FLUORESCENCE ASSOCIATED WITH CORN INFECTED WITH *ASPERGILLUS FLAVUS* AND *A. PARASITICUS* IN STORAGE[1]

G. RAMBO, J. TUITE, and G. L. ZACHARIAH[2], Purdue University, West Lafayette IN 47907

ABSTRACT

Two yellow dent corn hybrids were inoculated with *Aspergillus flavus* and *A. parasiticus* and stored in static and aerated systems. Two fluorescences were observed under black light in the inoculated grain: bright greenish yellow (BGYF) and blue white (BWF). The incidence of these fluorescences varied with each isolate in relation to storage conditions. The *A. parasiticus* inoculated grain had more BWF than BGYF, whereas corn inoculated with *A. flavus* had similar amounts of both fluorescences. The correlation between the incidence of either the BGYF or BWF and aflatoxin in individual kernels was 74–80% for *A. parasiticus*, a good aflatoxin producer, but very low for *A. flavus*, a poor toxin producer. Aflatoxin occurred in 6.7 and 19.6% of the nonfluorescing kernels selected from the static and aerated tests, respectively.

In 1955, Marsh and his associates (1) reported a bright greenish yellow fluorescence (BGYF) in cotton bolls infected with *Aspergillus flavus*, and in 1969, they (2) reported a positive correlation between aflatoxin and the occurrence of BGYF. A similar fluorescence has been detected in corn (3,4). Shotwell *et al.* (4) suggested using long-wave ultraviolet light to detect BGYF, with subsequent chemical analysis for aflatoxin. Preliminary evidence indicates a good but not 100% correlation between BGYF and aflatoxin in commercial corn (3).

There have been no controlled investigations on the formation of BGYF in storage of corn. The tests reported here explored the effects of moisture, temperature, and two species of the *A. flavus* group on the production of BGYF and aflatoxin. In one test, high storage temperatures suitable for the growth of *A. flavus* and *A. parasiticus* but unsuitable for the production of aflatoxin (5) were included to determine if fluorescence is induced without aflatoxin.

MATERIALS AND METHODS

Static Storage

Yellow dent corn, Ind. 814, was hand-harvested in 1969, dried at 40°C, and stored at 2°–3°C until 1972. Viability at time of storage was 96%. Four hundred grams were placed in 2-qt mason jars with lids having 0.5-in. holes plugged with Gaymar Identiplug size A. Water was added to adjust the corn to 22, 26, and 30% moisture. Three jars of corn at each moisture were placed in plastic bags containing moistened paper toweling and stored at 24°, 28°, and 32°C for 14 days.

Aerated Storage

Pioneer 3334 yellow dent corn, combine-harvested in 1972, was dried at 40°C and stored at 2°–3°C. Viability was 86%. The corn was adjusted to 26% moisture

[1]Purdue University Agricultural Experiment Station Journal Paper No. 5628.

[2]Respectively: Postdoctoral Researcher and Professor, Dept. of Botany and Plant Pathology, and Professor, Dept. of Agricultural Engineering.

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and inoculated. Three replicates of each isolate were incubated at 26°, 35°, 40°, and 45°C for 14 days. In addition, three replicates of high-temperature-dried corn, having a viability of 10%, were adjusted to 22% moisture and incubated at 26°C. The corn was aerated with saturated air at 0.3 cfm/bu.

Inoculation

The corn was inoculated with spore suspensions (5×10^4 spores/g) of either *A. parasiticus* NRRL 2999 or *A. flavus*. The latter was isolated from corn collected in Missouri by W. R. Wichser (Quaker Oats Co., Barrington, Ill.). The spore suspension was added to the sterile water used to adjust the corn moisture. The low-viability corn, high-temperature-dried, was inoculated with *A. parasiticus* only.

