CITRUS ADVANCED TECHNOLOGY PROGRAM

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

▶ INSTRUCTIONS

○ *Quarterly Report* ○ *Annual Report* ◉ *Final*

Required: What is the "headline" for this report (e.g. a one-sentence "newspaper headline" describing what you accomplished)

Tissue blot diagnostic method for detection of citrus greening

Proposal Title

Development of sensitive non-radioactive and rapid tissue blot diagnostic method for large-scale detection of citrus greening pathogen

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
2/12/13	Citrus Research and Development Foundation	Pathogen/Disease Detection

REPORT UPDATE (500 words; summarize your accomplishments)

Substantial progress in the detection of Candidatus Liberibacter asiaticus (CLas) in citrus tissues and psyllid vector using non radioactive digoxigenin labeled probes during the tenure of this project. We developed and used both digoxigenin-labeled Plabeled PCR probes as well as RNA probes. We produced digoxigenin-labeled PCR products using specific primers for the OMP, RNA polymerase beta subunit, DNA polymerase region, the r-DNA region, and the 23S and 16S ribosomal RNA intergenic regions. Citrus plants from the green house infected with HLB pathogen were used as the source plants for obtaining the tissue for the blots and to isolate total nucleic acids template necessary for the amplification of CLas specific DNA. Primers were designed based on the CLas sequence information and amplified specific amplicons from the DNA isolated from HLB infected plants, and amplification was not observed from the healthy control plants grown under similar conditions. In the initial tissue blot experiments we did not observe hybridization signals specific for HLB. The reason probably is the low titer of pathogen and/or the non uniform distribution of the pathogen in the infected tissues. It is also possible that the PCR probes failed to detect low titer of the pathogen. Therefore, the amplified regions of Las were also cloned in the transcription vector, and digoxigenin labeled strand specific RNA probes were generated by transcription and the probes generated substantially improved detection of Las in citrus tissues. In addition to tissue blots of the stem sections, we have used the midrib and petiole region and whole leaf and branch containing multiple leaves from healthy and infected citrus on nylon membranes. The hybridization observed with the midrib imprints and whole leaves showed clear signals compared to stem imprints. In other experiments we used imprints of the leaf midrib, petiole and stem from new flushes of infected citrus since the psyllids preferentially feed and acquire the pathogen from such tissues. We also imprinted on the membrane the inside surface of the bark which contains phloem tissue in which the HLB pathogen is located. This procedure, was observed to be to be very useful to the determine the distribution of CLas in infected tissue.

The second area of our focus is on the detection of Las in psyllid vector by tissue blots (squash blot) on nylon membranes. The procedure for isolating the nucleic acid from single psyllid was optimized, and we have been able to amplify CLas specific amplicons from single infected psyllids using pairs of Las specific primers. Conditions of amplifications were optimized with different primer pairs and now we have been able to amplify HLB specific amplicons without non-specific bands in PCR. In initial studies of whole psyllid tissue blots, hybridization signal was also observed with healthy psyllids (psyllid colony from the healthy psyllid containment facility). However use of specific primer pair corresponding to the EFTU gene of CLas has been promising and we will use the probe generated for this gene in tissue blots of psyllids henceforth. Success of this work would benefit the citrus industry by providing simple, sensitive and rapid detection method for large-scale detection of HLB under field conditions. Tissue imprints, once on the membrane could be stored for later use, can be shipped easily and generally are stable. The modified form of tissue blot, the 'squash-blot', for psyllids developed will be useful to understand the epidemiology of HLB. Two manuscripts have been submitted and have come back for revisions (one in Molecular and Cellular Probes and the second in the Journal of Bioscience).

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Project Duration (years) 2 *Year of Project* 2

Total Funds (current year) $119,200.00

SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.