



Mississippi Corn Promotion Board 2012 Progress Report

Project Title: Decreasing the Impact of Aflatoxin on Corn and Distillers Grains

PI: Ashli Brown

Department: Biochemistry, Molecular Biology, Entomology, and Plant Pathology

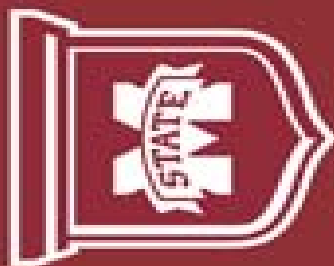
Project Summary (Issue/Response)

The occurrence of aflatoxin results in both an economic burden to agricultural stakeholders and an increased health risk to consumers. The information derived from the use of novel tools such as genomics, proteomics, and metabolomics will provide us with the best and the quickest opportunity to achieve a clear understanding of the survival of toxigenic fungi in the field, the ability of the fungus to invade crops, and the process of toxin contamination under various environmental conditions. Maize hybrids with varying aflatoxin resistance were inoculated with either atoxigenic or toxigenic strains of *A. flavus* or combinations of the two, in test plots in Mississippi. Using qRT-PCR and strain specific molecular marker we have begun to determine the *A. flavus* biomass. The volatile metabolome and other small non-protein molecules of maize in response to *A. flavus* infection and aflatoxin accumulation are currently being profiled with SPME and liquid extraction coupled to GC/MS and GC-QTOF technologies. Inoculated kernels, adjacent kernels, uninoculated kernels, and cob sections have been collected at various times after inoculation for several analyses: RNAseq technology is being used to examine the gene response of different germplasm lines to both toxin producing and non-toxin producing *A. flavus* strains and to identify genes that are integral to the host/pathogen interaction. Aflatoxin is being quantified by LC/MS-MS and cob samples are being analyzed via qRT-PCR for the expression of resistance related genes. To effectively manage corn and other crops, early detection and identification of plant pathogens is crucial. Specifically, the use of infrared spectroscopy is being evaluated as a means to detect the presence of toxigenic (aflatoxin-producing) strains of fungi in cornfields. Fall Army Worms (*Spodoptera frugiperda*) are a common pest that farmers across the southeast battle each year. Our earlier research found that the inbred variety of corn (Mp708) was resistant to infestation and consumption by Fall Army Worms, but the methods of this resistance were not fully understood. Analysis of the resistance conveyed by the Mp708 revealed a cocktail of volatiles being produced and given off by the plant that repelled the worms.

Project Results/Outcomes

We identified, cataloged and functionally classified proteins from a whole cell mycelial extract of *A. flavus* NRRL 3357 using 2-DE and MALDI TOF/TOF. We also established the first *A. flavus* 2-D proteome reference map. The description of *A. flavus* proteome provides a deeper insight into its basic biology and a basis for future proteomic investigations. We have shown that infrared spectroscopy has the capability to successfully differentiate *Aspergilli* at the species and strain level. In our preliminary studies, we have successfully differentiated two species of *Aspergillus* and separated toxigenic from non-toxigenic strains of *Aspergilli*. This differentiation could prove

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Project Results

valuable to plant pathologists in the monitoring of crops for toxigenic and non-toxigenic strains of *Aspergilli*, especially those crops treated with a competitive, non-toxigenic strain (such as Afla-Guard[®]) to insure that crop safety is being maintained. We have investigated the wound-response pathway of FAW resistant maize lines. One of these volatiles involved in this pathway, (E)- β -caryophyllene, was also determined to be the most potent. Trials conducted using this volatile showed a great repelling effect of Fall Army Worms away from susceptible corn (TX601) treated with (E)- β -caryophyllene. Other, less prominent volatiles are also present in the mixture given off by the Mp708 that is responsible for resistance. Two compounds in particular are the subject of this research, methyl jasmonate and jasmone. Resistance studies were conducted with these compounds individually and in combination. The volatile trials yielded mixed conclusions, however, at varying concentrations both volatiles seem to play a role in the overall resistance of the Mp708 variety. However, this role is not as prominent as the role of the (E)- β -caryophyllene. The identification of activated wound-response genes and produced volatile compounds will aid in the genetic engineering of more resistant maize lines.

