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	1/15/10

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

Primers amplify COI 850bp ; qPCR indicates titer fluctuation in colony psyllids; FISH optimization; SEM/TEM visualization

TITLE and CONTACT INFORMATION

Proposal Title Gross and fine structure localization of Liberibacter in citrus psyllid Diaphorina citri organs: elucidating the transmission pathway.

Principal Investigator Judith	*PI Last Name* Brown
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Phone 520-621-1402	*Project Duration (years)* 3 *Year of Project*
Organization The University of Arizona; University of Florida	*Total Direct Funds (current year)* 92,958 + sub 26,800

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

Obj. 1. Carry out DNA bar coding to establish the identity and diversity (if it exists) in south Florida (Stansly, Brown). PCR using mtCOI primers amplify a 850 bp fragment from colony psyllid adults; DNA sequencing confirmed. FL field collections from citrus and other spp. obtained from cooperator collections in previous years; 2010 spring and summer FL (and elsewhere) field collections are planned when psyllid dispersal is underway.

Obj. 2. Employ qPCR to detect Ca. Liberibacter presence (or absence) in the psyllid colony cohorts over different AAPs, for immatures and adults (Roberts, SWFREC, HLB diagnostics lab). The FL psyllid rearing system is fully functional and Ca. Liberibacter-infected and bacterium-free colonies are being maintained. Climatic factors affect growth of colony host plants (Citrus, Murraya) and seem to influence bacterial titer in plants and in the psyllid vector instars. Using qPCR (DNA isolation) we are monitoring over time the detectable bacterial titer in psyllid colonies and the host plant, toward quantitative detection in fresh flush plant tissue and in whole insect bodies. Obj. 3a. Carried out exploratory analysis of psyllid organs (guts) for HLB detection using qPCR (consulted methods of: Li et al. 2006 for plant material; Manjaunth et al. 2008 for psyllids).

Obj. 3. Using the colonies (analyzed above by qPCR), localize Ca. Liberibacter in whole psyllids and dissected organs to develop a gross anatomical road map of Ca. Liberibacter accumulation in organs, tissues, and cells (adult and 5th instar psyllids) (Cicero, Brown). [In our hands, D. citri does not render a signal when processed with the standard protocol proven for whiteflies (Gottlieb et al 2006)]. We have observed positive fluorescence by in-situ hybridization (FISH) with a Cy5-HLB 16S rDNA probe in variously prepared samples. We have observed a robust signal in infected adult psyllids cut in half to improve infiltration. These results represent extensive experimentation to develop and optimize fixation, dehydration, decolorization and hybridization procedures for this species. After replicated experimentation and further validation of this and several other probes, probes can then be applied to thick sections, which in turn can be compared to ultrathin TEM sections (gold probes) (Obj. 4) for precisely localizing interactions between HLB and the organs involved in transmission.

Obj. 4. Using the resultant thick section road map, elucidate at the TEM level, specific organs, tissues, and cells where Ca. Liberibacter accumulates (Cicero, Brown). Using TEM we observed bacterial cells on the outside of the esophagus and of the PSGs. By SEM, bacteria were observed on the outside and inside of the midgut epithelial cells. This suggests that Ca. Liberibacter bacterial colonies exist in multiple locations, perhaps adhering to the upper alimentary canal and midgut, and elsewhere. We also have observed by TEM groups of phage-like particles, intercellularly in Liberibacter-infected adult psyllids (unknown significance). We have compiled an anatomical, serial, ultrathin-section library of infected, 5th instar D. citri larvae. It is apparent that our focus must include both adult and late immature instars based on recent results of transmission studies. Therefore we are developing procedures for adult and immature instars to extirpate and SEM-stage digestive organs, followed by TEM. We also have carried out initial SEM exploration of extirpated guts from adults and 5th immature instars.