ASPERGILLUS FLAVUS INFECTION AND AFLATOXIN PRODUCTION IN MIXTURES OF HIGH-MOISTURE AND DRY MAIZE*

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Abstract—High-moisture (26.6-27.9\% m.c.) and dry (9.8\% m.c.) fractions of white and yellow maize were examined for fungal development and aflatoxin production during an 8-week incubation at 25°C. Treatment procedures included blending of either high-moisture white with dry yellow or high-moisture yellow with dry white maize fractions (average moisture in blend, 14\%) and inoculation of some test maize with A. flavus spores. At sampling time white and yellow components of maize blends were manually separated and all of the maize samples were analyzed for levels of moisture, fungal infection and aflatoxin. Moisture levels in maize blends equilibrated rapidly during the initial 2-4 days of incubation; neither dry yellow nor dry white exceeded 13\% moisture during the trial period. Only a limited incidence of A. flavus was observed on uninoculated maize, but in samples treated with A. flavus spores a high infection rate developed; from 58 to 98\% of the kernels in dry fractions of inoculated blends were infected with A. flavus during the trial. Aflatoxin was detected in high-moisture maize and in both high-moisture and dry fractions of inoculated maize blends. Up to 500 \(\mu\)g aflatoxin B\(_1\)/kg of corn was found after the 8-week incubation in a dry fraction of inoculated maize blends.

INTRODUCTION

Average moisture levels and the distribution of moisture in harvested grain are major factors determining storability (Christensen and Kaufmann, 1969; Koehler, 1938). Heterogeneity of moisture levels may reflect pre-harvest crop conditions, meteorological events during harvest, or handling techniques employed during and after harvest. Grain entering commercial channels is often intentionally mixed to achieve: (1) an average moisture level required for a specific grade, (2) a specific grade by blending lower quality with higher quality grain and (3) extension of storage time for high-moisture grain through blending with dry grain. Mixing of grain to achieve an average “safe” moisture level produces an initially heterogeneous blend in which some kernels contain sufficient moisture for fungal growth. In addition, grain fractions in a blend may be infected with fungi that continue to develop during moisture equilibration or drying and therefore become a source of inoculum for the entire bulk of the commodity.

Several studies (Hart, 1967; Hubbard et al., 1957; White et al., 1972) have shown that moisture levels of high-moisture and low-moisture grain fractions equilibrate after blending. However, moisture contents of the fractions vary at equilibrium depending on whether the grain was adsorbing or desorbing moisture during the equilibration. Retention of an elevated final moisture level by high-moisture grain relative to drier seed has been attributed to a hysteresis effect. Hart (1964) utilized dyed seed as a marker for post-incubation separation of wheat samples. He observed that fractions of a mixture having original moisture differences of 4\% or more possessed 0.76 of a percentage point difference at equilibrium. Other workers (White et al., 1972) have shown that most of the moisture exchange in maize blends occurs during the initial 12-24 hr after mixing. The rate of equilibration is dependent on the initial moisture levels of maize fractions and storage temperature.

* Mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over firms or similar products not mentioned.
HART (1967) observed that mixing of overdried, shelled maize with undried samples to a mean of 15.5% moisture provided conditions more conducive to fungal development than a homogeneous sample at the same moisture level. However, no specific information on fungal spp. was provided. Deterioration of grain in storage is generally attributed to infection by *Aspergillus* and *Penicillium* spp. (CHRISTENSEN, 1957; QASEM and CHRISTENSEN, 1958, 1960); these fungi predominate on grain stored at 13-18% moisture. In prior studies we have examined *A. flavus* infection of maize kernels and subsequent aflatoxin production (FENNELL et al., 1973). Other workers have characterized the moisture requirements in unblended maize samples for *A. flavus* infection (CHRISTENSEN and KAUFMANN, 1969; KOEHLER, 1938; LOPÉZ and CHRISTENSEN, 1967). Our objective in this investigation was to acquire information on the spread of *A. flavus* infection and aflatoxin production in high- and low-moisture fractions of maize blends with average moisture levels of 15.5% or below.

### MATERIALS AND METHODS

Maize was acquired directly from fields in Sangamon County, Illinois, on 2 October 1973. Five-bushel samples of white and yellow maize were harvested, shelled in a small laboratory-scale, mechanical sheller, and fractions of the shelled maize were dried immediately in forced-draft chambers at 60°C for 16 hr to below 10% moisture. Initial moisture determinations were made with a Motomco meter and subsequent moisture level tests were carried out by the air-oven method (ANON., 1962). Moisture levels of test maize at the start of the experiment were: high-moisture (h-m) white, 26.6%; dry (d) white, 9.8%; h-m yellow, 27.9%; and d yellow, 9.8%. Bushel lots of blended maize were prepared by blending: (1) 14 lb (6.3 kg) h-m white + 42 lb (19.0 kg) d yellow (mean moisture = 14.0%); and (2) 13 lb (5.9 kg) h-m yellow + 43 lb (19.5 kg) d white (mean moisture = 14.0%) in a Patterson-Kelley (P-K) twin-shell blender.

