Florida Citrus Advanced Technology Program

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

Instructions Complete the fields based on your project specs. When finished, save the form to your local disk using a unique name. Then, go to **http://research.fcprac.com**, and log in with your user name and password using Researcher Login in the lower left. Find this project title and click on **Submit a Report**. Update your profile information if needed, then upload this report as directed.

2009-2010 REPORT	CATEGORY (drop-down)	TODAY'S DATE (m/d/yr)
○ *Quarterly Report* ◉ *Annual Report* ○ *Final*	HLB Pathology	5/18/10

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 (HLB).

TITLE and CONTACT INFORMATION

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

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REPORT UPDATE (650 words; provide details about your headline)

During the first year we have made progress in developing the non radioactive digexigenin labelled probes for the detaection of Candidatus Liberibacter asiaticus in citrus tissues and psyllid vector. We 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. initially dot blot hybridizations were carried out using the known amount of HLB specific DNA from the infected plants. A linear relationship was observed with amount of input DNA and the degree of hybridization. Citrus plants from the green house infected with HLB pathogen were used as the source plants for obtaining the needed tissue for the blots and to isolate nucleic acid 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 plants. In the initial tissue blot experiments we did not observe hybridization signals specific for HLB. We used healthy plants grown under similar conditions as controls. 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 were not of sufficient specific activity so as 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. However, use of the high specific activity RNA probes did not improve hybridization of the tissue blot and occasionally non specific hybridization was also observed in healthy tissue blots. This suggested less than optimal hybridization regimen. We have subsequently focused on optimizing the hybridization conditions and use of multiple probes in an effort to increase the extent of signal. These changes have 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 imprints from the leaves of infected citrus on nylon membranes. The hybridization observed with the midrib imprints showed much clear signals compared to stem imprints. In forthcoming experiments we will use 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 imprint on the membrane the inside surface of the bark which contains phloem tissue in which the HLB pathogen is located. Although this procedure is not optimized, we believe this to be very useful since the distribution of Las in infected tissue could be easily documented.

The second area of our focus is on the detection of Las in psyllid vector by tissue blots (squash blot) on nylon membranes. At the outset, a 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. The observed background was probably due to the extraneous tissues of the psyllids like wings that attach to the nylon membranes during psyllid squash and very hard to exclude during membrane wash. Therefore we intend to separate the head and the abdomen regions of the psyllids in tissue blots and exclude the wings region to reduce the background signal.