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	10/1/09

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

Antibodies for the HLB pathogen are on the way!

TITLE and CONTACT INFORMATION

Proposal Title Preparation of antibodies against Candidatus Liberibacter asiaticus

Principal Investigator John S. Hartung	*PI Last Name* Hartung
Email john.hartung@ars.usda.gov	*FDACS Contract Number* Hartung-js1-95
Phone 301-504-6374	*Project Duration (years)* 3 *Year of Project* 1
Organization USDA ARS	*Total Direct Funds (current year)* $221,900

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

Administrative arrangements for funding the research and recruitment activities for staff were completed in the previous reporting period. During this reporting period Dr. Yuan Qing arrived from China August 10th, and began to work on the project. Citrus psyllids were obtained from collaborators at the University of Florida and at Ft Detrick, MD. These were both known to be infected with Ca. Liberibacter and field collected insects. These insects were used to work out details of the extraction protocol to be used to inject mice with Ca. Liberibacter asiaticus obtained from citrus psyllids. Two methods of grinding the psyllids to obtain Ca. Liberibacter were compared: Liquid nitrogen with ceramic mortar and pestle with resuspension in buffer or plastic microfuge tubes with plastic pestle in extraction buffer. The latter method gave better results as measured by q-PCR. These results were improved by the addition of a small amount of sand to the extraction buffer, which resulted in a finer grind of the insects. We also compared low speed centrifugation to simply letting sediments settle out by sitting on the bench in ice on the yield of Liberibacter in these extracts. Allowing sediments to settle under gravity gave better results than centrifugation at only 100 RPM. Therefore the protocol to obtain Ca. Liberibacter asiaticus from infected psyllids will be: grind in extraction buffer with sand in microfuge tubes, followed by allowing sediments to settle under gravity.

Permits were obtained to use an animal care facility to maintain the mice needed for the immunization experiments. Mice were also obtained and injected to produce antibodies for this project. For our first experiment we used Xylella fastidiosa (CVC strain) instead of Ca. Liberibacter. The X. fastidiosa was added to psyllids and treated as above, then injected into mice. This will allow us to work out the complex process of antibody library creation and screening with a cultured, 'positive control', before we begin to work with the non cultured Ca. Liberibacter. Antibodies against the CVC strain of X. fastidiosa would constitute a 'bonus' from the grant. All of the reagents and primers needed to construct the antibody library have been obtained. Antibody libraries will be created early in the next reporting period. USDA ARS at Beltsville has also accepted from the contractor (9/25/09)a new Insect Quarantine Facility which we will use to raise psyllids for use in these experiments. Permit from APHIS to operate the facility is pending approval.

We also obtained cloned genes encoding the outer membrane protein (omp) of Ca. Liberibacter asiaticus from Duanping Yuan. The OMP protein has been over expressed in E. coli and has been purified. We will raise antibodies against it to use them to capture Ca. Liberibacter cells from psyllid extracts if our initial methods are not successful. Dr. Yuan also will receive antibodies against this protein from us when available.