Moisture and Mold Determination

The moisture content (wet weight basis) was determined after 4 days' incubation by drying two 15-g samples from each replicate for 72 hr at 103° ± 1°C. The mycoflora was determined on the seventh day from the static test and the seventh and fourteenth days from the aerated test. One hundred kernels were submerged in 5% sodium hypochlorite solution, rinsed twice with sterile water,

TABLE I
Fungi in Dent Corn, Ind. 814[a], after 7 Days' Incubation with *Aspergillus parasiticus* or *A. flavus* at Three Temperatures and Moistures in a Static Condition

Treatments			% Kernels Infected with[b]				
Fungus	Moisture %	Temperature °C	*Aspergillus flavus* group	*Fusarium moniliforme*	*Penicillium* spp.	*Mucor*	Others
A. parasiticus	22	24	54	51	4	2	3
		28	82	36	1	1	4
		32	84	20	1	0	1
	26	24	26	68	4	6	12
		28	52	69	50	2	5
		32	82	24	8	2	2
	30	24	1	78	8	6	11
		28	2	94	4	7	7
		32	6	97	1	2	10
A. flavus	22	24	39	59	10	1	6
		28	97	33	3	1	4
		32	77	47	1	1	6
	26	24	3	79	13	3	11
		28	78	55	24	0	3
		32	54	74	12	1	11
	30	24	0	86	25	11	9
		28	4	96	6	13	7
		32	18	94	2	55	12

[a]Original corn sample 52% *F. moniliforme*, 8% *Cephalosporium acremonium*, 4% *Nigrospora oryzae*, and 4% *Gibberella zeae*.
[b]Average of three replications of 100 seeds, each disinfected with 5% NaC10, plated on PDTC, and incubated 6 days at 24°C.

plated on potato dextrose agar containing 30 mg/l. chlortetracycline and 100 mg/l. Tergitol NPX (PDTC), and incubated for 5 days at 24°C.

Fluorescence Analysis

Kernels from the static test were examined for fluorescence after 3, 6, 10, and 14 days. Fifty kernels were split longitudinally and observed with a long-wave ultraviolet light, 365 nm (Brinkman Instruments Model No. UVL-56). In the test with aerated corn, 100 kernels from each replicate were examined after 4, 8, and 14 days.

Aflatoxin Analysis

The isolates were screened for aflatoxin production by growing them on moistened autoclaved popcorn for 7 days at 26°C. Four 50-g portions were assayed for aflatoxins using the method of Pons *et al.* (6). After 7 and 14 days, 50 g of each replicate of the storage tests was similarly assayed for aflatoxins. Individual kernels were assayed for aflatoxins by the method of Shotwell *et al.* (4).

Kojic Acid Analysis

The isolates of *Aspergillus* were screened for kojic acid production using the method outlined by Hesseltine *et al.* (7).

TABLE II
Fungi in Dent Corn, Pioneer 3334[a], after 8 and 14 Days' Incubation with *Aspergillus parasiticus* or *A. flavus* at Four Temperatures in Aerated System

Treatments			% Kernels Infected with[b]		
Fungus	Days in Storage	Temperature °C	*Aspergillus flavus* group	*Fusarium moniliforme*	*Aspergillus fumigatus*
A. parasiticus	8	26	51	4	0
		35	69	5	0
		40	62	0	0
		45	13	0	0
	14	26	62	3	0
		35	79	7	0
		40	85	0	0
		45	11	0	36
A. flavus	8	26	52	4	0
		35	79	4	0
		40	77	0	0
		45	55	0	0
	14	26	75	2	0
		35	72	3	0
		40	81	0	0
		45	27	0	27

[a]Original sample yielded 33% *F. moniliforme* and 4% *Penicillium* spp.
[b]Average of three replications of 100 seeds, each disinfected with 5% NaC10, plated on PDTC, and incubated 6 days at 24°C.

