Aflatoxin Accumulation in Inoculated Ears of Field-grown Maize

G. A. PAYNE, Associate Professor, Department of Plant Pathology, W. M. HAGLER, JR., Associate Professor, Department of Poultry Science, and C. R. ADKINS, Research Assistant, Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616

ABSTRACT

Payne, G. A., Hagler, W. M., Jr., and Adkins, C. R. 1988. Aflatoxin accumulation in inoculated ears of field-grown maize. Plant Disease 72:422-424.

The rate of aflatoxin accumulation in maize ears that were silk- or wound-inoculated or naturally infected with *Aspergillus flavus* was examined at Rocky Mount, NC, in 1982 and at Clayton, NC, in 1982 and 1983. In both years, aflatoxin appeared in wound-inoculated ears within 1 wk and increased linearly before peaking 7–9 wk postinoculation (16–21% moisture). Aflatoxin accumulation in silk-inoculated ears followed a similar pattern except that levels of toxin were lower. In both years, toxin levels declined as kernel moisture decreased to 14–16% ($P = 0.05$). In 1983, aflatoxin concentration was positively correlated with kernel infection and both aflatoxin concentration and kernel infection were negatively correlated with kernel moisture. Two important considerations are evident from the results. First, harvesting maize as early as possible will limit aflatoxin contamination. Second, any comparison of lines of maize for resistance to aflatoxin accumulation should be done during the linear phase of aflatoxin accumulation.

Aflatoxin contamination of maize before harvest is a serious problem in the southern and southeastern United States. High temperature, water stress, and insect damage enhance infection and aflatoxin production by *Aspergillus flavus* Link ex Fries in field maize (2,6,9,13,17). Little is known about the rate of aflatoxin accumulation prior to harvest. It is not known when levels of aflatoxin are first detectable, when they peak, or if they plateau and remain constant for the rest of the season.

Thompson et al (16) examined the rate of aflatoxin accumulation in developing maize kernels on plants grown in a controlled environment. Aflatoxin appeared 2 days after inoculation with *A. flavus* and reached a maximum in 9 days. Elevated temperatures enhanced aflatoxin accumulation. Anderson et al (2) found aflatoxin in field-grown maize 6 wk prior to harvest but did not report an increase in aflatoxin levels in maize left standing after normal harvest time. Jones et al (6), however, found higher levels of aflatoxin in maize harvested at 18% kernel moisture than in maize harvested at 28%. Lillehoj et al (7) sampled maize grown in Illinois, Missouri, Georgia, and Texas four times throughout the season and concluded that most of the toxin production occurred during the first 30 days after inoculation. It is difficult to determine the kinetics of aflatoxin production from their study because of differences among locations. For example, kernel moisture content in Georgia at 30 days postinoculation (about 31%) was comparable to kernel moisture at Illinois at 70 days (34%).

Knowledge of the rate of aflatoxin accumulation is important in developing control strategies for aflatoxin contamination. If aflatoxin levels increase throughout the season, one control procedure may include early harvest followed by drying. Second, it is important to know the kinetics of aflatoxin production such that breeding lines can be effectively evaluated. The objective of this study was to examine the rate of aflatoxin contamination in naturally infected and inoculated ears in the field to determine the time of appearance and the kinetics of aflatoxin accumulation.

MATERIALS AND METHODS

Experimental design. This study was conducted in North Carolina at the Upper Coastal Plain Research Station near Rocky Mount in 1982 and at the Central Crops Research Station near Clayton in 1982 and 1983. Maize hybrid Pioneer Brand (Johnston, IA) variety 3147 was planted 4 May and 6 May 1982 at Clayton and Rocky Mount, respectively, and 2 May 1983 at Clayton. The experimental design was a split plot with four replications in 1982 and six replications in 1983. Whole plots were sampling dates and subplots were inoculation type. Each whole plot consisted of two rows in 1982 and three rows in 1983. Rows (6.1 m) within whole plots were chosen randomly for silk inoculation or wound inoculation in 1982 and for silk inoculation, wound inoculation, or no inoculation in 1983. In 1982, border rows were sampled to determine natural levels of aflatoxin contamination.

Inoculation procedures. Plants were inoculated with a conidial suspension of *A. flavus* NRRL 3357. The fungus was grown on potato-dextrose agar in petri plates for 10 days at 28 C. Cultures were flooded with 0.05% Triton X-100 and gently rubbed with a glass rod. The concentration of dislodged conidia was determined with a hemacytometer and diluted to 5×10^5 spores ml^{-1} in 0.05% Triton X-100. Spore suspensions were prepared the day before inoculation and stored at 4 C.