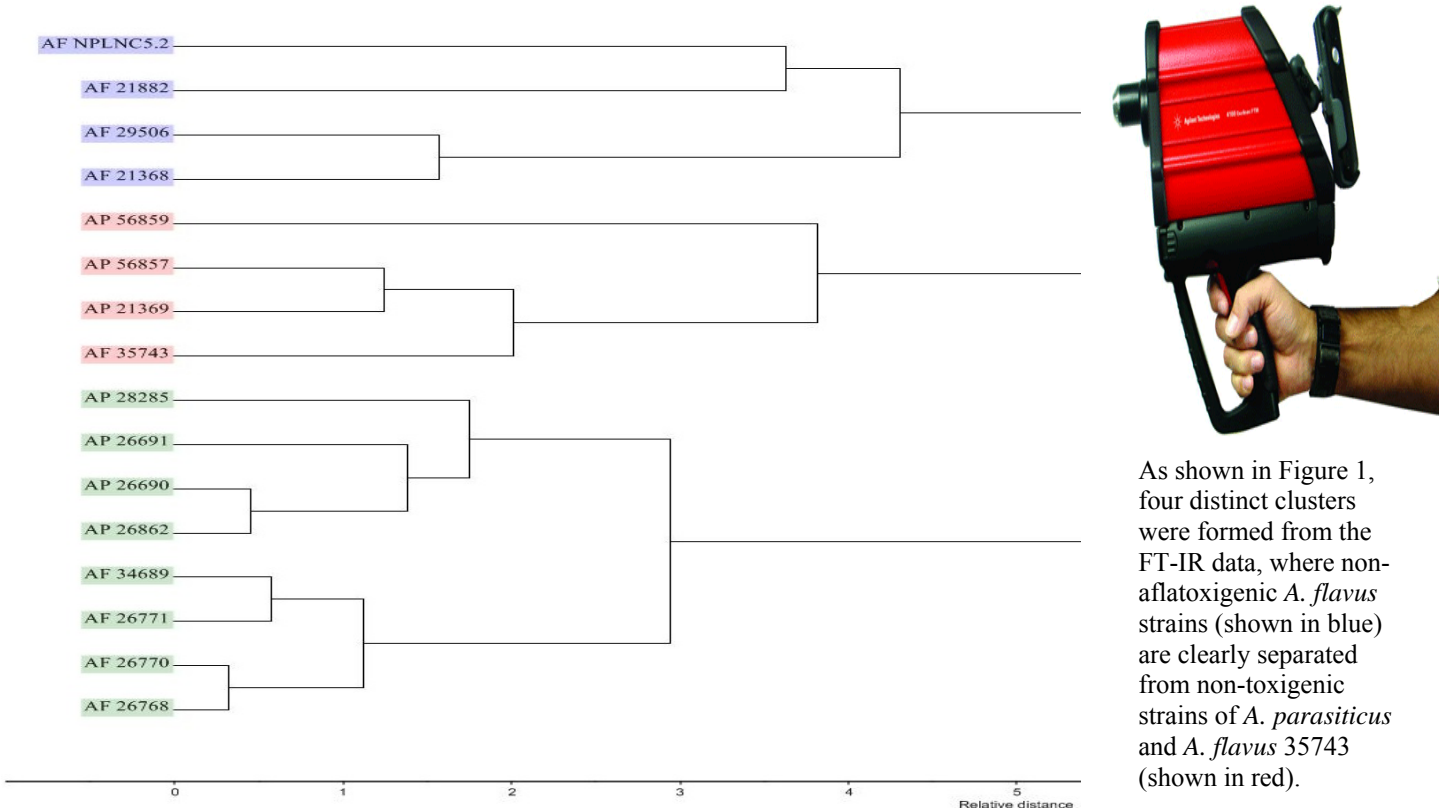


Fig. 1: Dendrogram of Complete Linkage Clustering by Squared Euclidean Distance. Agilent ExoScan Handheld FT-IR.



Fig. 2: Graduate student Cedric Reid pollinating maize hybrids at North Farm.

