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

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)
1/13/16	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) investigate the effect of heat stress on Las; 2) monitor healthy and HLB-affected citrus genome response to heat; and 3) optimize field thermotherapy.

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 (to study phage gene copy number) and RNA (to study gene expression) has been extracted. Changes in gene copy number for genes located in the phage region have been studied. Additionally, expression levels for Las genes were studied with RNA-seq and several genes of interested were identified. Reverse-transcription PCR has confirmed the results of the RNA-seq analysis and has suggested a possible mode of action behind the successful elimination of Las seen in thermotherapy.

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 were 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 (from new flush present before heat treatment and the new flush that developed after heat) were analyzed. 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. Additionally, potted Las-positive and negative citrus were exposed to 30 hours of 40°C, 85 % relative humidity and sampled during the exposure. RNA has been extracted from these samples. Time 0 before treatment and time 30 hours (of continuous heat) are being monitored for changes in gene expression for heat shock genes and other identified stress genes using RT-qPCR.

As for the objective 3, over 3 years of prior data (tree Las Ct values, treatment procedures, and temperature logs from one location) have been summarized. Extensive analysis of temperature and humidity data using KS nonparametric test, ANOVA, Tukey's HSD, and other measures have shown that each HLB-affected trees respond uniquely to heat treatment. The greatest decrease in Las titer and overall duration of this decrease varies for each tree (6-18 months) and is not solely dependent on heat but most likely affected by the biology of the tree. When comparing 7 versus 9 days of treatment, the longer treatment did not increase titer reduction. Also, six days was not more effective than a 4 day treatment. Regarding temperature, the greatest effect was present at 40, 41, and 42°C for 5 to 7 hours for 3 out of 4 test plots. A detailed correlation chart indicates other combinations of temperature and durations can also be effective at reducing Las. All statistical analyses shows that the response to heat stress is unique for each Las-infected tree. Although fruit drop did decrease for many of the treated trees, due to the large variation in data, the decrease was not statistically significant. Fruit was harvested and juice made. Volatile production analysis of juice from commercial grove 1 has been finished. A total of 63 aromatic volatile compounds were detected by HS-SPME-GC-MS. Discriminant analysis separated the "no heat" juice from the "heat" juice. Juice made from the product of the heat-treated trees had "fruity" and "pineapple fruit" top-notes. Juice is still being analyzed for sugar/acid, secondary metabolites, and Las titer. Juice quality taste panels are complete and panelists could distinguished between heat treated and no treatment juice sampled juice from the two commercial HLB positive Valencia groves ($p<0.05$). USDA Picos farm juice was not significantly different ($p=0.052$). Almost all panelists who differentiated between juice correctly said that heat-treated HLB juice was sweeter, less acidic, less bitter, and had more flavor and body than the juice produced from unheated trees.

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