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	11/13/09

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

Antibodies are now available to prepare disease-specific antibodies as a diagnostic for citrus greening disease.

TITLE and CONTACT INFORMATION

Proposal Title Integrative approaches to discover pathogenesis-associated proteins from the causal agent of citrus greening disease and build new diagnostic tools.

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Phone 352-392-5430	*Project Duration (years)* 3 *Year of Project* 1
Organization University of Florida	*Total Direct Funds (current year)* $400,000

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

We currently have in hand all IgYs that will be necessary to perform pCMAT. IgYs were raised in chickens against diseased plants at 3 months, 1 year and 2 years post-infection. The material from each time point was injected to two hens for IgY production. One of the two hens used for the 3 months time point unfortunately entered molting and had to be discarded. The last batch of antibodies was shipped to Oragenics on October 7th.

We have demonstrated by Western analysis that IgYs raised in hens are broadly immunogenic. To perform that assay, plant extracts obtained by sonication of healthy plants were separated by electrophoresis and transferred onto nitrocellulose filters. The filters were blocked and probed with the IgYs raised against individual hens. All seven hens had a strong reactivity against the healthy plant extracts using IgYs at a concentration of 1:5,000, and a secondary antibody (peroxydase-conjugated rabbit anti chicken IgY) at a concentration of 1:20,000. Several hundred bands were observed as well as a smear, which indicates a measurable reactivity against a large number of plant (and pathogen) proteins. We are currently developing an ELISA to further measure the exact titer of each hen.

In the absence of a convenient source of DNA or protein extracts from the infecting bacterial pathogen, we will perform all subsequent steps of IgY adsorbtion with healthy plant material. Plant fractions are currently being prepared for this step. We are currently optimizing the conditions necessary to bind healthy plant extracts to latex beads, which will be necessary for the specific adsorption of the IgYs. Adsorbed IgYs should be available by the end of the month.

We are also currently optimizing the conditions for proteomic identification of those proteins of the plant induced upon infection, and of the proteins of the pathogen expressed during the infection of the plant.

In summary, all material required to perform pCMAT are currently in hand and most quality control steps have been performed. As planned in the grant application, the next few month will be spent on optimizing the adsorption conditions and isolate in vivo induced proteins from diseased material using proteomics.

Our work on the etiology of citrus greening disease is now published:
Tyler, H.L., R.F.W. Roesch, S. Gowda, W.O. Dawson, and E.W. Triplett. 2009. "Candidatus Liberibacter asiaticus" and assessment of microbial diversity in Huanglongbing-infected citrus phloem using a metagenomic approach. Molecular Plant-Microbe Interactions 22:1624-1634.