Relation of Aflatoxins in Cotton Seeds at Harvest to Fluorescence in the Fiber

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In bulk samples of U. S. cotton seed collected to represent all harvestable bolls, aflatoxins were found infrequently and, when present, at very low levels. However, seeds from occasional individual bolls whose fiber exhibited an *Aspergillus flavus*–associated greenish yellow fluorescence contained variable but generally high levels of aflatoxin B_1. Seeds from visibly rotted bolls lacking the fluorescence also sometimes contained aflatoxin B_1 but at a much lower average level. In laboratory culture, 49 out of 50 isolates of *A. flavus* produced aflatoxin B_1, the amount being unrelated to the geographical origin of the culture, while other seed-invading field fungi produced little or no aflatoxins. All *A. flavus* isolates caused the greenish yellow fluorescence in living cotton fiber, whereas no other field fungi tested did so. The fiber fluorescence, therefore, appears to offer promise as a preliminary screening test to locate seeds containing high levels of aflatoxins at harvest. Penetration of *A. flavus* occurred through the chalazal end of the seed.

Aspergillus flavus Link was reported by Marsh *et al.* (1955) to cause a hitherto undescribed cotton boll rot characterized by a bright greenish yellow (BGY) fluorescence in the fibers attacked by the fungus. The fluorescence, which is sufficiently distinctive to allow rapid recognition of such infections in commercial lint, is seen only infrequently (Marsh and Simpson, 1968; Marsh and Taylor, 1958). Data from three laboratories (Ashworth and McMeans, 1966; Ashworth *et al.*, 1968; Marsh *et al.*, 1968; Whitten, 1966) suggested a relation between the fluorescence in the fiber and aflatoxins in the seeds. The present paper provides further information on this fluorescence-aflatoxin relation.

MATERIALS AND METHODS

Aflatoxin analyses were performed, after grinding of the fuzzy seed, by the method of Pons *et al.* (1966), with modification as follows. Acetone and chloroform were removed by flash evaporation rather than on a steam bath. Disposable Drummond microcaps (capacity 5 μl.) were used to apply solutions to the plates, a procedure which increased speed and decreased the possibility of contamination. The amount of solution drawn into the microcap by capillarity was reproducible to $\pm$ 4%. Two spots of single and double application were employed for each extract and comparisons made with similarly applied spots of the standards. The solvent for the development was the upper, benzene-rich phase from a system containing benzene-ethanol-water (46:35:19) (Association of Official Analytical Chemists, 1966). Recommended safety precautions for handling aflatoxins were followed (Fischbach and Campbell, 1965; Stoloff and Trager, 1965).

Seed cotton for analyses recorded in Table I was received at Beltsville as 1- to 5-pound samples, from each of which a subsample was ginned on a small laboratory gin to produce a 50-gram sample of fuzzy seed. The 1965-crop samples were from Auburn, Ala.; Brawley, Calif.; Florence, S. C.; Lubbock, Tex.; Phoenix, Ariz.; Portageville, Mo.; St. Joseph, La.; Shafter, Calif.; and Stoneville, Miss. The 1966-crop samples were from Baton Rouge, La.; Brawley, Calif.; Chickasha, Okla.; College Station, Tex.; El Paso, Tex.; Experiment, Ga.; Florence, S. C.; Knoxville, Tenn.; Lubbock, Tex.; Phoenix, Ariz.; Portageville, Mo.; Shafter, Calif.; Stoneville, Miss.; and Weslaco, Tex. The 1967-crop samples were from Auburn, Ala.; Brawley, Calif.; Chickasha, Okla.; College Station, Tex.; El Paso, Tex.; Experiment, Ga.; Florence, S. C.; Knoxville, Tenn.; Lubbock, Tex.; Phoenix, Ariz.; Portageville, Mo.; Shafter, Calif.; and Stoneville, Miss. Each sample concerned in the data of this table included all seeds from all bolls which could be harvested from each plant sampled. The samples listed under "Regional Variety" included seeds of the variety Deltapine 15 and one locally adapted variety at each location. The commercial samples were from locally popular varieties grown with locally suitable cultural practices. Both early and late harvest dates were involved.

