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	4/15/10

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

bar-coding underway; qPCR verification of transmission and psyllid infection; bacteria observed in alimentary canal

TITLE and CONTACT INFORMATION

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

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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.DNA bar coding in progress using mtCOI primers to amplify a 780 bp fragment from psyllid field and colony collections from various U.S. states and off-shore locations. The purpose of this objective is to examine diversity within and between varied populations in relation to the associated Liberibacter spp. based on 16S rDNA. Over 50 collections are in hand.

Obj. 2. qPCR has been applied to detect Ca. Liberibacter presence (or absence) in the psyllid colony cohorts used for light and TEM studies, and by FISH (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 routinely. About 200 field-collected adult psyllids testing 10% PCR positive were placed into 2 more cages each with 6 ungrafted plants and 100 psyllids. Grafted plants were held in an air conditioned greenhouse at (28 °C 64% RH) and psyllid inoculated plants were held in an air conditioned insectary under similar conditions and 14 h light. All plants tested negative for HLB before they were infected with either method. After about one to one and a half month 85-90% grafted plants tested HLB positive based on PCR conducted on dark green mature leaves, whereas, plants exposed to infected psyllids still tested negative. When grafted plants were pruned to induce new shoots, 31-35% tested HLB positive by PCR. Two weeks later when PCR was conducted on leaves from young shoots 90-100% were positive. Four weeks later 97% tested positive by (dark green mature leaves). Two months after psyllid exposure to infected (PCR +) plants, 83% of the psyllid infested plants tested HLB positive by PCR of the dark green mature leaves. Adult psyllids collected on 8/12/2009 and tested at the SWFREC HLB lab were 61% and 67% HLB positive from budded and psyllid infected plants, respectively. Adult psyllids collected on 8/25/2009 and PCR tested at the USDA-ARS Riverside lab were 36% and 13% HLB positive from budded and psyllid infected plants, respectively.

Obj. 3. Because it can be reared year-round and because of quarantine constraints we have adopted Bactericera cockerelli as a surrogate species for Diaphorina citri. All three sections, V1,V2 & V3, of the B. cockerelli ventriculus support bacterial growth and replication that advances into huge colonies in the epithelia and on the outside surfaces as well. The esophagus and external hindgut harbor small numbers on their outside surfaces. Thus focus on transmission is now shifted to the oral region. The salivary glands apparently do not support massive, organ-destroying cultures, however, high titers of bacteria occur in the hemolymph surrounding them. Their role in the terminal stages of transmission is still under study. FISH technique, involving coagulant fixation, adopted from prior works by various authors, have proven unsatisfactory for this species. New techniques are under development, including the rendering of individual organs on slides, and the de-shelling of aldehyde fixed whole bodies for acceptable in-situ localization.

Obj. 4. We are investigating whether bacteria gain lateral access to the salivary canal without invading the glands. The most likely site would be near the precibarium, where the maxillary stylets converge (Ullman and McLean 1989) and glandular secretions are present. Studies are underway to determine the configuration of the oral area to aid in interpretations of liberibacter-anatomical TEM visualization.