

# Citrus Industry Update

**Working  
To Keep You  
Informed**

Published by the University of Florida, Institute of Food and Agricultural Sciences, with the mission of keeping the Florida Citrus Industry informed of current research concerning canker and greening.

Welcome to a new effort from UF, IFAS to update progress in research on citrus greening and canker. This monthly newsletter is targeted to the Florida citrus industry and will provide ongoing progress in developing base understanding of these diseases, as well as advances in development of tools for their detection and management in Florida.

## GREENING DIAGNOSTICS



- Comprehensive comparisons of existing and new diagnostic methods are being performed in several labs to improve on current detection methods. These efforts are looking at molecular methods, but also trying to refine simple, field-ready techniques that might aid growers in identifying greening infected trees more readily or sooner in the infection process. Methods are being investigated to extract DNA/RNA from HLB infected citrus trees. Optimization of several diagnostic methods is under way.

- Improvements continue to be made in the US Sugar/IFAS Collaborative diagnostic lab in Clewiston and the numbers of diagnoses are increasing. Plans are progressing to develop an additional lab in Southwest Florida to meet grower needs.

- As a part of our continued efforts to develop sensitive detection methods for HLB, we compared the sensitivities of conventional PCR with Go Taq DNA polymerase (Promega) and SpeedStar HS DNA polymerase (Takara) with that of real-time PCR. We found that the conventional PCR with SpeedStar HS DNA polymerase is equally sensitive to real-time PCR. The conventional PCR with SpeedStar DNA polymerase can be used as a substitute to real-time PCR if expensive real-time PCR equipment and reagents are not available.

(S. Tatineni, CREC, tasa@crec.ifas.ufl.edu)

## HLB BACTERIAL INTERACTION WITH THE PLANT



- Quantitative study of HLB bacteria populations inside the phloem is underway. Specific fluorescent probes targeting 16r RNA were designed and tested. Anatomical analysis of the phloem is underway.

- Progress was made in comparing the gene expression of HLB-infected citrus trees and non-infected trees from the grove. Preliminary data are being developed. This research is also focusing on HLB-infected citrus trees and non-infected trees available for investigation from the greenhouse.

- Series of citrus plants are being infected with HLB under controlled conditions to understand infection and symptom development. A range of responses is being observed in potted plants, including onset of visible symptoms within three months following infection. Other plants are testing positive for infection after three months but are not yet showing symptoms. Some information on varietal response to greening should come from this work.

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- Location of citrus greening bacteria in plant tissues is indicating occurrence of greening in most tissues investigated. This research is shedding some light on infection processes.

### CULTURING OF GREENING BACTERIA



• Culturing methods are the focus of several projects, using a variety of techniques and approaches. We have not yet cultured the HLB pathogen. Progress has been made in the optimization and defining parameters for growth of the babaco bacterium that is being used as a model system. We still do not have a consistent source of HLB bacterium in plants in greenhouses to support extensive attempts at culturing it. In the interim, infected materials from the field are being used to supplement greenhouse sources of bacteria for culturing efforts. We have identified a couple of potential systems for co-culture of the HLB pathogen with other organisms as alternatives to axenic culture (Mike Davis - mjedavis@ufl.edu)

### OTHER BACTERIAL SPECIES INSIDE THE CITRUS PHLOEM

• Species of bacteria other than *Liberibacter* are being isolated from citrus cell culture inoculated with HLB infected samples. We are currently investigating those bacteria to determine their identity and role in citrus tissues. We are also working to separate internal from external bacteria from citrus tissues.

### THE ROLE OF PSYLLIDS IN GREENING SPREAD

Psyllid Transmission of HLB pathogen is an important area of study. It includes:

- Work on acquisition of HLB by adult psyllids is ongoing
- Acquisition rates ranging from 10-40% (very preliminary)

- No evidence to date of transovarial transmission (very preliminary)
  - Study of psyllid transmission rates ongoing but will take some time before results can be collected due to latency period
  - Research on prevention of transmission using insecticides is ongoing but again will take time to determine if transmission has been prevented due to latency period. (Michael Rogers - MRogers@ufl.edu)

### PSYLLID MANAGEMENT

#### **Research**

#### *Pesticide efficacy evaluations*



- We continue to test new compounds and timing of existing pesticides for effectiveness in controlling psyllids
  - Demonstrated effectiveness of adicarb (Temik) for psyllid control in large-scale trials with evidence that rainfall can affect the success of Temik applications
  - Several new products have been identified as having effects on psyllids and are being tested further, including products that could be sprayed during bloom.

#### *Pesticide resistance management*

- Determination of the LC50 values for currently used products for psyllid control is ongoing
  - We have successfully determined the LC50 values for Danitol (fenpropathrin)
  - We have preliminary LC50 data for imidacloprid
  - With additional funding support, we expect to have LC50 data for all pesticides used for psyllid control completed with 12 months
  - This is important for monitoring pesticide resistance in psyllids in the coming year(s) to maintain the effectiveness of the few products available for psyllid control.

#### *Transgenic Approaches to Psyllid Management*

- Drs. Rogers, Dawson and Boucias

are collaborating to screen more than 100 insect toxins for effects on psyllids that can be expressed in plants transgenically. Some preliminary results appear to show some activity with some toxins.

### ASIAN CITRUS PSYLLID SUPPRESSION



•Timely reduction in Asian Citrus Psyllid (ACP) populations is key to reducing spread of greening in citrus crops. The highest labeled rate (33lbs/acre) of aldicarb 15% (Temik 15G) and reduced rates (16.5lbs and 8.25lbs per acre) were evaluated for their impact on ACP infestations in 2006.

