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	10/15/09

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

D.citri from newly built quarantine houses processed at different ages/AAPs for qPCR, fluorescence and electron microscopy

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
Email jbrown@ag.arizona.edu	*FDACS Contract Number* 34
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)

Objective (2) Employ qPCR to detect Ca. Liberibacter presence (or absence) in the experimental adult psyllid cohorts given a range of acquisition-access periods as immatures or adults.
Objective (3) Employ qPCR to quantify Liberibacter titer in psyllids and dissected organs, and in subsamples of the 'titered' cohort, define the gross association of Ca. Liberibacter in thick sections by fluorescent in situ hybridization to develop a 'gross anatomical road map' of Ca. Liberibacter accumulation in key organs, tissues, and cells.

With the completion of our new quarantine greenhouse, we are now maintaining 12 colonies of infected psyllids, 8 on budded Citrus, and 4 on Citrus infected by psyllids collected in the field. These colonies allow for a baseline of high HLB titer when sampling all instars as needed.

New information from a recent publication (Inoue et al 2009) indicates that the best approach for determining organ-to-organ movement of C. Liberibacter in the host body is to work with 4th and 5th instars transplanted from noninfected plants to infected plants.

To accommodate this finding, single branches were selected with enough leaves to allow for removing some for qPCR while at the same time hosting adjacent, transplanted larvae. These larvae were confined to the branches in such a way as to maintain control and vigilance during their AAP. Cohorts were given AAP on post-flush leaves for 1 hr to 24 hrs, then processed for qPCR and both flourescence and electron microscopy.

Accompanying this approach, cohorts of 2nd, 3rd, 4th, 5th and adult instars were sampled directly off of different portions of infected plants and processed the same way. This series of samples will best represent actual proliferation profiles, since, in the field, larval mobility is negligible, and late instars are theoretically preinoculated from their early instar ontogeny. This inherited inoculum would build up to high titers along with the inoculum they imbibe themselves.

We expect that amidst such a broad series of confined, transplanted larvae, incipient infiltration of bacteria into the digestive system will be seen, and will allow us to determine the organ-to-organ, or organ section, sequence of proliferation. We expect that in samples coming directly off of infected plants, the bacteria will have had time to build up to high titers inside the host, and the routes bacteria took during proliferation will be obfuscated using the current FISH labeling system.

Twenty specimens of leaves and psyllids were sampled for qPCR from areas of the host plants from which the study specimen lots came. Eleven lots of psyllids were collected for microscopy, with each lot divided into two sublots, one for FISH, the other for TEM. The first TEM investigation of guts and glands of infected 5th instar larvae is in progress at this time.

Inoue, H., Ohnishi, J., Ito, T., Tomimura, K., Miyata, S., Iwanami, T. and Ashihara, W. 2009. Enhanced proliferation and efficient transmission of Candidatus Liberibacter asiaticus by adult Diaphorina citri after acquisition feeding in the nymphal stage. Annals of Applied Biology 155:29-36.