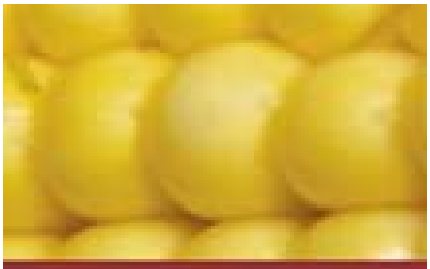
Mississippi Corn Promotion Board
2012 Progress Report



Project Title: Identification and quantification of *Aspergillus flavus* in resistant and susceptible maize genotypes using molecular methods.

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Project Summary (Issue/Response)

The primary aim of this project was to determine population densities using QPCR in inoculated maize tissues. Another goal was to determine if there was a correlation between necrosis from the inoculation procedure (stem lesion length) to population density (QPCR/ng of DNA). This correlation could be used to determine the susceptibility or resistance of maize to *Aspergillus flavus*. A novel Q-PCR TaqMan probe (manuscript in process) has been designed in this laboratory and utilized to detect and quantify toxigenic isolates in culture and in inoculated maize tissue. Fungi from other taxa were used as a control to ensure the primer-probe set did not amplify any other fungi present within the greenhouse or field setting. Standard curves were generated from known amounts of *Aspergillus flavus* (strain NRRL 3357) DNA amplified by the TaqMan probe (R^2=0.9714) and by the *A. flavus* specific-primers designed by Santiago et al (2010) (R^2=0.9999). Maize DNA was used as an internal standard. A total of seven greenhouse tests, two previous growth chamber tests, and two field studies were conducted, were combined for analysis. Data suggests from these studies that stem lesion length and DNA fungal density levels found within inoculated maize seedlings can possibly be correlated with resistance and susceptibility to the fungus. By utilizing a standard curve generated from these studies correlating the stem lesion length and the amount of fungal biomass at harvest. Four genotypes obtained from the USDA-ARS-CHPRRU, susceptible genotypes, GA209 and GA212M (S) and the putative resistant genotypes, Mp313E and Mp717E (R) were used for greenhouses tests through seven (2010-2011), two field tests (2010, 2011) and two growth chamber tests (2009- 2010). All remaining tissue samples from the above studies (approximately1500 to 2000 samples) were processed as outlined using traditional isolations, DNA extractions and QPCR (2012). All data from research conducted (2009-2012) were pooled and analyzed in 2012.

Project Results

Briefly, a novel QPCR TaqMan probe was designed. Greenhouse, previous growth chamber and field tests were performed. All data for S genotypes were combined and all date for R genotypes were combined because there was no significant difference between the two S genotypes or between the R genotypes, respectively (P<0.0001).

In three of the seven greenhouse tests, necrotic lesions observed in the S genotypes inoculated with the toxigenic isolate were significantly larger than lesions observed in both R genotypes and the control (P<0.0001). In the remaining three greenhouse tests and two field tests, there were no significant differences observed between the length of the lesion, and/or the genotype (P<0.0001). In the greenhouse and field tests, necrotic lesions were primarily limited to the phloem. Interestingly, plants grown in growth chambers inoculated were significantly different from all greenhouse and field tests (P<0.0001). Plants were much taller due to inadequate lighting and had to be inoculated (20-25 DAP) and harvested earlier (40-45 DAP), but necrotic lesions were much longer in both S and R plants and were observed through the xylem and the pith. Data also suggests that the fungus is able to spread faster and much further within the stem tissues in susceptible genotypes. This may be due to specific phenolic compounds released in response to wounding, a thicker cell wall found within the pith or a response to maize growing in light conditions not conducive to the growth of maize. Quantitative PCR estimates indicated a gradient in the amount of fungal biomass with the greatest amount of DNA (ng) found immediately adjacent to the inoculation point in both R and S seedlings (Figure 1). Using QPCR, the fungus was detected the pathogen in tissue beyond the border of the visible stem lesion in both genotypes inoculated with the fungus. The fungus was detected at either sampling sites below or above the inoculation site (3 or 6 cm) by traditional isolation with the toxigenic isolate in both the S and R seedlings. Utilizing QPCR, the fungus was detected at all sites sampled (Table 1, Figure 1). Replicated QPCR confirmed that toxigenic isolates were amplified with the novel TaqMan probe, as well as amplified with the *A. flavus*-specific primers. Maize-specific primers were used as an internal standard (Santiago et al 2010).

	Susceptible		Resistant	
Stem Location	QPCR Toxigenic DNA only	QPCR *A. flavus* and Toxigenic DNA	QPCR Toxigenic DNA only	QPCR *A. flavus* and Toxigenic DNA
6CM ABOVE	59%	71%	36%	53%
3CM ABOVE	89%	73%	55%	67%
Inoculation Point	87%	73%	62%	78%
3CM BELOW	89%	39%	37%	56%
6CM BELOW	37%	37%	28%	29%

Table 1. Total percentage of DNA amplified by using novel TaqMan Probe to detect the toxigenic-only amount of DNA[1] and toxigenic-only amount of DNA plus the total amount of *A. flavus*[2] found within seven greenhouse trials. Greenhouse data was pooled (2009-2012) for analysis to show a trend separately between both S genotypes and both R genotypes.

