Early Appearance of Aflatoxin in Developing Corn Kernels After Inoculation with *Aspergillus flavus*

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ABSTRACT

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 Disease 67:1321-1322.

Developing kernels of corn (*Zea mays* L.) of the cultivar Gaspe × W103 were inoculated with *Aspergillus flavus* Link ex Fr., grown in four postinoculation regimes, harvested at weekly intervals from 2 to 37 days after inoculation, and assayed for aflatoxin B_1. Aflatoxin B_1 levels averaged 238 ppb 2 days after inoculation and 2,482 ppb for all other dates. Aflatoxin levels were near maximum 9 days after inoculation and accumulations were minimal after that time. There was no evidence of a temperature effect in the range of 13.5–21.5 C thermal units per day.

Aflatoxin contamination of preharvest corn (*Zea mays* L.) continues to be a serious problem in the southeastern United States (13). Infection of developing kernels by *Aspergillus flavus* Link ex Fr. and subsequent production of aflatoxin is influenced by many factors. Temperature is one factor that appears to influence aflatoxin production in grain. The warmer temperatures of the Southeast have been implicated as a major reason for higher levels of aflatoxin in the Southeast than in the Midwest (13). In support of this hypothesis, Thompson et al (12) found a general trend toward increased toxin with increased temperature over a range of 9.5–17.5 thermal units per day and suggested that temperatures higher than those used may give higher toxin levels.

The purpose of this study was to examine aflatoxin accumulation in developing kernels as affected by four temperature regimes and six harvest dates after inoculation.

Cooperative investigations of USDA, ARS, and the North Carolina Agricultural Research Service, Raleigh 27650; the Southern Regional Research Center, New Orleans, LA 70170; and the Missouri Agricultural Experiment Station, Columbia 65201.

Paper 8789 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh 27650.

Accepted for publication 26 May 1983.

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MATERIALS AND METHODS

This study was conducted in controlled-environment rooms at the Southeastern Plant Environment Laboratories, Raleigh, NC. Lighting and other environmental aspects of the rooms have been described (3).

Plants of the corn cultivar Gaspe × W103 were grown singly in 20.3-cm plastic pots (4,000 ml) in a substrate of one-third gravel and two-thirds Peat-lite. Before inoculation, plants were grown at the day/night temperature regime of 26/22 C with an interrupted night to provide long day length. Plants were irrigated twice each day with a modified half-strength Hoagland's solution (3). A treatment unit consisted of five plants. Each of two replicates was grown at a different time.

At silking, Gaspe × W103 plants averaged 169 cm in height with 11.4 leaves per plant and an average ear height of 61 cm. Pollen shedding and silking occurred at an average of 38 and 41 days after planting, respectively. W103 is an early yellow dent inbred and Gaspe is a partly inbred line from the accession PI 214279 Gaspe Flint.

Ears were inoculated 24 days after silking, when the kernels were in the late milk to early dough stage. Husks were pulled back on one side of the ear and the two adjacent rows of kernels were wounded with a pinboard containing a single line of pins spaced 5 mm apart. After wounding, the injured kernels were sprayed with 0.5 ml of an *A. flavus* (NRRL 3357) spore suspension containing 10^6 spores per milliliter. Husks were repositioned, secured, and covered with plastic and paper bags to maintain humid conditions. After 3 days, the plastic bags were removed. Plants were subjected to four postinoculation temperature regimes immediately after inoculation.

Four postinoculation temperature regimes were defined in thermal units (Table 1), which were calculated as $\{[(\text{day temp.} \times 9 \text{ hr}) + (\text{night temp.} \times 15 \text{ hr})]/24\} - 10$ C. For each replicate, half of the plants were moved morning and evening between the two rooms to achieve the four temperature regimes. One room was set to a day/night temperature of 26/22 C and the other at 34/30 C with day/night times of 9/15 hr and uninterrupted night to give a short day length effect.

Ears were harvested in the shuck, dried for 1 wk at 60 C, bulked, and shelled for each cultivar-replicate treatment. Kernels were ground, blended, and assayed for aflatoxin by the Official First Action Method of AOAC, Sections 26.049–26.051, and 26.075 (1). Quantities of aflatoxin B_1 were determined on activated thin-layer chromatographic (TLC) plates coated with 0.5 mm of Absorbosil-1. Plates were developed with water:acetone:chloroform (1.5:12:88, v/v/v) in unequilibrated tanks and fluorescent zones were measured densitometrically (2). Aflatoxin B_1 was confirmed in representative positive samples by formation of water adduct (1).

