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*	Psyllid	3/4/10

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

TMV-based anti- psyllid cDNA sequences are available and RNAs can be delivered to psyllids

TITLE and CONTACT INFORMATION

Proposal Title Controlling HLB by controlling psyllids with RNA interference

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Organization University of California, Davis, CA 95616	*Total Direct Funds (current year)* $198,274

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

Our longterm goal is to identify and then use specific psyllid RNA sequences to induce RNA interference (RNAi) activity resulting in a negative phenotype (even death) in recipient psyllids. We envision that successful application of RNAi towards the Asian citrus psyllid (ACP, D. citri) will negatively impact their ability to colonize and/or reproduce on selected plants and thereby interfere with their ability to transmit C. Liberibacter spp., the causal agents of HLB. This strategy could then be an important component of HLB and/or ACP control. To make rapid progress we are using the tomato psyllid (Bactericerca cockerelli), which colonizes herbaceous plants (tomatoes, tobaccos) and transmits another Liberibacter spp. (C. L. psyllaurous; C. L. solanacearum) to several plant species. This psyllid:Liberibacter systems is very similar to the ACP:L. asiaticus complex associated with HLB.

During this year we have established high quality colonies of B. cockerelli and identified three solanaceous plant species (tomato, Nicotiana benthamiana, N. tabacum) for use in our experiments. These plants are good hosts for the virus we will use to deliver interfering RNAs to plants, Tobacco mosaic virus (TMV), and for B. cockerelli and C. L. solanacearum. Furthermore, C. solanacearum induces specific and characteristic symptoms in these plants. We are using all three plant species for our ongoing efforts and by using recombinant TMV we have shown that we can induce production of specific siRNAs corresponding to the recombinant sequence in these plant species. We can also detect the presence of the recombinant RNAs in psyllids after feeding on these plants.

We have utilized two complementary approaches to generate psyllid interfering RNAs for our work. The first has been by mining existing GenBank data for potentially useful D. citri sequences, and the second has been by constructing a new, normalized cDNA library for B. cockerelli mRNAs directly in the TMV-based virus delivery system. Both approaches have yielded a number of potential RNAi targets. We analyzed the D. citri EST database and identified 179 RNAs which are predicted to be expressed in D. citri midguts. Based on these analyses we cloned 80 midgut sequences (partial sequences varying in size between 500-1000bp) from D. citri. By BLAST comparative sequence analyses we next cloned 36 D. citri homologs from B. cockerelli. Sequence comparisons showed these to be highly homologous (80 – 90%) to corresponding D.citri sequences. RT-PCR analysis was used to assess RNA presence in psyllids and confirmed our predictions showing that 25 of these were abundant in B. cockerelli gut RNA extracts. We believe that gut RNAs are potentially useful targets, and their knockdown effects can be specifically monitored in gut tissues. Sequence-based analyses identified some, including ion transporter and ATPase sequences, which are ideal candidates for RNA interference. We have developed three approaches for delivering candidate interfering RNAs to psyllids including TMV-based expression, micro-injection of dsRNAs into the psyllid hemocoel, and artificial membrane feeding in vitro. We are presently using all three to determine their abilities to induce RNAi effects in B. cockerelli. RNAi effects are monitored by RNA knockdown (northern hybridization and qRT-PCR) and by phenotype in recipient psyllids.

We also generated and are presently analyzing a normalized B. cockerelli cDNA library. The library sequences were cloned directly into our TMV-based plasmid for expression in plants. Nucleotide sequence analysis has allowed us to confirm the quality of the library. Homologs corresponding to NADH dehyrogenase subunit, elongation factor Tu, glutathione peroxidase, peritrophin, alpha-keto reductase etc. have been identified within the library and these are being tested.