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	12/21/09

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

Callose deposition and inhibition of phloem loading in the Liberibacter-infected citrus phloem cells

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)* $109,211

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

We have examined callose accumulation in the Liberibacter-infected citrus phloem tissues by epifluorescence microscopy since the last quarterly report. Although it is a low-resolution technique, epifluorescence microscopy has an advantage over transmission electron microscopy (TEM) in that it enables callose imaging across larger leaf areas, facilitating extensive and thorough callose measurement. In healthy leaves, no callose was detected in the phloem. In contrast, callose deposition in sieve pores and plasmodesmata pore units (PPUs) was observed in huanglongbin (HLB) symptomatic leaf phloem cells. It was shown that callose accumulates in the phloem cells of senescing citrus leaves (1), raising a question as to whether the callose deposition is a secondary phenomenon following leaf deterioration by Liberibacter infection. To address this question, we examined callose deposition in citrus leaves that are positive for Liberibacter infection (assayed by PCR) but display no HLB signs yet. Callose was detected in the sieve pores and PPUs of the asymptomatic leaves almost as much as those in the symptomatic leaves. This demonstrates that callose synthesis in the Liberibacter-infected phloem precedes development of HLP symptoms.

Callose deposition in the plasmodesmata is a natural plant defense reaction but it reduces solute transport efficiency through the plasmodesmata (2). This led us to hypothesize that the callose synthesis in the PPU accompanying Liberibacter infection may affect phloem loading in the citrus leaves. To test the possibility, we injected carboxyfluorescein into intercellular spaces of healthy leaves, Liberibacter-infected asymptomatic leaves, and Liberibacter-infected symptomatic leaves. When we imaged carboxyfluorescein at 24 hours after injection, the dye was seen to concentrate in the vein and spread through the vein in healthy leaves. However, dye remained at the injection site in the Liberibacter-infected leaves, indicating that phloem loading is inhibited in the infected leaves. The massive starch accumulation in the parenchyma cells of HLB symptomatic leaves suggests a blockage in photosynthetate export and inhibition in phloem loading of the infected leaves provides an explanation for the blockage. Our results also suggest that cell death in the HLB citrus trees is caused by inappropriate plant response against Liberibacter rather than damages actively done by the bacterial cells, which is consistent with the fact that this phloem-limited bacterium devastates the entire tree at a very low titer.

As stated in the second report, we have identified phloem cells containing bacterial cells in HLB symptomatic leaves. We examined their bacterial cell shapes by serial section 3D reconstruction. (An image from our 3D models is shown at http://news.ifas.ufl.edu/2009/12/09/uf-researchers-find-lone-culprit-behind-greening/) We found two morphologically distinct bacteria cells in periwinkle leaf phloem cells. (Please see the first progress report.) But all the bacterial cells in the citrus leaf phloem cells displayed uniform morphological features including diameter, length, cytoplamic staining and cell wall staining, and so on. This observation suggests that Liberibacter vastly outnumbers other bacteria, if there is any, in the HLB-infected citrus phloem tissue and agrees with a recent metagenomic sequencing result by (3).

1. M. J. Jaffe, R. Goren, Physiologia Plantarum 72, 329 (Feb, 1988).
2. Y. L. Ruan, S. M. Xu, R. White, R. T. Furbank, Plant Physiol 136, 4104 (Dec, 2004).
3. H. L. Tyler, L. F. W. Roesch, S. Gowda, W. O. Dawson, E. W. Triplett, MPMI 22, 1624 (Dec, 2009).