CITRUS ADVANCED TECHNOLOGY PROGRAM

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

▶ INSTRUCTIONS

(●) *Quarterly Report* ( ) *Annual Report* ( ) *Final*

Required: What is the "headline" for this report (e.g. a one-sentence "newspaper headline" describing what you accomplished)

RNAi effects observed in psyllids

Proposal Title

Controlling HLB by controlling psyllids with RNA Interference

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
10/14/10	Citrus Research and Development Foundation	Vector Management

REPORT UPDATE (500 words; summarize your accomplishments)

We are attempting to develop an RNAi-based approach for controlling psyllids. Our goal is to identify and then use specific psyllid RNA sequences to induce RNA interference activity in recipient psyllids. To make rapid progress we are using the tomato psyllid (Bactericerca cockerelli), which colonizes herbaceous plants and is the vector of Liberibacter spp. (Ca. L. psyllaurous), which is believed to cause psyllid yellows in tomato.

Currently, sequence information of B. cockerelli is very limited in available databases. However, we were able to clone 28 gene fragments from B. cockerelli by using D. citri homologous primers from the D. citri midgut cDNA library database. We also generated a B. cockerelli normalized cDNA library and so far have sequenced less than 100 targets.

We have developed three approaches for delivering candidate RNAs to psyllids including a Tobacco mosaic virus (TMV)-based expression system and tomato plants; micro-injection of dsRNAs directly into the psyllid hemocoel; and artificial diet membrane feeding in vitro.

We have used our artificial diet system for screening candidate dsRNAs for B .cockerelli. Psyllid acquisition of the artificial diet was confirmed by food dye and Cy3-labelled dsRNA visualization. Both are obviously apparent in the psyllid gut tissues. When the dsRNAs for psyllid sequences were added into the food, we saw increased mortality of B. cockerelli for some sequences at higher concentrations of dsRNA. Four of ten sequences caused substantial psyllid mortality as compared with GFP dsRNA controls. Consistent and specific down-regulation of endogenous mRNA also was revealed by qPCR, and corresponding siRNAs were detected by Northern blot analysis. These latter two analyses demonstrate RNAi activity in psyllids.

Second, we are using a TMV-based plant expression system which is efficient for producing dsRNAs/siRNAs for insert psyllid sequences in tomato plants. So far, decreased survival of B .cockerelli was detected for 2 from 6 sequences after 10 day feeding on plants. We are in the process of analyzing psyllids for RNA hallmarks of RNAi activity.

Direct intrathoracic injection of dsRNAs into the psyllid hemocoel is also being used to test candidate sequences. Survival for injected psyllids ranges from 50 – 60% three days post injection. We are using injection only as a positive control for comparison with the other two approaches, and as an alternative approach to identify candidate dsRNAs for subsequent analysis by oral feeding.

In conclusion, we developed an applicable system for screening of psyllid sequences for RNAi effects, and efforts for transgenic plants are also underway. We have cloned the promising sequences into plant expression vector to produce dsRNA/SiRNA by transgenic approaches, and we will use these vectors to get transgenic tomato.

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Organization University of California, Davis

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SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.