CALIFORNIA CITRUS NURSERY BOARD

Progress Report for 2009
California Citrus Nursery Board
Lee-09; Agreement # 58-5310-9-244

Project Year 2009 Anticipated Duration of Project 3 years

Progress Report for year 1 of a 3 year project (Final report for CY 2009)

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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

From the USDA ARS Exotic Disease Quarantine Collection, the following DNA extracts using Qiagen DNeasy kits have been made:

Witches' broom disease of lime, from Oman
Phytoplasma from India, isolate 1
Phytoplasma from India, isolate 2 (probably a genetic variant rather than phytoplasma as this disease is graft perpetuated rather than graft transmitted, and no PCR products have been obtained.)
Phytoplasma from Jamaica that causes "galls" or bud proliferation, appears to be easily insect vectored in Jamaica
Phytoplasma from Jamaica that causes juvenility traits to be expressed in citrus (upright growth and no fruit set)

From Brazil, a cooperator is sending DNA extracts of the phytoplasma which causes HLB-like symptoms (see figure below).

From Florida, a Phytoplasma has been found in *Bergera koenigii* (curry leaf plant) and is being included in this study.

From California,
Four different Phytoplasmas have been established in planta at the Repository in the past two years, these have been extracted and used for PCR amplification. Two of these cultures induce witches' broom symptoms on sweet orange, and the other two induce bud proliferation symptoms.

From all of the above DNA extractions, the 16 S rRNA region has been amplified using conserved primers, cloned into the Topo 4 vector, and clones selected for sequencing. The sequencing is being done using 96 well format plates at the core facility, University of Florida. We have received the sequences from the first sequencing plate, and found sequences related from Pigeon Pea phytoplasma (similar to those reported from Brazil which produce huanglongbing symptoms on citrus). We have performed a second set of DNA extractions and are awaiting a second set of sequences from the UF core facility (which shuts down for 3 weeks over the holidays). Utilizing the sequence information already obtained, primers are being designed which will be ordered early in 2010. This coming year, emphasis will be on objective 2) Optimization of real time PCR assays and development of multiplex assays with stubborn and HLB.

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

While this activity was planned for year 3 of the project, the finding of the Asian citrus psyllid (ACP) in San Diego and Imperial Counties prompted our collection of trees planted in the Citrus Variety Collection (CVC) for analysis for HLB, and selected trees were tested for Phytoplasma using conventional PCR assays. Samples from over 2000 trees were collected from in the CVC and DNA extracted. Using real time PCR for HLB, no HLB was found although the internal control to monitor the quality of DNA from the host indicated the DNA quality was very good. Two trees were found in Block 18 with Phytoplasma, one a Citrus sp. and the other is a *Murraya paniculata*. The DNA extracts will be stored frozen for future analyses using real time PCR assays, once they are developed, and as a source of pre-ACP DNA from the collection.

Examples of some of the Phytoplasmas:

Figure 1 Bud proliferation phytoplasma from California.

Figure 2 Witches' broom disease of lime from Oman, from the Beltsville collection.

Figure 3 Phytoplasma from Jamaica that induces juvenility (on left) and from the Beltsville collection (on right).

Figure 4 Phytoplasma from Murraya (left) and on a citrus tree (right) from the Citrus Variety Collection, Riverside.