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	7/23/09

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

Liberibacter genome sequence confirmed in citrus and Liberibacter is the sole pathogen in infected phloem.

TITLE and CONTACT INFORMATION

Proposal Title Integrated approaches to discover pathogenesis-associated proteins form the causal agent of citrus greening disease and build new diagnostic tools.

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Email ewt@ufl.edu	*FDACS Contract Number* 163
Phone 352-392-5430	*Project Duration (years)* 3 *Year of Project* 1
Organization University of Florida, IFAS	*Total Direct Funds (current year)* $400,000

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

Below is the abstract of a paper recently accepted for publication in Molecular Plant-Microbe Interactions.

Title: "Confirmation of the sequence of "Candidatus Liberibacter asiaticus" in citrus and assessment of microbial diversity in Huanglongbing-infected citrus phloem using a PCR-independent, metagenomic approach".

Authors: Heather L. Tyler, Luiz F.W. Roesch, Siddarame Gowda, William O. Dawson, and Eric W. Triplett

The citrus disease, Huanglongbing (HLB), is highly destructive in many citrus-growing regions of the world. The putative causal agent of this disease, Candidatus Liberibacter asiaticus, is difficult to culture and Koch's postulates have not yet been fulfilled. As a result, efforts have focused on obtaining the genome sequence of Ca. L. asiaticus in order to give insight on the physiology of this organism. In this work, three next generation high-throughput sequencing platforms, 454, Solexa, and SOLiD, were used to obtain metagenomic DNA sequences from phloem tissue of Florida citrus trees infected with HLB. A culture independent, PCR-independent analysis of 16S rRNA sequences showed the only bacterium present within the phloem metagenome was Ca L. asiaticus. No viral or viroid sequences were identified within the metagenome. By reference assembly, the phloem metagenome contained sequences that provided 26-fold coverage of the Ca L. asiaticus contigs in GenBank. By the same approach, phloem metagenomic data yielded less than 0.2-fold coverage of five other alphaproteobacterial genomes. Thus, phloem metagenomic DNA provided a PCR-independent means of verifying the presence of Ca. L. asiaticus in infected tissue and strongly suggest no other disease agent was present in phloem. Analysis of these metagenomic data suggest that this approach has a detection limit of one Liberibacter cell for every 52 phloem cells. The phloem sample sequenced here is estimated to have contained 1.7 Liberibacter cells per phloem cell.

In addition to the manuscript abstract above, we have begun the PCMAT work described our proposal. We have extracted protein from infected and uninfected phloem tissue for the purpose of making antibodies. We expect to have the antibodies delivered to us in a few weeks. We are also training a new student in the nanosensor approach described in the proposal.