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	1/13/10

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

Limited media cultivation of Liberibacter species being improved through phloem chemistry and genomic analysis

TITLE and CONTACT INFORMATION

Proposal Title Cultivation and Identification of the Causal Agent of Huanglongbing Disease of Citrus

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Organization FDWSRU NAA ARS USDA	*Total Direct Funds (current year)* 227,000.00

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

The main focus thus far has been to improve the current media to increase growth of the group of bacteria known as Candidatus Liberibacter. Our main focus for improving the growth has focused on understanding the phloem chemistry of citrus. The completed phloem and genomic metabolic analysis indicated several key growth factors to be tested. The nitrogen sources ethanolamine and micronutrient zinc have shown improve growth. Further improvement of the media is ongoing with quantification now possible using microscopy. With the improved media, we are also quantifying differences between different DNA extraction methods using phase contrast and light field microscopy.

In collaboration with Ft. Pierce, FL, the testing of Koch's postulates under field conditions has continued this quarter. Symptoms have not appeared, but are not expected for six to nine months (March-June). RT-PCR assays have not found Ca. L. asiaticus beyond the inoculation point and more tests are planned for the coming quarter.

The second focus has been sequencing the genomes of the three Liberibacter species from pure culture. In cooperation with the National Center for Genome Resources (NCGR), we used Illumina sequencing to sequence the strains used in the published pathogenicity trials (China1 Ca. L. asiaticus). The current genome is in the alignment and annotation. The second strain used in the pathogenicity trials (Brazil1 Ca. L. americanus) is also in the alignment and annotation phase at NCGR. Currently we are ensuring the quality and purity of the representative strain of Ca. L. africanus (Africa2) which will be shipped by the end of January.