# Identification of Aflatoxin-Producing Atmospheric Isolates of *Aspergillus flavus*

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## ABSTRACT

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Qualitative and quantitative air samplers were used to collect aflatoxin-producing isolates of *Aspergillus flavus* from several corn production sites in Missouri. Coconut agar provided rapid, and effective qualitative identification of isolates that produced aflatoxin. During the 3-yr period 1976–1978, aflatoxin-producing isolates were obtained from all sampling sites in the state. Of 31 isolates collected by air samplers in 1977, 20 (65%) produced aflatoxin.

*Additional key words:* aerobiology, spore detection.

Air samplers are very effective collectors of spores of *Aspergillus flavus* Link ex Fries and closely related species that will infest corn kernels (6) and may produce aflatoxin (7). Some strains of *A. flavus* do not produce detectable aflatoxin, so a method of determining the percentage of isolates of the fungus from a given area that produce aflatoxin is essential to predicting the potential hazard to corn. In this investigation, a method was sought for the rapid detection of such isolates so that accurate predictions of the potential hazard might be issued to corn growers. *Aspergillus* differential medium (ADM) (4) and a baiting method employing "decapped" corn kernels (10) were used to identify *A. flavus* spores caught by air samplers; and the aflatoxin-producing ability of the isolates was determined by chemical analyses of infested "decapped" corn kernels or by a qualitative test with coconut agar (9).

## MATERIALS AND METHODS

The Rotorod, Burkard, and Andersen air samplers used in this study have been described elsewhere (1,2,5). These samplers were operated adjacent to corn fields at several sites in Missouri during a 3-yr period, 1976–1978 (Fig. 1). The Rotorod sampler was operated at the .3- and 1.8-m levels above the soil surface, for 15 min, 2 days per week during the growing season, usually from the 1st wk of May through August or the last of September depending upon the season. A Burkard sampler was operated continuously during the growing season adjacent to the Rotorod samplers at Novelty in all 3 yr, and at the Bradford farm in 1977. An Andersen sampler was operated for 5 min, once per week at Novelty in 1977 and twice per week at Novelty, McCredie, and the Bradford farm in 1978. Collection surfaces were strips of double-stick cellophane tape for the Burkard and Rotorod samplers, and petri dishes filled with *Aspergillus* differential medium (ADM) for the Andersen sampler.

In 1976, only the Rotorod and Burkard samplers were operated. The presence of *A. flavus* spores on collection tapes was detected by use of "decapped" corn kernels to induce germination, colonization, and sporulation during incubation for 1 wk at 27 C in moist petri dishes. The aflatoxin-producing ability of *A. flavus* identified in this manner was determined by chemical analyses of the corn kernels that exhibited sporulation under incandescent light

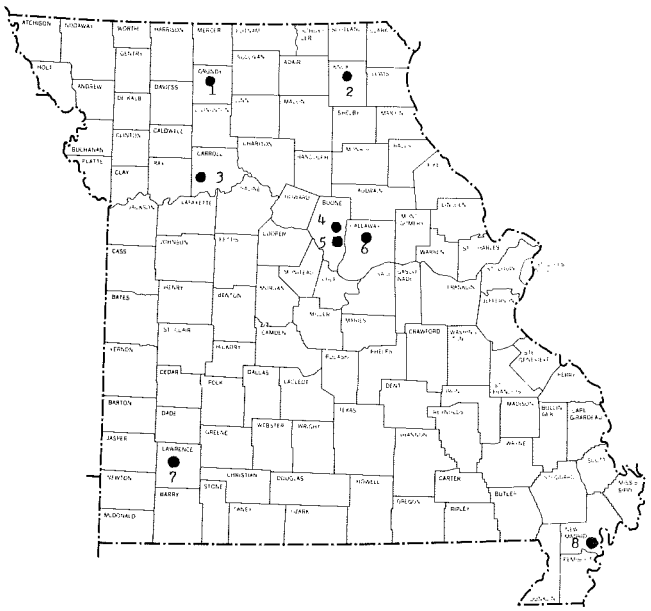


**Fig. 1.** Sites of atmospheric samplers in Missouri (●). Locations: 1–Spickard, 2–Novelty, 3–Norburne, 4–Columbia, 5–Bradford Farm. 6–McCredie, 7–Mt. Vernon, and 8–Portageville. Rotorod air samplers were operated at locations 1, 2, 4, 7, and 8 in 1976; 1, 2, 3, 4, 5, 6, 7, and 8 in 1977; and 1, 2, 4, 5, 6, 7, and 8 in 1978. Burkard air samplers were operated at location 2 in 1976, locations 2 and 5 in 1977, and location 2 in 1978. Andersen air samplers were operated at location 2 in 1977 and locations 2, 5, and 6 in 1978.