Smaller samples were analyzed in obtaining the data of other tables. "Visibly damaged" refers to bolls in which the fiber was discolored and incompletely fluffed, symptoms usually accompanied by microbial infection.

Aspergillus flavus NRRL 2999 was obtained from C. W. Hesseltine, USDA Northern Regional Research Laboratory. Other cultures were from isolations made at Beltsville. Particular care was taken in the experiment of Table V to grow each fungus on the shredded wheat promptly after isolation, to avoid any change in aflatoxin-producing capability in culture.

Pure culture experiments with living cotton fiber involved the incubation of whole bolls in a quart jar assembly as previously described (Marsh *et al.*, 1955).

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In the shredded wheat cultures (Hesseltine *et al.*, 1966), 10 grams of a commercial shredded wheat breakfast food was placed in a cotton-plugged 500-ml. Erlenmeyer flask and 50 ml. of water was added. The vessel and contents were sterilized in an autoclave at 15 pounds' steam pressure for 15 minutes, cooled, inoculated by needle with a pure culture of a fungus, and incubated for 7 days at 30° C. in a room with a 12-hour/12-hour light/dark cycle. The medium was removed at the end of the incubation, dried for 16 to 48 hours at 40 to 50° C., and analyzed. Prior to washing, glassware was treated with a 5% solution of sodium hypochlorite to inactivate aflatoxins.

Surface sterilization of seeds was performed by placing 25 ml. of Clorox (NaOCl, 5.2% available chlorine) in a 50-ml. beaker in the bottom of an 11-liter desiccator, putting the delinted seed on the desiccator plate, tipping 2 ml. of 1*N* HCl into the Clorox, and allowing the seeds to remain in the gas for 24 hours. The seeds were then placed on water agar at 30° C., and the fungi present in them were identified when they emerged after 2 or 3 days.

RESULTS

Field Samples. Seed cotton samples were collected across the U. S. cotton belt from the crops of 1965, 1966, and 1967 in such a manner as to include all harvestable cotton from each plant. The seeds in such bulk collections exhibited aflatoxin B_1 levels above 20 p.p.b. in only a few instances (Table I). The data revealed no clear trend toward association of aflatoxin content with variety or date of harvest.

Only extremely limited fluorescence of the type associated with *A. flavus* was noted in the fiber of the samples of Table I, but samples with aflatoxin B_1 above 200 p.p.b. all came from an area where such fluorescence had been observed (Marsh and Taylor, 1958). Twelve additional samples from the 1965 crop at Brawley, not listed in Table I and described only as coming from "fertilizer plots," contained small amounts of fluorescent fiber and included two samples in the 201- to 2000-p.p.b. range.

Exposure to wet weather in the field did not necessarily lead to aflatoxins in the seeds. Thus, with fiber pH as an index to such weathering (Marsh *et al.*, 1951), an extensive series of samples of seed cotton harvested on an individual boll basis at Baton Rouge was segregated according to the pH of the fiber of each boll into low-pH, medium-pH, and high-pH groups and analyzed accordingly. Analyses of samples in such groupings, made within two varieties and two harvest dates, resulted only in a single sample with measurable aflatoxin content—namely, a 5-p.p.b. level of aflatoxin B_1 in a single sample from the pH range of 6.4 to 6.6.

The freedom from contamination with aflatoxins of most cotton seeds at harvest (Table I) was not surprising, since *A. flavus* only very infrequently infects cotton seeds in the field. Thus, examination of the internal fungal flora of 928 visibly damaged bolls from the crop of 1964 revealed the following frequencies of occurrence of the different fungi: *Fusarium* sp., 43; *Alternaria* sp., 20; *Aspergillus niger*, 13; *Nigrospora oryzae*, 13; *Colletotrichum gossypii*, 7; *Diplodia gossypina*, 6; *Aspergillus flavus*, 4; *Rhizopus stolonifer*, 2. These frequencies add up to more than 100% because two or more fungi sometimes infected seeds in a single boll. Very similar data were obtained from the crops of 1965 and 1966.

