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	7/15/10

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

More potent promoters used to drive antimicrobial transgenes

TITLE and CONTACT INFORMATION

Proposal Title Production of Transgenic Commercial Cultivars Resistant to HLB and Canker

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Phone 772-462-5951	*Project Duration (years)* 3+ *Year of Project* 2nd
Organization USDA/ARS/USHRL	*Total Direct Funds (current year)* $104, 325

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

Huanglongbing (HLB) and Citrus Bacterial Canker (CBC) present serious threats to the future success of citrus production in the US. Insertion of transgenes conferring resistance to these diseases or the HLB insect vector is a promising solution. Genes for antimicrobial peptides (AMPs) with diverse promoters have been used to generate transformants of rootstock and scion genotypes. More active promoters, derived from ubiquitin genes, have been identified and used in recent transformations. A wide series of promoters driving a reporter gene are being tested in transformed citrus and show very different levels of expression.

Liberibacter sequence data are being used to develop a transgenic solution for HLB-resistance, targeting a transmembrane transporter. Peptide has been made corresponding to the extra-membrane sequence and a phage display array system is being used to identify structures which are specific to this epitope, with tests against an E. coli containing the Liberibacter protein underway. When identified, transgenics will be constructed and challenged with Las. Collaboration with a USDA team in Albany, CA is providing constructs with enhanced promoter activity, minimal IP conflicts, and reduced regulatory and consumer concerns. Genes are being identified from citrus genomic data, from Carrizo citrange generated using USDA funds, to permit transformation and resistance using citrus-only sequences.

Antimicrobial peptides (AMPs) continue to be 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, along with the Tachyplesin (which is among the most effective AMPs in Dr. Dawson's CTV expression vector study), 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, and some of these were constructed using key functional elements from the horseshoe crab-derived Tachyplesin. Four new and very potent variants have been tested in the last few months. Transformation constructs will be prepared to produce citrus with these AMP transgenes.

Transgenes are being developed to suppress a lectin-like protein produced in the phloem of HLB-infected citrus. It is possible that suppression of this protein may significantly reduce disease symptoms.

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. After 1 year in the field, the first trial shows similar levels of infection across all three methods of Liberibacter transfer. The complete experiment is being repeated and planted in February 2010. the greenhouse complement to this study is showing earlier symptom development than field trees, especially from graft-inoculation.