High-moisture and dry white and yellow maizes were examined for *A. flavus* at the start by plating 100 surface-sterilized kernels of each. Throughout the experiment, kernels for microbiological tests were surface-sterilized with 1% sodium hypochlorite (1 min), rinsed twice with sterile water and placed on ME agar (malt extract, 30 g/l and agar, 15 g/l) in Petri plates; test kernels were incubated at 28°C for 5 days and examined for fungal development with a stereoscopic microscope.

Spore inoculum of an aflatoxin-producing strain of *A. flavus*, NRRL 3357, was prepared on cracked maize in 500 ml Erlenmeyer flasks (25 g maize, 9 ml water). The substrate was autoclaved with subsequent inoculation of each flask with a 1-ml spore suspension. The inoculated maize was incubated at 28°C for 2 weeks and dried at 60°C for 3 days before use. The contents of a single flask of *A. flavus*-contaminated maize were distributed in 28 lb (12.7 kg) of test maize by mixing in the P-K blender.

Ten treatment procedures were employed: (1) d white; (2) h-m white; (3) d yellow; (4) h-m yellow; (5) blend, h-m yellow and d white; (6) blend, d yellow and h-m white; (7) blend inoculated with *A. flavus*, h-m yellow and d white; (8) blend inoculated with *A. flavus*, d yellow and h-m white; (9) h-m white inoculated with *A. flavus*; and (10) h-m yellow inoculated with *A. flavus*. Test maize for each treatment was distributed in 12 2-l. beakers (900 g/beaker), covered with cheesecloth and incubated at 25°C. Samples of h-m and d fractions of blends were separated manually after the incubation periods of 2, 6, 9, 16, 30 and 58 days; at these sampling times, 25 kernels were selected at random from two replicate beakers of each treatment for microbiological tests, and representative 50-g samples were taken from each beaker for moisture determination and aflatoxin assay. The aflatoxin detection technique was essentially the one described by DANTZMAN and STOLOFF (1972); a 50-g sample of maize was extracted with 250 ml of chloroform and 25 ml water in a Waring Blender for 3 min. The extract was filtered, dried with anhydrous sodium sulfate and reduced in volume by vacuum evaporation. Aflatoxin levels were determined by comparison with reference standards on developed thin-layer chromatographic plates (0.5 mm Adsorbisil-I, silica gel). Aflatoxins B₁ and
Infection and Aflatoxin Production in Dry Maize

RESULTS

Moisture determinations were made on maize samples of freshly harvested, h-m and d, white and yellow maize, singly and in blends during an 8-week test period (Fig. 1). In treatments 2 and 4 (Fig. 1a) moisture levels in h-m white (26.6%) and yellow (27.9%) maize decreased during the test to about 15%. Both white and yellow dry maize (treatments 1 and 3) decreased gradually from an initial level of 9.8% to slightly below 8% moisture. In the blend of h-m yellow and d white of treatment 5, most of the moisture equilibration occurred during the initial 2-4 days of incubation (Fig. 1b), with h-m yellow decreasing to a final level of about 10%. The moisture level in the d white fraction increased to 11.6% during the initial 6 days of the test, then decreased during the remainder of the incubation. Moisture decrease during the later stages of incubation was attributed to aggregate drying in the ambient atmosphere. A similar moisture pattern was observed in the h-m white and d yellow blend of treatment 6 and in the inoculated blends of treatments 7 and 8. Neither the d yellow nor the d white constituent of any blend exceeded 13% moisture during the trial period.

Germination tests were carried out on maize from individual treatments to evaluate the physiological effects of fungal infection on the seed during the incubation period (Christensen and Kaufmann, 1969; Qasem and Christensen, 1958). The initial 90% germination rate exhibited by freshly harvested h-m white maize (Fig. 2a) was reduced...
only slightly during incubation in maize blends (treatments 6 and 8) but to a greater degree in non-blended samples (treatments 2 and 9). In contrast, germination levels of d white maize declined more in blended samples (treatments 5 and 7) than in non-blended maize of treatment 1. Similar germination patterns were observed for yellow maize fractions during the incubation (Fig. 2b).

