CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD March 2015

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Optimizing heat treatment in the fields and understanding the molecular mechanism behind the success of thermotherapy for the control of citrus HLB

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
4/16/15	Citrus Research and Development Foundation	Unclassified

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

The objectives of this research are 1) to develop cost effective thermotherapy protocols for field application by optimizing temperature and relative humidity conditions in the tent; 2) to develop a mathematical model derived from our data and grower's data which will be used to determine the best treatment duration in future applications; and 3) to study gene expression of HLB-affected citrus plants that received heat treatment, and identify critical citrus genes that may be induced by heat stress for the benefit of suppressing HLB.

To reach our goals in objective 1, we have exposed both HLB-affected and healthy periwinkle (40°C) and citrus (42°C) to heat stress. DNA has been extracted and amplified for Las 16S rRNA and certain phage genes. A standard curve for a normalization gene has been established and data is being analyzed using the delta delta Ct method. Additionally, changes in expression levels for these genes are being monitored. As that Las cannot be cultured, a kill curve of Liberibacter crescens was determined. A dramatic decrease in viability was shown after L. crescens was exposed to 10 minutes of 46°C.

For objective 2, we have conducted a comparison study between field heat-treated and non-heat-treated citrus plants. There were 31 consistent up-regulated genes and 47 down-regulated genes in the citrus trees treated with heating. Additionally, potted Las-positive and negative citrus was exposed to 4 hours for 4 days of 40°C, 85 % relative humidity (similar to heat exposure in field setting) in a controlled greenhouse. RNA-Seq data was analyzed using DESeq2 with a FRD of 0.5 and fold change above 2. Using new tender flush as a sample, there were 3,722 differently expressed genes (DE) between Las-negative and positive trees not exposed to heat. Flush that appeared after heat treatment on the positive plants had 294 DE as compared to flush on unheated positive plants and 1308 DE as compare to flush on healthy trees. Some heat shock and oxidative proteins were identified in the DE lists. Analysis is ongoing.

As for the third objective, over 3 years of prior data (tree Las Ct values, treatment procedures, and temperature logs from one location) have been summarized and are currently being used to determine an algorithm that relates environmental conditions with decreases in Las titer. This initial data indicates that longer durations of temperatures greater than 41-42°C is more effective with reduction of Las titer. Although, leaving the tent on the tree longer than a week to achieve this duration does not appear to improve thermotherapy results. Eighteen months samples are being analyzed. Extensive analysis of temperature and humidity data is being analyzed to identify why results at plot 2 do not fit the trend usually observed. Despite increasing titers, trees continue to grow well. By April 17th, Valencia will be harvested from these three plots and juiced. Fruit drop (based off of two marked branches) will be determined. Brix, chemical analyses, and a taste panel are in the process.

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Total Funds (current year) $199,727.00

Form PR-12 Quarterly Reports **SUBMIT REPORT**

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.