Increased Aflatoxin Contamination in Nitrogen-Stressed Corn

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ABSTRACT

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Aflatoxin concentration in corn kernels wound-inoculated with *Aspergillus flavus* was correlated negatively with corn yield, silk leaf nitrogen, and grain nitrogen in a 2-yr study in North Carolina. At two of three locations in 1982, aflatoxin concentration in silk-inoculated ears was also correlated negatively with yield, silk leaf nitrogen, or grain nitrogen. Percent kernel infection of silk-inoculated ears was correlated with aflatoxin concentration but not with yield, silk leaf nitrogen, or grain nitrogen. Silk- or wound-inoculated ears from plants receiving no added nitrogen contained an average of 28% more aflatoxin than ears from plants that received optimum nitrogen fertilization. We conclude from these studies that nitrogen stress may be a factor contributing to aflatoxin contamination of corn.

Aflatoxin contamination of corn (*Zea mays* L.) before harvest is a concern to corn growers each year in the southern United States. In certain years, aflatoxin contamination of kernels by *Aspergillus flavus* Link ex Fries may be extensive enough that the crop is not marketable or must be sold at reduced prices. For example, direct costs to farmers due to aflatoxin contamination in the Southeast in 1980 was estimated to be $97 million (9).

Not all of the factors leading to aflatoxin contamination of corn are known. Conditions that stress the plant, such as high temperature and drought, increase aflatoxin contamination (1,4,6,10–13,15,16). Nitrogen imbalance also has been associated with increased aflatoxin contamination. Anderson et al (1) reported that plants fertilized with 67 kg/ha of nitrogen contained more than twice as much aflatoxin as plants fertilized with 157 kg/ha of nitrogen. Jones and Duncan (5) also observed nearly twice as much aflatoxin in corn grown with 11 kg/ha of nitrogen than in corn grown with 146 kg/ha of nitrogen. Although these reports provide initial indications of effects of nitrogen stress on aflatoxin contamination, each study was done for only 1 year at one location. The objective of our study was to critically examine the effect of nitrogen fertilization on aflatoxin contamination of corn by examining a series of nitrogen rates at several locations in a 2-yr study.

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MATERIALS AND METHODS

Experiments were conducted in 1981 and 1982 at the Central Crops Research Station near Clayton on Dothan loamy sand soils (fine loamy, siliceous thermic, Plinthic Paleudults) and at the Lower Coastal Plain Tobacco Research Station near Kinston on Goldsboro sandy loam soils (fine loamy, siliceous thermic, Aquic Paleudults). In 1982, an experiment was also conducted at the Tidewater Research Station near Plymouth on Portsmouth very fine sandy loam soil (fine loamy, mixed thermic, Typic Umbraquults).

Nitrogen treatments. Nitrogen treatments consisted of a control (no nitrogen application) and NH_4NO_3 applied at rates of 56, 112, 168, and 224 kg of nitrogen per hectare, of which 28 kg was applied at planting and the remainder as a sidedressing 4 wk after emergence of the corn. The phosphorus and potassium were applied at planting at the rate of 22 kg of phosphorus and 45 kg of potassium per hectare. Recommended herbicides were applied preemergence. The corn hybrid Pioneer Brand variety 3320 was planted 17.5 cm apart in four-row plots 14 m long with row widths of 96 cm. Each treatment was replicated four times in a randomized complete block design.

Samples for total nitrogen analyses were taken from the leaf opposite and below the ear at silking (SLN) and the grain at harvest (GN). Nitrogen was determined by the Kjeldahl method in the Analytical Laboratory of the Soil Science Department, North Carolina State University. Grain yields were calculated at 15.5% moisture.

Inoculum preparation. Plants were inoculated with a conidial suspension of isolate NRRL 3357 of *A. flavus* grown on potato-dextrose agar for 10 days at 28 C. Culture plates were flooded with 0.05% aqueous Triton X-100, the spores were dislodged with a glass rod, and the concentration of conidia was adjusted to 5×10^5 per milliliter. Conidial suspensions were prepared the day before inoculation and stored in the dark at 4 C.

