Incidence and Distribution of Airborne Spores of *Aspergillus flavus* in Missouri

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ABSTRACT

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Airborne spores of *Aspergillus flavus*, a fungus that may produce aflatoxin, were collected with three air samplers at several sites near cornfields in Missouri during 1976–1978. "Decapped" corn kernels and *Aspergillus* differential medium (ADM) were used to detect *A. flavus* spores. The percentage of days on which spores were collected at each site differed from year to year but was never below 17%. In 1976 and 1977, spores were caught on the highest percentage of days at opposite ends of the state (northwest and southeast Missouri). ADM was more efficient than decapped kernels for detecting and estimating the concentration of airborne *A. flavus* spores.

In 1964, a summary of airborne mold surveys (13) ranked *Aspergillus* fourth among dominant airborne spores in frequency; no species were distinguished. Airborne spores of *Aspergillus flavus* Lk. ex Fr. have been identified at various sites (4,8,9,11,14–17). In most cases (4,11, 14–17), spores were collected and identified on solid agar growth medium in petri dishes exposed to the air. In this method, spores are deposited on the agar by gravity and wind; they are not differentiated according to their ability to germinate and grow in corn kernels and produce aflatoxin.

A new method (18) uses "decapped" corn kernels in a presumptive test of material collected by air samplers for growth of *A. flavus* and production of aflatoxin. Furthermore, *Aspergillus* differential medium (ADM) (3,6) has been used for rapid detection and identification of *A. flavus* and related organisms in the *A. flavus* group. We used the decapped kernel technique and ADM to estimate the incidence and distribution of airborne spores of the fungus near Missouri cornfields before, during, and after harvest.

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MATERIALS AND METHODS

Spores were collected with three atmospheric samplers: the Rotorod sampler (Metronics, Palo Alto, CA) (1), the Burkard version of the Hirst volumetric spore trap (Burkard, Rickmansworth, Herts., England) (7), and the Andersen six-stage viable particle sampler (Andersen 2000 Inc., Atlanta, GA) (2).

For the decapped kernel method used in 1976 and 1977, the collecting surfaces (double-stick cellophane tape) from the Rotorod and Hirst samplers were placed exposed-side-up on filter paper in sterile 15-cm petri dishes. Corn kernels were surface disinfested by soaking for 2 min in a 1% solution of sodium hypochlorite, then rinsed twice with sterile distilled water. Tops of the kernels were cut off with a sterile razor blade, and kernels were placed cut-side-down on the tapes in the petri dishes. The filter paper was moistened with sterile distilled water, and the plates were incubated at 27 C for 7 days. Cut ends of kernels were then checked microscopically for *A. flavus* growth and examined under long-wave ultraviolet light for bright greenish yellow fluorescence, which is regarded as a presumptive test for *A. flavus* infestation and aflatoxin contamination (5,12,19).

Aspergillus differential medium was used to detect *A. flavus* on the collecting tapes of the Rotorod and Hirst samplers

in 1978. Sterile toothpicks were aseptically placed on the unexposed surface of tapes to facilitate the rapid removal of the tapes from the Rotorod collecting arms or Hirst collection drum and to aid in the later handling of the sticky tapes. The tapes were placed exposed-side-down (toothpick-side-up) on the surface of ADM agar in plastic petri dishes. *A. flavus* colonies on plates were counted after a 72-hr incubation at 28 C.

In 1977 and 1978, we used the Andersen sampler to test the usefulness of ADM for estimating *A. flavus* spore concentrations. The six collection plates of the sampler each contained 27 ml of ADM. The sampler was operated for exposure periods of 1 and 5 min (5 min only in 1978) on each sampling day. After each exposure, plates were incubated for 72 hr at 28 C, then examined for the characteristic yellow orange pigment under tan, nonsporulating colonies. The number of spores per cubic meter of air was calculated by dividing the total number of *A. flavus* colonies on the six plates by the number of minutes the sampler was operated (flow rate of sampler = 1 ft^3/min) and then multiplying the resulting number by 35.315 to convert from cubic feet to cubic meters (35.315 ft^3 = 1 m^3).

When characteristic colonies had been counted, mycelial plugs from individual colonies were transferred to Czapek solution agar for species verification. Isolates obtained from the air samplers were also tested for their ability to produce aflatoxin; results of these tests have been published (10).

Samplers were located next to cornfields at sites indicated in Table 1. Rotorod samplers were placed 0.3 and 1.8 m above the soil surface and were operated for 15-min periods 2 days each week from time of planting until a few weeks after harvest (1 day per week at Norburne in 1977 and McCredie in 1977 and 1978). At some locations, a Hirst sampler was placed next to the Rotorod samplers and operated continuously from time of planting until after harvest (about 15 May–1 November). Hirst collection drums were changed weekly, so that exposed tapes of the Hirst sampler represented spores collected from 8:00 a.m. Monday to 8:00 a.m. the following Monday.

RESULTS

A. flavus was a common spore component of the air in Missouri during the 1976–1978 growing seasons. The fungus was collected each year from every location sampled. The percentage of days on which spores were collected varied from year to year but never fell below 17%. The number of days the samplers were operated and the percentage of days that spores were detected at each location in each year are presented in Table 1.

