



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)
<input checked="" type="radio"/> <i>Quarterly Report</i> <input type="radio"/> <i>Annual Report</i> <input type="radio"/> <i>Final</i>		HLB Pathology ▼	10/21/2009
WHAT IS THE "HEADLINE" FOR THIS REPORT (e.g. a one-sentence "newspaper headline" describing what you accomplished)			
one-step nested PCR works well for detection Liberibacter from plant tissue, but it needs be optimized for psyllid Lib. detection			
TITLE and CONTACT INFORMATION			
<i>Proposal Title</i> Diagnosis of Candidatus Liberibacter asiaticus in plant and vector based on molecular and serological approaches.			
<i>Principal Investigator</i> Helvécio Della Coletta-Filho		<i>PI Last Name</i> Coletta-Filho	
<i>Email</i> helvecio@centrodecitricultura.br		<i>FDACS Contract Number</i> 061	
<i>Phone</i> 55 19 35461399		<i>Project Duration (years)</i> 2 <i>Year of Project</i>	
<i>Organization</i> Centro de Citricultura		<i>Total Direct Funds (current year)</i> 32500,00	
REPORT UPDATE (650 words; provide details about your headline)			
<p>As proposed on the last report, our next step was to focus the development of an one-step nested PCR to minimized cross reactions that sometimes occur in two-step nested PCR assay. We designed two primers (LEFT_INTER - ACGGGTTATTTTTCTGAAGTCAAT; RIGHT_INTER -GATCATACTTAAACTTGTCGATATGC) that amplify a fragment of 406 bp. This pair was used together with another primers set (OMP1F-tgtaattcggcgtgaacttg and OMP1R-cacgcgacctataccctta) in the same reaction tube for amplified Liberibacter asiaticus (Clas) from plants tissues. Although the one-step nested PCR yielded good reactions, we are still optimizing the PCR conditions for samples obtained from psyllids. The non-amplification of Clas from psyllid samples by one-step nested PCR intrigued us by the fact that the samples were amplified by the two-step Nested PCR. Different primer concentrations, number of cycles and annealing temperature between the external and internal sets of primers are being tested for optimization of reactions.</p> <p>Also mentioned in the last report, we still had not received any funds for this project as a consequence of legal problems with Bank of Brazil. But at last September funds were finely made available and allowed us to hire and pay a student to work full time in the serological components of the project. This student is looking for other protein targets in addition to the OMP protein for antibody production. Besides heterologues expression to obtain proteins, we will send to companies the amino acids sequences of proteins for peptide synthesis. Within the nest 3 months we expect to have the target proteins expressed as well some peptide synthesized for the next steps of antibody production. At the same time the one-step nested PCR for Liberibacter amplification from psyllids will be in progress too.</p>			