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	9/30/09

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

Callose accumulation in the companion cells and sieve elements in the Liberibacter-infected phloem tissue

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)

Since the last update, we focused on characterizing callose accumulation in the Liberibacter-infected phloem tissues. First, we localized callose in the infected phloem tissues by immunogold labeling. The main purpose of the experiment was to verify whether callose over-accumulation is responsible for the swelling of the plasmodesmata pore units (PPUs). We utilized a monoclonal callose-specific antibody from Dr. Bacic. This antibody has been the favorite callose antibody in the plant cell wall community during the last two decades thanks to its specificity and low background (Meikle et al., 1991). The swollen PPUs were intensely labeled by callose-specific immunogold particles indicating an excessive callose buildup. In addition to the PPUs, immunogold particles were associated with plasmodesmata between the sieve elements and with sieve pores. An interesting observation is that the inner surface of the sieve elements was covered with callose. In uninfected samples, callose was detected only in the plasmodesmata and sieve plates where callose is naturally deposited. However, numbers of associated gold particles were far smaller (less than 30%) than those in the infected samples. Callose accumulation was not seen in non-phloem cells such as mesophyll sponge cells and parenchyma cells in both infected and uninfected samples. These results indicate that sieve element cells synthesize callose in response to Liberibacter infection and the callose often swells plasmodesmata of the cells.

Recently it was reported that sieve plate pores are plugged by callose in Liberibacter infected sieve elements (Kim et al., 2009). The sieve pore plugging is the first wound reaction against phloem damages, so that plants do not lose their precious photosynthetate (Ehlers et al., 2000). We examined sieve plates from infected and uninfected mid-vein samples by scanning electron microscopy (Mullendore and Knoblauch., 2009). The advantage of this microscopy technique over TEM is that cell wall structures including sieve plates, plasmodesmata, and callose are seen in three-dimension, facilitating comparison sieve pore size and callose accumulation in the phloem. In the face-on views of sieve plates, diameters of sieve pores in the infected samples were smaller than those of uninfected sieve plate pores. However, we have not found any sieve pores that are completely blocked by callose in the infected phloem samples. The pore sizes in the infected sieve plates were still larger than the width of regular Liberibacter cells. These data suggest that callose synthesis in the sieve plates is not efficient enough for completely sequestering Liberibacters to their infection sites.

For the next quarter, we will investigate callose distribution by fluorescence microscopy to combine with what we have learned from high resolution electron microscopy imaging. We will also finish 3D reconstruction of Liberibacter cells and plasmodesmata deformed by callose from serial sections of citrus sieve element samples. (We have collected 3 serial sections from citrus sieve element cells.)

Ehlers, K., Knoblauch, M., and van Bel, A.J.E. (2000). Ultrastructural features of well-preserved and injured sieve elements: minute clamps keep the phloem transport conduits free for mass flow. Protoplasma 214, 80-92.

Kim, J.S., Sagaram, U.S., Burns, J.K., Li, J.L., and Wang, N. (2009). Response of sweet orange (Citrus sinensis) to 'Candidatus Liberibacter asiaticus' infection: microscopy and microarray analyses. Phytopathology 99, 50-57.

Meikle, P.J., Bonig, I., Hoogenraad, N.J., Clarke, A.E., and Stone, B.A. (1991). The Location of (1-]3)-Beta-Glucans in the Walls of Pollen Tubes of Nicotiana-Alata Using a (1-]3)-Beta-Glucan-Specific Monoclonal-Antibody. Planta 185, 1-8.

Mullendore, D. L. and Knoblauch, M. (2009). A new method to investigate the cell wall of living cells by high-resolution scanning electron microscopy. Annual Meeting of American Society of Plant Biologists Abstract number: P14001.