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	10/14/09

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

Homologous sequence cloning and randomized cDNA library construction of the psyllid, Bactericerca cockerelli

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* 1
Organization University of California, Davis	*Total Direct Funds (current year)* $198,274.00

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

We are attempting to identify and then deliver to psyllids, RNAs capable of inducing RNA interference (RNAi) activity in recipient psyllids. Our goal is to use RNAi to confer a negative phenotype (even death) in psyllids, such that they cannot colonize and/or reproduce on selected plants. We believe that by controlling the psyllid vector this will aid other efforts to control HLB/citrus greening. We don't know which RNA sequences will prove to be the best for our effort, and we are using both directional cloning of specific sequences and a random cDNA library to identify effective sequences. In order to test and identify effective interfering RNAs, we are attempting to develop an efficient, high throughput screening approach that can be used with random and/or specific potential interfering RNA sequences.

We are using the tomato psyllid (Bactericerca cockerelli), which colonizes herbaceous plants and is the vector of another Liberibacter spp. (C. L. psyllaurous) in our studies. B. cockerelli readily feeds on and colonizes most tomato cultivars tested by us so far, and on potatoes. It also appears to transmit C. L. psyllaurous to these plants based on our PCR-based detection analyses. We also showed that pJL36 Tobacco mosaic virus-based expression vector can be used to deliver specific RNAs into the plant phloem, and that psyllids can acquire some of these RNAs by feeding on corresponding TMV-infected tomato plants. Moreover, by using recombinant TMV we can induce production of specific siRNAs corresponding to the recombinant sequence in tomatoes. Thus, we will use this approach to induce production of siRNAs corresponding to psyllid genes in plants and to evaluate candidate sequences for RNAi activity against the tomato psyllid.

We have already cloned sequences for eight psyllid genes. These are highly conserved insect gene sequences such as for actin, and we will use these in our initial experiments. Our initial sequences were obtained using primer sequences based on the Asian citrus psyllid (D.citri), but using B. cockerelli RNA as the template. We cloned these sequences into pGEM-T easy, and the dsRNAs for these sequences were synthesized. We are attempting to use microinjection to deliver these dsRNAs into psyllid nymphs to assess their abilities to induce RNAi effects. Initial efforts showed that the survival of psyllid nymphs after injection is low but acceptable. Further experiments are needed to improve injection and increase psyllid survival. Also, we are attempting to orally deliver dsRNAs to psyllids. We also have cloned eight gene sequences into pJL36 and have infiltrated tomato plants using Agrobacterium tumefaciens. We will place B. cockerelli psyllids on these plants and to determine we can induce specific RNAi effects in recipient psyllids.

We have also identified 1904 contig sequences from a D. citri EST database and identified 179 midgut sequences among these. Primers for 80 midgut contigs, which are immediate genes of interest for us, were synthesized and are being used to clone homologous sequences from B. cockerelli. Our hypothesis is that the midgut genes will be important to be targeted by orally-acquired siRNA or dsRNA. We have also taken efforts for an unbiased, high-throughput approach to evaluate and identify additional B. cockerelli target sequences. The normalized cDNA library construction is underway by Bio S&T inc. In this library, the random sequences from B. cockerelli will be directly cloned ino the pJL36 plasmid, and transformed into Agrobacterium tumefaciens GV3101. This will allow us to test them directly by tomato inoculation and psyllid feeding experiments.