- The high rate was most effective in reducing the density of ACP as assessed by the proportion of citrus flush infested with eggs and nymphs, numbers of these per flush and adult number estimates by a beat method.

- Another study in 2006 demonstrated that application on the one side (bed side) of the trees was as effective as the application on both sides (bed and swale). Application of 33lbs/acre in about 30 days prior to spring flush in January 2007 was most effective compared with applications in November or February.

- Reductions in psyllid populations were detectable for over five months. No impact of Temik was observed on populations of ladybeetles known to be responsible for high levels of predation on ACP. (Phil Stansly, SWFREC, pstansly@ufl.edu)

### IMPACT OF INSECTICIDES ON ASIAN CITRUS PSYLLID



•Asian Citrus Psyllid (ACP) suppression is a key component of greening management. ACP can only reproduce on young flush but survives as adults in winter by feeding on mature foliage. Broad spectrum foliar and soil applied insecticides

were applied either alone or in concert with soil application of aldicarb during the dormant season to suppress adult populations. The immediate impact on adult populations and subsequent effect during the growing season were evaluated as well as effects on natural enemies. One application of dormant sprays, significantly reduces psyllid populations for over 5 months. Reinforcement with aldicarb generally improved control. Few ladybeetles were present when sprays were applied but reappeared later to feed on aphids and psyllids in the spring flush. Maintenance of these presatios may explain the prolonged suppression of ACP observed. Thus, dormant sprays appear to be providing pest suppression for many months with apparently minimal ecological liability. (Phil Stansly, pstansly@ufl.edu)

### GENETICS AND SEQUENCING HLB AND CITRUS

- Several independent labs in IFAS are working to isolate and sequence the DNA of greening bacteria, and some progress in isolating clean preparations of DNA has been made. Partial sequences are forthcoming, but a complete sequence is not yet available.

- We are proceeding with efforts to lead and coordinate the International Citrus Genome Consortium's project to sequence a citrus genome by the end of 2008 (one of the top three highest impact priorities from the Int. HLB/ACC conference, November 2005) (Fred Gmitter - fgg@ufl.edu);

### TOWARDS GREENING RESISTANT CITRUS PLANTS

- Transgenic grapefruit plants have been produced containing the 'Lima' anti-bacterial construct that has been used in grapes to create resistance to Pierce's Disease (Jude Grosser Lab - jwg@ufl.edu).

- Transgenic grapefruit and lime plants have been produced, containing a construct that elevates pathogenesis-related proteins up to 10-fold (Jude Grosser Lab - jwg@ufl.edu)

- Successful testing of a phloem-limited gene promoter from *Arabidopsis* in Citrus - for targeting expression of anti-bacterial genes in transgenic citrus (Jude Grosser Lab - jwg@ufl.edu).

- Collection of seedlings of *Citrus latipes* (purported genetic resource for HLB tolerance), to assess tolerance and inheritance of the trait; (Fred Gmitter - fgg@ufl.edu)

- Somatic hybridization experiments have been initiated with the purportedly tolerant *Citrus latipes*, and somatic embryos recovered have been recovered. Grafted and ungrafted *C. latipes* seedlings are being propagated for greening challenge to validate this information (Jude Grosser Lab - jwg@ufl.edu).

#### SUPPRESSION OF CITRUS CANKER IN THE GROVE

- Evaluation of copper Materials for rates, timing and efficacy continues in Florida and in Brazil through cooperators



- Foliar antibiotic materials labeled for other agricultural crop use are being evaluated for their role in canker management. (James Graham - jhg@ufl.edu)

#### MANAGEMENT OF CITRUS LEAFMINER TO MINIMIZE CANKER INOCULUM

- IPM approaches are being pursued to enhance management of Citrus Leafminer

- Foliar pesticide applications are being conducted to provide best management practices for pesticide

use for leafminer, psyllid and other arthropod management

- Significant effort is being made to address inquiries regarding the recent concern over honeybee decline which is occurring nationwide. IPM strategies developed for citrus include avoidance of applications of pesticides when possible during periods of bee activity (bloom).



#### WINDBREAKS TO REDUCE CANKER SPREAD

- Research to identify species and planting characteristics for implementing windbreaks in existing as well as new planting are underway, with experimental plantings being installed at several locations.

- Dynamics of air flow through natural windbreaks are being evaluated in existing plantings of candidate windbreak species.

#### NEW CITRUS PRODUCTION SYSTEMS TO RESPOND TO PRESENCE OF GREENING AND CANKER

- Evaluating new rootstocks that are excellent candidates for use with 'OHS' style new productions systems that feature high early yields and shortened grove rotations. Such rootstocks feature excellent nursery performance, ability to control tree size, precocious bearing, and wide soil adaptation (Jude Grosser Lab - jwg@ufl.edu).

- Propagation of several dozen new size-controlling rootstocks is underway for experiments to examine their suitability for new production systems (high-density, intensive management, early economic returns), and their potential to facilitate vegetative flush control in the Florida environment; (Fred Gmitter - fgg@ufl.edu)

- Evaluating high quality processing sweet orange clones with reduced juvenile phases, making them excellent candidates for genetic engineering in terms of speeding up the process of commercialization. These clones feature early fruit production, with even first generation trees showing high juice quality and minimal thorniness (Jude Grosser Lab - jwg@ufl.edu).