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	7/13/10

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

Evaulation of anti-psyllid interfering RNAs is underway

TITLE and CONTACT INFORMATION

Proposal Title Controlling HLB by controlling psyllids with RNA interference

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Phone 530-752-0302	*Project Duration (years)* 3 *Year of Project* 2
Organization University of California, Davis	*Total Direct Funds (current year)* $198,274.00

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

We are beginning the second year of our project. During this year we are focused on evaluating psyllid-derived sequences which can be used as double-stranded RNAs to yield RNA interference-induced negative effects (even death) in recipient psyllids. We are using the tomato/potato psyllid, Bactericerca cockerelli, and its solanaceous plant hosts as an herbaceous plant model system. This herbaceous system is much easier and faster to manipulate than is the perennial citrus system, but we believe our efforts will lead to subsequent application toward the asian citrus psyllid, Diaphorina citri. We have well over 100 candidate sequences identified from last years work, and several thousand more cloned and stored. We are now comparing specific sequences for RNAi activity via three delivery methods: direct injection into the psyllid hemocoel, in vitro acquisition via feeding through parafilm membranes, and by feeding on tomato plants infected with recombinant Tobacco mosaic virus (TMV) constructs containing the candidate sequences.

B. cockerelli midgut cDNAs, and those generated last year from the B. cockerelli normalized cDNA library, were engineered for use as templates to generate dsRNAs in vitro (via T7 RNA polymerase transcription) and for insertion into TMV. In vitro-generated dsRNAs were adjusted to specific concentrations and used for subsequent direct delivery experiments while TMV was used to express the sequences in whole plants. The in vitro-generated dsRNAs were tested by feeding psyllids through stretched parafilm membranes containing the candidate dsRNAs in a solution of 15% sucrose. B. cockerelli psyllids readily feed on this solution for up to 7 days, thus allowing adequate time for acquiring the test dsRNAs and assessing potential RNAi effects. We have demonstrated that fluorescent, Cy3-labelled dsRNAs were acquired by membrane feeding. We were able to visualize these in psyllid guts by using fluorescence microscopy after feeding. We also used qPCR to identify acquired dsRNAs and to quantify target mRNAs in psyllids. In some experiments dsRNAs evaluated in initial membrane feeding experiments caused mortality in psyllids. However, we are investigating now the quantitative effects of these treatments to ensure that phenotypic effects, including psyllid mortality, are due to the specific sequence and not due to overly abundant dsRNAs.

The dsRNAs also were evaluated via micro-manipulator driven intra-thoracic injection (200 nL/psyllid). Mortality is fairly high, ~50%, due to injection even when injecting buffer controls. Therefore, we are injecting large numbers of insects (at least 15 per treatment) and including buffer controls so as to have sufficient numbers for statistical analysis. We are using injection here only as a comparator, intra-thoracic injection is a standard means to induce RNAi effects in insects so it is a very good positive control for comparison against oral delivery via membrane feeding and TMV-infected plant acquisition.

The same psyllid sequences are being evaluated via psyllid feeding on recombinant TMV-infected tomato plants. Our data show that psyllids feeding on these plants acquire the specific test RNAs, as determined by RT-PCR and qRT-PCR. However, this approach is still a little problematic as TMV sometimes loses the inserted test RNA sequence during tomato plant infection, therefore all plants used for feeding experiments are carefully evaluated for inserted sequence retention. We are also assessing how much and what forms of RNAs are acquired during whole plant feeding.