Nitrogen Fertilizer Influence on Aflatoxin Contamination of Corn in Louisiana

K. M. Tubajika,*,† H. J. Mascagni, Jr.,‡ K. E. Damann,† and J. S. Russin†,§

Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, Louisiana 70803; Northeast Research Station, Louisiana State University Agricultural Center, St. Joseph, Louisiana 71366; and Department of Plant, Soil, and General Agriculture, Southern Illinois University, Carbondale, Illinois 62901

Studies were conducted in 1997 and 1998 on a Gigger silt loam at the Macon Ridge Research Station at Winnsboro, LA, to determine the influence of nitrogen (N) rate, timing, and starter nitrogen fertilizer on aflatoxin contamination in corn. Fertilizer N (0, 50, 100, 150, 200, and 250 lb of N/acre), two timings (at planting and six-leaf stage), and starter N fertilizer (a control and 10 lb of N/acre applied in furrow) were evaluated. Application of starter, N rates, and the interaction of starter with N timing and N rates significantly affected aflatoxin levels. Rates of 50−250 lb of N/acre were 34−43% lower in aflatoxin contamination than plots receiving no N. The application of 10 lb of N/acre starter reduced the aflatoxin levels by 20% compared to the no-starter control.

Keywords: *Corn; Aspergillus flavus; nutrition; thin-layer chromatography*

INTRODUCTION

Aflatoxins, toxic secondary metabolites produced by *Aspergillus flavus* Link ex. Fries and *Aspergillus parasiticus* Speare, are potent carcinogens to animals and have been linked to liver cancer in humans. Preharvest contamination of corn (*Zea mays* L.) grain with aflatoxin is a chronic problem in Louisiana as well as other southern states. Research has demonstrated that corn produced in the southeastern United States has levels of aflatoxin in mature kernels ≥20 ng/g higher than those in corn produced in the midwestern states of the corn-belt (Zuber et al., 1976). Because corn acreage has increased recently in Louisiana, there is a growing concern among corn producers regarding levels of aflatoxin. Conditions that favor the preharvest production of aflatoxin in corn include reduced fertilization and dense plant population (Anderson et al., 1975; Jones and Duncan, 1981) as well as high temperature, high humidity, drought stress, and insect damage (Payne, 1983), particularly during the silking to late dough stages of grain development (Zuber and Lillehoj, 1979).

Factors leading to aflatoxin contamination are not well understood. There is limited information on the influence of nitrogen (N) fertilizer on preharvest aflatoxin contamination. Previous work (Anderson et al., 1975; Wilson et al., 1989; Payne et al., 1989) has indicated that N deficiency is associated with increased aflatoxin contamination in the field. Because crops are fertilized to obtain maximum productivity and quality, the effect of N fertilization on disease becomes an important consideration.

Soil types vary widely among Mississippi River alluvial soils. Optimal N rate and time of application for specific soil types are needed to enhance N fertilizer efficiency, increase corn profitability, and minimize environmental pollution concerns. Research has indicated that N fertilizer efficiency is greatest for sidedress versus at-planting applications (Fox et al., 1986; Stanford, 1973). Greater N fertilizer efficiency should result in reduced application rate. Corn plants may suffer N deficiency prior to sidedress if no previous N is applied. Because only small amounts of N are needed during the early seedling stage in corn (Fox et al., 1986; Stanford, 1973), a starter fertilizer may supply sufficient N for early growth, thereby reducing the risk of an early-season N deficiency. This study examines the influence of N rate, time of N application, and N starter fertilizer on aflatoxin accumulation in corn.

MATERIALS AND METHODS

Field Plots. Field experiments were conducted during 1997 and 1998 on Gigger silt loam soil (fine-silty, mixed, thermic Typic Fragiudalf) at the Louisiana State University Agricultural Center's Macon Ridge Research Station located near Winnsboro, LA. Treatments were as follows: two levels of N starter fertilizer (0 and 10 lb/acre applied in furrow; six rates of N fertilizer (0, 50, 100, 150, 200, and 250 lb of N/acre); and two times for N fertilizer application (at planting or at six-leaf stage). The fertilizer N source for all fertilizer treatments was commercial urea/ammonium nitrate solution containing 32% N. Potassium was applied at rates of 80 and 75 lb/acre in 1997 and 1998, respectively. Corn and cotton have been grown on the field. During the 2 years preceding these experiments, the field was not fertilized with N.