Aflatoxin B_1 values were transformed to logarithms for analyses of variance according to a fixed-effects model. The factorial effects (harvest dates and thermal units per day) were tested. The least significant ratio (LSR) was calculated as the antilogarithm of the LSD of the transformed data (11) for comparison of harvest means and is presented to reflect the precision of the experiment. Summary estimates were retransformed into original units (ppb) for Table 1.

RESULTS AND DISCUSSION

Means for aflatoxin B_1 concentrations are shown in Table 1. The only significant response in the treatments ($P = 0.01$) was the increase in aflatoxin between 2 and 9 days after inoculation; therefore, the response surface is adequately represented by a single step from the low level of 238 ppb 2 days after inoculation to a plateau of about 2,000 ppb at 9 days and beyond. There were no differences in response after 9 days or over the four levels of thermal units per day, and there was not a significant interaction. Levels among these five dates and four temperature

Table 1. Mean concentrations of aflatoxin B_1 in developing corn kernels grown in four postinoculation temperature regimes and harvested at weekly intervals from 2 to 37 days after inoculation

Postinoculation temperature regime day/night[a] (C)	Thermal units per day (C)	Aflatoxin (ppb) at the following days after inoculation						Mean
		2	9	16	23	30	37	
26/22	13.5	297	1,662	2,179	1,909	1,548	4,527	1,553
34/22	16.5	244	3,479	2,218	2,518	2,720	1,489	1,636
26/30	18.5	220	1,983	3,411	1,930	3,995	3,347	1,837
34/30	21.5	201	1,965	2,003	2,187	2,671	1,902	1,436
Mean		238	2,166	2,397	2,122	2,589	2,559	1,609

LSR:[b] harvest means 2.03

[a] Regime represents 9 hr at the day temperature and 15 hr at the night temperature.
[b] LSR = least significant ratio. Means whose ratios exceed the LSR values are significantly different ($P = 0.05$).

regimes ranged from 1,489 to 4,527 and averaged 2,482 ppb.

These observations of detectable toxin levels in developing corn kernels at 2 days are consistent with observations on aflatoxin production in culture by *A. flavus* and *A. parasiticus* Speare. Detectable levels of aflatoxin have been reported at 2 days on a defined medium (8) and on autoclaved cottonseed, peanuts, and rice (10).

Similarly, our finding of maximum toxin production at 9 days in developing corn kernels is consistent with studies on toxin production in culture. Reddy et al (8) reported maximum aflatoxin in a defined, stationary medium in 8 days. Schroeder and Hein (10) found toxin production to be maximum on cottonseed and peanuts in 9 days and on rough rice in 10 days.

About 9 days after inoculation appears to be sufficient time for aflatoxin accumulation in developing kernels. One should be cautious when relating these results to field conditions, however, because secondary spread of the fungus can occur in the field.

Our temperature regimes ranged from 13.5 to 21.5 C thermal units per day but differences among them for aflatoxin were not significant ($P = 0.05$). In a previous study (12), 17.5 C thermal units per day (the highest included) gave the highest aflatoxin levels; however, toxin levels for 14.5 and 17.5 C thermal units per day were not significantly different ($P = 0.05$). Apparently, increasing temperatures above about 13.5 C thermal units per day has little effect on aflatoxin production.

These temperature observations are in the range reported for the fungus in culture. Both *A. flavus* and *A. parasiticus* produce maximum toxin near 25 C and have an optimum temperature for toxin production lower than the optimum temperature for growth (5,9). A constant temperature of 25 C equals 15 C thermal units per day as defined in this paper.

Plant condition ratings, which were made to describe plants at harvest as to the percentage of green tissue relative to full green, generally decreased as time and temperature increased. There was no apparent association between plant condition ratings at harvest and aflatoxin levels.

Although there was no increase in aflatoxin production at temperatures greater than 13.5 thermal units per day in this study, temperatures higher than this may be important in aflatoxin contamination in the field. Because *A. flavus* has a temperature optimum of 36–38 C for growth (5), up to 28 thermal units per day should provide conditions more conducive for fungal growth and inoculum production. Furthermore, heat inputs as high as 21.5 thermal units per day are required for maximum silk colonization and direct kernel infection by *A. flavus* (6). Neither inoculum level nor direct infection was important in this study because kernels were wounded and inoculated with high levels of inoculum applied directly to the wounds. Another important aspect of high temperature is its role in plant stress. Drought stress, which is intensified by high temperatures, has been shown to increase toxin levels (4,7).

The main findings of this study were that aflatoxin was detected in developing kernels 2 days after inoculation, that levels were near maximum 9 days after inoculation with minimal accumulations thereafter, and that concentrations were not significantly affected by temperatures that ranged from 13.5 to 21.5 C thermal units per day.

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