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	7/29/10

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

Survey of HLB in seedlings from seed of HLB-affected trees from groves and citrus nursery seed blocks is in progress

TITLE and CONTACT INFORMATION

Proposal Title Transmission of HLB by citrus seed

Principal Investigator James H. Graham	*PI Last Name* Graham
Email jhg@crec.ifas.ufl.edu	*FDACS Contract Number* 78049
Phone 863-956-1151	*Project Duration (years)* 2 *Year of Project* 2
Organization UF, Citrus Research and Education Center	*Total Direct Funds (current year)* $22,783

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

Objective 1: Survey and confirmation of HLB in seedlings from HLB-affected trees. In 2009, 500 seedlings grown from seed extracted out of mild to severely HLB-affected fruit from Pineapple orange and Murcott tangor groves in Hendry Co. were assayed for HLB detection. All seedlings were negative for HLB detection by PCR in repeated assays in July and Nov. 2009. Objective 2: Thermotherapy of HLB-affected seed for the same seed sources under Objective 1 were treated at 125F, 130F and 135F to test for the effect of heat treatment on HLB detection in seedlings. Thermal treatments were lethal to Murcott, but not Pineapple seed. The emergent seedlings at 125F (142), 130F (98) and 135F (97) were PCR tested. All seedlings were negative for HLB in repeated PCR assay in July and Nov. 2009. Objective 3: In October 2009, seed source trees in two Florida nurseries were found positive for HLB by FDACS-DPI (Nursery 1) or Southern Gardens Diagnostic Lab (Nursery 2). Discovery of infected seed source trees in two Florida citrus nurseries identifies a potential (but unconfirmed) risk of outdoor seed source trees acting as a source of inoculum for introduction into nursery propagations. In Nursery 1, seed was collected from symptomatic branches of two sources of Swingle citrumelo, four sources of 'Kuharske' Carrizo citrange and one source of Cleopatra mandarin. In Nursery 2, seed was collected from one source of Sekwasha mandarin. From 189 to 278 seedlings from each source provide enough leaves for PCR testing in February 2010 and June 2010. The June test yielded a single questionable PCR detection for Cleopatra mandarin. Follow-up testing of this seedling from two separate midrib DNA extractions resulted in inconsistent positive results from 16S qPCR analysis with a mean detected Las titer of 6.9 and 4.3 16S copies/reaction (76.7 and 47.8 bacteria/midrib or 3652 and 2020 bacteria/g of midrib) for the two DNA extractions. Even in this case, detection by qPCR only occurred in only 75 and 33 percent of all 16S qPCR runs for the two DNA extractions. Las was not detected using beta-operon qPCR primers in either DNA extraction even after multiple runs. False amplification at very high Ct's, 43-50 (5 to 0.001 copies/rxn by standard curve), occurs about 33 percent of the time with the 16S primer/probe system from water and healthy controls, whereas false amplification has not been observed with the beta-operon primer/probe system. Because the positive detections are within the range of false amplifications in water and healthy DNA extracts, we are currently using nested PCR in the 16S rDNA, beta-operon gene, and OMP gene to attempt confirmation of Las presence by sequence analysis.