Corn hybrid Pioneer brand 3167 was planted in four-row 12 m plots on March 27, 1997, and April 7, 1998, at ∼11500 seeds/ha. The experimental design was a randomized complete block with a factorial arrangement of treatments with four replications. Furrow irrigation was used each year. Irrigations were initiated May 20, 1997, and May 16, 1998, and ∼38 mm of water was applied each week if rainfall did not occur until physiological maturity. Atrazine (1.5 lb of ai/acre), Lasso (1.0 lb of ai/acre), and 0.5% surfactant were applied preemergence weed control. The insecticide Counter 20CR (1.0 lb of ai/acre) was applied in furrow at planting.

* Corresponding author (e-mail Ktubajika@agctr.lsu.edu).
† Louisiana State University.
‡ Louisiana State University Agricultural Center.
§ Southern Illinois University.

10.1021/jf990535f CCC: $18.00
Published on Web 12/02/1999

Inoculum and Inoculations. Strain AF13 of *A. flavus*, which produces abundant aflatoxin B_1 (Brown et al., 1991; 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 °C in darkness. Cultures were flooded with 0.05% Triton X-100 and gently rubbed with a glass rod. The concentration of dislodged conidia was determined using a hemocytometer and diluted to 10^6 conidia/mL. Conidial suspensions were prepared the day before inoculation and stored at 4 °C. The next day the inoculum was kept at room temperature before inoculation.

At ~20 days after silk emergence, top ears in a 10-ft section of row in each plot were inoculated before dusk with a conidial suspension of *A. flavus* AF13 using the pinbar technique. The pinbar technique is a wound-inoculation technique (King and Scott, 1982).

Harvest and Grain Handling. Ten ears per plot were collected from inoculated sections of rows. Each ear was harvested by hand at ~18% moisture and shelled, and the moisture content was recorded with a digital moisture computer model 700 (Burrows Equipment Co., Evanston, IL). Shelled grain was bulked and dried at 60 °C to ~13% moisture in a forced-air dryer. Subsamples of 500 g from each plot were removed, ground in a Wiley Mill (model 4) to a particle size of 1 mm, and stored at 4 °C until used for aflatoxin extraction.

Aflatoxin Extraction and Thin-Layer Chromatography (TLC). Levels of aflatoxin B_1 were determined using official methods of the American Oil Chemists' Society (AOCS, 1988). Aflatoxin was extracted from 50 g of ground corn. The ground corn was combined with 100 mL of methylene chloride in a 250 mL flask and placed in a rotary shaker for 30 min. The contents of the flask were filtered through Whatman No. 1 paper, and solvent was allowed to evaporate to dryness under a fume hood. The residues were dissolved in 2 mL of benzene/acetonitrile (98:2), spotted (10 μL) on silica gel TLC (EM Science, Gibbstown, NJ), and developed in ether/methanol/water (96:3:1). Aflatoxin B_1 was quantified using a scanning densitometer with a fluorometry attachment (model CS-930; Schimadzu Scientific Instruments, Inc., Tokyo, Japan). 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 a fluorometry attachment can detect aflatoxins (B_1, B_2, G_1, G_2) at concentrations as low as 1 ng/g.

Statistical Analysis. Aflatoxin data were analyzed using the analysis of variance procedure of the Statistical Analysis System (SAS Institute, Cary, NC). Prior to 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 N rate on aflatoxin levels.

RESULTS AND DISCUSSION

Statistical analyses showed no significant year by treatment interaction for aflatoxin levels; therefore, aflatoxin data were combined across years. During the 2-year study, only slight kernel pericarp damage was observed, suggesting that insect activities were negligible. Analysis of variance for aflatoxin contamination indicated statistically significant ($P = 0.05$) main effects of starter N fertilizer and N rate and the interaction of starter N fertilizer with N timing and starter × N rates × N timing (Tables 1−3). Aflatoxin levels from all N rate levels were different compared to the nontreated control. The main effect of N starter fertilizer treatments indicated that plants receiving 10 lb of N/acre significantly ($P \leq 0.05$) reduced aflatoxin levels by 20% overall compared with those not receiving starter (Table 1). However, the main effect of N timing indicated that aflatoxin levels were not affected by time of fertilizer application at planting versus sidedress (applied at six-leaf growth stage).

Table 1. Effect of Starter N Fertilizer, N Timing, and N Rates on Aflatoxin Levels Averaged across Years at Winnsboro, LA, during 1997 and 1998 Growing Seasons

mean effect	aflatoxin[a] (ng/g)
starter fertilizer (S)	
control	15777
10 lb of N/acre[b]	12429
LSD ($P = 0.05$)[c]	2580
timing (T)[d]	
at planting	14808
six-leaf stage	13398
LSD ($P = 0.05$)	NS
N rate (R)[e]	
0	21688
50	15548
100	13523
150	14729
200	8788
250	10343
LSD ($P = 0.05$)	4469
contrast	
linear	**[f]
quadratic	**
interaction	
N × T	NS
N × S	NS
T × S	*
N × T × S	**

[a] Analyses were done on transformed [$\log(X + 1)$] data, but the means presented are not transformed. [b] Applied in furrow as urea/NH_4NO_3 solution (32% N). Means of 60 observations. [c] Statistical differences based on Fisher's least significant differences test at $P = 0.05$. NS = Not significant. [d] Means obtained from 60 observations. [e] Means obtained from 20 observations. [f] *, **, significant at 5 and 1% levels, respectively.

