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	7/15/10

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

PCR amplification COI 1450 bp; PCR and qPCR detection of Liberibacter in adults, immatures, eggs; SEM/TEM

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)

DNA bar coding was initiated by developing and optimizing a pair of primers that amplify 1500 bp of the mitochondria COXI psyllid gene. A nested primer pair was designed to be specific to the Asian citrus COXI gene, and validated. FL field collections were obtained from cooperators that represent 2006 and onward. PCR amplification and sequencing of field collections is underway.

We optimized qPCR to detect Ca. Liberibacter presence (or absence) in the psyllid colony cohorts over different AAPs, for immatures and adults (Roberts, SWFREC; Brown lab UA). Ca. Liberibacter-infected Asian citrus psyllids are now routinely maintained at SWFREC, and the potato psyllid study system is in place in AZ to explore as a more tractable system for optimizing parameters under study in Obj 3, 4 e.g. localization and time course feeding for FISH, SEM/TEM.

Our FISH probes against Liberibacter support our earlier suggestion that the pathogen exists in multiple locations in the abdomen of D. citri. Distinguishing them from a potentially broader, extra-bacteriosome flora requires the bringing together of numerous visualization techniques. After fixing specimens in 2 different ways, coagulative and noncoagulative, 4µ paraffin through-sections of the same insect are mounted for three different techniques to give the best bouquet of results – ISH/FISH, haematoxylin/eosin and SEM. ISH indicates areas of infection, H&E helps with anatomical interpretation, and SEM shows gross bacterial morphology as well as any possible mix of flora. These techniques are compared with SEM of exposed organs and/or extirpated organs of all life cycle stages, including the pharate adult and the teneral. These results are compared with ISH using a 10nm colloidal gold-labeled probe and TEM. TEM allows for ultrastructure of labeled bacteria.

SEM of extirpated organs of infected potato psyllid has shown that one component of the flora multiplies inside and outside the gut of young adults to the extent that it overwhelms old adults and completely replaces epithelial cytoplasm. Such a scavenging species has not been found yet in uninfected psyllids. First views of the external salivary gland surface from these old adults do not show such tissue scavenging. The condition of the salivary gland internally is being investigated using SEM.

The oral box, 0.06mm3 in size, delimited by the tentoria and their arms, is an extremely complex conglomerate of esophageal, pharyngeal, salivary ducal and stylet tissues that converge to allow passage of ingested, egested and secreted materials. Because of the potential ability of bacteria to proliferate in the hemocoel, and burrow through epithelia, this box has been given considerable attention, along with other, well defined, models of transmission through the gut and salivary glands, taking the study of their organization well beyond classical groundwork laid down using other model Hemiptera.