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

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ABSTRACT

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. Aflatoxin B1 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.

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Four postinoculation temperature regimes were defined in thermal units (Table 1), which were calculated as $\left[\frac{(\text{day temp.} \times 9 \text{ hr}) + (\text{night temp.} \times 15 \text{ hr})}{24}\right]$ 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 B1 were determined on activated thin-layer chromatographic (TLC) plates coated with 0.5 mm of Absorbsil-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 B1 was confirmed in representative positive samples by formation of water adduct (1).

Aflatoxin B1 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 B1 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
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* Spear. 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.

<table>
<thead>
<tr>
<th>Postinoculation temperature regime day/night*</th>
<th>Thermal units per day</th>
<th>Aflatoxin (ppb) at the following days after inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>(C)</td>
<td>(C)</td>
<td>2</td>
</tr>
<tr>
<td>26/22</td>
<td>13.5</td>
<td>297</td>
</tr>
<tr>
<td>34/22</td>
<td>16.5</td>
<td>244</td>
</tr>
<tr>
<td>26/30</td>
<td>18.5</td>
<td>220</td>
</tr>
<tr>
<td>34/30</td>
<td>21.5</td>
<td>201</td>
</tr>
<tr>
<td>Mean</td>
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<td>238</td>
</tr>
</tbody>
</table>

LSR:* harvest means 2.03

*Regime represents 9 hr at the day temperature and 15 hr at the night temperature.

*LRS = least significant ratio. Means whose ratios exceed the LSR values are significantly different (*P* = 0.05).

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Aflatoxin production (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.5</td>
<td>2,003</td>
</tr>
<tr>
<td>16.5</td>
<td>2,187</td>
</tr>
<tr>
<td>18.5</td>
<td>2,671</td>
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<tr>
<td>21.5</td>
<td>1,902</td>
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<tr>
<td>Mean</td>
<td>2,166</td>
</tr>
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</table>

LITERATURE CITED