Averaged across starter N fertilizer and N timing, aflatoxin responses to increasing N fertilizer were quadratic (Table 1), with aflatoxin levels decreasing 28% for the 50 lb/acre rate, 38% for the 100 lb/acre rate, and 59% for the 224 lb/acre rate compared to those from nontreated controls. Similar findings were reported by Jones and Duncan (1981), Anderson et al. (1975), Payne et al. (1989), and Asghari et al. (1984). Jones and Duncan (1981) suggested that inadequate N fertilization increased aflatoxin levels by altering the nutritional status of preharvest corn, making it a good substrate for aflatoxin production. Anderson et al. (1975) reported in their study that stresses induced by dense plant population and reduced fertilization were responsible for increased aflatoxin contamination in the field. Younis et al. (1965) demonstrated the drought stress may alter the uptake and translocation of nitrogen in corn.

Averaged across N rate, the interaction of starter N fertilizer with N timing revealed decreasing aflatoxin levels in plants receiving starter N fertilizer either at planting or at six-leaf growth stage (Table 2). The significant interaction between starter, timing, and N rates indicated that the difference in aflatoxin levels between N starter and no starter was not the same for each time of N application and each N rate. Comparison of means revealed that the plots receiving 10 lb of N/acre starter in addition to 200 lb of N/acre had lower aflatoxin levels compared to nontreated controls (Table 3). Overall, plants receiving no N had 53% more aflatoxin compared with those in plots receiving N fertilizer.

Table 2. Effect of Starter N Fertilizer and N Timing on Aflatoxin Contamination in Corn Sampled Inoculated with *A. flavus* at Winnsboro, LA

starter	timing	aflatoxin[a] (ng/g)
0	planting	16749
	six leaf	14802
10[b]	planting	12866
	six leaf	11992
	LSD ($P = 0.05$)[c]	1039

[a] Analyses were done on transformed [log($X + 1$)] data, but the means presented are not transformed. Means obtained from five replications. [b] Applied in furrow as urea/NH_4NO_3 solution (32% N). [c] Statistical differences based on Fisher's least significant differences test at $P = 0.05$.

Table 3. Effect of Starter N Fertilizer, N Timing, and N Rates on Aflatoxin Contamination in Corn Sampled Inoculated with *A. flavus* at Winnsboro, LA

N rate	starter	timing	aflatoxin[a] (ng/g)
0	0	planting	31456
		six leaf	23221
	10[b]	planting	15452
		six leaf	16622
50	0	planting	16454
		six leaf	15891
	10	planting	16725
		six leaf	13119
100	0	planting	14909
		six leaf	15566
	10	planting	14834
		six leaf	9019
150	0	planting	16268
		six leaf	15348
	10	planting	14834
		six leaf	12463
200	0	planting	11780
		six leaf	8111
	10	planting	6780
		six leaf	8478
250	0	planting	9628
		six leaf	10686
	10	planting	8808
		six leaf	2248
		LSD ($P = 0.05$)[c]	2144

[a] Analyses were done on transformed [log($X + 1$)] data, but the means presented are not transformed. Means obtained from five replications. [b] Applied in furrow as urea/NH_4NO_3 solution (32% N). [c] Statistical differences based on Fisher's least significant differences test at $P = 0.05$.

Aflatoxin contamination is a complex problem, and N nutrition is one of many factors that can be manipulated to reduce plant stress and reduce aflatoxin contamination. N fertilizer has an effect on plant vigor, and that can influence the microclimate in a crop and so affect infection and sporulation of a pathogen such as *A. flavus*. It is important to recognize that it is difficult to single out any individual factor in the control of aflatoxin, therefore making it important to study each combination (of host and pathogen).

N fertilizer availability in the developed countries is not a problem. The amount to be applied and the N timing should be of concern in the regions where drought conditions are likely to occur. The cost of using N fertilizer is less important relative to the health costs associated with aflatoxin and crop losses due to kernel ear rot by *A. flavus*.

Results from this study indicate that N fertilizer and the use of a starter fertilizer may reduce aflatoxin levels. High aflatoxin levels obtained from nontreated plots are evidence that failure to apply N can be a risk regardless of other recommended practices. Results also show that the time of application of N is of limited consideration in the 2 years of this study.