When seeds were analyzed from individual bolls in which the fiber exhibited the characteristic fluorescence associated with *A. flavus* infection, the aflatoxin levels were variable but often very high (Table II). The

Table I. Summary of Aflatoxin Contents of Bulk Samples of Cotton Seed from U. S. Crops of 1965, 1966, and 1967[a]

Type of Sample	Year	No. of Samples with P.P.B. of Aflatoxin B_1			
		"Zero"[b]	1–20	21–200	201–2000
Regional variety	1965	46	10	3	0
Commercial field	1965	15	8	0	0
Regional variety	1966	129	5	0	2
Commercial field	1966	72	4	2	0
Regional variety	1967	35	8	0	0
Commercial field	1967	108	4	3	2

[a] Samples from across cotton belt at locations listed in "Materials and Methods."
[b] Below limit of detection.

Table II. Occurrence of Aflatoxins in Seeds from 30 Visibly Damaged Bolls at a Single Location, Including 20 Bolls with Typical *Aspergillus flavus*–Associated Fluorescence in Fiber and 10 without Fluorescence

Boll No.	Estimated % of Fiber with Fluorescence	P.P.B. of Aflatoxin	
		B_1	B_2
31.002	0	0[a]	0
31.003	0	0	0
31.005	0	0	0
31.006	0	0	0
31.007	0	0	0
31.008	0	0	0
31.009	0	0	0
31.010	0	0	0
31.004	0	260	0
31.001	0	13,000	0
31.105	1	139	0
30.109	4	0	0
30.104	5	0	0
30.107	6	285	143
31.102	7	5,700	3,400
31.103	9	0	0
30.108	10	127	0
31.101	15	0	0
30.101	15	2,750	0
30.103	15	80,000	3,350
30.106	22	0	0
31.110	23	34,300	20,580
31.108	31	0	0
30.102	32	400	0
31.107	33	60,300	36,180
31.106	44	173,000	86,500
30.110	50	960,000	0
31.109	54	23,700	14,220
30.105	62	0	0
31.104	62	81,000	0

[a] Zeros indicate nondetectable amounts.

Table III. Occurrence of Aflatoxin B_1 in Seeds from Visibly Damaged Bolls without Greenish Yellow Fluorescence in Their Fiber

Boll No.	P.P.B. Aflatoxin B_1 in Bolls from: Brawley, Calif. (1965)	College Station, Tex. (1966)	Experiment, Ga. (1966)	Lubbock, Tex. (1966)	Phoenix, Ariz. (1966)	St. Joseph, La. (1965)	Stoneville, Miss. (1966)
1	0	0	0	0	0	100	0
2	803	0	0	0	0	220	0
3	780	0	0	0	0	67	0
4	0	0	0	0	0	650	120
5	0	0	0	0	0	0	74
6	0	0	0	0	10,000	0	0
7	0	250	0	0	0	0	2700
8	0	0	0	9900	0	0	2700
9	0	0	0	0	0	0	0
10	0	0	0	210	0	0	170

chance for high levels of aflatoxins was greatest in seeds whose fiber showed the typical *A. flavus* fluorescence. In a series of 10 bolls with BGY fluorescence from the location involved in Table II but from a previous year, the toxin content of the seeds averaged 53,814 p.p.b. of aflatoxin B_1 and 777 p.p.b. of aflatoxin B_2. In a series of 11 bolls with the same fluorescence from another location, analyses of the seeds revealed an average of 2491 p.p.b. of aflatoxin B_1 and 41 p.p.b. of aflatoxin B_2.

In seeds from visibly damaged, partially rotten bolls without greenish yellow fluorescence, collected at several locations, aflatoxins occurred only sporadically and usually then at relatively low levels (Table III).

Table IV shows further data on the occurrence of aflatoxins in seeds from visibly damaged locks from the field which exhibited no greenish yellow fluorescence in the fiber. (Cotton bolls are divided into four or five radial segments, the seed and attached fiber in each segment being termed a "lock.") The data, like those of Table III, suggest that although moderate to high concentrations of aflatoxins are particularly common in locks with the BGY fluorescence, aflatoxins may also be found at least occasionally and sometimes at fairly high concentrations in individual damaged bolls in the absence of such fluorescence.

