# CITRUS ADVANCED TECHNOLOGY PROGRAM

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

**SELECT PERIOD** September 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 completed the correlation studies for the blight related pararetrovirus. For the correlation study leaves and roots were collected from over 50 trees from five geographically distinct locations. The majority of these trees were identified as being blight affected by water uptake testing, but putatively healthy trees were also sampled. In some cases, bark tissue from trunks was also collected for testing. RNA extractions were completed for all samples from all trees, and the presence of active pararetrovirus was assessed using the two primer sets selected in the optimization study from the previous quarter. Every tree that showed diminished water take up using the syringe injection test was positive for citrus blight associated pararetrovirus DNA. In the previous quarter the root samples were tests, and all but 1 tree tested positive for pararetroviral RNA. In this quarter those results were confirmed again using more stringent treatments to eliminate genomic DNA. In addition, leaf samples from all 50 trees (along with healthy controls) were tested for the presence of active pararetrovirus RNA. Active pararetrovirus RNA was found in all blight affected tree leaf samples and none of the healthy control samples. At this stage we consider that we have met the goals of this objective, and there are two important conclusions. First, there is a very strong correlation between the reduced water uptake via the syringe test and the presence of active citrus blight associated pararetrovirus. Secondly, the active pararetrovirus can be found in leaf samples, eliminating the need for laborious root sampling as part of blight surveys.

Our efforts now turn towards the remaining project objectives. Progress continues to be made towards generation of a complete genome sequence for the blight associated pararetrovirus. A primer walking strategy is being implemented to extend known sequences in both directions to generate a full genome. Sequencing efforts are being complicated by the need for complete removal of genomic DNA from the template RNA. In addition, the sequence data clearly indicates that there are more than one active citrus blight associated pararetrovirus. 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 50 % of the genome at this point.


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