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	2/16/10

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

Callose accumulation at the phloem plasmodesmata inhibits phloem loading in the Liberibacter-infected citrus leaf vein.

TITLE and CONTACT INFORMATION

Proposal Title Correlative Microscopic and Molecular Characterization of the Microbiome in the Citrus Phloem Tissue

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Phone 352-846-0952	*Project Duration (years)* 2 *Year of Project* 1
Organization University of Florida	*Total Direct Funds (current year)* 109,211.00

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

The objectives of our research project are:
Objective 1. Characterize the microbial community of healthy and Liberibacter-infected citrus phloem tissue by serial-section electron microscopy; Objective 2. Localize antigens and DNA sequences specific to Liberibacter in citrus phloem tissues by immunofluorescence microscopy, immunoelectron microscopy, and electron microscopic in situ hybridization.
For objective 1, we completed three-dimensional (3D) serial-section electron microscopy analysis of 7 sites in the citrus phloem where Liberibacter cells are detected (One of the sites reconstructed into a 3D model is shown at http://news.ifas.ufl.edu/2009/12/09/uf-researchers-find-lone-culprit-behind-greening). In all the sites, the bacterial cells displayed uniform morphological features including diameter, length, cytoplasmic staining, and cell wall staining, suggesting that Liberibacter is the sole bacterial species in the citrus phloem or it vastly outnumbers other bacteria, if there are any. While we were examining the Liberibacter-infected (Las+) citrus phloem cells by 3D reconstruction, we discovered that the plasmodesmata in the Las+ phloem cells were swollen and heavily labeled by anti callose-specific immunogold particles. In parenchyma cells of Huanglongbing (HLB) symptomatic leaves, chloroplasts are disrupted by massive accumulation of starch granules and the cells eventually die. By fluorescence microscopy with aniline blue staining, we demonstrated that the callose accumulation at the phloem plasmodesmata precedes starch build up in the parenchyma cells of Las+ leaves. Callose deposition in the plasmodesmata reduces their transport efficiency and photosynthetate loading into the phloem is mediated by phloem plasmodesmata. So we tested phloem-loading in the Las-, callose-rich Las+ leaves by injecting carboxyfluorescein dye into their intercellular space. Phloem loading was inhibited in asymptomatic Las+ and symptomatic Las+ leaves while phloem loading was not inhibited in the Las- leaves. This phloem occlusion in the Las+ citrus leaves is likely to interfere with export of photosynthetate from the leaf parenchyma cells and provides an explanation for their excessive starch accumulation. Our results also suggest that the death of non-vascular cells in Las+ leaves is due to an inappropriate plant response rather than damages directly done by the phloem-limited bacterial cells. These results were presented in an international meeting (Plasmodesmata 2010) and a manuscript is being prepared for publication in the Plant Physiology. For objective 2, we have prepared citrus phloem samples ready for immunogold labeling and in situ hybridization. We were able to detect polysaccharides by immunogold labeling but several antibodies against bacterial proteins (from Dr. Duan) have not provided consistent immunogold labeling results. We are testing more antibodies from Dr. Duan. We tried two oligonucleotides for localizing Liberibacter by in situ hybridization but their specificity has not been satisfactory. We are improving our protocols and designing new oligonucleotides.
During the first year, we focused on structural characterization of Liberibacter cells in the citrus phloem and elucidating plant responses against Liberibacter infection. In the second year, we will carry out in situ hybridization and immuno-microscopy research using molecular markers of Liberibacter strains as planned in the original proposal. We will also elaborate callose detection/phloem loading research to characterize citrus varieties with different HLB susceptibility. The second year research will complement our structural research of HLB, help understanding the HLB disease development process, and improve current microscopy tools that will be applied not only to HLB but also to the "Zebra Chip" disease, a Liberibacter-associated disease of potato.