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	4/15/10

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

Citrus transgenics expressing D4E1 AMP show greater growth than controls following Las infection.

TITLE and CONTACT INFORMATION

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

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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. Thousands of putatively transformed shoots have been developed to produce citrus resistant to HLB and CBC or citrus psyllid. Many hundreds have been micrografted. 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 displayed Las development and show HLB symptoms. More active promoters have been identified and used in recent transformation. A wide series of promoters driving a reporter gene are being tested in transformed citrus. Tests of garlic-lectin transformed citrus are underway to determine effect on psyllid feeding and development.

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 the final steps 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 to permit transformation and resistance using citrus-only sequences. Border sequences and promoters from citrus are now in a construct driving GUS and will soon be inserted into citrus.

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, 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. 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.

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. 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. Very sensitive methods have been derived and leaf extracts with and without spiked AMP have been tested.

A material transfer agreement has been established with Texas A&M University and we have received their 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.