Florida Citrus Advanced Technology Program

QUARTERLY & FINAL REPORTS: Control of Citrus Greening, Canker & Emerging Diseases of Citrus

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2009-2010 REPORT	CATEGORY (drop-down)	TODAY'S DATE (m/d/yr)
● Quarterly Report ○ Annual Report ○ Final	Management	October 23, 2010

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

Phloem specific promoters cloned from citrus drive phloem specific expression for sap-sucking insect RNAi

TITLE and CONTACT INFORMATION

Proposal Title MANAGEMENT OF PSYLLA IN TREE FRUIT CROPS, USING RNA INTERFERENCE

Principal Investigator Kerik D Cox	*PI Last Name* Cox
Email kdc33@cornell.edu	*FDACS Contract Number* NAS ID NUMBER 38
Phone 315-787-2401	*Project Duration (years)* 3 *Year of Project* 2
Organization Cornell University	*Total Direct Funds (current year)* $106,915.00

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

We proposed to identify and assess gene sequences for their negative effects on sap-sucking Hemipteran insects via RNAi using both in vitro and in planta dsRNA feeding assays. Objective 1 of our proposal intended to evaluate candidate genes for dsRNA-induced lethality of Diaphorina citri and our model organism, M yzus persicae, using artificial feeding assays. To date, we have cloned sequences at least 400 bp in length from nine homologous D. citri and M. persicae transcripts. In addition, we have carried out artificial feeding assays on M. persicae using dsRNA derived from the salivary gland-specific Coo2, midgut-specific glutathione-S-transferase S1 (GSTS1) and constitutively expressed S4e ribosomal protein from M. persicae, as well as a control derived from green fluorescent protein (GFP) sequence. While our results suggest that the M. persicae-specific dsRNA has a negative effect on both the lifespan of the insects and the number of offspring generated, additional independent assays are needed to enhance treatment replication to better resolve statistically significant differences among dsRNA treatments.

Since the annual report, we have nearly completed our evaluation of the RNAi strategy in planta for its effects against our model insect, M. persicae (objective 2). Since recent evidence suggests that RNAi in sap-sucking insects may operate more effectively in planta than in vitro, this approach may prove to be critical to the success of this study. This research requires the use of Gateway-based vectors that express the selected insect dsRNA either constitutively (35S promoter) or in a phloem-specific manner. We have cloned and characterized two SUS1 promoter alleles (CsSUS1-1 and 2) from Citrus sinensis cv. valencia and have found them to drive phloem-specific expression in both Arabidopsis and tobacco when fused to a reporter gene. Deletion analyses have shown that two separate phloem-specific enhancer regions (nucleotides -1153 to -462, and -410 to -268) exist in CsSUS1-1. Conversely, only the latter enhancer region promotes phloem-specific expression in CsSUS1-2, while the former region drives constitutive expression. We have also successfully generated Gateway vectors that will result in the constitutive (35S promoter) or phloem-specific (CsSUS1 promoter) expression, respectively, of M. persicae-specific Coo2, GSTS1 and S4e dsRNA, as well as a control derived from GFP.

We have also commenced objective 3: to transform citrus with RNAi-inducing transgenes against D. citri. Initially, 3• rapid amplification of cDNA ends was utilized to decipher additional nucleotide sequence from vacuolar ATP synthase subunit G, S4e and β-tubulin transcripts from D. citri. We are currently in the process of inserting sequences of the aforementioned transcripts into Gateway vectors downstream of both the constitutive 35S and phloem-specific CsSUS1 promoters. The resulting D. citri-specific gateway vectors will subsequently be transformed into citrus for subsequent feeding assays with D. citri. Initial attempts suggest that transformed Citrus sinensis cvs. Valencia and Hamlin can be regenerated.

In summary, we have cloned a number of transcripts from both D. citri and our model organism, M. persicae, and have analyzed a subset of derived dsRNAs to test their effect on M. persicae using in vitro assays (objective 1). We have also cloned and characterized several novel phloem-specific promoters from C. sinensis, and have evaluated their expression patterns. We have also developed new Gateway-derived vectors bearing a native citrus phloem-specific promoter, for use in RNAi and are evaluating them in planta (objective 2). Finally, we are in the process of generating similar vectors specifically designed against D. citri (objective 3), and preparing use them with our developing citrus transformation infrastructure.