Silks were inoculated when yellow-brown (8) at Rocky Mount and Clayton on 15 July and 12 July 1982, respectively, and at Clayton on 22 July 1983. A 1.0-ml sample of spore suspension was sprayed on each silk (13). After inoculation, ears were enclosed in a plastic bag for 3 days. Ears at the late-milk to early-dough stage were wound-inoculated by first dipping a paring knife into a spore suspension and then jabbing the knife through the husk once into the ear approximately 4 cm from the ear tip. Wound inoculation was done on 26 July at Rocky Mount and 27 July at Clayton in 1982 and on 29 July in 1983. For each method, all the dominant ears of each plant in the row were inoculated.

Aflatoxin analysis. At each sampling date, all ears in the row were hand-harvested, bulked, and machine-shelled. Kernels were dried at 60 C (kernel moisture <10%) and stored in a low-humidity room. At the early sampling dates (5–26 August) ears had to be dried before they could be shelled from the cob. Kernel moisture was determined by drying ears to a constant weight at 75 C.

Aflatoxin concentrations were determined within 60 days of the last harvest date. The stored samples were ground to pass through a 20-mesh (1-mm) screen and thoroughly blended, and a 20-g subsample was extracted for aflatoxin. Extraction and analyses were done as described by Hutchins and Hagler (4). Concentrations are presented as aflatoxin $B_1 + B_2$ (ng/g).

Determination of kernel infection. In 1983, a 600-ml subsample of kernels was withdrawn before grinding for deter-

Paper 11133 of the Journal Series of the North Carolina Agricultural Research Service.

Use of trade names does not constitute endorsement by the North Carolina Agricultural Research Service of the products named and does not imply criticism of similar ones not mentioned.

Accepted for publication 21 December 1987 (submitted for electronic processing).

mination of kernel infection. Two hundred kernels were selected randomly from each sample, surface-disinfested for 3 min in a solution of 95% ethanol:sodium hypochlorite:water (10:20:70), and plated on malt agar containing 6% NaCl. Only kernels without visible injury and free from sporulation by *A. flavus* were plated. The plates were incubated at 34 C for 4 days and the number of seeds with visible sporulation of *A. flavus* was counted.

Data analyses. Data were subjected to analysis of variance with comparisons made between sampling dates within inoculation treatments by the least significant difference comparison test. Simple correlations were conducted between dependent variables for the 1983 field plot at Clayton.

RESULTS

Aflatoxin accumulated in non-inoculated ears at each test. Levels of contamination peaked at 43 and 10 ng/g at Rocky Mount and Clayton, respectively, in 1982 and at 784 ng/g at Clayton in 1983. In contrast, levels in wound-inoculated ears peaked at 1,199 and 673 ng/g at Rocky Mount and Clayton, respectively, in 1982 and at 2,764 ng/g at Clayton in 1983. The maximum concentration of aflatoxin and the rate of accumulation differed among sites, but in wound-inoculated ears aflatoxin appeared at a similar time and accumulated at a nearly linear rate until reaching a peak (Figs. 1–3). Aflatoxin was first detected 1 wk after inoculation and reached a maximum after 7 wk. At Clayton in 1982 (Fig. 1), aflatoxin reached a peak in wound-inoculated ears at 21.7% kernel moisture (13 September) and decreased ($P = 0.05$) as the moisture dropped to 18.2% (20 September). Aflatoxin concentration peaked at Rocky Mount (Fig. 2) between 16 September and 7 October (18.2–17.0 kernel moisture). From this broad peak the level of aflatoxin decreased ($P = 0.05$) as the moisture dropped to 15.6% (21 October). In 1983, kernel moisture decreased linearly from 5 August to 16 September. From 16 September until 21 October, only 52.6 mm of rain fell. An increase in kernel moisture from 16 September to 23 September was associated with 23.4 mm of rain, and the increase in kernel moisture from 7 October to 21 October was associated with 24.6 mm of rain. Aflatoxin levels reached a peak 16 September at 15.6% moisture and decreased ($P = 0.05$) as the moisture fell to 14% (Fig. 3). However, an increase in kernel moisture late in the season coincided with an increase ($P = 0.05$) in aflatoxin accumulation. For the entire 1983 season there was a significant negative correlation between kernel moisture content and aflatoxin levels (Table 1).