Project Impacts/Benefits

This research will verify that population densities (pathogen DNA) can be used to determine which genotypes have greater resistance to the fungus. The stem inoculation procedure can shorten the amount of time needed to screen varieties for resistance as researchers delay until plant maturity. Genotypes can be grown and screened in a greenhouse or controlled environmental growth chamber year round.

Another novel use from the research includes evaluating biocontrols such as Aflaguard®. The primers and the novel TaqMan probe developed in this laboratory can distinguish the toxigenic strain NRRL 3357 currently used in field resistance trials by the USDA-ARS-CHPRRU from the Aflaguard® strains. This information can provide additional and valuable molecular data to possibly understand the interaction between toxigenic and non-toxigenic strains in the field. In a broader sense, the data obtained from this investigation can then be used as new or novel tools to enhance the development for instance by plotting data on a standard curve, for screening of maize breeding programs for resistance to aflatoxin contamination throughout the mid-south.

Project Deliverables

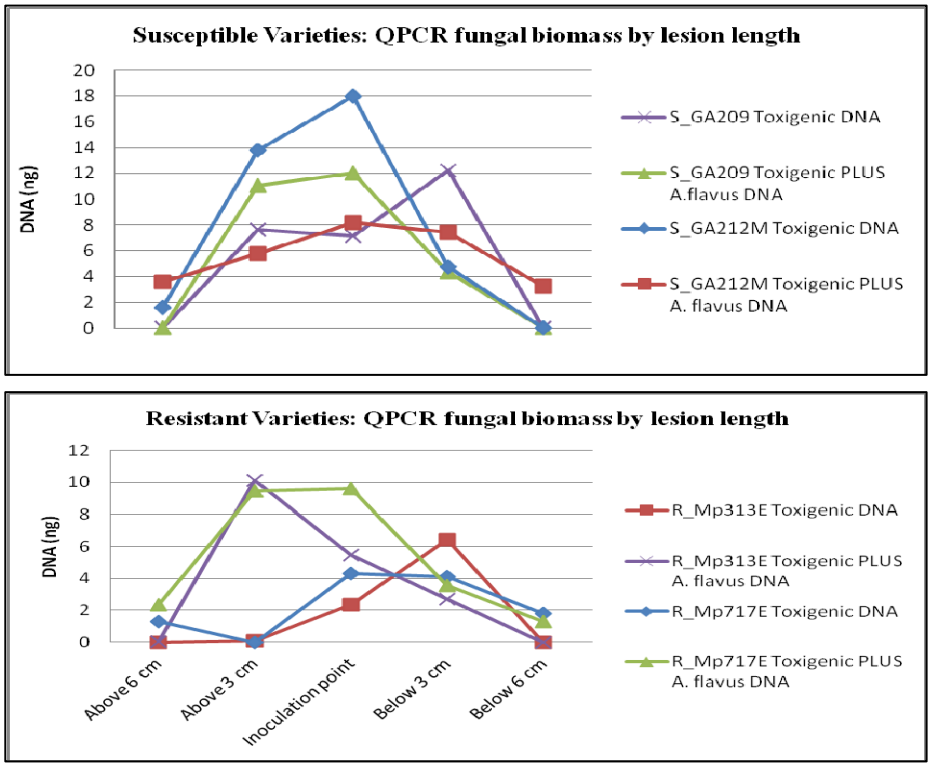
Farm Bureau Board of Directors Tour. May 10, 2012. Tour of ongoing research and discussion of project including progress, results and benefits.

Wood-Jones, A.K., Pilgrim, C. and Baird, R.E. Evaluating for resistance to *Aspergillus flavus* in maize genotypes utilizing a novel TaqMan Probe and stem inoculations. (*In preparation).*

Wood-Jones, A.K. and Baird, R.E. 2011. Evaluating resistance to *Aspergillus flavus* in maize genotypes using stem inoculations. American Phytopathological Society and the International Association for Plant Protection Services, National meeting.

Wood-Jones, A.K. and Baird, R.E. 2010. Screening for resistance to *Aspergillus flavus* in maize genotypes using a QPCR and stem inoculations. Mississippi Association of Plant Pathologists and Nematologists Meeting. Corpus Christi, TX.

Figure 1. Quantitative PCR estimates indicated a gradient in the amount of fungal biomass in and S genotypes (GA209 and GA212M) and R genotypes (Mp313E and Mp717E) grown in the greenhouse (2009-2012).



[1] The 'Toxigenic DNA' shown in the legend is the DNA amplification of *A. flavus* strain NRRL 3357, an aflatoxin producer (toxigenic) used in inoculations.

[2] In order to overcome the outside contamination of the ubiquitous nature of *Aspergillus* spp., the TaqMan probe was used to verify the amplification of only the toxigenic isolates. The 'Toxigenic PLUS *A. flavus* DNA' in the legend is the DNA amplification of all *A. flavus* isolates detected within the greenhouse.