Inoculation procedures. Corn ears were either silk- or wound-inoculated. The dominant ear on each plant was silk-inoculated using a hand-held atomizer to spray 1 ml of spore suspension on silks. Nitrogen stress delayed silk emergence and development. In 1981, silks on plants in all plots were inoculated when silks on plants receiving 168 kg/ha of nitrogen were yellow-brown (7). Silk inoculations in 1982 were staggered and were applied in each nitrogen treatment when silks were yellow-brown. Silk inoculations were done between 28 June and 7 July for the 2 yr. After inoculation, ears were enclosed in a plastic bag and covered with a paper bag. Three days later the plastic bag was removed but the paper bag remained on the ear until harvest. For the wound-inoculation method in 1981, kernels were injured once, after peeling back the husks on one side of the ear, by applying a pinboard with a line of 23 pins spaced 5 mm apart to four adjacent rows of kernels. In 1982, the method was modified such that all kernels in the four rows were wounded. This was done to provide a similar ratio of wounded kernels to uninjured kernels even though the length of the ear differed among nitrogen treatments. The wounded area was sprayed with 1 ml of conidial suspension. Husks were repositioned, secured with rubber bands, and covered with plastic and paper bags to maintain humid conditions. After 3 days, the plastic bags were removed but the paper bag remained on the ear until harvest. Ears were wound-inoculated in the late-milk to early dough stage. For

Table 1. Influence of five nitrogen rates on yield and percent of nitrogen in the silk leaf and grain of maize at two locations in 1981 and three locations in 1982

Nitrogen rate (kg/ha)	Clayton			Kinston			Plymouth		
	Yield[w]	SLN[x]	GN[y]	Yield	SLN	GN	Yield	SLN	GN
1981									
0	5.483	1.46	1.11	3.727	1.22	0.95			
56	7.811	2.22	1.05	6.136	2.06	0.97			
112	9.197	2.63	1.23	7.886	2.62	1.14			
168	10.151	2.90	1.31	8.953	2.82	1.23			
224	9.787	2.97	1.42	9.229	3.07	1.32			
R^2	0.83*[z]	0.90*	0.87*	0.92*	0.92*	0.96**			
1982									
0	5.753	1.45	1.07	3.833	1.59	1.10	4.555	1.63	1.07
56	9.241	2.50	1.24	7.158	2.73	1.14	7.108	2.43	1.08
112	11.236	3.29	1.27	7.930	3.17	1.30	8.884	2.80	1.21
168	12.196	3.34	1.28	8.056	3.38	1.47	10.013	2.88	1.29
224	12.077	3.45	1.42	7.955	3.50	1.50	10.484	3.01	1.31
R^2	0.83*[z]	0.82*	0.87*	0.65	0.83*	0.95**	0.93**	0.83*	0.94**

[w] Yield = megagrams per hectare.
[x] Percent of nitrogen in silk leaf at silking.
[y] Percent of nitrogen in grain at harvest.
[z] Regression coefficient of determination for linear models: * = significant at $P = 0.05$, ** = significant at $P = 0.01$.

each method, approximately 20 ears in a 14-m section of row were inoculated.

Aflatoxin analysis. All inoculated ears in each 14-m row were hand-harvested, bulked, and machine-shelled. Yield was recorded and a 4.5-kg subsample was dried at 60 C to a kernel moisture of 10% and was stored for 2 mo in a low-humidity room. The entire sample was ground to pass through a 20-mesh screen, was blended thoroughly, and a 50-g subsample was extracted for aflatoxin (3). Aflatoxin concentrations are reported as the sum of aflatoxin B_1 + B_2. Aflatoxin concentrations were determined for noninoculated ears only at Kinston in 1981.

Determination of kernel infection. All silk-inoculated ears in each 14-m row were hand-harvested, bulked, machine-shelled, and a 600-ml subsample was withdrawn. The subsample was dried and stored as above. Two hundred kernels were selected randomly from each subsample, rinsed thoroughly with running water, surface-sterilized for 3 min in a solution of 95% ethanol:sodium hypochlorite (5.25%):water (10:20:70), and plated on malt agar containing 6% NaCl. Only kernels free of visible injury and sporulation of the fungus were plated. Kernels were incubated at 34 C for 4 days and seeds with visible *A. flavus* were counted. No kernels were plated from the wound-inoculated treatments.

RESULTS AND DISCUSSION

At all locations in both years there was a response in corn to nitrogen application. Average grain yield over all locations ranged from 4.7 Mg/ha where no nitrogen was applied to 9.9 Mg/ha where 224 kg/ha of nitrogen was applied (Table 1). Although the lowest yield was always in plots receiving no nitrogen, maximum yield was obtained in plots receiving 224 kg/ha of nitrogen only at Kinston in 1981 and at Plymouth in 1982.