Aflatoxin accumulation in silk-inoculated ears was similar to that in wound-inoculated ears except concentrations were lower. At Clayton in 1982, aflatoxin was detected at the first sampling date but the levels remained low throughout the season (Fig. 1). At Rocky Mount, peak concentrations of aflatoxin occurred on 23 September and 14 October, corresponding to kernel moisture contents of 18.2 and 15.8%, respectively (Fig. 2). In 1983 (Fig. 3), the highest concentrations were detected in samples taken at 9 September and 21 October. Aflatoxin accumulated in naturally inoculated plots in a similar pattern, but the concentrations were lower.

The highest incidence of *A. flavus* was found in kernel samples from wound-inoculated plots, followed by the silk-inoculated and naturally infected plots (Fig. 4). Incidence peaked 16 September in the silk-inoculated plots and 30 September in the wound-inoculated plots. In both, incidence decreased as kernel moisture fell to 14% and increased as kernel moisture increased late in the season. Kernel moisture was negatively correlated with the percentage of infected kernels in all treatments, and aflatoxin concentration was positively correlated with the number of infected kernels (Table 1).

DISCUSSION

Aflatoxin began accumulating in kernels of field-grown maize before the first sampling, and the accumulation was characterized by a near linear increase up to a peak. Peak levels of aflatoxin occurred around the reported minimum kernel moisture (16%) required for growth of *A. flavus* (15). A distinctive observation in our study was a decline in aflatoxin concentration and percentage of kernel infection late in the season. We also observed a second increase in aflatoxin concentration, which in 1983 was associated with late-season rains and an increase in kernel moisture.

There is only one report related to the kinetics of aflatoxin accumulation in field-grown maize. Lillehoj et al (7) conducted a study in Illinois, Missouri, Georgia, and Texas. In their study, ears were either wound-inoculated with *A. flavus*, mechanically damaged, or left untreated and sampled 15, 30, 45, and 70 days later. When the data from their study and our study are compared with respect to time, we wound-inoculated 1 wk earlier and continued sampling approximately 1 wk longer, until 98 days postsilking. Overall, we sampled eight additional times over the season, and three of these sampling periods fell between their 45- and 70-day sampling

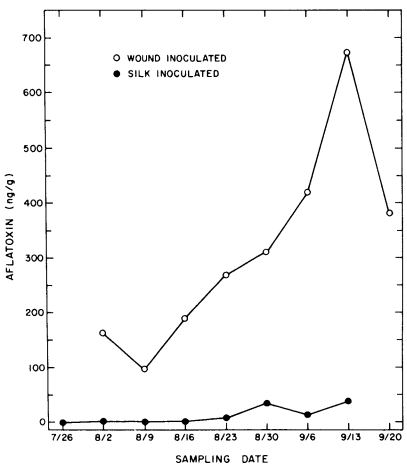

Fig. 1. Aflatoxin accumulation in maize ears silk-inoculated (12 July) and wound-inoculated (27 July) with *Aspergillus flavus* in 1982 at Clayton, NC.

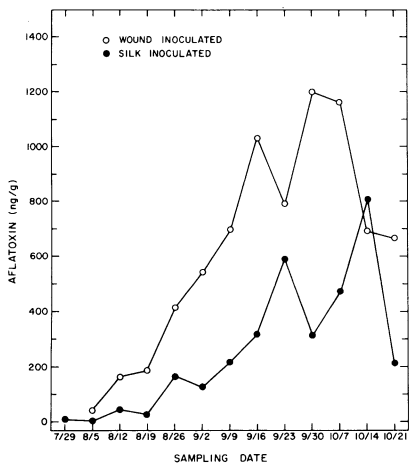

Fig. 2. Aflatoxin accumulation in maize ears silk-inoculated (15 July) and wound-inoculated (26 July) with *Aspergillus flavus* in 1982 at Rocky Mount, NC.

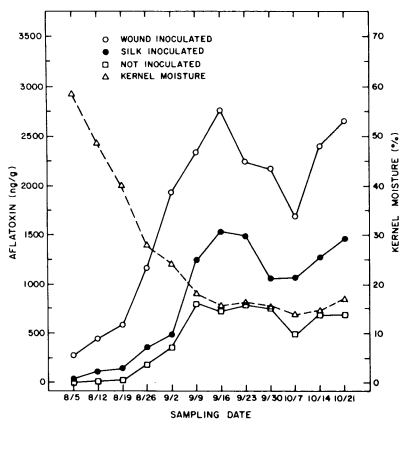

Fig. 3. Aflatoxin accumulation in maize ears silk-inoculated (22 July), wound-inoculated (29 July), and naturally infected with *Aspergillus flavus* in 1983 at Clayton, NC.

times. In their study, they found aflatoxin at 15 days postinoculation and concluded that most of the aflatoxin had accumulated by 30 days after inoculation. Their 30-day postinoculation sampling corresponded to our fifth sampling (50 days postsilking). We also observed high levels of aflatoxin at this time but found higher concentrations at later sampling dates (Figs. 1–3). Accumulation peaked 65 days postsilking (seventh sampling).

