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	10/15/09

WHAT IS THE "HEADLINE" FOR THIS REPORT (e.g. a one-sentence "newspaper headline" describing what you accomplished)

Diagnostic method for the detection of citrus greening pathogen (HLB)

TITLE and CONTACT INFORMATION

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

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Organization CREC- IFAS, University of Florida	*Total Direct Funds (current year)* $ 41,040.00

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

Specific hybridization technology using non-radioactive digoxigenin-labeled probes specific for HLB pathogen to detect the onset of HLB infection and its progression in citrus trees in the field is being developed. As outlined in the previous quarterly report, several regions of the HLB pathogen were identified, based on the sequence information of the HLB pathogen and were used to design specific primer pairs for the detection of HLB in infected citrus trees and psyllids. The amplified products were highly specific for the HLB DNA from HLB infected citrus plants. Probes were designed to these regions. Digoxigenin labeled single stranded RNA probes and PCR based DNA probes were generated and used in dot blot hybridization against nucleic acids isolated from the HLB infected citrus and Psyllids carrying the HLB pathogen. A linear relationship of the signal was observed in the dot blots with increasing amounts of DNA used. However, in the tissue blots from HLB positive citrus plants (from the green house) the hybridizations are not consistent. Tissues blots from some sections were positive but some were not. This probably is because of the uneven distribution of Las that is normally observed with HLB infection and the low titer of the pathogen. We are currently optimizing the tissue blot hybridization using high specific activity probes and multiple probes if necessary. The procedure for isolating the nucleic acid from single psyllids were optimized, and we have been able to amplify Liberibacter specific amplicons from single Infected psyllids using pairs of Las specific primers. The primer pair designed based on the tufB region of the Las genome was observed to be very specific and gave consistent amplification from the psyllids. We plan to use this primer pair to generate non-radioactive probes for the detection of Las in psyllid populations under laboratory and field conditions.

Current work: Optimization of the citrus tissue blot hybridization on nylon membranes using Las specific non-radioactive DNA and RNA probes. Develop similar technology with psyllids.