Test kernels from every h-m and d maize treatment were examined at each sampling time for the presence of *A. flavus*. The incidence of the fungi routinely found in maize (*Fusarium*, *Cephalosporium* and *Penicillium*) was also studied in an attempt to gain some knowledge on the possible interaction between *A. flavus* and the natural mycoflora of the seed. At the outset of the experiment, 1% of both d and h-m yellow maize was infected with *A. flavus* (Fig. 3) but the fungus was not found in white maize. Dry white maize (treatment 1) remained essentially free of *A. flavus* throughout the experiment but some development of the fungus was observed in h-m white samples (treatment 2). Similarly, d yellow maize (treatment 3) did not support significant *A. flavus* proliferation during the trial but the fungus infected h-m yellow seed of treatment 4. A limited increase in *A. flavus* infection occurred in blends of treatments 5 and 6 with the naturally occurring inoculum. However in the inoculated blends (treatments 7 and 8), the incidence of *A. flavus* inside the kernel increased significantly during the initial stage of the incubation. After 8 weeks the levels of *A. flavus*-contaminated kernels in treatments 7 and 8 ranged from 78 to 80% in the h-m fractions and 58 to 98% in the dry constituents. In h-m, unblended, inoculated white and yellow maize (treatments 9 and 10) the internal *A. flavus* rate increased to 100% during the trial.

The number of fungus-free kernels ranged initially from 0 to 5% and increased in dry fractions during the experiment. Initial incidence of *Fusarium* spp. was significantly higher on both d and h-m white maize than on comparable yellow kernels. Percentage
Infection and Aflatoxin Production in Dry Maize

![Graph showing fungal infection distribution](image)

**Fig. 3.** Distribution of fungal-infected test kernels during an 8-week incubation period. 1w = dry white, 2w = high-moisture white, 3y = dry yellow, 4y = high-moisture yellow; 5w = dry white (blend), 5y = high-moisture yellow (blend), 6w = high-moisture white (blend), 6y = dry yellow (blend), 7w = dry white (blend), 7y = high-moisture yellow (blend), 8w = high-moisture white (blend), 8y = dry yellow (blend), 9w = high-moisture white and 10y = high-moisture yellow. Treatments 1w, 2w, 3y, 4y, 5w, 5y, 6w and 6y were not inoculated with *A. flavus*. Treatments 7w, 7y, 8w, 8y, 9w and 10y were inoculated with *A. flavus*. Each bar represents the percentage of test kernels infected with certain fungi or fungal-free at the following stages of incubation; 0, 2, 6, 9, 30 and 58 days.

of seeds internally infected with this fungus declined in dry grain of treatment 1 and both h-m and d fractions of treatments 5 and 8. Comparison of the number of *Fusarium*-infected kernels with the number of fungus-free kernels in the dry maize of treatment 1 suggests that the decreased incidence of *Fusarium* spp. accounts for the increased number of kernels with no fungi.

In contrast to *Fusarium* infection rates, the occurrence of *Cephalosporium* sp. increased on dry grain (treatments 1 and 3) and remained nearly constant or decreased on h-m maize of treatments 2 and 4 (Fig. 3). The common *Fusarium* spp. outgrow most other fungi on test plates; in their presence enumeration of *Cephalosporium*-infected kernels...
Table 1. Aflatoxin production on dry or high-moisture, yellow and white maize and mixtures of maize during an 8-week incubation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Aflatoxin B₁ (µg/kg)**</th>
<th>Incubation (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>6</td>
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<tr>
<td>1. d white</td>
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<tr>
<td>2. h-m white</td>
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<td>20</td>
</tr>
<tr>
<td>3. d yellow</td>
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</tr>
<tr>
<td>4. h-m yellow</td>
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<td>20</td>
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<tr>
<td>5. Blend; d white, h-m yellow</td>
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<tr>
<td>6. Blend; d yellow, h-m white</td>
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<tr>
<td>7. Inoc. blend; d white</td>
<td>---</td>
<td>20</td>
</tr>
<tr>
<td>h-m yellow</td>
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</tr>
<tr>
<td>8. Inoc. blend; d yellow</td>
<td>&lt;20</td>
<td>&gt;20</td>
</tr>
<tr>
<td>h-m white</td>
<td>&lt;20</td>
<td>20</td>
</tr>
<tr>
<td>9. Inoc.; h-m white</td>
<td>100</td>
<td>1000</td>
</tr>
<tr>
<td>10. Inoc.; h-m yellow</td>
<td>200</td>
<td>2500</td>
</tr>
</tbody>
</table>

*At the outset of the experiment (0 day), none of the four test maizea contained detectable levels of aflatoxin; d = dry, h-m = high-moisture, blend = mixtures of d and h-m maize fractions, inoc. = inoculated with A. flavus.