The third cluster (shown in green) contains the aflatoxigenic *A. parasiticus* strains and the fourth cluster (also in green) contains the aflatoxigenic *A. flavus* strains. In the second cluster, the non-aflatoxigenic *A. flavus* NRRL 35743 grouped within the non-aflatoxigenic strains of *A. parasiticus*. This result indicates that NRRL 35743 isolate has a spectral profile very similar to non-toxigenic *A. parasiticus* strains and yet is still different from the analyzed aflatoxigenic strains of both *Aspergillii* species. Overall, this method is able to differentiate between the *Aspergillii* species on the basis of aflatoxicity. It was also able to distinguish *A. flavus* from *A. parasiticus*.

Project Impacts/Benefits

Our research holds the potential to minimize the entry of aflatoxins into the food chain, which would increase profit margins for producers and provide the agricultural community with effective research strategies for fight against the aflatoxin contamination and insect attack. We are using a systems biology approach to better understand the complex host-pathogen interaction, and develop a conceptual model to predict the occurrence of emerging toxins. We have analyzed 410 corn samples from 6 counties, with soil pH ranging from 5.88-6.99, and aflatoxin concentrations of N.D.- 100 ppb. Coupling this data to weather conditions and agronomical practices we hope to elucidate an aflatoxin outbreak model. Using SPME-GC-MS we have identified a volatile analyte that can be linked to aflatoxin production. We have constructed a 2D proteome reference map of the aflatoxigenic fungus *A. flavus*, which the majority of proteins were functionally annotated as related to cellular metabolic processes, biosynthesis, and enzymes from aflatoxin synthesis pathway. Additionally, using DGE and qRT-PCR we have identified several genes that show an increase in expression during aflatoxin reduction. To control aflatoxin contamination of crops, non-toxicogenic isolates have been employed as bio-control agents. With techniques using live organisms to combat aflatoxin contamination, a necessity arises in the monitoring of local populations of *Aspergillus* to insure that the desired competitive inhibition occurs. A rapid and cost effective technique, such as FT-IR spectroscopy is necessary to effectively identify strains of fungi in the field and establish that inhibition of toxigenic strains. This differentiation could prove valuable to plant pathologists in the monitoring of crops for toxigenic and non-toxicogenic *Aspergilli* strains to ensure crop safety.

Project Deliverables

Abstracts and Presentations:

Ashli Brown, Darrell Sparks, Paul Williams, Jose Rodriguez, Stephanie Ward, Kevin Armbrust (2012) Monitoring Aflatoxin in Mississippi. Joint IFSH/FRI Mycotoxin Symposium, Rosemont, IL. April 12, 2012.

Cedric X. Reid, Christopher I. Johnston, Karl Bell, Erick Larson, Ashli Brown, Darrell Sparks (2012) Monitoring Aflatoxin Contamination of Corn in Mississippi. AOAC Southern Section Annual Meeting, Atlanta, Georgia. April 30- May 1.

*Alka Tiwari, W. Paul Williams, Xueyan Shan (2012) Comparative Structural Analysis of Maize Homologous Proteins Tentatively Associated with Host Plant Resistance to *Aspergillus flavus* Infection and Aflatoxin Accumulation. Corn Utilization and Technology Conference, Indianapolis, Indiana. June 4-6.

*Ashli Brown, Darrell Sparks, Cedric Reid, Andrew McDaniel, Olga Pechanova, Jose Rodriguez, Paul Williams (2012) Decreasing the Impact of Aflatoxin in Mississippi. Florida Pesticide Residue Workshop, St. Pete Beach, Florida. July 15-18.

Gale Hagood, Ashli Brown, Darrell Sparks, Cedric Reid, Andrew McDaniel, Olga Pechanova, Jose Rodriguez, Paul Williams (2012) Research Decreasing the Impact of Aflatoxin in Mississippi. AOAC International Annual Meeting and Expo, Las Vegas, Nevada. September 30- October 3.

*Award winning presentations.

Publications:

Olga Pechanova, Tibor Pechan, Jose M. Rodriguez, W. Paul Williams, Ashli E. Brown (2012) A two-dimensional proteome map of the aflatoxigenic fungus *Aspergillus flavus*. *Proteomics*. (In Press)

Johnston, I., Singleterry, R., Reid, C., Sparks, D., Baldwin, B., Hill-Ward, S., Williams, W.P., Brown, A. (2012) Fermentation of Aflatoxin Inoculated Corn. *Natural Resources*. 3(3) DOI10:4236/nr.2012.33017 .

