TITLE: Rapid Identification of Soybean Fungi by Spectroscopic Techniques

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PROGRESS/ACTIVITY

The current available methods for detection of fungi are time consuming and not always very specific. Therefore, our research focused on rapid detection methods.

Fourier transform infrared (FT-IR) microscopy has proved to be a reliable and sensitive method for detection of molecular changes in cells. Fungi pathogens display typical infrared spectra that differ from the spectra of substrate material such as soybean. Our research team has generated spectral libraries that can be used by technicians to identify the types of fungi. Now this technology needs to be applied to field samples using a portable handheld FT-IR so the generated data can be effectively used to manage crop areas and losses.

Our primary objective was to evaluate the feasibility of using mobile Fourier Transform Infrared Spectroscopy (FT-IR) as an onsite field diagnostic tool for determining the presence of phytopathogenic fungi that cause severe economic damage to soybeans.

OBJECTIVE(S): Using an interdisciplinary approach and in keeping with our original time line put forward last, year we plan to address the goals outlined in year two of our three-year study. Stage 1. Soybean Cultivation and Infection. Stage 2. MALDI-TOF. Stage 3. Microscope FT-IR. Stage 4. Chemometrics.

GRADUATE STUDENT SUPPORT: This funding helped to support 3 different graduate students:

Curtis Atkinson, MS Biochemistry (MS Defended August, Degree Conferred. December 2013); Karl Bell, MS Biochemistry (In Progress); Candace Williams, PhD Molecular Biology (In Progress).
UNDERGRADUATE STUDENT SUPPORT:
Olivia Crow, Emily Davis (Drake), Jeffery Robins, Will Ford

GRADUATE STUDENT AWARDS:

Asilomar Research Student Travel Grants: Curtis Atkinson received travel grants for exceptional research.

WORKSHOP:

MSU-Agilent FT-IR workshop. Working with Agilent technologies, Darrell Sparks and I hosted a mobile FT-IR workshop. (MULTIDISCIPLINE AGILENT FTIR WORKSHOP. Clay Lyle RM 110, Mississippi State University, November 31, 2012, Attendance-22.) This workshop brought together researchers and field agents to discuss and showcase the ability of this instrument to detect fungal strains in the field.

PUBLICATIONS:


ABSTRACTS AND PRESENTATIONS:


Emily Davis, Curtis Atkinson, Ashli Brown, and Darrell Sparks (2013) Developing NALDI-TOF MS for Aflatoxin Screening of Corn. AOAC Southern Section Annual Meeting, Atlanta, GA. (April 22-23).


LEVERAGING:

January 2013: Mississippi Corn Promotion Board. DECREASING THE IMPACT OF AFLATOXIN ON CORN AND DISTILLERS GRAINS ($41,980 for 1yr) PI

January 2012: Research Initiation Program. DEVELOPMENT OF A RAPID AND ADVANCED NADLI-MS METHOD FOR MYCOTOXIN ANALYSIS ($42,200 for 1yr) PI

January 2012: Mississippi Corn Promotion Board. DECREASING THE IMPACT OF AFLATOXIN ON CORN AND DISTILLERS GRAINS ($59,758 for 1yr) PI

September 2012: FDA. MISSISSIPPI STATE CHEMICAL LABORATORY FOR MFRPS LAB ACCREDITATION. ($1,447,102 for 5yrs) PI

November 2012: Agilent Technologies-Instrument Partnership. MONITORING FUNGAL CONTAMINATION. (FT-IR $50,000) PI

December 2012: Texas Corn Producers Board/USDA ARS. RAPID DETECTION OF ASPERGILLUS FLAVUS IN CORN. ($35,000 for 1yr) Co-PI

December 2012: Special Research Initiative. RAPID FIELD DETECTION OF PHYTOPATHOGENIC FUNGI IN AGRICULTURAL AND BIOFUEL CROPS. ($43,200 for 1yr) Co-PI

March 2011: Agilent Technologies-Instrument Partnership. MONITORING MYCOTOXIN CONTAMINATION IN MISSISSIPPI. (LC-QQQ $500,000) PI

August 2011: AMCOE Aflatoxin Program. IDENTIFICATION OF GENE-BASED MARKERS FOR RESISTANCE TO AFLATOXIN ACCUMULATION IN CORN BY EXAMINATION OF THE PLANT/PATHOGEN/ENVIRONMENT INTERACTIONS. ($180,000 for 2yrs) PI
Stage 1. Soybean Cultivation and Infection.

Small, black microsclerotia present in soybean root tissue after inoculation with Macrophomina phaseolina.
**Stage 2.** MALDI-TOF. Classification and identification of microorganisms was achieved with fast high-throughput using MALDI-TOF mass spectrometry and FT-IR.

MALDI-TOF Spectrum of *T. basicola*  
MALDI-TOF spectrum of *M. phaseolina*

MADLI data has been used to generate 3D scatter plots of the first three PCA scores for the two individual fungal species. This multivariate analysis of *T. basicola* and *M. phaseolina* is shown below.
Stage 3. FT-IR. Classification and identification of microorganisms was achieved with fast high-throughput using MALDI-TOF mass spectrometry and FT-IR. Corn and soybeans were artificially infected.

![Cluster Analysis](image1.png)  ![Mid-IR Spectra](image2.png)

Stage 4. Chemometrics.
