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## Aflatoxin Production in Corn by *Aspergillus flavus* Relative to Inoculation, Planting Date, and Harvest Moisture in Louisiana.

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### Introduction

Contamination of food and feed grains by aflatoxins is a problem throughout the world. Aflatoxins, which are toxic secondary metabolites produced by *Aspergillus flavus* Link ex. Fries and *A. parasiticus* Speare, are potent carcinogens to animals and have been linked to liver cancer in humans (Castegnaco and McGregor, 1998; Moreno and Kang, 1999).

Corn produced in the southeastern United States has higher levels of aflatoxin than corn produced in the Corn Belt states of the Midwest (Zuber et al., 1976). Because corn acreage has increased recently in Louisiana, there is a growing concern among corn producers regarding levels of aflatoxin in their grain. Although the most effective control of *A. flavus* and aflatoxin contamination is through the development of genetically resistant hybrids (Scott et al., 1991), successful management of aflatoxin in the field will require host resistance combined with management strategies such as appropriate N fertilization and population density, planting date, harvest moisture, insect control, and irrigation (Jones and Duncan, 1981; Jones et al., 1980; Payne, 1992; Wilson and Payne, 1994; Tubajika et al., 1999). Previous work indicated that early planting and harvesting reduced aflatoxin contamination in the field (Jones and Duncan, 1981; Jones, Duncan, and Hamilton, 1980). This study examines the interaction of inoculation, planting date, and harvest moisture on the production of aflatoxin in Louisiana corn.

### Materials and Methods

Planting date X harvest moisture experiments were conducted during 1997 and 1998 at the Louisiana State University Agricultural Center's Northeast Research Station located near Winnsboro on Gigger silt loam soil (fine silty, mixed, thermic-Typic Fragiudalf). Three planting dates (March 7, April 2, and May 1 in 1997 and March 13, April 2, and May 7 in 1998) and four targeted harvest moistures (15, 20, 25, and 30%) were used.

The experimental design was a split-plot with four replications. Planting date was the main plot, and harvest moisture was the subplot. Experimental units consisted of a single row 20 ft long and 1 m apart. Corn hybrid used was Pioneer brand 3167. Furrow irrigation was used each year. Irrigations were initiated May 20, 1997, and May 16, 1998, and approximately 38 mm of water was applied in all plots each week until physiological maturity if rainfall did not occur. Plots were topdressed with 200 lb/A of nitrogen (32% N solution). Weeds were controlled using standard practices.

Strain AF13 of *Aspergillus flavus*, which produces abundant aflatoxin B1 (Cotty, 1989), was used each year. The fungus was grown on V-8 juice agar (5% V-8 juice, 2% agar) for 10 days at 28 degrees C in darkness. Cultures were flooded with 0.05% Triton X-100 and gently rubbed with a glass rod. The concentration of conidia was determined using a hemacytometer. Conidial suspensions (10<sup>6</sup> conidia/ml) were prepared the day of inoculation.

The top ear of each plant in the first half of the row was inoculated 20 days after mid silk (50% of ears with emerged silks) with a conidial suspension of *A. flavus* AF13 using the pinbar wound-inoculation technique (King and Scott, 1982).

Ten ears per plot were collected from inoculated and uninoculated control plots. Each ear was hand harvested at approximately 15 [15-17.5], 20 [17.6-22.5], 25 [22.6-27.5],

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and 30% [ $> 27.6$ ] moisture, machine shelled, and moisture content was recorded with a digital moisture computer model 700 (Burrows Equipment Co., Evanston, IL). Shelled grain was mixed and dried at 60 degrees C to about 13% moisture in a forced-air dryer. Subsamples of 500 g from each plot were collected, ground in a Wiley Mill (model 4 with a 20-mesh screen), and stored at 4 degrees C until used for aflatoxin extraction. The subsamples were replicated four times (50 g ground corn/replicate).

The ground corn (50 g) was extracted with 100 ml of methylene chloride in a 250 ml flask by rotary shaking for 30 min. The contents of the flask were filtered through Whatman no. 1 paper, and the solvent was evaporated to dryness under a fume hood. The residues were dissolved in 2 ml of benzene:acetonitrile (98:2), spotted (10  $\mu$ l) on silica gel TLC (EM Science, Gibbstown, NJ), and developed in ether:methanol:water (96:3:1). A commercial aflatoxin B

and G mixture (Sigma, St. Louis, MO) served as a standard. Criteria for purity of aflatoxin primary standards and determination of concentration of diluted working standards for TLC plates have been described (AOCS, 1988). The scanning densitometer with fluorometry attachment can detect aflatoxins ( $B_1$ ,  $B_2$ ,  $G_1$ ,  $G_2$ ) at concentration as low as 1 ng/g. Aflatoxin  $B_1$  was quantified using a scanning densitometer with a fluorometry attachment (Model CS-930; Shimadzu Scientific Instruments, Inc., Tokyo, Japan).

