CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD December 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 focused our efforts on the remaining objectives: generating complete genome sequences for any and all active blight associated pararetroviruses and developing a active virus specific assay comprehensive enough to detect all blight associated pararetroviruses. Progress towards the generation of a complete genome sequence for the blight associated pararetroviruses was stymied by the discovery of a variable region in the genome. The previously developed primer walking strategy that was implemented to generate a full genome was insufficient to cross this variable region and additional approaches were needed to complete the 5' and 3' ends of the sequence. An adapted strategy was employed to generate higher levels of coverage in variable regions of the genome, and an inverted PCR strategy will be developed to complete the 5' and 3' ends. As was suspected, the sequence data clearly indicates that there are three different active citrus blight associated pararetroviruses with clear differences in sequence identity. The active viruses are more closely related to each other than they are to the non-active endogenous pararetroviruses, and genome sequence has been generated for roughly 90% of the genome at this point.

As we move into the next quarter, we hope to align the active pararetrovirus sequences with the non-active pararetrovirus sequences, to identify regions and PCR primers that will amplify from all active but no inactive pararetroviruses. These primers will then be tested on the 50 tree sample set to determine specificity, sensitivity and inclusivity as a usable blight assay.

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