CALIFORNIA CITRUS NURSERY BOARD

Progress Report for 2010
California Citrus Nursery Board
Lee-09; Agreement # 58-5310-9-244

Project Year 2010 Anticipated Duration of Project 3 years

Progress Report for year 2 of a 3 year project (Final report for CY 2010)

Project Leader Richard Lee

Phone: 951-827-4399 FAX: 951-827-4398

E-Mail: Richard.Lee@ars.usda.gov

Location USDA ARS Germplasm, 1060 Martin Luther King Blvd, Riverside, CA 92507

Co-Project Leader Manjunath Keremane

Phone: 951-827-4399 FAX: 951-827-4398

E-Mail: Manjunath.Keremane@ars.usda.gov

Location USDA ARS Germplasm, 1060 Martin Luther King Blvd, Riverside, CA 92507

Project Title Development of reliable detection methods for Phytoplasmas from citrus and insect vectors for use in California nurseries

Objectives:

While we are preparing to combat the arrival of the psyllid in California, it is concerning to note that HLB symptoms in Brazil are associated with Phytoplasmas as well. In order to succeed in combating against emerging new disease, we believe that our efforts need to be directed at the detection of all associated organisms: Liberibacters associated with HLB, Phytoplasmas and stubborn. We are proposing to develop reliable diagnostic methods of Phytoplasma which may also be present in California and possible even associated with symptoms similar to those reported for HLB and to develop multiplex real time assays to permit concurrent detection of HLB and stubborn.

Specific objectives:

1. To conduct molecular characterization and determine the classification groups for Phytoplasma from California and isolates from Oman and Jamaica established in the *in planta* collection at the USDA ARS Exotic Disease of Citrus Quarantine facility, Beltsville, MD.

2. To develop real time PCR assays for detection of all Phytoplasma reported in citrus and additionally for specific Phytoplasmas identified from California. Develop real time assays so that they may be performed as a multiplex assay with assays for HLB, and stubborn, *Spiroplasma citri*.

3. Conduct a survey in Riverside and San Diego areas and in Lindcove area to determine if Phytoplasma occurs and if so, the incidence. Assays for HLB and *S citri* will be performed concurrently.

Progress:
Objective 1) Molecular characterization and classification of Phytoplasma
And
Objective 2) To develop real time PCR assays for detection of all Phytoplasma reported in citrus and additionally for specific Phytoplasmas identified from California.

Since July 2010 new DNA extractions have been made from the USDA ARS Exotic Disease Quarantine Collection, Beltsville, MD. These include several exotic phytoplasma from India, Jamaica, and Oman. From Brazil, we have received DNA extractions from the pigeon pea related phytoplasma which produces huanglongbing (HLB) like symptoms. From Florida, several phytoplasma-like symptoms have been recovered from citrus relatives, and DNA extractions have been made. In California, we have established at least five phytoplasmas from citrus, and in one case a Rhizobium-like bacterium which produces positive results when tested for HLB using the APHIS approved protocol and probes. The California phytoplasmas have been established in planta in the quarantine greenhouse. After the holidays, we will received sequence information for PCR products amplified from "universal" 16S rDNA primers from these DNA extractions. This sequence information will be used to design phytoplasma specific probes for use in real time and conventional PCR. The reaction components for both real time PCR for HLB and citrus stubborn are being optimized at the present time.

Objective 3) conduct surveys in Riverside, San Diego, and Lindcove areas utilizing the multiplex assays for phytoplasmas, stubborn, and HLB.

Additional surveys (about 2000 samples) have been conducted in the Riverside area, and DNA has been extracted and presently is stored in the ultracold freezer for later use for testing with the real time PCR assay for phytoplasmas, HLB, and citrus stubborn. At present we have over 4000 DNA extractions from surveys in Riverside, Orange, Los Angeles, San Diego, and Imperial counties ready for testing in the future.