Table IV. Occurrence of Aflatoxins in Damaged Boll Samples from Various Field Locations[a]

Location	Crop Yr.	Aflatoxin, P.P.B. B_1	B_2	G_1
Brawley, Calif.	1964	0	0	0
Brazos Valley, Tex.	1964	0	0	0
Clayton, N. C.	1964	0	0	0
College Station, Tex.	1964	82	0	0
Florence, S. C.	1958	0	0	0
	1958	210	0	130
	1961	404	0	207
	1961	0	0	0
	1961	0	0	0
	1964	0	0	0
	1965	910	810	0
Knoxville, Tenn.	1965	0	0	0
Portageville, Mo.	1965	0	0	0
Raleigh, N. C.	1964	0	0	0
	1965	0	0	0
Stillwater, Okla.	1964	0	0	0
Stoneville, Miss.	1965	0	0	0
Tempe, Ariz.	1964	0	0	0
Tifton, Ga.	1964	0	0	0

[a] Each sample consisted of seeds from 10 locks. No greenish yellow fluorescence present in fiber.

Laboratory Samples. When 50 isolates of *A. flavus* were made from cotton seeds from various U. S. field locations and incubated in the laboratory on a standard shredded wheat medium, 49 proved to have the basic capability of producing one or more aflatoxins (Table V). The data revealed no tendency for high aflatoxin producers to come from any special part of the country. The average production of B_1 was approximately 100,000 p.p.b. Some strains produced either B_2 or G_1 in addition to B_1. Although the depth of green color on the shredded wheat varied greatly from one isolate to the next, no relation was seen between this color and aflatoxin production.

Some further insight into the comparative aflatoxin-producing potential of the isolates of Table V may be gained from figures obtained on aflatoxin production by the standard strain NRRL 2999 under the same culture conditions. The isolate as received produced 540,000 p.p.b. of aflatoxin B_1, somewhat over five times the average for the cultures of Table V. To determine whether strain NRRL 2999 had components with different aflatoxin-producing capabilities, 21 single spore isolates were taken from it and incubated by the method of Table V. These 21 isolates produced 540, 420, 410, 430, 710, 1200, 1200, 890, 350, 220, 690, 1100, 1200, 960, 450, 530, 640, 760, 1200, 280, and 690 p.p.m. of B_1. Thus, these single spore isolates were variable in B_1 production but none fell as low as the average for the isolates of Table V.

Aflatoxins appeared to be produced on the shredded wheat during incubation in constant darkness quite as well as in the usual 12-hour/12-hour light/dark cycle. Thus, production of B_1 and G_1 by *A. flavus* USDA 227 was 170,000 and 580,000 p.p.b., respectively, in both constant darkness and in light/dark. Comparable data for *A. flavus* USDA 248 were 630,000 and 380,000 in constant darkness and 870,000 and 390,000 in light/dark. Presumably, therefore, variations in the intensity of light reaching the internal part of a seed in the field or in storage do not greatly influence aflatoxin production.

Table V. Aflatoxins in Shredded Wheat Medium after Incubation for 7 Days with Cultures of *Aspergillus flavus* Isolated from Cotton Seeds Collected in Field at Various Locations in U. S.

