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*	Genetics	10/15/09

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

Production of Transgenic Commercial Cultivars Resistant to HLB and Canker

TITLE and CONTACT INFORMATION

Proposal Title Numerous citrus transgenics now express anti-microbial peptides. Decreased canker susceptibility is documented. HLB challenges are underway. In-vitro- tests show many AMPs with strong activity.

Principal Investigator Ed Stover	*PI Last Name* Stover
Email ed.stover@ars.usda.gov	*FDACS Contract Number* 221
Phone 772-462-5951	*Project Duration (years)* 3+ *Year of Project* 1st
Organization USDA/ARS/USHRL	*Total Direct Funds (current year)* $104, 325

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

The diseases Huanglongbing (HLB) and Citrus Bacterial Canker (CBC) present serious threats to the future success of citrus production in the US. Insertion of genes conferring resistance to these diseases or the HLB insect vector is a promising way to solve these problems. Transformation vectors, suitable for incorporating genes into citrus trees, have been prepared for five antimicrobial peptides (AMPs) with many promoters have been used to generate transformants of rootstock and scion genotypes. Thousands of putatively transformed shoots have been developed to produce citrus resistant to HLB and CBC or citrus psyllid. Many hundreds have been micrografted and some further propagated for replicated evaluation. D35S/D4E1 transformed rootstocks have been challenged with HLB and CBC. Initial trials on CBC resistance were inconclusive. HLB-inoculated transformed plants grew significantly better than controls but developed HLB symptoms. More active promoters are in the pipeline to achieve better results. Tests of garlic-lectin transformed citrus are underway to determine effect on psyllid feeding and development.

Efforts are underway to use Liberibacter sequence data to develop a transgenic solution for HLB-resistance, targeting a transmembrane transporter. Collaboration is underway with a USDA team in Albany, CA to provide constructs with enhanced promoter activity, minimal IP conflicts, and reduced regulatory and consumer concerns. Genes are also being identified from citrus genomic data to permit transformation and resistance using citrus-only sequences.

39 antimicrobial peptides (AMPs) have been assessed in-vitro for activity in suppressing growth of the bacteria causing CBC and two bacteria related to Liberibacter. In the initial studies, the synthetic AMPs D4E1 and D2A21 were among the most active, with minimum inhibitory concentrations at 1 µM or less across all test bacteria. An additional 20 synthetic AMPs were assessed, revealing several AMPs that were highly active against all test species, with negligible hemolytic activity. Transformation constructs will be prepared to produce citrus with these AMP transgenes, having completed an agreement with entities who posses the rights to these AMPs.

The tomato cultivar 'M82' was transformed with the AMP D4E1 and Garlic Lectin as a model system for more quickly assessing resistance than is possible using citrus. D4E1-transformed tomatoes were challenged by inoculations with Agrobacterium tumefaciens: no immune plants were identified, but some produced only very small galls and overall gall mass was 30% lower in D4E1-transformed vs. control 'M82'. Transformed plants have been propagated and D4E1-transformed vs. control plants will be challenged with Xanthomonas campestris pv. vesicatoria to assess resistance. Garlic-lectin-transformed tomatoes vs. control 'M-82' will be tested for effects on the phloem-feeding whitefly.

High throughput evaluation of HLB resistance will require the ability to efficiently assess resistance in numerous plants. Graft-inoculation, controlled psyllid-inoculation, and "natural" psyllid inoculation in the field are being compared. High-throughput CBC screening methods are being compared, with the hope that CBC-resistance will be correlated with HLB resistance in transgenics driven by constitutive promoters.

A material transfer agreement has been established with Texas A&M University to permit lab and greenhouse comparisons with the spinach defensin expressing grapefruit and 'Hamlin'. They also are providing the spinach defensin AMPs for in-vitro analysis. Since this material is well down the regulatory pathway, it makes no sense to move forward with any transformed citrus which is not markedly superior to this benchmark material.