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	7/1/10

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

Membrane Uptake and micro-injection provide alternate pathways for infecting psyllids

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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Email vern.damsteegt@ars.usda.gov	*FDACS Contract Number* 58-1920-9-925 40
Phone 301-619-7307	*Project Duration (years)* *Year of Project*
Organization Foreign Disease-Weed Science Research Unit	*Total Direct Funds (current year)* No cost extension

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) was successfully completed when the second year funds were terminated and a no cost extension was granted to complete loose ends. 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 would complete Koch's postulates. We have been able to cultivate a strain of 'Candidatus Liberibacter asiaticus' (Las) Taiwan (B239) in our laboratory using different media components and it has been shown to be Las by PCR and sequencing. We have followed the procedure previous described in our last quarterly report to transmit the bacteria produced in culture media or from infected dodder using stretched parafilm membrane sachets containing 10% sucrose solutions in 1X TE buffer in which the cultured bacteria were suspended. The titer of the bacteria in sucrose was roughly assessed using real time PCR (Ct values ranged from 20 to 38). Seedlings were grown at 25 C with natural daylight supplemented with HID lamps to extend the daylength to 18 hr. No symptoms were observed at 3 months, however, those positive by PCR at 3 months developed typical blotchy mottle symptoms by 6 months. We have repeated membrane inoculations with cultivated bacteria from infected dodder and citrus plants but will not be able to complete the final steps of Koch's postulates because of lack of funding.

A second approach used direct microinjection of the culture fluid into the hemolymph of adult psyllids. Psyllids were immobilized with a low velocity stream of C02 and approximately 0.01µl of bacterial culture was injected into the abdomen of each adult psyllid as described in the last quarterly. After 6 months, we have observed 2 of 7 sweet orange seedlings inoculated with injected bacteria exhibiting HLB symptoms. This has been repeated and we are waiting for results. 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.

We have been continuing to refine our membrane and micro-injection techniques to obtain additional confirmatory data on the ability of D. citri to transmit cultivatable (HLB causing) bacteria obtained from HLB infected sweet orange, dodder, or infectious psyllids.