RESULTS

Mycoflora

In the static system, after 7 days the lowest moisture (22%) had the most infection by *A. flavus* and *A. parasiticus* with few other fungi isolated (Table I). At 30%, the highest moisture, invasion by either species was slight with *Fusarium moniliforme* predominating. As expected, the higher temperatures of 28° and 32°C favored *A. flavus* and *A. parasiticus* (5). In the aerated grain, 35° and 40°C favored invasion for both species (Table II). *A. flavus* was more prevalent at 45°C than was *A. parasiticus*. *A. fumigatus* was frequent at 45°C. In general, competition from other fungi was much less in aerated storage than in static storage.

Fluorescence

Two fluorescences were observed in the grain inoculated with either *A. flavus* or *A. parasiticus*: BGYF and blue white (BWF). The BGYF appeared similar to the BGY described by Marsh *et al.* (1) on cotton and the greenish gold fluorescence described by Shotwell *et al.* (4) in commercial lots of corn. The BWF has not been previously reported in corn.

Both fluorescences were observed after 4 days in the aerated test and essentially after 6 days in the static test (Tables III and IV). Only two kernels, from a total of 1350, had BGYF after 3 days under static conditions. After 7 days, 96% of the kernels in the test using the low viability seed exhibited BWF, but BGYF was not observed. The fluorescences were detected only in the endosperm or in an area between the germ and the endosperm; neither was clearly observed

TABLE III
Incidence of BWF and BGYF in Kernels Incubated 6, 10, and 14 Days under Static Conditions

			Moisture								
			22%			26%			30%		
Fungus	Fluorescence	Storage Time	24°C	28°C	32°C	24°C	28°C	32°C	24°C	28°C	32°C
A. parasiticus	BWF	6	0[a]	1	3	0	3	9	0	0	3
		10	1	12	17	4	10	14	0	3	2
		14	5	37	38	4	19	49	4	0	0
A. parasiticus	BGYF	6	0	0	0	0	5	12	0	0	0
		10	0	2	0	0	4	10	0	0	0
		14	0	4	1	1	19	29	0	0	0
A. flavus	BWF	6	0	0	1	0	1	3	1	3	5
		10	1	2	2	0	4	2	4	7	7
		14	4	0	1	1	16	19	1	5	8
A. flavus	BGYF	6	0	0	0	0	9	11	0	1	4
		10	0	0	0	2	15	9	0	2	10
		14	0	1	3	9	11	12	3	4	5

[a]Figures represent the average of three replicates. Percentage fluorescence in 100 kernels.

in the germ or the horny endosperm. This is similar to the results of Fennell *et al.* (3) for a commercial corn sample.

The incidence of fluorescence (BWF and BGYF) varied with each isolate according to temperature and moisture (Tables III and IV) and in the static test appeared to be associated with the incidence of the two species (Tables I and III). Thus, in the static test, the lower two moistures (22 and 26%) and the higher two temperatures (28° and 32° C) yielded more BWF and BGYF than did the highest moisture, 30%, and the lowest temperature, 24° C. In the aerated test, which included higher temperatures, kernel infection was substantial at 40° C (Table II) but both BWF and BGYF dropped off substantially (Table IV). In both tests, *A. parasiticus* induced much more BWF than BGYF and much more BWF than did *A. flavus*. *A. flavus* induced about as much BGYF as BWF. The index of BWF and BGYF production in relation to infection after 14 days of aerated storage (Table V) reflects the differential fluorescent induction by the two species.

TABLE IV
Incidence of BWF and BGYF in Kernels Incubated 4, 8, and 14 Days in an Aerated Storage System at 26% Moisture

Fungus	Fluorescence	Storage Time	Temperature 26° C	35° C	40° C	45° C
A. parasiticus	BWF	4	7[a]	15	1	0
		8	27	50	4	0
		14	49	68	6	0
A. parasiticus	BGYF	4	0	3	1	0
		8	2	5	0	0
		14	11	7	3	1
A. flavus	BWF	4	3	2	2	2
		8	6	8	0	5
		14	11	3	1	6
A. flavus	BGYF	4	1	0	0	3
		8	1	3	4	5
		14	5	16	1	8

[a]Figures represent the average of three replicates. Percentage fluorescence in 100 kernels.

