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	6/30/09

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. Several methods of extracting the phloem, including micro-dissection of the phloem elements have been performed. Samples are being analyzed at the UC Davis MetaCore facility using GC/MS. A metabolic analysis of the available Ca. L. asiaticus genome was also performed to indicate which metabolic pathways are present and may be important for the growth of the bacteria. The analysis indicated that the bacteria had specialized transport mechanisms for the micronutrient zinc and the amino acid proline. A previous study of the xylem content (includes the phloem) of citrus indicated a preponderance of proline and the symptoms of HLB have been compared to zinc deficiency. Current formulations of the published media are focusing on optimizing the pH of the media, and the additions of zinc and proline. Media improvements will be on-going during the course of the project.

Our lab has successfully shown that the bacteria known as Ca. L. asiaticus, Ca. L. americanus and Ca. L. africanus are a causal agent of HLB by completing Koch's postulates. To replicate the experiment under field conditions and study symptoms in the flower and fruiting body, a study has started at Ft. Pierce, FL. Healthy plants are in place and a Florida-derived culture of Ca. L. asiaticus has been started at Ft. Pierce. Inoculations should occur 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, we will use Illumina sequencing to sequence the strains used in the published pathogenicity trials (China1 Ca. L. asiaticus and Brazil1 Ca. L. americanus) and a representative strain from Ca. L. africanus (Africa2). An agreement is now being processed. Currently we have grown enough bacteria to be used in the Illumina process for all three of these strains and are focused on ensuring quality and purity before sequencing. Barring any sequencing and alignment issues, all three genomes should be publicly available by the end of the year.