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*	Management	10/19/09

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* 1
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 evaluate potential candidate genes for RNAi-induced lethality of sap-sucking Hemipteran insects using both in vitro and in planta dsRNA feeding assays. Since July 9th we've continued to make strides and have nearly completed Objective 1: the identification of target genes for oral delivery of RNAi-inducing dsRNA to Diaphorina citri and our model organism, Myzus persicae

To date, we have developed several RNAi constructs for candidate targets. These include constructs targeting six Hemipteran specific mRNAs for essential proteins (e.g. actin, α-tubulin, β-tubulin, L19e ribosomal protein, S4e ribosomal protein and ATP synthase) , and a construct targeting a vacuolar-ATPase subunit G , which is higly expressed in midgut tissues (encoding vacuolar-ATPase subunit G). We have also developed RNAi constructs for supplementary targets exhibiting preferential expression in the midgut (e.g. glutathione-S-transferase S1 and hexose transporter 1) and salivary glands (Coo2). These were developed to allow the examination of possible effects resulting from tissue-specificity of the dsRNA on RNAi efficiency. All resulting cloned sequences are in the process of being submitted to genbank. Since it has been shown that dsRNAs longer than 134bp have a high success rate we are continually working to create longer (400bp) dsRNAs for all of candidate targets. We are nearing the completion of our first objective as we have just established a working dsRNA feeding system for our sap sucking insect modelr, Myzus persicae, and will begin feeding assays with completed dsRNA constructs in November and December.

New scientific evidence has come to light suggesting that insect RNAi may only work effectively in planta, so we are proceeding with our in planta assay for RNAi induced resistance to sap-sucking insects using our Arabidopsis thaliana and Prunus domestica model systems (Objective 2). Previously, we mentioned making progress on cloning of phloem-specific promoters (sucrose synthase 1 and sucrose H+ symporter 2) from citrus. Indeed we have cloned SUS1 homologues from Citrus sinensis, which we have used to selectively localize transcripts (GUS) to phloem tissue in our Arabidopsis model system. This will allow us to ensure the localization of dsRNA to the feeding site of Hemipteran insect pests in any RNAi system we develop in the future. With this development we will begin to run in planta feeding assays (although slower) alongside the in vitro assay (comparably quick) allowing us to develop the ideal RNAi system more quickly.

In summary, we have successfully developed several RNAi candidate constructs for use D. citri (objective 1), and are beginning to evaluate those constructs on sap-sucking insects in vitro and in planta (objective 2) in a phloem specific expression system.