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or bright greenish-yellow fluorescence (BGYF) under longwave ultraviolet light. Such BGYF has been reported to be presumptive evidence of aflatoxin production (8). Aflatoxin analyses were conducted at the USDA Northern Regional Research Center, Peoria, Illinois, by the official method of the AOAC (3).

In 1977 all three kinds of samplers were used. *A. flavus* spores on collection tapes of the Rotorod and Burkard samplers were detected by using the same method as in 1976. Viable *A. flavus* spores collected by the Andersen sampler resulted in characteristic colonies on the ADM collection plates after incubation for 3 days at 28 C. Mycelial plugs from these *A. flavus* colonies, and "decapped" kernels infested with *A. flavus* from the Rotorod and Burkard tapes were transferred to plates containing coconut agar.

In 1978 all three samplers were used again. However, *A. flavus* spores on collection tapes of the Rotorod and Burkard samplers were detected by placing the tapes exposed-side down on the agar surface of ADM plates, incubating, and observing characteristic *A. flavus* colonies. *A. flavus* spores collected by the Andersen sampler were detected as in 1977 by using ADM. Mycelial plugs from *A. flavus* colonies on ADM were transferred to coconut agar plates.

The recipe for the coconut agar used in this study (9) is: Coconut extract is prepared by blending meat and milk from one fresh coconut with hot water and straining the mixture through cheesecloth. Add 200 ml coconut extract to 600 ml distilled water, adjust pH to 6.9, add 16 g agar, autoclave, and pour the plates when sufficiently cooled.

Coconut agar plates were incubated at 28 C and examined after 3 and 7 days for a characteristic yellow pigment under incandescent light and for blue fluorescence under longwave ultraviolet light—a qualitative test for the presence of aflatoxin (9). The coconut agar from the plates supporting the fungus was analyzed to confirm the presence of aflatoxin.

In 1977, 31 random *A. flavus* isolates from the samplers were cultured and conidial suspensions were sprayed on kernels of five commercial corn hybrids. The kernels were incubated for 1 wk in moist petri dishes, examined for *A. flavus* sporulation, and viewed under longwave ultraviolet light to detect BGYF. Isolates exhibiting BGYF were cultured on coconut agar that was then analyzed for aflatoxin.

## RESULTS

In 1976, aflatoxin was detected in kernels from 11% of the groups of decapped kernels incubated on air sampler tapes from Spickard and 33% of the groups of kernels tested from Mr. Vernon, which indicated the presence of airborne aflatoxin-producing strains at these locations (Table 1). No aflatoxin was detected in decapped kernels incubated on sampler tapes from Portageville, Novelty, or Columbia.

In 1977, examination of isolates incubated on coconut agar demonstrated that isolates from all eight sampling locations produced aflatoxin. The percentage of aflatoxin-producing isolates ranged from 50% at Norburne and McCredie to about 20% at Columbia and Portageville (Table 1).

In 1978, coconut agar again was used to test *A. flavus* isolates for aflatoxin-producing ability. Aflatoxin-producing isolates were collected from the air at six of seven locations (Table 1). The one isolate obtained from Portageville was negative. The percentage of aflatoxin-producing isolates from the other six locations ranged from 50% at Spickard to 86% at Columbia. Chemical analyses of the fluorescing agar confirmed the presence of aflatoxin.

The 31 isolates collected from air sampler tapes in 1977, cultured, sprayed on kernels of five commercial corn hybrids, and incubated, were evaluated for sporulation and the production of BGYF (Table 2). All 31 isolates sporulated and produced BGYF on kernels of the five hybrids. Tests of the ability of the isolates to produce aflatoxin in coconut agar revealed that 20 (65%) of the isolates produced aflatoxin in this qualitative test (Table 2). Chemical analyses of the blue-fluorescing agar confirmed the presence of aflatoxin.