TABLE V
Index of BWF and BGYF after 14 Days of Aerated Storage at 26% Moisture

Temperature °C	*A. parasiticus* BWF	*A. parasiticus* BGYF	*A. flavus* BWF	*A. flavus* BGYF
26	79.0[a]	17.7	14.6	6.6
35	86.1	8.8	4.2	22.2
40	7.0	3.5	1.2	1.2
45	0	9.1	22.2	29.6

[a]Percentage of fluorescent kernels divided by per cent infection with *A. flavus* or *A. parasiticus*.

Aflatoxin Analysis

In the autoclaved moist popcorn, *A. parasiticus* produced an average of 18,500 ppb of aflatoxin B_1 while *A. flavus* yielded only 68 ppb. In both storage tests, *A. parasiticus* produced greater amounts of aflatoxin than did *A. flavus* (Fig. 1). The greatest amounts of aflatoxin were detected at 32° C for the static test and 35° C for the aerated test. Aflatoxins were not detected in the samples stored at 30% moisture and inoculated with *A. parasiticus* or *A. flavus*. In the aerated tests, only small amounts were detected in samples inoculated with *A. parasiticus* and incubated at 40° and 45° C and none were detected in the corn inoculated with *A. flavus* (Fig. 1).

Fluorescence and Aflatoxin

In both storage systems, individual kernels exhibiting BWF yielded aflatoxin slightly more often and usually in higher amounts than did BGYF kernels (Table VI). The *A. parasiticus* inoculated kernels, which exhibited BWF or BGYF, yielded aflatoxins from 61 of 78 kernels, 17/23 (73.9%) with BGYF and 44/55 (80.0%) with BWF (Table VI). The *A. flavus* inoculated kernels yielded aflatoxins from only 3 out of 56 kernels with either BWF or BGYF (Table VI).

Both fluorescences occurred in small amounts in corn inoculated with *A. flavus*, at 30% moisture, at all temperatures, in the static test (Table III) and 45° C in the aerated test (Table IV), but no aflatoxins were detected in 50-g samples from these replicates (Fig. 1).

A number of kernels, infected with either isolate from the static and aerated test but not exhibiting BWF or BGYF, were also extracted. Aflatoxins were

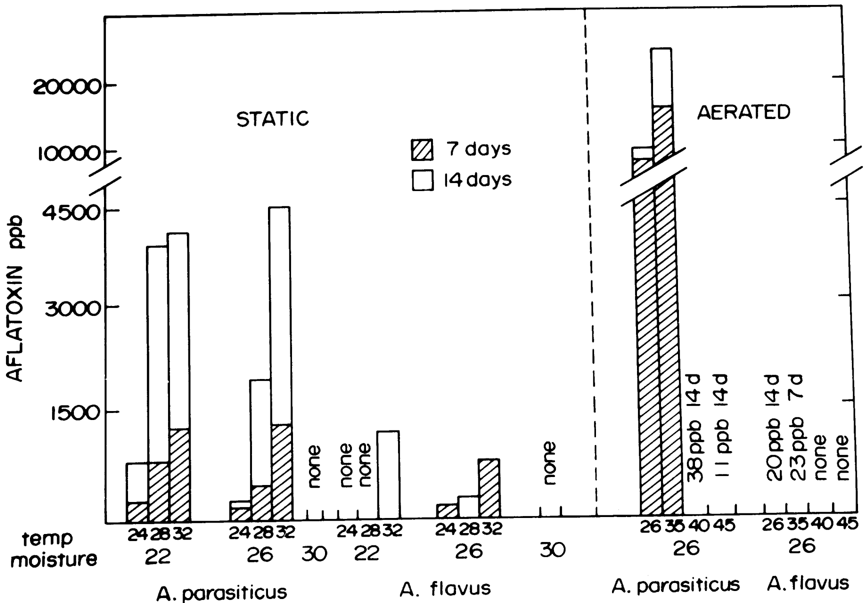

Fig. 1. Amounts of aflatoxin detected in 50-g samples from cultures of *A. flavus* and *A.*

detected in 2/30 (6.7%) in the static test and 9/46 (19.6%) in the aerated test (Table VI) and are so-called hidden positives.