ACKNOWLEDGMENT

We thank Drs. S. Wegulo and O. M. Olanya for reviewing the manuscript and Dr. Peter Cotty for providing the AF13 isolate.

LITERATURE CITED

Anderson, H. W.; Nehring, E. W.; Wichester, W. R. Aflatoxin contamination of corn in the field. *J. Agric. Food Chem.* **1975**, *23*, 775−782.

AOCS. Aflatoxin in maize. In *Official Methods and Recommended Practices*; American Oil Chemists' Society: Champaign, IL, 1988; A13-87.

Asghari, M.; Hanson, R. G. Climate, management, and N effect on corn leaf N, yield, and grain. *N. Agron. J.* **1984**, *76*, 911−916.

Ayers, P. G. Interaction between environmental stress injury and biotic disease physiology. *Annu. Rev. Plant Pathol.* **1984**, *22*, 53−75.

Brown, R. L.; Cotty, P. J.; Cleveland, T. E. Reduction in aflatoxin content of maize by atoxigenic strains of *Aspergillus flavus*. *J. Food Prot.* **1991**, *54*, 623−626.

Cotty, P. J. Virulence and cultural characteristics of two *Aspergillus flavus* strains pathogenic on cotton. *Phytopathology* **1989**, *79*, 808−814.

Fox, R. H.; Kern, J. M.; Piekielek, W. P. Nitrogen fertilizer source and method of application effects on no-till corn yields and nitrogen uptake. *Agron. J.* **1986**, *78*, 741−746.

Jones, R. K. The influence of cultural practices on minimizing the development of aflatoxin in filed maize In *Aflatoxin in Maize: Proceedings of a Workshop*; Zuber, M. S., Lillehoj, E. B., Renfro, B. L., Eds.; CIMMYT: Mexico City, 1987; pp 136−144

Jones, R. K.; Duncan, H. E. Effect of nitrogen fertilizer, planting date, and harvest date on aflatoxin production in corn inoculated with *Aspergillus flavus*. *Plant Dis.* **1981**, *65*, 741−744.

Jones, R. K.; Duncan, H. E.; Hamilton, P. B. Planting date, harvest date, and irrigation effects on infection and aflatoxin production by *Aspergillus flavus* in field corn. *Phytopathology* **1981**, *71*, 810−816.

King, S. B.; Scott, G. E. Field inoculation techniques to evaluate maize for reaction to kernel infection by *Aspergillus flavus*. *Phytopathology* **1982**, *72*, 782−785.

Payne, G. A. Nature of field infection of corn by *Aspergillus flavus*. In *Aflatoxin and Aspergillus flavus in Corn*; Diener, U. L., Asquith, R. L., Dickens, J. W., Eds.; Southern Cooperative Series Bulletin 279; Alabama Agriccultural Experiment Station: Auburn, AL, 1983; pp 16−19

Payne, G. A. Aflatoxin in Maize. *Crit. Rev. Plant Sci.* **1992**, *10*, 423−440.

Payne, G. A.; Kamprath, E. J.; Adkins, C. R. Increased aflatoxin contamination in nitrogen stressed corn. *Plant Dis.* **1989**, *73*, 556−559.

Stanford, G. Rationale optimum nitrogen fertilization in corn production. *J. Environ. Qual.* **1973**, *2*, 159−166.

Wilson, D. M.; Walker, M. E.; Gascho, G. J. Some effects of mineral nutrition on aflatoxin contamination of corn and peanuts. In *Soilborne Plant Pathogens: Management of Diseases with Macro- and Micro-element*; Englehard, A. W., Ed.; American Phytopathological Society: St. Paul, MN, 1989; pp 137−151.

Younis, M. A.; Pauli, A. W.; Mitchell, H. L.; Sticker, F. C. Temperature and its interaction with light and moisture in nitrogen metabolism of corn seedlings. *Crop Sci.* **1965**, *5*, 321−326.

Zuber, M. S.; Lillehoj, E. B. Status of the aflatoxin problem in corn. *J. Environ. Qual.* **1979**, *8*, 1−5.

Zuber, M. S.; Calvert, O. H.; Lillehoj, E. B.; Kwolek, W. F. Preharvest development of aflatoxin B1 in corn in the United States. *Phytopathology* **1976**, *66*, 1120−1121.

Received for review May 18, 1999. Revised manuscript received August 29, 1999. Accepted October 6, 1999. This project was supported in part by the Louisiana Soybean and Small Grain Research and Promotion Board. Approved for publication by the Director of the Louisiana Agricultural Experiment Station as Manuscript 99-38-0247.

JF990535F