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*	Management	8/1/10

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

Integrated Prototype Development for a Rapid Molecular Diagnostic Device

TITLE and CONTACT INFORMATION

Proposal Title An Integrated Low Cost Nucleic Acid Analysis Platform for the Rapid Detection of Plant Pathogens

Principal Investigator R. Bruce Cary, Ph.D.	*PI Last Name* Cary
Email rbcary@mesabiotech.com	*FDACS Contract Number* 5300-141
Phone	*Project Duration (years)* 3 *Year of Project* 1
Organization Mesa Tech International, Inc.	*Total Direct Funds (current year)* $153,802

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

Mesa Tech International (MTI) has developed low cost heating systems employing microcontroller-based temperature control. Circuit boards supporting heating elements and microcontroller interfaces have been designed, fabricated and tested. These systems have been found to support isothermal amplification in low cost plastic housings.

Sample preparation methods based on novel passive buffer exchange technologies developed at Los Alamos National Laboratory and refined for field deployment and application to crude citrus tissue extracts at MTI, provide an effective means of isolating amplification ready nucleic acids without complex user interventions or external instrumentation. The integration of this sample preparation technology with amplification and detection sub-systems will result in an easily used molecular diagnostic device suitable for use under field conditions with no need for additional specialized equipment. In this period, MTI has developed an integration strategy for incorporating passive buffer exchange-based sample preparation with isothermal amplification and lateral flow detection. Development of the strategy has prioritized methods that can be realized without moving parts such as valves or pumps, yet accomplish the required fluidic manipulations to transport amplification reactions from a temperature controlled chamber to a lateral flow detection strip. In addition to eliminating any need for automated moving parts, the strategy employs low cost components that are suitable for incorporation into a device designed for disposal after a single use. Prototypes are fabricated from laser cut sheet plastic using prototyping equipment available in-house at MTI, reducing prototype development costs and turn around times. Initial test systems have been fabricated to experimentally determine physical design parameters and to allow testing of material properties. These breadboard integration test platforms are now being refined to incorporate circuit boards supporting both temperature control and fluidic control mechanisms.

To assure designs are relevant to applications most needed in the citrus community, MTI's efforts are focused on the development of a functional first generation prototype that will target the etiologic agent of citrus greening or HLB (Candidatus Liberibacter). Toward this goal we are currently assessing assay chemistries for their utility in the detection of C. Liberibacter nucleic acids both from laboratory purified material and from infected plant tissue processed with MTI's passive buffer exchange sample preparation system. A small number of greenhouse infected tree samples were processed using established RNA purification methods in Norm Schaad's laboratory at the USDA Fort Detrick facility. These RNA preparations were assayed at MTI using MTI designed primers and probes targeting sequences identified by Schaad's group as useful in the detection of C. Liberibacter. These initial studies demonstrated that MTI's primer sets were capable of amplifying C. Liberibacter derived targets and detecting the resulting amplicons on lateral flow detection strips. More recently, a larger number of citrus nucleic acid samples were obtained from Mike Irey (United States Sugar Corporation). These samples consisted of 20 field samples collected in Florida and found by Irey's team to be positive for HLB by real-time PCR, 10 greenhouse samples positive for HLB and 20 control samples found to test negative in Irey's laboratory. We have conducted an initial screening of these samples with a DNA-based assay and have found near perfect agreement between our assay results and those anticipated on the basis of real-time PCR tests conducted in Irey's facility. One sample negative by real-time PCR exhibited borderline positive test results in our assay.