DISCUSSION

The isolate of *A. parasiticus* and of *A. flavus* used in our test induced BWF and BGYF in corn of original high viability. *A. parasiticus* produced much more BWF than did *A. flavus*. Both fluorescences were produced over a wide range of moisture and temperature but were markedly reduced for *A. parasiticus* and low for *A. flavus* at 40° and 45°C. The reduction at these high temperatures coincided with little or no production of aflatoxin. BGYF was not produced in corn of very low viability (10%), supporting the idea that BGYF production is dependent on living tissue or at least functional enzyme systems (8). Because of their low viability, some commercially dried samples, if rewetted or independently dried and subsequently invaded by *A. flavus*, would be unlikely to produce a high incidence of BGYF. They, however, could be contaminated with aflatoxin. Kernels not exhibiting BWF or BGYF yielded aflatoxin in both storage tests. The percentages of 7.0% for the static and 19.6% for the aerated tests may be attributed in part to the amount of nonviable seed in these tests (4 and 14%, respectively).

For individual kernels, the correlation of BWF and BGYF incidence and aflatoxin was good for viable samples inoculated with *A. parasiticus*, 80 and 74% respectively, but was very poor, 6.2 and 5%, for the *A. flavus* isolate. The lack of correlation was probably a result of the poor aflatoxin-producing ability of this isolate. However, it would be expected to induce BGYF as it is a good producer of kojic acid which is needed for the production of the BGYF compound (8). Some isolates of *A. flavus* produce kojic acid but little or no aflatoxin (7,9).

TABLE VI
Amounts of Aflatoxin in Individual Kernels Incubated in Aerated and Static Systems with *A. parasiticus* and *A. flavus*

Fungus	Fluorescence	Aflatoxin (μg/g) 0	<5	<50	>50–100	Total	% with Aflatoxin
Static test							
A. parasiticus	BWF	1	1	8	8	18	94
	BGYF	2	1	5	1	9	78
	None	8	2	0	0	10	20
A. flavus	BWF	6	0	0	0	6	0
	BGYF	16	1	0	0	17	6
	None	20	0	0	0	20	0
Aerated test							
A. parasiticus	BWF	10	3	24	0	37	73
	BGYF	4	2	8	0	14	71
	None	22	3	2	0	27	18
A. flavus	BWF	9	0	1	0	10	10
	BGYF	22	1	0	0	23	5
	None	15	3	1	0	19	36

The inconsistencies in the correlation of BGYF production and aflatoxin, the occurrence of false and hidden positives in individual kernels and cottonseed (3,10), and reports of a "BGYF" not associated with *A. flavus* (11) do not appear to invalidate the black light test as a presumptive screening test for aflatoxin. As commercial samples are often extensively blended, it would be expected that if the corn is invaded by the *A. flavus* group, it would, in ordinary size samples, be invaded by a mixture of isolates and probably consist of corn stored and handled under different circumstances. Quickly differentiating false "BGYF" appears feasible and would remove a weakness of the black light technique. In a conscientious monitoring of corn lots, one may be forced to blindly collect samples and run chemical analysis for aflatoxin. The BGYF test could be used as a basis for a rational selection of samples for assay. BWF, because it occurs in nonviable kernels, may also be of use in screening for potential aflatoxin. Its detection, however, would need to be more readily and precisely made since it is not as distinctive a fluorescence as BGYF.

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