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	January 16, 2010

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

Antibodies have been made that will allow for the discovery of proteins for early diagnosis of 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.

Principal Investigator Eric W. Triplett	*PI Last Name* Triplett
Email ewt@ufl.edu	*FDACS Contract Number* 163
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 continue to make progress in our goal to identify proteins that are specific to Liberibacter-infected citrus. These proteins, and the antibodies made against them, will be used in the development of a rapid diagnostic tool for the disease in the field.

We have previously reported that all IgYs that will be necessary to perform pCMAT are in hand. Briefly, 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.

It was demonstrated by Western analysis that the IgYs that were raised against diseased plants are broadly immunogenic. All pools of hen serum had a strong reactivity against plant extracts. Several hundred bands were observed as well as a smear, which indicates a broad and measurable reactivity against a large number of plant (and presumably pathogen) proteins.

The next phase of this study aimed at optimizing the steps of IgY adsorption using healthy plant material. This was to deplete the IgYs from those antibodies that are reactive with proteins constitutively expressed in healthy and in diseased plant tissues. This was performed using a proprietary method called PCMAT. Briefly, IgYs were adsorbed against insoluble fractions of homogenized and sonicated healthy plant tissues. To monitor the adsorption process, sequential absorption of pooled IgYs was performed using PCMATTM against extracts from healthy plant tissues. The IgYs that did not bind to healthy plant tissue were collected and purified, and used to probe cellular extracts in an ELISA format. Clearly, the adsorption process is not yet complete after 5 rounds of adsorptions with insoluble material, yet resulted in a significant decrease of reactivity against healthy plant extracts. We are currently optimizing the conditions necessary to bind soluble healthy plant extracts to solid matrices, which will be necessary for further absorb the IgYs; until the reactivity against healthy plant extracts reaches background levels. Thoroughly adsorbed IgYs which specifically retain their reactivity against extracts from diseased specimens should be available by the end of the month.

In summary, we are on schedule to perform the experiments described in the original application. The next few months will be spent on refining the adsorptions process with soluble plant extracts. The resulting IgYs will then be used as a probe to isolate proteins of citrus that are specifically induced during infection as well as gene products of the bacterial pathogen produced during that process. This will be performed by immunoaffinity and LC-MS/MS, as originally proposed.