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*	Management	3/10/10

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

Plugging materials involved in HLB symptom development and some Liberibactor virulence factors successfully identified

TITLE and CONTACT INFORMATION

Proposal Title Characterizing the Roles of Callose and Phloem Proteins in HLB Symptom Development

Principal Investigator Leo Gene Albrigo	*PI Last Name* Albrigo
Email albrigo@ufl.edu	*FDACS Contract Number* NAS ID 2
Phone 863 956-1151	*Project Duration (years)* 3 *Year of Project* 1
Organization Citrus Research & Education Center	*Total Direct Funds (current year)* 125,000

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

A manuscript on the sequence of symptom development and the identification of callose and phloem protein 2 (PP2) as the amorphous and filamentous plugging materials was accepted: DS Achor, et al. 2010.Plant Pathology J. 9(2): in press. The upset of normal phloem translocation of carbohydrates to other plant parts and ultimately the starvation of the root system (Etxeberria et al, 2009, Physiol. Mol. Plant Pathol. 74:76-83) may be the main reason for tree decline from HLB infection. Field samples of several cultivars were collected and prepared to determine the amounts of amorphous versus filamentous plugs disrupting phloem sap flow. If one type of plugging is more prevalent, that pathway for plugging may be more important. Earlier work by a colleague indicated that three common rootstocks do not express HLB symptoms when infected with HLB and grown in a greenhouse. One of these, Swingle, was found infected in the field, but it developed typical leaf mottle symptoms and phloem plugging. Carrizo trees are being monitored until they become PCR positive. Poncirus trifoliate trees are being monitored in two field sites also as they may have HLB tolerance. Since insufficient bacteria are present to directly cause phloem plugging, work is underway to understand the mechanism by which the bacterial infection leads to this phloem plugging. To determine how the bacteria elicits the over expression of phloem plugging materials, one Agilent microarray was designed based on the genome sequence of Candidatus Liberibacter asiaticus. Bioinformatics analysis was performed to identify potential virulence factors. Six potential virulence factors were cloned into pGEMT-easy vector. The insertions were confirmed using PCR and three virulence factors were expressed in tobacco; one of these caused plant death. These factors and others will be inserted into citrus with a phloem promoter to study their potential roles in virulence. Transgenic approaches to achieve over-expression of the citrus ß-1,3-glucanase gene using different promoters, though protoplast/GFP co-transformation and Agrobacterium-mediated transformation are underway in order to minimize HLB associated callose-plugging. Initial transgenic grapefruit trees that over-express 1, 3-β glucanase were grown out on Macrophylla rootstocks and challenged with HLB by bud inoculation. Only 4 of 44 plants had successful bud take and none of these show symptoms 4 months after the inoculation attempt. All these plants were re-inoculated with infected buds in February. The citrus ß-1,3-glucanases gene from Valencia embryogenic callus and young leaves (McCollum et al., 1999) was cloned. Citrus β-1,3 glucanase cDNA (GenBank accession number AJ000081) was synthesized from Valencia leaf and embryogenic callus. A 1011 bp citrus β-1,3 glucanase gene fragment was amplified. To obtain the suitable restriction site and additional cMyc tag (to facilitate subsequent western analysis) on the cDNA, another PCR reaction was performed using a new primer set with a different restriction site including the cMyc tag. The final PCR product (BG3) was purified, cloned into the pGEM T-Easy vector (Promega) and sequenced. The cloned BG3 fragment was ligated into a vector (pUCLON) between the 35S promoter and 35S terminator, and was transformed into host E coli DH5α cells. Plasmid DNA was isolated using 5prime kit and checked through restriction digestion and PCR analysis. From the ligated plasmid (pUBG3), the HindIII fragment was excised and ligated into the HindIII site on another vector pCIT101 holding the GFP/NPTII fusion gene. The final vector , pCITBG3, was transformed into Agrobact. Co-transformation experiments can now be done. Plasmid vector pARS108 with the ER-targeted GFP gene was used to make a new construct for efficient protoplast transformation. Another vector, pGASS, was constructed to target BG3 expression in phloem tissues only. Two new sweet orange callus lines are available to carry out transformations. >Antisense or other knockout methods for phloem protein 2 and callose genes will be used in year 2.