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	8/13/10

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

Use of psyllids provides alternate means of proving pathogenicity of cultivated 'Ca. 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)* No cost extension

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

This is the final report of the proposal (FDACS Contract Number 58-1920-9-925 [40]). 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 cultivated 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. Short term cultivation of a strain of 'Candidatus Liberibacter asiaticus' (Las) from Taiwan (B239) was accomplished in our laboratory using two different published methods, and determined to be Las by conventional and real-time PCR and sequencing. We prepared stretched parafilm membrane sachets containing sucrose solutions in 1XTE buffer in which the cultivated bacteria were suspended. Sucrose solutions ranged from 5-20%, however, most experiments were conducted with 10% sucrose. The titer of the bacteria in sucrose was roughly assessed using realtime 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 acquisition feeding, psyllids were transferred onto sweet orange seedlings and the remaining 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.

A second approach used direct microinjection of the bacterial cultivation fluid into the hemolymph of adult Diaphorina citri. Psyllids were immobilized with a low velocity stream of C02 and approximately 0.01µl of bacterial culture (Ct value of the bacterial suspensions ranged from 32.9 to 38.3) was injected into the abdomen of each adult psyllid. Psyllids were allowed to recover and then placed on young sweet orange seedlings for inoculation feeding for 14 days. Surviving psyllids were captured, assayed by realtime PCR for the presence of Las, and plants were observed for 3 to 6 months for symptom development. Sweet orange plants inoculated in this manner also became infected, and showed symptoms typical of HLB.

Our primary objective was to complete Koch's postulates by transmitting a pure culture of Las using the psyllid vector. Although a stable pure culture was not attained, we did obtain short term cultivation of a fastidious bacterium which reacted to Liberibacter primers/probe in conventional and realtime PCR assays, produced positive infections of healthy sweet orange seedlings, and could be re-isolated. Our research helps to cement the causal relationship of Las to HLB and provides important insights into the nature of the pathogen-vector interaction.