Aflatoxins data were analyzed using the analysis of variance procedure of Statistical Analysis System (SAS Institute, Cary, NC). Before analysis, aflatoxin data were transformed using the  $\log(x+1)$  transformation to equalize variances. Means were separated using least significant difference ( $P \leq 0.05$ ). Linear and quadratic models were used to elucidate the effect of harvest moisture on aflatoxin levels.

**Table 1. Effect of planting date, harvest moisture, and inoculation on aflatoxin concentration of corn<sup>x</sup> grown at Winnsboro, La., in 1997 and 1998.**

Effect	Aflatoxin (ppb) <sup>y</sup>			
	Control plots		Inoculated plots	
	1997	1998	1997	1998
Planting date				
March	10 b	297 b	16,033 a	16,713 a
April	27 b	694 ab	3,302 b	15,225 a
May	87 a	768 a	4,106 b	11,538 a
Harvest moisture <sup>y</sup>				
1	89 a	1,328 a	12,662 a	32,721 a
2	23 b	883 b	6,809 b	15,638 b
3	7 b	333 c	14157 a	12,168 b
4	7 b	133 c	1376 b	4,293 c
contrast				
linear	**z	**	NS	**
quadratic	**	**	**	**

<sup>x</sup>Pioneer 3167.

<sup>y</sup>parts per billion. Means within columns followed by the same letter are not significantly different by the least significant difference test ( $P = 0.05$ ). Each mean was obtained from four observations.

<sup>x</sup>1 = 15-17.5%, 2 = 17.6-22.5%, 3 = 22.6-27.5%, and 4 =  $> 27.6\%$  grain moisture.

<sup>z</sup>\*\* significant at 1% level. NS = Not significant.

## Results & Discussion

The interaction of planting date with harvest moisture was not significant in this study. Analysis of variance indicated significant ( $P \leq 0.05$ ), main effects of planting date and harvest moisture on aflatoxin contamination. Averaged across planting dates, aflatoxin responses to decreasing harvest moisture were linear and quadratic in most cases, with aflatoxin levels increasing with decreased harvest moisture in control as well as in inoculated plots (Table 1). Aflatoxin contamination was higher in 1998 than in 1997, probably because mean temperatures for May through August were the highest on record while the monthly rainfall was far below normal during 1998. In 1997, inoculated samples from the March planting contained the highest aflatoxin levels and samples from the April planting had numerically lower, but not significantly different, aflatoxin levels than those from the May planting. In 1998, the aflatoxin levels in the March planting did not differ from those of April planting, but both were higher than those from the May planting.

Under natural infection conditions, May planting contained the highest aflatoxin levels, and limited contamination was observed in samples from the March and April plantings (Table 1). In 1998, similar trends were observed. Our results under natural infection conditions concur with results of Jones et al. (1980), who reported reduced levels of aflatoxin B<sub>1</sub> in April compared to May planting under North Carolina conditions. They attributed this reduction to less plant stress during the pollination and grain filling stage of corn development. Jones et al. (1980) reported that reduction in aflatoxin was caused by additional water supply (irrigation) or by other unknown factors.

Under artificial inoculation conditions, the aflatoxin levels decreased with delayed corn planting. Our results in Louisiana confirm the results of Widstrom et al. (1990) that later planting dates sustained less aflatoxin at harvest. They

indicated that the reduction in aflatoxin in later planting dates was associated with exposure of corn plants to lower temperature during kernel filling. Our results from inoculated plots do not agree with the trend shown by Jones (1981) in the inoculated planting date study, however.

Results show that the highest aflatoxin level was observed at 15-17.5% moisture and lowest at 27.6-30% moisture in both years (Table 1). The aflatoxin levels increased with lower grain moisture, confirming the previous result of Payne et al. (1988). They argued that the increase in aflatoxin production observed late in the season is most likely the result of reduced physiological activity of the corn kernel and lack of its ability to express active defense against *A. flavus*.

Aflatoxin levels ranged from 7 to 89 ppb and from 1,376 ppb to 12,662 ppb in uninoculated and inoculated samples, respectively, in 1997. These levels were two and 10 times higher in inoculated and uninoculated samples, respectively, in 1998. This increase in aflatoxin in 1998 was most likely the result of drought stress and high temperatures favoring increased infection by *A. flavus*.

The differing trends in aflatoxin contamination between inoculated and naturally infected corn with regard to planting date suggest that data obtained from inoculated experiments should be interpreted cautiously. Early harvest presents some problems, however. Grain elevators may not take corn over 20% moisture, so a grower would have to dry corn down to an acceptable level. Test weight is also high at high moistures, which can result in dockage when test weights are lower than 53 lbs/bu. Future research should be directed to the identification of corn germ plasm with greater tolerance to drought and high temperature. Results indicate that March planting and early harvesting at moisture  $\geq 20\%$  may be an effective strategy to reduce aflatoxin contamination in Louisiana corn.

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