Table 2. Linear correlation coefficients for yield, silk leaf nitrogen, and grain nitrogen of maize and percent of infection and aflatoxin production by *Aspergillus flavus* at three locations in 1981 and 1982

Variables[x]	1981		1982			Across sites
	Clayton	Kinston	Clayton	Kinston	Plymouth	
Yield vs. % SLN	0.99**[y]	0.99**	0.99**	0.98**	0.98**	0.99**
Yield vs. % GN	0.75	0.93*	0.90*	0.75	0.94*	0.90*
% SLN vs. % GN	0.80	0.92*	0.91*	0.87*	0.87*	0.90*
Wound-inoculated ears						
Aflatoxin vs. yield	−0.89*	−0.79	−0.34	−0.27	−0.81+	−0.99**
Aflatoxin vs. % SLN	−0.88*	−0.93*	−0.40	−0.33	−0.85+	−0.99**
Aflatoxin vs. % GN	−0.89*	−0.87*	−0.20	−0.55	−0.64	−0.90*
Silk-inoculated ears						
Aflatoxin vs. yield	...[z]	...	−0.78	0.10	−0.77	−0.04
Aflatoxin vs. % SLN	...	...	−0.73	0.30	−0.83+	−0.03
Aflatoxin vs. % GN	...	...	−0.64	0.65	−0.59	0.39
Aflatoxin vs. % I	...	...	0.52	0.91*	−0.84+	0.77
% I vs. % yield	0.13	−0.33	−0.85+	0.04	0.69	−0.16
% I vs. % SLN	0.13	−0.31	−0.87*	0.25	0.66	−0.18
% I vs. % GN	0.41	−0.09	−0.59	0.68	0.69	−0.17
Noninoculated ears						
Aflatoxin vs. yield	...	−0.94*	...	...	...	−0.94*
Aflatoxin vs. % SLN	...	−0.77	...	...	...	−0.77
Aflatoxin vs. % GN	...	−0.83+	...	...	...	−0.83+

[x] SLN = silk leaf nitrogen at silking, GN = grain nitrogen at harvest, aflatoxin = micrograms per kilogram $B_1 + B_2$, % I = percent of kernels infected with *A. flavus*.
[y] ** = Significant at $P < 0.01$, * = significant at $P < 0.05$, + = significant at $P < 0.10$.
[z] Data not taken.

Maximum yields in the other tests were in plots receiving 168 kg/ha of nitrogen (Table 1). There was a significant relationship between nitrogen rate and either yield, SLN, or GN (Table 1). Further, corn yield was correlated significantly with SLN and GN (Table 2). The responses of corn to added nitrogen in this study are consistent with those in previous studies (2,8,14). Thus, a range of nitrogen levels in corn tissue was established in our treatments.

Aflatoxin contamination was also related to nitrogen fertility. In 1981 and 1982, the highest concentration of aflatoxin in wound-inoculated ears occurred in plants receiving 0 or 56 kg/ha of nitrogen, whereas the lowest concentration was in plants receiving optimum nitrogen rates (i.e., rates that gave maximum yields) (Fig. 1A,B; Table 1). In 1981, there was a significant ($P \leq 0.05$) linear and negative relationship between nitrogen rate and aflatoxin contamination of wound-inoculated ears (Fig. 1A). Further, aflatoxin content in these ears was negatively correlated with yield, SLN, and GN (Table 2). In 1981, aflatoxin contamination of noninoculated ears was negatively correlated with yield and GN (Table 2). Percent kernel infection of silk-inoculated ears was not correlated with yield, SLN, or GN.

In 1982, the relationship between nitrogen rate and aflatoxin contamination of wound-inoculated ears was not