In 1976 and 1977, spores were collected on more than 70% of the days on which air samplers were run at Spickard and Portageville (at the northwest and southeast ends, respectively, of the state), but on less than 41% of the days at Mt. Vernon. In 1978, spores were collected on 88% of the sampling dates at McCredie. In contrast to the two earlier years, spores were collected on 30% and 17% of the sampling days at Spickard and Portageville, respectively, in 1978, and on 54% of the days at Mt. Vernon.

Atmospheric spore concentrations. In 1977 at Novelty, *A. flavus* spores were collected on ADM with the Andersen sampler on 45% of the sampling days; on days when the fungus was detected, viable spores per cubic meter of air averaged 22.7 (Table 2). Spore concentrations were highest in June and October and lowest in August and September (Fig. 1).

On days in 1977 when the concentration calculated with the Andersen sampler was greater than 35.0 spores per cubic meter, qualitative data from the Hirst and Rotorod samplers also indicated the presence of *A. flavus* spores in the air. When the concentration was less than 35.0, data from the samplers were not in complete agreement. However, because the Andersen sampler was run infrequently (1 day/wk), great weight cannot be placed on these comparisons.

In 1978, *A. flavus* spores were collected on 43% of the days the Andersen sampler was operated at Novelty (Table 2); viable spores per cubic meter of air averaged 23.9, and spore count was highest (56.5) on 11 July. At McCredie, spores were collected on 38% of the days, viable spores per cubic meter averaged 14.1, and the count was highest (28.3) on 1 August. At Bradford, spores were collected on 30% of the days, viable spores per cubic meter averaged 12.4, and the count was

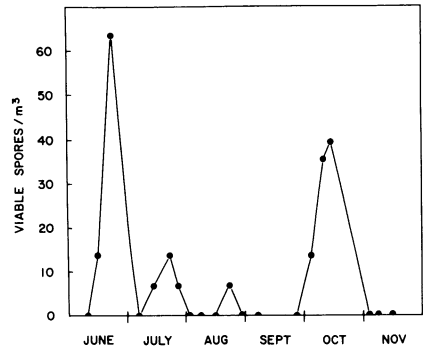

Fig. 1. Atmospheric spore concentration of *Aspergillus flavus* at Novelty, Missouri, in June–November 1977, as determined with an Andersen six-stage viable particle sampler.

Table 1. *Aspergillus flavus* spores collected by Rotorod, Hirst, and Andersen air samplers at eight locations in Missouri, 1976–1978

Location	Sampler[a]	No. of days operated			Days *A. flavus* was detected (%)		
		1976	1977	1978	1976	1977	1978
Spickard	R	35	30	30	80	73	30
Novelty	R	34	64	24	56	67	17
	H	126	154	70	47	41	24
	A	···	20	7	···	60	43
	T	128	154	76	54	48	30
Norburne	R	···	21	···	···	48	···
Columbia	R	38	46	21	34	57	48
Bradford Farm	R	···	46	22	···	67	50
	H	···	71	···	···	41	···
	A	···	···	13	···	···	22
	T	···	97	22	···	54	59
McCredie	R	···	6	8	···	67	75
	A	···	···	8	···	···	38
	T	···	6	8	···	67	88
Mt. Vernon	R	22	30	24	36	41	54
Portageville	R	18	30	6	78	77	17

[a] R = Rotorod sampler; H = Hirst volumetric spore trap; A = Andersen six-stage viable particle sampler; T = total for all samplers.

Table 2. Atmospheric spore concentration[a] of *Aspergillus flavus* at three locations in Missouri during the 1977 and 1978 growing seasons, as determined with an Andersen six-stage viable particle sampler

Location	No. of days sampler operated	Days *A. flavus* was detected (%)	Viable spores of *A. flavus*/m^3[a]
	1977		
Novelty	20	45	22.7
	1978		
Novelty	7	43	23.9
McCredie	8	38	14.1
Bradford	13	30	12.4

[a] Average spore concentration on days when spores were detected.

highest (21.2) on 1 August. Because the Andersen sampler was operated on only a few days in 1978, we did not compare these data with the qualitative data from the other two samplers.

DISCUSSION

Airborne spores of *A. flavus* were present at all sampling sites in each growing season. Collected spores were viable and are potential inoculum for infesting corn kernels before harvest.

Our results indicate greater incidence of *A. flavus* spores in the air by Missouri cornfields than was found in 1975 in Georgia, Illinois, Maryland, South Carolina, and Virginia, when only 13 of 260 exposed ADM plates yielded characteristic *A. flavus* colonies after 30-min exposure periods (4). The higher incidence found in Missouri could have been caused by differences in location; more likely, however, our air samplers were more effective in collecting *A. flavus* spores than the exposed plate method, which relies on gravity and wind.

The decapped kernel technique, though effective, was slow. Using ADM reduced the work load and enabled direct and rapid identification of *A. flavus* spores. Moreover, ADM provided an excellent method of estimating the concentration of *A. flavus* spores in the atmosphere from samples collected with the Andersen sampler. Because *A. flavus* does not develop secondary colonies on ADM during the 3 days of incubation, spore concentration can be estimated more accurately on ADM than on media that do not hinder sporulation.

The incidence and concentration of *A. flavus* spores fluctuated with the year and season. In 1977 at Novelty, highest spore counts in June and October (Fig. 1) coincided with the greatest tillage and harvesting activities. More frequent sampling with the Andersen sampler and more complete documentation of field tillage activity will help to clarify the relationship between the two phenomena.

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