A. flavus Culture No.	Geographical Source of Culture	Aflatoxin Content of Medium,[a] P.P.B. B_1	B_2
262	Brawley, Calif.	31	0
261	Brawley, Calif.	50	0
244	Brawley, Calif.	5,900	0
245	Brawley, Calif.	31,000	0
247	Brawley, Calif.	56,000	4,500
260	Brawley, Calif.	57,000	4,500
248	Brawley, Calif.	59,000	5,900
327	Brazos Valley, Tex.	40,000	4,000
326	Brazos Valley, Tex.	190,000	0
284	College Sta., Tex.	16,000	1,000
285	College Sta., Tex.	376,000	0
224	Fayetteville, Ark.	5,000	0
226	Fayetteville, Ark.	8,300	1,900
242	Fayetteville, Ark.	20,000	0
225	Fayetteville, Ark.	85,000	1,900
228	Fayetteville, Ark.	150,000	0
227	Fayetteville, Ark.	417,000	0
335	Florence, S. C.	490	40
295	Knoxville, Tenn.	27	0
296	Knoxville, Tenn.	49	0
292	Knoxville, Tenn.	19,000	0
301	Knoxville, Tenn.	43,000	7,200
291	Knoxville, Tenn.	44,000	5,500
294	Knoxville, Tenn.	100,000	21,000
293	Knoxville, Tenn.	150,000	0
304	Portageville, Mo.	0	0
305	Portageville, Mo.	15,000	1,900
306	Portageville, Mo.	33,000	6,600
269	Portageville, Mo.	36,000	0
268	Portageville, Mo.	44,000	5,500
324	Portageville, Mo.	240,000	0
325	Portageville, Mo.	260,000	0
297	Raleigh, N. C.	15	0
302	Raleigh, N. C.	19,000	3,100
305	Raleigh, N. C.	26,000	3,200
299	Raleigh, N. C.	37,000	11,000
300	Raleigh, N. C.	55,000	5,800
270	Stillwater, Okla.	50	0
271	Stillwater, Okla.	140,000	18,000
272	Stillwater, Okla.	925,000	0
263	Tempe, Ariz.	120,000	16,000
264	Tempe, Ariz.	130,000	13,000
248	Tempe, Ariz.	732,000	0
334	Tifton, Ga.	400	40
333	Tifton, Ga.	1,100	0
329	Tifton, Ga.	20,000	10,000
331	Tifton, Ga.	160,000	21,000
330	Tifton, Ga.	530,000	25,000
326	Tifton, Ga.	640,000	25,000
332	Tifton, Ga.	730,000	21,000

[a] Aflatoxin G_1 present in cultures 245, 228, 227, and 248 at 8600, 7800, 1,670,000, and 275,000 p.p.b., respectively; absent from all other cultures. No aflatoxin G_2 in any culture.

When several isolates of cotton boll rot organisms other than *A. flavus* were tested on the shredded wheat medium for ability to produce aflatoxins, the results were generally negative (Table VI). A few isolates produced fluorescing substances which registered as a small amount of aflatoxin B_1 in the analyses, even though there was no certainty that the true aflatoxin B_1 was involved. Repeat tests with the "positive" organisms invariably resulted in failure to reproduce the positive results in a second incubation. In incubations

Table VI. Results of Test on Cotton Boll Rot Organisms Other than *Aspergillus flavus* for Production of Aflatoxins on a Standard Shredded Wheat Medium

Fungus	No. of Isolates Tested	Producing no detectable aflatoxins[a]
Alternaria sp.	7	5
Colletotrichum gossypii	6	5
Diplodia gossypina	10	10
Fusarium sp.	10	9
Nigrospora oryzae	6	6
Rhizopus stolonifer	8	5

[a] "Positive" isolates for the several genera produced "aflatoxin B_1" in parts per billion as follows: *Alternaria* (61, 31); *Colletotrichum* (2590); *Fusarium* (115); *Rhizopus* (104, 34, 19). No "positive" results could be reproduced in repeat incubations.

Table VII. Aflatoxin Content of Cotton Seed from Bolls of Different Ages Incubated with *A. flavus* NRRL 2999 in a Humid Atmosphere

Age of Boll, Days[a]	Aflatoxin Content, P.P.B. B_1	B_2	G_1	G_2
24	24,000	0	7,900	16,000
26	38,000	0	25,000	18,000
30	27,000	0	18,000	18,000
33	92,000	0	61,000	2,500
35	120,000	0	82,000	10,000
43	16,000	0	17,000	2,700
44	270	0	180	0
44	3,600	0	4,800	1,200
45	13,000	0	11,000	2,900

[a] Bolls from flowers tagged on date of flowering.

of one isolate from each of the genera of Table VI on live seed in cotton bolls, all analyses of seeds failed to show positive results.

