 150 trees/plot
- Three replications per treatment. Treatments located with two row or two tree buffer minimum
- Five treatments: Minimal control, Insecticide vector control, Roguing, Roguing via PCR+, and Comprehensive
- Main diseased areas were located adjacent to HLB+ trees in adjacent F-4 block to north



Detection Protocols

- How do different primer sets and testing protocols affect detection sensitivity?
  - Head to head comparisons, multiple primer sets, conventional PCR vs. real time
    What extraction protocols can be adapted to high throughput testing?
  - Test multiple extraction protocols
- How do we deal with the possibility of other species being present?
  - Test multiple *Liberibacter* specific primers vs generic 16S primers followed by sequencing



Expected outcome:

- Most sensitive testing protocol for routine testing will be identified.
- More sensitive methods means more thorough identification and better control.

Interactions in Mixed Infections of HLB and Other Systemic Citrus Pathogens

Viroids

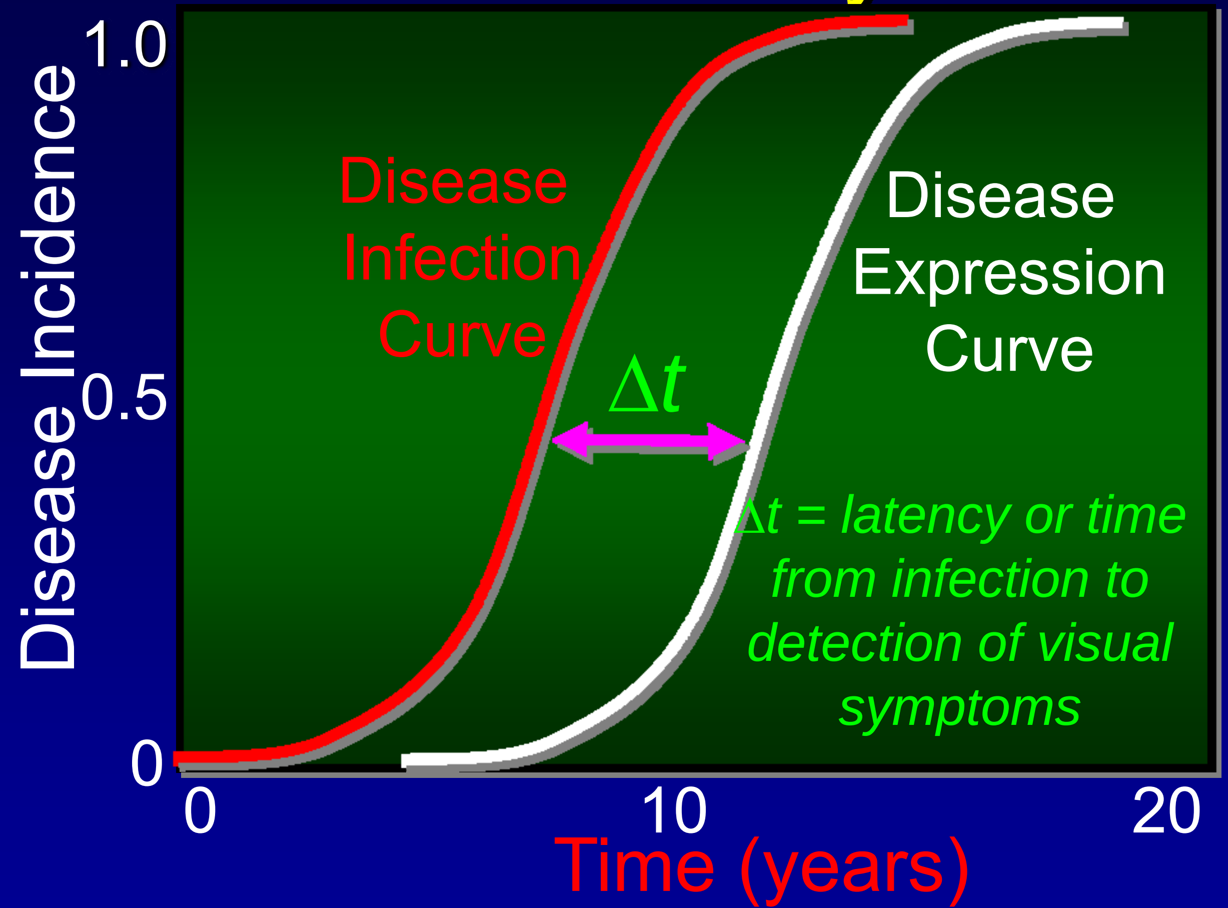
Psorosis

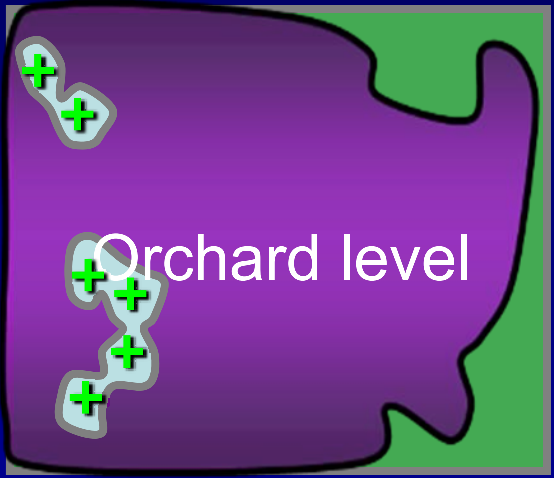
