CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD June 2015

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Determining the role of a novel virus in Citrus Blight

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
4/6/15	Citrus Research and Development Foundation	Pathogen/Disease Detection

REPORT UPDATE (500 words-It is not necessary in this public report to disclose your institution's proprietary information or intellectual property.)

Citrus blight continues to be a major economic problem in citrus groves in Florida. Thousands of trees each year succumb to citrus blight, with estimated losses at over $60 million per year. The disease can occur on all common citrus cultivars, and Carrizo citrange are especially susceptible. Early symptoms are zinc deficiency in the leaves which may disappear, zinc accumulation in the phloem and eventually high zinc levels in the xylem. Blockage of xylem tissues with amorphous plugs follows with reduced water uptake. The causal agent of citrus blight is unknown. However, symptoms and all of the characteristics associated with citrus blight can be reproduced by root graft inoculations. Therefore in a project previously funded by CRDF we used NGS RNA sequencing protocols to look for novel viruses in roots of sweet orange with blight, but not present in roots of healthy trees, or trees affected by HLB. We identified several related endogenous pararetroviruses related to Petunia Vein Clearing Virus (PVCV) using a collection of 10 RNA libraries prepared from 10 different root samples collected from healthy trees or those with blight or HLB.

In the quarter just ending we have built on this start by using the same ten RNA libraries to compare global gene expression in the roots of trees with blight or HLB as compared to the healthy controls. These analyses were performed using citrus genome coding sequences and mapping the short RNA reads with BowTie to the coding sequences. Each Blight library was compared to the healthy control library separately. The numbers of short RNA reads that mapped to each known citrus coding sequence were tabulated. The differences in expression were determined using Gfold, a program that can identify differentially expressed genes in such datasets without replications, by using the Poisson distribution. We have found large numbers of genes are differentially expressed in blight vs healthy roots. These include genes known to be involved in response of plants to both disease and stress. We also have identified proteins containing NAC domain-2 that are up regulated in roots with blight. These protein are active in the secondary strengthening of cell walls and when they are over expressed polyphenolic compounds accumulate in the lumen of xylem vessels. This could lead to the occlusion of the xylem vessels seen in blight.

We have designed primers for qPCR of these genes that appear to be differentially expressed to validate the differences by qPCR using each RNA library as true replicates.

PI First Name William

PI Last Name Schneider

Email william.schneider@ars.usda.gov

Phone 301-619-7312

Organization USDA-ARS FDWSRU

Project Number 921

Project Duration (years) 2 *Year of Project* 1

Total Funds (current year) $177,902.00