Population Ecology of *Aspergillus flavus* in Mississippi Delta Soils

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ABSTRACT

Populations of *A. flavus* propagules and other soil microflora (*Fusarium* spp., total fungi) associated with Mississippi Delta soils were determined in soils from 12 and 15 sites collected in 2000 and 2001, respectively. Soil characteristics were correlated with fungal populations. *A. flavus* ranged from log (10) 1.97 to 4.31 colony forming units (cfu) g^{-1} soil, while total fusaria ranged from log (10) 2.99 to 5.37 cfu g^{-1} soil. The highest *A. flavus* populations were associated with higher organic matter soils, especially in sites under no-tillage management. In 2001, there was a highly significant correlation between *A. flavus* propagules and history of maize cultivation, but not in 2000. Soil fertility factors such as organic matter content, nitrate and extractable phosphorus correlated with the populations of *A. flavus* as well as *Fusarium* spp., and total fungi, but little relationship was observed with soil texture. The greatest potential for maize aflatoxin contamination may be in the most fertile and productive soils.

INTRODUCTION

Aflatoxin contamination is a food safety concern that can limit economical maize production in the southern US. Understanding the source of *Aspergillus flavus* is required to manage within-field aflatoxin contamination. Populations of *A. flavus* have been characterized in the Southern US (Horn & Dorner, 1998; Jamie-Garcia & Cotty, 2006). This study focused on the Mississippi Delta region, where cotton was the dominant crop. During the last ten years there has been increased maize production, and the need for this research. In addition to *A. flavus*, total fungi and fusaria were also enumerated.

MATERIALS & METHODS

Soils: Surface 0 to 5 cm soils were collected from experimental and producer farms in Leflore, Washington, and Sunflower counties. Sites were sampled in the spring (before planting) with four replicate samples collected from each site and position geo-referenced (GPS). Most soils have been described elsewhere (Zablotowicz et al., 2006).

Fungal Populations: *Aspergillus propagules* were enumerated according to Horn and Dorner (1998) on MDRB agar. Total fungi and *Fusarium* propagules were determined by serial dilution plating on RB-PDA and Peptone-PCNB media (Nash & Snyder, 1965). *Aspergillus flavus* isolates were assessed for aflatoxin production using ELISA (Abbas et al., 2004).

Soil dilutions spread on MDRB plate (Left) Purified *A. flavus* grown on PDA (Right).

MATERIALS & METHODS

Soil Characteristics: Soil moisture was determined gravimetrically and soil texture determined by the hydrometer method. Soil chemical analysis was conducted by the University of Arkansas Soil Analysis Lab.

Statistics: Pearson's correlations with soil properties and fungal populations were done using SAS Proc Corr (Cary, NC).

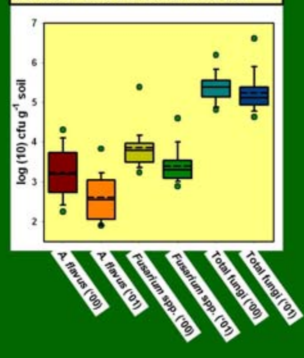
RESULTS & DISCUSSION

A summary of fungal populations are presented as box plots(Fig.1), showing the range and skewness of these populations. In 2000 samples, mean *A. flavus* propagules ranged from log (10) 2.31 to 4.31 cfu g^{-1} soil. *Fusarium* spp. ranged from log 3.29 to 5.37 cfu g^{-1} soil, while total fungal propagules ranged from log 5.13 to 6.19 cfu g^{-1} soil. In 2001 soils, *A. flavus* mean populations ranged from 1.90 to 3.83 log cfu g^{-1} of soil, while *Fusarium* spp. ranged from 2.99 to 4.65 log cfu g^{-1} of soil. Populations of *A. flavus* represented from < 0.1 to 8.1% of the total fungal population, while *Fusarium* spp. populations ranged from about 0.8 to 6.6% of the total fungal population. Populations of *A. flavus* enumerated from these soils were consistent to populations reported for soils from warm temperate climates (Horn & Dorner, 1998; Jamie-Garcia and Cotty, 2006). The frequency of aflatoxin production among *A. flavus* isolates ranged from 18 to 81% depending on soil. In soils with maize history, the frequency of aflatoxigenic isolates was 59 and 44% in 2000 and 2001, with a similar range in soils never cultivated in maize (data not shown). Most (>80%) aflatoxigenic isolates produced greater than 1,000 ng aflatoxin g^{-1} fresh weight of fungal biomass

