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*	Other	7/1/09

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

Rapid Field Deployable Nucleic Acid Isolation

TITLE and CONTACT INFORMATION

Proposal Title Novel Immunocapture Technology for Field Deployable Nucleic Acid-Based Detection of Plant Pathogens

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Phone 505-424-1114	*Project Duration (years)* 2 *Year of Project* 2
Organization Mesa Tech International, Inc.	*Total Direct Funds (current year)* $180,000.00

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

The primary hypothesis guiding this project is that a highly simplified and field deployable nucleic acid sample preparation system can be realized using low cost and easily used lateral flow chromatography technologies. The proposed device will enable highly sensitive and accurate pathogen detection and identification under field conditions. Toward this end we are developing and testing lateral flow chromatographic immuno-capture methods, novel passive fluid exchange systems, and integration of these systems to form the basis of a field deployable diagnostic platform for citrus pathogen detection in trees and insect vectors.

In this period of performance, we have demonstrated methods for the affinity isolation of nucleic acids from crude plant tissue extracts using a proprietary thin layer chromatography method. These studies have shown: 1) that integration of our passive buffer exchange method with a nucleic acid (NA) affinity matrix enables washing and buffer replacement to attain NA in amplification compatible buffers, and 2) that the isolated NAs are suitable for polymerase chain reaction and isothermal amplification of target sequences. Taken together with our prior work demonstrating immuno-affinity capture of pathogens for enrichment from complex sample matrices, these more recent findings suggest that integration of immuno-affinity enrichment with a subsequent bulk nucleic acid isolation will allow efficient sample processing under field conditions with minimal user intervention. Additionally, the ability to directly isolate NA without reliance upon immuno-affinity enrichment ligands will enable the use of the test device for the detection of pathogens for which immuno-affinity reagents are unavailable.

Concomitant with the development and optimization of nucleic acid isolation methods, we are developing approaches to the integration of sample preparation, isothermal amplification and detection that are consistent with the realization of a low cost, easily used field deployable test device. Toward this end, we have initiated an engineering effort to prototype and test varying integration approaches. These efforts are now focused on two integration approaches that will be subjected to laboratory testing and comparative analysis. As these efforts mature, we anticipate the development of a first generation prototype that will allow NA isolation and amplification to take place without user intervention following the initial introduction of crude sample.