** --- = Aflatoxin B₁ not detected.

might be underestimated. In the maize blends of treatments 5 through 8, there was a gradual increase in the number of Cephalosporium-infected kernels with no clear differences between inoculated and uninoculated samples.

The initial incidence of Penicillium spp. was distinctly higher than that of A. flavus and lower than Fusarium spp. except in d yellow maize. During the experiment Penicillium infection decreased on dry samples of both white and yellow maize and increased on high-moisture seed of treatments 1 through 4. Although the number of Penicillium-infected kernels did not change extensively in uninoculated maize blends of treatments 5 and 6 (the exception was d yellow of treatment 6), a decreasing trend was observed in both d and h-m fractions of inoculated blends (treatments 7 and 8).

Elaboration of aflatoxin was followed during the 8-week incubation period (Table 1). Toxin was detected in both uninoculated and inoculated h-m maize (treatments 2, 4, 9 and 10) and in both h-m and d constituents of inoculated blends (treatments 7 and 8); the fungal metabolite was found after 2 days in treatments 8, 9 and 10 and after 6 days in treatments 2, 4 and 7, with 9 days of incubation required before the toxin was detected in treatment 2. Highest yields occurred in the h-m maize of treatments 9 and 10, with final levels exceeding 15,000 µg B₁/kg. Although aflatoxin was detected early in the incubation of both the h-m white and d yellow maize of treatment 8, the d yellow fraction of the blend yielded toxin levels of 100 µg/kg after 9 days whereas the h-m white produced a comparable quantity of toxin only after 58 days. Aflatoxin was produced in both the d and h-m fractions of treatment 7 but yields were lower than in treatment 8. In test maize that supported maximum aflatoxin production, the toxin was elaborated rapidly during the initial 16 days of incubation but proceeded at a reduced rate thereafter.
DISCUSSION

The results of our laboratory tests show that *A. flavus* will infect and subsequently produce aflatoxin in the dry fractions of heavily inoculated maize blends that have a mean moisture level of 14% or less. Both dry and high-moisture components of inoculated blended maize in treatments 7 and 8 became heavily infected by the fungus during the trial, and aflatoxin accumulation was observed in all of the maize fractions. Dry maize of treatments 1 and 3 and both dry and high-moisture fractions of the uninoculated blended maize in treatments 5 and 6 failed to support significant *A. flavus* infection or detectable toxin production. The highest final incidence of internal infection by *A. flavus* and the maximum yields of aflatoxin occurred in inoculated high-moisture maize of treatments 9 and 10. A limited *A. flavus* increase and aflatoxin production of 500-1000 μg B<sub>1</sub>/kg were observed in uninoculated, high-moisture maize of treatments 2 and 4.

A critical observation made in this study was the increase in *A. flavus* infection and aflatoxin production in the dry maize fractions of inoculated maize blends (treatments 7 and 8) although the moisture levels in the dry components did not exceed 13% moisture during the trial period. Previous studies of *A. flavus* moisture requirements in unblended maize had shown that levels of 17.5% were necessary for growth of the fungus (Lopez and Christensen, 1967). It appears that distinctly lower average levels of moisture will support *A. flavus* development in maize blends that are heterogeneous in terms of moisture distribution. Pre-requisite conditions for spore germination and germ tube growth such as relative humidity, temperature, and spore densities, may determine critical aspects of initial *A. flavus* growth requirements with a subsequent decrease in environmental restrictions on the developing fungus. Christensen and Kaufmann (1969) relate, "Once the fungi have invaded grain, they will continue to grow in it at a lower moisture content than they otherwise would."

We found significant differences in aflatoxin content between the components of the two inoculated maize blends of treatments 7 and 8. High-moisture yellow maize of treatment 7 contained more aflatoxin at the end of the trial period than the dry white component; the reverse was true in treatment 8, i.e. h-m white had a lower level of aflatoxin than d yellow. Similarly, yellow maize in treatments 7 and 8 whether high-moisture or dry, contained more kernels infected with *A. flavus* at the end of the trial than did white maize. The enhanced occurrence of *A. flavus* in yellow maize of inoculated blends may reflect its initial 1% natural incidence in this maize. Yellow maize in all treatments had a higher incidence of *A. flavus* infection and supported larger yields of aflatoxin than white maize; this together with the 1% natural incidence of the fungus in yellow corn suggests that the variety of yellow maize utilized in the test is inherently more susceptible to *A. flavus* infection and aflatoxin contamination than the white maize variety.

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REFERENCES


