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*	Psyllid	4/2/10

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

Successful transmission by psyllids following membrane uptake of cultured Liberibacter asiaticus

TITLE and CONTACT INFORMATION

Proposal Title Psyllid mediated completion of pathogenicity tests (Koch's postulates) with a pure culture of the associated Huanglongbing causal bacterium

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Phone 301-619-7307	*Project Duration (years)* 2 *Year of Project* 1
Organization Foreign Disease-Weed Science Research Unit	*Total Direct Funds (current year)* $175,000

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

The first year of a 2-year research proposal (FDACS Contract Number 58-1920-9-925 #40) has been completed. Our research objectives were: (1) Devise and perform alternative methods (microinjection and membrane uptake) to complete Koch's postulates using a pure culture of bacteria isolated and cultured in our laboratory and healthy psyllids as a transmission tool; and, (2) following successful inoculation or loading of the psyllids, we will complete Koch's postulates. The cultures of two strains of 'Candidatus Liberibacter asiaticus' (Las) from China (China1) and Taiwan (B239) were produced in our and a collaborator's laboratory and determined to be Las by PCR and sequencing. We prepared stretched parafilm membrane sachets containing sucrose solutions in 1XTE buffer in which the cultured bacteria were suspended. Sucrose solutions from 5-20% were tested, however, most experiments were done with 10% sucrose. The titer of the bacteria in sucrose was roughly assessed using real time PCR (Ct values ranged from 20 to 38). Membrane sachets were suspended over 15 ml or 50 ml conical tubes within which 15-20 healthy adult psyllids and 5th instar nymphs were allowed to probe the membranes. After 48 - 72 hr the feeding psyllids were transferred onto sweet orange seedlings and the remaining culture fluid in the sachets was assayed by real time PCR. Psyllids and nymphs were allowed to feed on the sweet orange seedlings for 14 days after which they were removed, and assayed for Las by real time PCR. From more than 30 membranes attempted, nine test membranes, each containing 250 µls of culture in 0.25 – 0.5 ml sucrose solution, were successful in uptake of bacterial culture by the psyllids. The orange seedlings were observed for symptom development and assayed three months post-inoculation by real time PCR. Although no symptoms were observed at 3 months, Madam Vinous sweet orange seedlings inoculated with infectious culture had Ct values ranging from 19.5 to 43.8. Typical HLB leaf symptoms were observed by six months. Test plants are being monitored for additional symptoms and plans are to re-isolate the bacteria from positive seedlings, assay for Las titer, sequence the bacteria to validate the strain originally inoculated, and grow the re-isolated bacteria in culture media. This would complete Koch's Postulates for Las as the causal agent of Huanglongbing.

A second approach used direct microinjection of the culture fluid into the hemolymph of adult and 5th instar nymphs of Diaphorina citri. Psyllids were immobilized with a low velocity stream of C02 and approximately 0.01µl of bacterial culture (Ct value of the bacterial culture suspensions ranged from 32.9 to 38.3) was injected into the abdomen of each adult psyllid. Microinjection of 5th instar nymphs was attempted but mortality was very high. Psyllids were allowed to recover and then placed on young sweet orange seedlings for recovery feeding for 14 days. Surviving psyllids were captured, assayed by real time PCR for the presence of Las, and plants were observed for 3 months for symptom development. While this does not serve as additional confirmation of Koch's postulates for Las, it does provide important insights into the interaction of Las with the vector. Continued refinement of the microinjection technique is underway.

We have developed a usable membrane uptake mechanism of transferring cultured bacteria of strains of Las. Our primary objective for year 1 was to complete Koch's postulates on a pure culture of Las and we are on the threshold of completing that objective. This work is not only demonstrating the causal relationship of Las to HLB, it is providing important insights about the nature of the pathogen-vector interaction.