Seeds from bolls incubated for 7 days in the living condition with *A. flavus* NRRL 2999 displayed highly variable aflatoxin contents. It was suspected that this variability might be related to the maturity of the seed, but experiments did not confirm this hypothesis (Table VII). The data for the table were drawn at random from a larger experiment, none of the results of which showed any clear relation between seed maturity and amount of aflatoxins produced. BGY production occurred in the fiber in all cases.

The variability in aflatoxin production by a single isolate in seeds of the same age is of unknown origin. In our data as a whole with different isolates on near mature bolls, a range of aflatoxin B_1 contents from 0 to 140,000 p.p.b. has been observed in the presence of strong fluorescence. Some uncertainty exists as to whether *A. flavus* penetrated into the interior of the seeds in all cases.

Many strains of *A. flavus* have been tested for their ability to produce the BGY fluorescence during incubation on a cotton boll. The following isolates were incubated on never-dried fiber and all produced the fluorescence: USDA isolates 152, 210, 227, 248, 261, 262, 263, 268, 269, 270, 271, 272, and 330; FDA 1, FDA 2, FDA 3; NRRL 2999; and QM 8378. Further, 23 single spore isolates from *A. flavus* NRRL 2999 all produced the fluorescence.

Isolate 2999.5 grown on locks at 15°, 20°, 25°, and 30° C. produced the fluorescence at each temperature;

the fluorescent color became obvious in about 2 days at 30° C. The fluorescence developed as well as in continuous darkness at 30° C. as in alternating light and darkness.

Since a shaded cotton boll may sometimes remain in a cracked condition for several days in the field, a question arose as to whether such a period of standing might result in loss of the fiber's capability for allowing formation of the BGY fluorescence when subsequently infected by *A. flavus*. Cotton locks taken from mature but unopened greenhouse-grown bolls were incubated sterile over water for 7 days at 30° C. Subsequent inoculation and incubation with *A. flavus* resulted in lesser amounts of BGY, but the fluorescence was still easily visible.

Aflatoxins may be produced not only in seeds of *Gossypium hirsutum*, the common species to which most U. S. cotton belongs, but also in seeds of other species of the genus. Table VIII shows aflatoxin production by *A. flavus* NRRL 2999 in seeds of *G. herbaceum*, an Indian type of cotton very different in many aspects from American Upland types. BGY formation occurred in the fiber. Both high aflatoxin production in the seeds and bright fluorescence of the usual appearance in the fiber occurred also with *G. arboreum* and with Pima cottons of the species *G. barbadense* (Table VIII). As with American Upland cottons, the absolute levels of aflatoxins in the seeds exhibited great variability from one boll to the next.

Further information on the penetration of *A. flavus* into cotton seeds seems needed. Indirect information revealed in Figure 1 suggests that the fungus may enter the seed through the chalazal opening. The experiment whose results are shown in Figure 1 was performed as follows: Ordinary fuzzy cotton seeds were sterilized in chlorine gas, placed on water agar, inoculated by spore suspension with *A. flavus* NRRL 2999, and incubated for 7 days at 30° C. The seeds were dried, acid-delinted, surface-sterilized in chlorine gas, placed without further inoculation onto water agar, and incubated

Table VIII. Production of Aflatoxins in Living Seeds of Various Gossypium Species Incubated with *Aspergillus flavus* NRRL 2999[a]

Species	Boll No.	Aflatoxin, P.P.B. B_1	B_2	G_1	G_2
G. arboreum	1	100,000	11,000	21,000	2,500
No. 3046	2	7,100	710	3,600	240
	3	12,000	3,600	8,200	2,000
	4	16,000	4,800	10,000	2,500
G. barbadense	1	86,000	10,000	57,000	1,100
Pima S-2	2	430,000	43,000	29,000	950
	3	2,800,000	110,000	730,000	7,300
	4	870,000	140,000	580,000	3,200
G. herbaceum	1	110,000	3,800	57,000	1,300
No. 3033	2	77,000	2,600	69,000	1,700
	3	210,000	5,100	140,000	3,400
	4	64,000	14,000	29,000	9,400

[a] Variable but generally high levels of aflatoxins produced under similar incubation conditions with *Gossypium arboreum* of genetic types 16, 19, 21, and 53, as well as in *G. barbadense* types Pima S-1, Pima S-3, and Pima S-4, and in *G. herbaceum* 3006.

