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

Infrastructure Support for Research on Detection and Management of Huanglongbing and Asian Citrus Psyllid

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
3/30/15	California Citrus Research Board	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.)

This project provides the infrastructure to support six CRB-funded projects that investigate multiple methods of detection of Candidatus Liberibacter asiaticus (CLas), the causal agent of huanglongbing (HLB), in asymptomatic plants or in psyllids. Early detection of CLas in asymptomatic plants uses a systems approach that includes transcriptomics, proteomics, and metabolomics. Detection of CLas using digital drop PCR uses preserved psyllids and CLas-infected plant material from colonies maintained in the Contained Research Facility. Colonies of both CLas-negative and CLas-positive Asian citrus psyllids and a culture of CLas (Hacienda Heights strain) are maintained for use in the above studies.

The first set of studies on the early detection of CLas in asymptomatic plants using the systems approach was conducted using Washington navels, Lisbon lemons and Tango mandarins on Carrizo rootstock. The plants were assigned to one of the following treatments: Control-control (no manipulation); graft-control (grafted with clean scion with 3 grafts per plant); and HLB-graft (grafted with scion that tested positive by qPCR for CLas and grafted with 3 grafts per plant). The plants used to get CLas-infected plant material had the following Ct values: Washington navel – 30.17; Lisbon lemon – 24.00; and Tango mandarin – 27.39. The clean scion used for the graft-control all had Ct values of 40. The plants were grafted on the following days – February 25, 2014 – navel graft-control and mandarin graft-control; February 26, 2014 – lemon graft-control, and navel – HLB-graft; and February 27, 2104 – lemon HLB-graft, and mandarin HLB-graft. All plants were housed in the same greenhouse under the same environmental conditions (80°F, 16L: 8D). The plants were sampled regularly by each group, and samples for qPCR analysis were taken at approximately monthly intervals. The qPCR samples were taken such that they did not interfere with sampling by any of the research groups. The plant tissue was held in a -80 freezer until it was sent to the Citrus Research Board Laboratory for qPCR testing using USDA-APHIS standard methods.

The number of plants becoming CLas-positive and the time to the first CLas-positive reading by qPCR varied with cultivar. Of the 22 lemons graft-inoculated with CLas, 10 plants became CLas positive after 319 days. For the navels, 15 plants out of 19 inoculated plants became positive after 320 days. Of the 16 mandarins graft inoculated with CLas, only 2 had at least one positive Ct value, and 2 had suspect Ct values. However, the sample immediately following the positive or suspect value gave a negative value. A small number of these plants are still being held and sampled in an attempt to get consistent positive Ct values. The time to the first Ct value that indicated the plant was CLas positive was the shortest for the lemons, followed by the navels, and then the mandarins. The mean days to the first positive Ct value for lemons was 121.1 days (±17.424); for the navels, 160.93 days (± 14.36); and for the mandarins, 267 days (± 10.99).

A second set of experimental plants has been generated and include 25 Washington navels, 42 Lisbon lemons, and 31 Tango mandarins, all on Carrizo rootstock. These plants will be subjected to one of the following treatments: control-control (no manipulation); CLas-negative psyllid feeding; and CLas-positive psyllid feeding. The psyllids will be caged on the plants and allowed to feed for 10 days, after which the psyllids will be removed. All plants will be treated with insecticides to insure removal of the psyllids. The insect inoculation is expected to occur in April 2015. A third set of experimental plants have been started, and there are 236 Carrizo plants waiting to be grafted with the appropriate scion in September 2015.

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

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.