There was no correlation between maize cultivation and any of groups of the fungi in 2000 (Table 1). However, in the 2001 soils, populations of *A. flavus* were highly correlated with years of maize cultivation. In 2001, there was a wider range of maize history (0 to 4 of the previous 10 years). Typically, all groups of fungi were more highly correlated with increased fertility. The higher populations were associated with soils of high organic matter, abundant nitrate, phosphate, and potassium. The correlation of *A. flavus* propagules in 2000 soils versus soil organic matter content is presented in Fig. 2. Adoption of no-tillage practices generally increases soil organic matter in the surface soil, and increased populations of *A. flavus* were found in no-tillage fields compared to adjacent conventionally tilled soils (data not shown). The greatest potential for maize afalatoxin contamination may be in the most fertile and productive soils.

In addition to an understanding of soil factors regulating *A. flavus* populations, this study generated cultures used in 3 studies assessing the diversity of *A. flavus* in Mississippi soils (Abbas et al., 2004a; 2005 Baird et al., 2006). The major impact of this study, however, was a source for non-aflatoxigenic strains that have potential to control aflatoxin in maize (Abbas et al., 2006).

Figure 1. Box plots of fungal populations, Boxes = 25 & 75%, whiskers = 10 & 90%, circles = 5 & 95% distribution. Median and mean = solid and dashed line in box.



Aspergillus Ear Rot of maize

Figure 2. Relationship between propagule density of *A. flavus* and soil organic matter content (2000 soils).

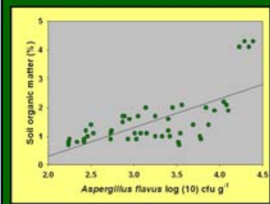


Table 1. Pearson correlations of fungal populations with various properties

Soil Parameter	Year	*A. Flavus*	*Fusarium* spp.	Total Fungi
Maize history	2000	0.0989	-0.1163	0.1168
	2001	**0.4118*** **	0.0981	0.1672
Organic matter	2000	**0.6781*** **	**0.8259*** **	**0.6716*** **
	2001	**0.5513*** **	**0.6604*** **	**0.6233*** **
Moisture	2000	**0.4461** **	**0.4611** **	**0.4048** **
	2001	-0.2192	0.0080	0.1489
Nitrate	2000	**0.3756** **	**0.6249*** **	**0.5727*** **
	2001	**0.3835** **	**0.6085*** **	**0.6098*** **
Phosphorus	2000	**0.5467*** **	**0.7619*** **	**0.4547** **
	2001	**0.4937*** **	**0.6169*** **	**0.4599*** **
Potassium	2000	**0.4001*** **	**0.7515*** **	**0.7219*** **
	2001	0.0854	0.2340	0.2648*
Clay	2000	0.3245*	-0.0478	0.1479
	2001	-0.1784	0.0357	-0.0025

*** = Pr > 0.05, ** = Pr > 0.01, *** Pr > 0.001**

LITERATURE CITED

Abbas HK, Zablotowicz RM, Weaver MA, Horn B, Xie W, Shier WT. (2004) *Can. J. Microbiol.* 50:193-199.

Abbas HK, Weaver MA, Zablotowicz RM, Horn B, Shier WT (2005) *European J. Plant Pathology* 112:283-287.

Abbas, H.K., Zablotowicz, R.M., Bruns, H.A., & Abel, C.A. (2006) *Biocontrol Sci & Technol.* 16: 437-449.

Baird R, Trigiano RN, Windham GL, Williams WP, Kelly R, Abbas HK, Moulton JK, Scruggs ML. (2006) *Mycopathologia* 161: 93-99.

Horn BW, Dorner JW. 1998. *Mycologia* 90:767-776.

Jamie-Garcia R, Cotty PJ. 2006. *Phytopathol.* 96:599-607

Nash SM , Snyder WC. (1965) *Phytopathol.* 52:567-572.

Zablotowicz, R.M., Weaver, M.A., Locke, M.A. (2006) *Weed Sci*. 54:538-547.

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