In our study, aflatoxin levels in corn ears declined after reaching a peak and increased again later in the season. Lillehoj et al (7) also observed a peak and decline in aflatoxin in three of four states: Georgia, Texas, and Missouri. They may have missed the late-season increase in aflatoxin levels because of their sampling schedule or because there was no late-season increase in moisture.

The decline in the number of kernels infected with *A. flavus* and the decrease in concentration of aflatoxin were unexpected. Such a trend has been reported for another mycotoxin in corn. Miller et al (12) found the counts of *Fusarium graminearum* Schwabe and the concentration of deoxynivalenol to increase rapidly up to 6 wk after inoculation and then to decline sharply. The degradation of deoxynivalenol is thought to be due to its metabolism by the plant, as wheat cell cultures have been shown to degrade deoxynivalenol (11). There is little substantial evidence for degradation of aflatoxin by maize plants. Mertz et al (10) were unable to detect degradation products in maize seedlings grown in Hoagland's solution containing ^{14}C-labeled aflatoxin B_1. Reiss (14) was also unable to show degradation of aflatoxin after exposure of pea and wheat seeds to a solution containing aflatoxin. It is possible that *A. flavus* or another ear-inhabiting fungus degraded the aflatoxin. *Aspergillus parasiticus* Speare, another producer of aflatoxin, has been reported to degrade aflatoxin in culture (3,5).

It is not obvious to us how *A. flavus* could be eliminated from kernels once infection occurred. Because the decline began at different dates in silk- and wound-inoculated ears, it is unlikely the result of errors in sampling or plating procedures. We did not plate injured kernels or kernels with visible sporulation of *A. flavus*. We are unaware, however, of any evidence that insects preferentially feed on kernels infected with *A. flavus*. A more plausible explanation is that some kernels were not extensively colonized by *A. flavus* and the fungus could not compete with other fungi in the ear. There is precedent for a decline in fungal colonization of corn kernels. Miller et al (12) observed a late-season decline in the viable count of *F. graminearum*. They postulated that the decline was due to end-product inhibitions. We are unaware of any evidence for this type of inhibition of *A. flavus*.

The results of our study indicate that aflatoxin can be produced early in the development of kernels of field-grown maize. Maximum levels, however, occurred as the kernel moisture initially declined to approximately 16–21%. This indicates that an effective way to limit aflatoxin contamination in maize is to harvest as early as possible. Maize can be harvested at 30% kernel moisture with little or no yield reduction (1). In 1983, harvesting maize at 28% moisture rather than 16% resulted in 58% less aflatoxin in wound-inoculated ears and 76% less aflatoxin in ears either silk-inoculated or not inoculated.

A second conclusion derived from the data presented is that maize lines should be compared for resistance to aflatoxin contamination near the peak of the linear phase of aflatoxin accumulation. Comparisons made after kernel moisture falls below 21% moisture may be misleading.

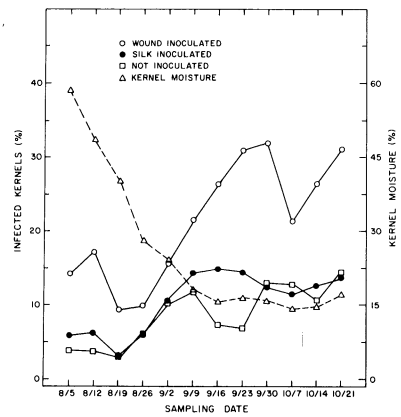

Fig. 4. Percentage of kernel infection in maize ears silk-inoculated, wound-inoculated, and naturally infected with *Aspergillus flavus* in 1983 at Clayton, NC.