## DISCUSSION

The testing of the isolates obtained from the samplers revealed that over the 3 yr, a large percentage produced BGYF and aflatoxin. Hence, there is a high potential for significant preharvest aflatoxin contamination of corn in the state if favorable environmental conditions prevail.

Coconut agar is useful for qualitative identification of isolates that produce aflatoxin. This method accelerates the testing process

TABLE 1. Percentage of aflatoxin-producing *Aspergillus flavus* isolates from spores collected from the atmosphere at Missouri corn-production sites, 1976–1978

| Location | 1976 | | 1977 | | 1978 | |
|---|---|---|---|---|---|---|
| | Isolates tested (no.) | Isolates producing aflatoxin[a] (%) | Isolates tested (no.) | Isolates producing aflatoxin[a] (%) | Isolates tested (no.) | Isolates producing aflatoxin[a] (%) |
| Spickard | 9 | 11 | 21 | 38 | 2 | 50 |
| Norburne | ... | ... | 8 | 50 | ... | ... |
| Bradford | ... | ... | 48 | 44 | 12 | 75 |
| Portageville | 11 | 0 | 22 | 23 | 1 | 0 |
| McCredie | ... | ... | 4 | 50 | 8 | 75 |
| Mt. Vernon | 6 | 33 | 12 | 33 | 6 | 83 |
| Novelty | 22 | 0 | 39 | 48 | 11 | 64 |
| Columbia | 4 | 0 | 10 | 20 | 7 | 86 |

[a] Qualitative aflatoxin determinations based on thin-layer chromatogrphy of extracts from ground "decapped" corn kernels, which had been incubated on Rotorod or Burkard tapes in a moist chamber.

[b] Qualitative aflatoxin determination based on incubation of *A. flavus* isolates on coconut agar, followed by examination of the agar for characteristic yellow pigment under incandescent light and blue fluorescence under longwave ultraviolet light.

TABLE 2. Aflatoxin-producing ability of 31 *Aspergillus flavus* isolates from spores collected by air samplers at eight locations in Missouri, 1977

| Location | Sampler | Date | Aflatoxin production[a] |
|---|---|---|---|
| Portageville | Rotorod | 27 July | − |
| | | 22 Aug | + |
| | | 12 Sept | − |
| McCredie | Rotorod | 1 Aug | + |
| | | 20 Sept | + |
| Mt. Vernon | Rotorod | 4 July | + |
| | | 8 Sept | + |
| | | 15 Sept | + |
| Bradford | Rotorod | 25 July | + |
| | | 31 Aug | − |
| | | 7 Sept | + |
| | | 7 Sept | + |
| | Burkard | 5 Aug | − |
| Spickard | Rotorod | 12 July | + |
| | | 21 July | + |
| Columbia | Rotorod | 5 Aug | − |
| | | 7 Sept | − |
| Norburne | Rotorod | 13 Sept | + |
| Novelty | Rotorod | 15 July | + |
| | | 2 Aug | + |
| | Burkard | 1 July | + |
| | | 7 Sept | − |
| | | 9 Sept | − |
| | Andersen | 18 July | + |
| | | 26 July | − |
| | | 15 Aug | − |
| | | 15 Aug | − |
| | | 23 Aug | + |
| | | 23 Aug | + |
| | | 4 Oct | + |
| | | 11 Oct | + |

[a] + = aflatoxin production qualitatively indicated after incubation of inoculated corn kernels on coconut agar by observation of characteristic yellow pigment under normal light and blue fluorescence under ultraviolet light, − = yellow pigment and blue fluorescence not detected.

by eliminating the chemical analysis of the corn kernel, growth medium, or other substrate for aflatoxin.

The fact that aflatoxin-producing isolates were obtained from all sampling sites in 1977 and six of seven sites in 1978 is significant, since the samplers were operated for relatively few days and for comparatively few minutes during the growing seasons, which severely reduced the chances of trapping aflatoxin-producing strains in a given location. Given greater resources, the sample size could be expanded considerably over a greater number of days, thus providing a broader data base for predicting the aflatoxin hazard in an area and warning the farmers likely to be affected.

Lastly, these data illustrate that aflatoxin-producing strains of the fungus are omnipresent and pose a potential threat to preharvest corn in Missouri.

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