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	10/8/09

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 USDA-ARS-NAA-FDWSRU	*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. A phloem analysis of sweet orange was completed by UC Davis MetaCore facility using GC/MS. Along with the genomic metabolic analysis of the available Ca. L. asiaticus genome, several key growth factors were indicated for further testing. Several nitrogen sources were tested as additions to the published media; ethanolamine has shown to improve initial growth. The seven micronutrients were tested with only the addition of zinc showing any improved growth. None of the amino acids or carbohydrates tested thus far have shown any improved growth, but several remain to be added to the media. Our current focus is to combine the different metabolites that show improved growth to optimize the media. In addition to these metabolites, sterilized fresh juice from uninfected plants was added to media in place of the citrus vein extract. We found that bacteria isolated from sour plants (lemons, limes) grew better on plates with sour juice compared to sweet juice and vice versa. From this information, we plan to do phloem analyses on multiple species and cultivars of citrus to identify commonalities and differences in the phloem chemistries.

In collaboration with Ft. Pierce, FL, the testing of Koch's postulates under field conditions began this quarter. A Florida-derived culture of Ca. L. asiaticus was initiated at Ft. Pierce last quarter. Inoculations using original isolations and transfers occurred this quarter. Symptoms are not expected for several months, but we will use RT-PCR assays to determine if Ca. L. asiaticus is present in the next 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 strain used in the published pathogenicity trials (China1 Ca. L. asiaticus). The current genome is in the alignment phase and we plan to release the sequence in the third quarter. The second strain used in the pathogenicity trials (Brazil1 Ca. L. americanus) is ready for shipment to the NCGR and sequencing will begin once the alignment of China1 is complete. Currently we are ensuring the quality and purity of the representative strain of Ca. L. africanus (Africa2).