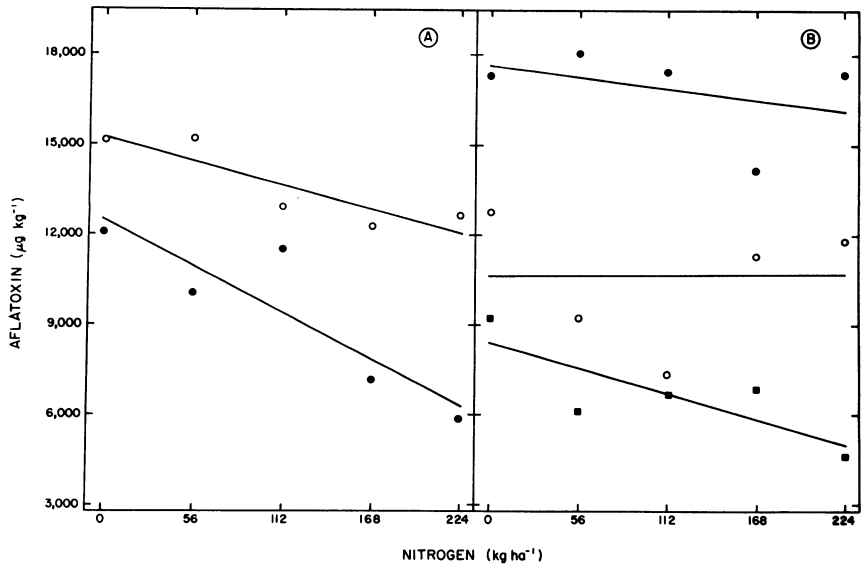

Fig. 1. Relationship between rate of nitrogen application and aflatoxin content of ears of maize wound-inoculated with *Aspergillus flavus* at **(A)** two locations in 1981, where lines represent regression equations $Y = 15{,}199.3 - 15.6X$ ($R^2 = 0.77$, $P \leq 0.05$) for Clayton (open circle), and $Y = 12{,}388.0 - 30.7X$ ($R^2 = 0.79$, $P \leq 0.05$) for Kinston (closed circle) and **(B)** three locations in 1982, where lines represent regression equations $Y = 10{,}450.3 + 13.7X$ ($R^2 = 0.0001$) for Clayton (open circle), $Y = 17{,}984.7 - 368.9X$ ($R^2 = 0.14$) for Kinston (closed circle), and $Y = 9{,}221.1 - 843.6X$ ($R^2 = 0.65$) for Plymouth (closed square).

as pronounced as in 1981. The slopes of the regression lines were negative at both Kinston and Plymouth, but the relationship between the rate of nitrogen application and aflatoxin contamination was not significant (Fig. 1B). Correlations between aflatoxin content and yield and SLN were significant only at Plymouth (Table 2). However, when correlations were made between variables over all locations for the 2 yr, aflatoxin contamination was significantly and negatively correlated with yield, SLN, and GN (Table 2).

In silk-inoculated ears, percent kernel infection was correlated with yield and SLN in one location in 1982 (Table 2). Furthermore, percent kernel infection of silk-inoculated ears was correlated with aflatoxin contamination.

Correlation coefficients between aflatoxin contamination of silk-inoculated ears and yield, SLN, and GN were high and negative at Plymouth and Clayton, but the only significant correlation was between aflatoxin concentration and SLN at Plymouth (Table 2). When correlations were made across sites for silk-inoculated ears, no trends were evident for aflatoxin concentration and yield, SLN, and GN (Table 2).

Based on tests at three sites over 2 yr, we conclude that aflatoxin contamination in noninoculated, silk-inoculated, and wound-inoculated kernels is greater for plants receiving low levels of nitrogen than for plants with optimum nitrogen fertilization. Silk- or wound-inoculated ears from plants receiving optimum nitrogen fertilization contained an average of 28% less aflatoxin than ears from plants that received no added nitrogen.

The effect of nitrogen treatments on aflatoxin concentration was not consistent between years or among locations. The correlation between aflatoxin contamination and nitrogen levels was more pronounced in 1981 than 1982 (Fig. 1). Differences in aflatoxin concentration between years in the wound-inoculated treatments could be due to inoculation technique. In 1982, we inoculated approximately the same proportion of kernels on an ear in each nitrogen treatment. In contrast, in 1981 the same number of kernels on an ear was inoculated, irrespective of size. Because ears from nitrogen-stressed plants are smaller, the technique used in 1981 resulted in a proportionally greater number of wound-inoculated kernels on ears in the lower nitrogen treatments. This procedure may have resulted in high concentrations of aflatoxin in the lower nitrogen treatments. Differences in aflatoxin concentration between 1981 and 1982 also could be due to other factors that are known to influence aflatoxin. High temperatures, drought stress, and insect injury are known to contribute to high concentrations of aflatoxin. It is possible that these factors were more important than nitrogen stress in influencing aflatoxin contamination at some locations.

Although our results are consistent with those of others (1,5), the differences we observed in aflatoxin concentrations between treatments were smaller than previously reported. We cannot explain why our differences were smaller, but it could be due to different environmental conditions. We did observe higher concentrations of aflatoxin in wound-inoculated corn than did Jones and Duncan (5). Whereas aflatoxin concentrations in wound-inoculated kernels from the low nitrogen treatment of Jones and Duncan (5) was approximately 4,000 μg/kg, concentrations of aflatoxin in kernels wound-inoculated with the same isolate in our low nitrogen treatment were greater than 13,000 μg/kg. Anderson et al (1) examined only noninoculated plants and reported 1,800 μg/kg of aflatoxin in the low nitrogen treatment. We had an average of 112 μg/kg of aflatoxin in silk-inoculated ears from our low nitrogen treatment.

We conclude from our studies that nitrogen stress can significantly increase aflatoxin contamination in corn kernels. It is unlikely, however, that nitrogen stress due to insufficient nitrogen application is of concern in developed countries where adequate nitrogen is usually applied. Nitrogen imbalance may result for reasons other than low nitrogen application. In his review, Jones (4) discusses how nitrogen stress may also result from drought stress. Reduced uptake of water results in decreased translocation and mobilization of nitrogen. Thus, a portion of the effect of drought stress on aflatoxin contamination may be the result of nitrogen stress.

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