Florida Citrus Advanced Technology Program

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

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2009-2010 REPORT	CATEGORY (drop-down)	TODAY'S DATE (m/d/yr)
● *Quarterly Report* ○ *Annual Report* ○ *Final*	HLB Pathology	7/9/09

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

Massive swelling of plasmodesmata-pore units in the sieve element-companion cell complex infected with Liberbacters.

TITLE and CONTACT INFORMATION

Proposal Title Correlative microscopic and molecular characterization of the microbiome in the citrus phloem tissue

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Organization University of Florida	*Total Direct Funds (current year)*

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

Objectives of our research proposal are 1) characterizing the microbial community of healthy and Liberibacter-infected citrus phloem tissue by serial-section electron microscopy; 2) localizing antigens and DNA sequences specific to Liberibacter in citrus phloem tissues by immunofluorescence microscopy, immuno-electron microscopy, and electron microscopic in situ hybridization.

During the first quarter of this year, we carried out transmission electron microscopy (TEM) analyses of periwinkle phloem tissues infected with Liberibacters by serial sectioning (Objective 1). Periwinkle leaf samples were first examined because periwinkles exhibit high Liberibacter titer, their leaf phloem architecture is simpler than that of citrus plant leaves, and they display starch accumulation symptoms when infected with Liberibacters as citrus trees do. The periwinkle samples were from Dr. Duan's lab (co-PI). The samples displayed disease symptoms and presence of Liberibacter was verified by PCR prior to TEM imaging.

Followings are the main findings. These findings are, however, still preliminary and require further investigation.

1) The plasmodesmata-pore units at the sieve element-companion cell interface are swollen.

In the phloem, long distance solute transport is mediated by the sieve tube that is made of continuous linear array of sieve elements. Sieve elements are live cells but they are devoid of most organelles including the nucleus. Their survival is dependent on companion cells that are associated with sieve elements and provide materials required for sieve elements. The primary connections between sieve elements and companion cells are modified plasmodesmata, termed plasmodesmata-pore units (PPUs) and they are thought to be the principal transport pathway into and out of the sieve element, important for phloem loading and unloading. This symplastic transport apparatus comprises a wide channel at the sieve element side that is linked to multiple desmotubules at the companion cell side. The PPU sites in the cell wall are often thickened to accommodate the branching twig-shaped plasmodesmata structure.

From our serial section analyses, we observed that PPU units from the sieve elements where Liberibacters inhabit are highly swollen due to insertion of non-filamentous substance around the PPU. The swelling occurs mostly in the companion cell side cell wall, widening the the companion cell side half cell wall 2~3 times thicker than the half cell wall on the sieve element side. We speculate that this alteration in the PPU structure could impede phloem loading as well as normal transport function of the phloem tissue. When compared with uninfected phloem samples and phloem samples infected with phytoplasma, the companion cell wall thickening at the PPUs was most severe in the Liberibacter-infected sieve elements.

2) Bacteria cells are seen exclusively in the sieve elements and two morphologically distinct bacteria cell types were identified.

The two groups of bacteria cells were differentiated by their diameters and staining properties. The first group consists of thick (diameters: 225±92 nm) and lightly stained bacteria cells. The second group consists of thin (diameters: 103±16 nm) and darkly stained cells. To determine whether these two groups correspond to different bacterial species, molecular characterization such as immunogold labeling with a Liberibacter-specific antibody or in situ hybridization need to be carried out (Objective 2). We have prepared samples embedded in LR white resin that is compatible with immunogold labeling. We are planning to try two Liberibacter specific antibodies from Dr. Duan.