Florida Citrus Advanced Technology Program

QUARTERLY & FINAL REPORTS: Control of Citrus Greening, Canker & Emerging Diseases of Citrus

Instructions Complete the fields based on your project specs. When finished, save the form to your local disk using a unique name. Then, go to **http://research.fcprac.com**, and log in with your user name and password using Researcher Login in the lower left. Find this project title and click on **Submit a Report**. Update your profile information if needed, then upload this report as directed.

2009-2010 REPORT	CATEGORY (drop-down)	TODAY'S DATE (m/d/yr)
(●) *Quarterly Report* ( ) *Annual Report* ( ) *Final*	HLB Pathology	7/13/09

WHAT IS THE "HEADLINE" FOR THIS REPORT (e.g. a one-sentence "newspaper headline" describing what you accomplished)

Establishment of research plots, validation of data collection techniques, and collection of preliminary and initial data.

TITLE and CONTACT INFORMATION

Proposal Title Spatial and Temporal Incidence of Ca. Liberibacter in Citrus and Psyllids Detected Using Real Time PCR

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Organization SWFREC, University of Florida	*Total Direct Funds (current year)* 90,120

REPORT UPDATE (650 words; provide details about your headline)

Spatial and Temporal Incidence of Ca. Liberibacter in Citrus and Psyllids Detected Using Real Time PCR

Funds for this project were released on 19 March although research began earlier in anticipation of funding. In order to assess seasonal patterns of the pathogen in citrus trees and psyllids in regions of high HLB incidence, a 12 acre block of 'Valencia' orange trees was selected at a commercial grove in 2008. The block was divided into 16 plots, half of which were treated with insecticides as needed to suppress psyllid populations while the other 8 plots were left untreated. A sample of psyllid adults collected in the block in November 2008 and analyzed at USDA-ARS Riverside labs contained 21% HLB positive psyllids. Samples of the most symptomatic leaves from every fifth tree were taken on 13 Nov 2008 and analyzed for the presence of Ca. Liberibacter asiaticus at the Huanglongbing Diagnostic Laboratory, Southwest Florida Research and Education Center (SWFREC), Immokalee, FL. DNA was isolated using a commercially available kit (Qiagen; Valencia, Califorina, USA) for plant (DNeasy Plant kit) and psyllids (DNeasy Blood and Tissue kit). Quantitative PCR analysis was conducted using specific TaqMan primer/probe assays for plant (Li et al. 2006) and psyllid (Manjuanth et al. 2008). Thirty-four percent of the samples tested PCR positive. A tree determined to be PCR positive and one PCR negative tree in each plot was trimmed to induce new flush. On 3 June, 10 psyllid adults from our HLB negative colony on orange jasmine (Murraya paniculata) were released on a new shoot that had been caged immediately after trimming. An additional cage was placed on a previously uncaged and psyllid-infested shoot on the same tree. All cages along with branches and psyllids were collected 2 weeks later for PCR analysis. Nineteen percent of shoots caged with psyllids from the HLB negative colony tested positive for HLB on trees, regardless of whether trees had previously tested HLB positive or negative. In contrast, shoots that were naturally infested with psyllids when caged were 63% and 56% positive on previously HLB positive and negative trees, respectively. It would appear that infestation with infected psyllids from the field was over 2.5 times more likely to result in a positive plant sample, whereas the previous history of testing had little bearing. We are processing the adult psyllid samples taken at the same time. We are also caging infested shoots on symptomatic trees at SWFREC and commercial groves and collecting monthly samples of psyllid adults found. Adults emerging from shoots on HLB symptomatic trees caged with negative psyllids during Feb-March 2009 and analyzed at US Sugar and SWFREC were found to be 5% HLB positive by both labs, although psyllids collected at large at the same time and location were 14% positive. Psyllids that were caged on these trees during July-August 2008 were 27% positive according to the USDA-ARS Riverside lab. Therefore, psyllid origin and time of collection are major sources of variation on PCR results that we will need much more data to sort out. As our methods for addressing the first objective are refined as described above, the second objective is being address by the identification of additional sites to survey for psyllids which differ with respect to tree age, variety, and rootstock, and block size as described in grant proposal. A survey to administer to growers for tracking HLB and grove management tactics in the selected sites is in preparation.

(Refs: Li, W., J.S. Hartung, and L.Levy. 2006. Quantitative real-time PCR for detection and identification of Candidatus Liberibacter species associated with citrus huanglongbing. Journal of Microbiological Methods. 66:104-115.
Manjuanth, K.L., S.E. Halbert, C. Ramadugu, S. Webb, and R.F. Lee. 2008. Detection of Candidatus Liberibacter asiaticus in Diaphornia citri and its importance in the Management of Citrus Huanglongbing in Florida. Phytopathology. 98(4):387-396.)