Table 1. Relationships between aflatoxin concentration, kernels infected, and kernel moisture at Clayton, NC, in 1983

Comparison	Correlation coefficient (*R*)		
	Natural	Silk	Wound
Aflatoxin concentration (ng/g) × kernel moisture (%)	−0.889**[a]	−0.877**	−0.914**
Kernels infected (%)[b] × kernel moisture (%)	−0.780**	−0.820**	−0.677**
Kernels infected (%) × aflatoxin concentration (ng/g)	0.743**	0.942**	0.785**

[a] ** = Significance at the 1% level.

[b] Percentage of 200 surface-sterilized kernels plated on malt agar + 6% NaCl infected with *Aspergillus flavus*.

LITERATURE CITED

1. Aldrich, S. R., Scott, W. O., and Hoeft, R. G. 1986. Modern Corn Production. 3rd ed. A & L Publications, Inc. Champaign, IL. 358 pp.
2. Anderson, H. W., Nehring, E. W., and Wichser, W. R. 1975. Aflatoxin contamination of corn in the field. J. Agric. Food Chem. 23:775-782.
3. Doyle, M. P., and Marth, E. H. 1978. Aflatoxin is degraded by heated and unheated mycelia, filtrates of homogenized mycelia and filtrates of broth cultures of *Aspergillus parasiticus*. Mycopathologia 64:59-62.
4. Hutchins, J. E., and Hagler, W. M., Jr. 1983. Rapid determination of aflatoxin in heavily contaminated corn. J. Assoc. Off. Anal. Chem. 66:1458-1465.
5. Huynh, V. L., and Lloyd, A. B. 1984. Synthesis and degradation of aflatoxins by *Aspergillus parasiticus*. I. Synthesis of aflatoxin B_1 by young mycelium and its subsequent degradation in aging mycelium. Aust. J. Biol. Sci. 37:37-43.
6. Jones, R. K., Duncan, H. E., and Hamilton, P. B. 1981. Planting date, harvest date, and irrigation effects on infection and aflatoxin production by *Aspergillus flavus* in field corn. Phytopathology 71:810-816.
7. Lillehoj, E. B., Kwolek, W. F., Vandergraft, E. E., Zuber, M. S., Calvert, O. H., Widstrom, N., Futrell, M. C., and Bockholt, A. J. 1975. Aflatoxin production in *Aspergillus flavus* inoculated ears of corn grown at diverse locations. Crop Sci. 15:267-270.
8. Marsh, S. F., and Payne, G. A. 1984. Scanning EM studies on the colonization of dent corn by *Aspergillus flavus*. Phytopathology 74:557-561.
9. McMillian, W. W., Wilson, D. M., and Widstrom, N. W. 1985. Aflatoxin contamination of preharvest corn in Georgia: A six-year study of insect damage and visible *Aspergillus flavus*. J. Environ. Qual. 14:200-202.
10. Mertz, D., Lee, D., Zuber, M., and Lillehoj, E. 1980. Uptake and metabolism of aflatoxin by *Zea mays*. J. Agric. Food Chem. 28:963-966.
11. Miller, J. D., and Arnison, P. G. 1986. Degradation of deoxynivalenol by suspension cultures of the *Fusarium* head blight resistant wheat cultivar Frontana. Can. J. Plant Pathol. 8:147-150.
12. Miller, J. D., Young, C. J., and Trenholm, H. L. 1983. *Fusarium* toxins in field corn. I. Time course of fungal growth and production of deoxynivalenol and other mycotoxins. Can. J. Bot. 61:3080-3087.
13. Payne, G. A., Cassel, D. K., and Adkins, C. R. 1986. Reduction of aflatoxin contamination in corn by irrigation and tillage. Phytopathology 76:679-684.
14. Reiss, J. 1984. Failure of plant tissues to metabolize aflatoxin B_1? Mycopathologia 85:43-44.
15. Sauer, D. B. 1978. Contamination by mycotoxins: When it occurs and how to prevent it. Part 5. Pages 145-158 in: Mycotoxic Fungi, Mycotoxins, Mycotoxicoses. Vol. 3. Mycotoxicoses of Man and Plants: Mycotoxin Control and Regulatory Practices. T. D. Wyllie and L. G. Morehouse, eds. Marcel Dekker, Inc., New York.
16. Thompson, D. L., Payne, G. A., Lillehoj, E. B., and Zuber, M. S. 1983. Early appearance of aflatoxin in developing corn kernels after inoculation with *Aspergillus flavus*. Plant Dis. 67:1321-1322.
17. Zuber, M. S., and Lillehoj, E. B. 1979. Status of the aflatoxin problem in corn. J. Environ. Qual. 8:1-5.