How does infection with HLB affect greenhouse and lab diagnosis of these other systemic pathogens, and of HLB?

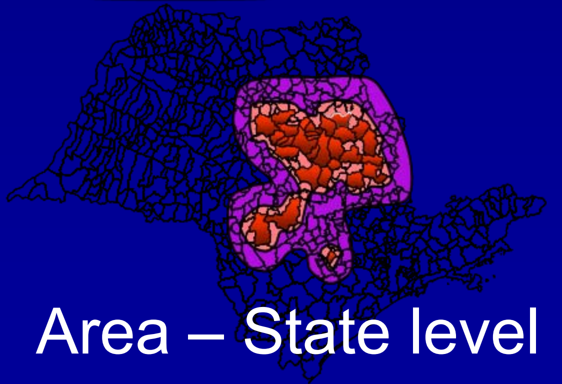
CTV

HLB

Latency of infection and Spatial Hierarchy







- For HLB latency period can be 3 months to multiple years depending on cultivar, tree age, health, environment, number of psyllid transmissions/infections, distribution of infections within tree, etc.

Host plant resistance is the ideal disease management strategy

- Highly effective (when available)
- Environmentally benign
- No reliance on pesticides
- Little or no additional expense to producers

Unfortunately… conventional breeding for HLB resistance is not a viable option

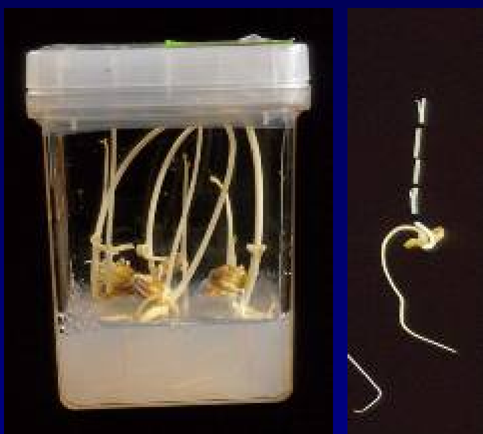
- No documented resistance to greening among edible citrus types
- *Citrus latipes* and *Citrus indica* may have some resistance – BUT THEY ARE NOT COMMERCIALLY ACCEPTABLE

A **transgenic** approach is the most viable option to develop citrus with resistance to HLB

- Many potential sources of resistance
- Change a single trait
- Much less time required than conventional breeding

Citrus Transformation

Transformation



Selection

Regeneration

Evaluation