Figure 1. Cotton seeds with *A. flavus* emerging from chalazal ends

Seeds inoculated and incubated with fungus and then delinted, surface-sterilized, and re-incubated on water agar for 2 days. The fungus regularly grew back out of the chalazal ends of the seeds.

When we placed a dozen fluorescing locks, from laboratory incubations with *A. flavus*, upon an outside window ledge in direct exposure to summer sunlight for 6 hours, a striking loss of fluorescence occurred upon the exterior surfaces of the locks without any exposure to rain. An even much greater loss could be seen if such fluorescing fiber were moistened in water and placed under the Blak-Ray ultraviolet lamp. Dry fiber resisted loss of fluorescence under the lamp but moist fiber lost fluorescence greatly in a very few minutes. Such a combination of circumstances—simultaneous exposure to both water and sunlight—could possibly have accounted for part or all of the fluorescence losses noted in field exposures by Ashworth *et al.* (1968).

DISCUSSION

Figure 2 shows certain tentative conclusions from the work to date. When the typical greenish yellow fluorescence is seen in field-collected cotton fiber, one can be fairly certain that *A. flavus* infected the fiber at the time of boll opening. Other fungi are common causes of infection in the seeds but cannot cause a greenish yellow fluorescence and apparently produce little if any aflatoxins. The relation between the estimated percentage of fiber with fluorescence and the amount of aflatoxins in the seeds of Table II was an imperfect one, probably for several reasons. Seed infection may have occurred in some bolls after the fiber was dead and thus no longer capable of forming the

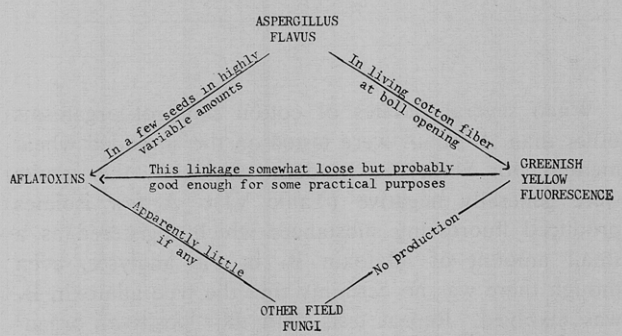

Figure 2. Probable relation of greenish yellow fluorescence to aflatoxins in cotton seeds at harvest

fluorescent substance. Or, infection of the fiber may have resulted in fluorescence in the fiber but may not have been followed by infection of the seed, because of rapid drying of the seed or for some other reason. Even the lower amounts of aflatoxins detected in the seeds of some of the bolls of Table III were much higher than the levels in unselected or bulk samples of seed from the same areas. In bolls such as those of Table III, some fungus other than *A. flavus* often seemed to be the dominant or at least the most evident organism present, but *A. flavus* may have followed as a secondary invader. By this time the fiber was presumably dead and unable to form the BGY fluorescence. The variability in aflatoxin-producing potential in different strains of the fungus (Table V) seems to offer another possible reason for variability in the fluorescence-aflatoxin relation in cotton seeds in the field. A much lower percentage of aflatoxin-producing strains was observed by Parrish *et al.* (1966), possibly because their cultures were not freshly isolated.

Aflatoxin production in cotton seeds may be influenced by factors affecting penetration of water and/or the fungus into them. A barrier to water penetration exists in the chalazal region of some genetic types of cotton (Christiansen and Justus, 1963). The property is also environmentally influenced, is variable from one seed to the next, occurs to some degree in present commercial cotton types, and might conceivably impede penetration by *A. flavus*. Evidence presented by Mayne *et al.* (1967) indicates that the impermeability characteristic may influence production of aflatoxin.

An accurate estimate of the average aflatoxin content of all seeds from a field of cotton probably requires extensive analytical data, possibly a prohibitively expensive procedure for practical purposes. In the absence of such analyses, surveillance of seed cotton for the presence of the typical *A. flavus*-associated fluorescence in the fiber may be useful.

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