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)

Anti-psyllid artificial miRNAs are made and being tested

Proposal Title

Transgenic RNAi-bases psyllid control

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
7/16/13	Citrus Research and Development Foundation	Vector Management

REPORT UPDATE (500 words; summarize your accomplishments)

We have constructed artificial mirco RNA interfering RNAs and are evaluating them as a strategy to deliver specific anti-psyllid RNAs to psyllid targets. Our belief is that these will be highly specific, and we know that miRNAs traffic in the phloem so should be available for phloem-feeding hemipterans such as psyllids. Seven amiRNAs targeting BC-ATPase were made and cloned into pBluescript SK, which are pAMIRA1, pAMIRA1c, pAMIRA2, pAMIRA2c, pAMIRA3, pAMIRA3c, and pAMIRA2PE. Four amiRNAs targeting GFP were made and cloned into pBluescript SK as well, which are pAMIRG1, pAMIRG1c, pAMIRG2, and pAMIRG2c. All 11 amiRNAs were then cloned into pGWB2, a gateway binary vector for expression driven by 35S promoter and into a viral vector with one extra stemloop from amiRA2 for amiRNAs of BC-ATPase and extra stemloops from amiRG1 and G2 for amiRNAs of GFP. The viral vector carrying amiRNAs were cloned into binary vector pCB301. We know that we can test RNA constructs on D. citri by in vitro feeding, but our hope is to also transiently express interfering RNAs in citrus such that they can move in the phloem and be acquired by feeding D. citri. We are utilizing a variety of approaches to test this possibility. So far we have performed agroinfiltration experiments on citrus and tobacco plants (the latter for the potato psyllid, Bactericera cockerelli). We used 35S promoter driven constructs and plant viruses in attempts to achieve gene expression. GFP assays failed to show detectable fluorescence in 5 different citrus types, but high level GFP expression was seen in tobacco.

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Contract Number 531

Project Duration (years) 3 *Year of Project* 1

Total Funds (current year) $139,859.00

SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.