Bright Greenish-Yellow Fluorescence and Associated Fungi in White Corn Naturally Contaminated with Aflatoxin

D. I. FENNELL, R. J. BOTHAST, E. B. LILLEHOJ, and R. E. PETERSON, Northern Regional Research Laboratory[1], Peoria, Illinois 61604

ABSTRACT

Good correlation was observed between the presence of *Aspergillus flavus* Link ex Fr., a bright greenish-yellow (BGY) fluorescence, and aflatoxin in a naturally contaminated white corn. However, *A. flavus* was observed on nonfluorescent whole undamaged kernels that assayed at <20 p.p.b. and on nonfluorescent whole damaged kernels and fragments that assayed at 3,000 p.p.b. Whole damaged kernels that failed to fluoresce externally yielded some fluorescing fragments when coarse ground. Routinely, BGY fluorescence in whole corn was observed on the face of the kernels immediately above the germ, and in sectioned kernels the emission was generally restricted to a narrow region outlining the germ. Usually, initial development of *A. flavus* was confined to the germ region. Isolates of *A. flavus* from aflatoxin-contaminated corn could be separated into four groups on gross cultural characteristics. Two of four isolates selected as representative of these groups exhibited aflatoxin-production capability, whereas all four synthesized kojic acid, the presumed precursor of the BGY fluorescence.

A Food and Drug Administration seizure of aflatoxin-tainted white corn (1) was ordered during the latter part of 1971. Although various corn surveys have shown a limited incidence of aflatoxin contamination in corn (2,3,4), this action dramatized the need for a simple, rapid, presumptive test that could be used routinely by corn processors to detect aflatoxin.

Marsh and co-workers (5,6,7) have studied extensively the relationship between the appearance of a bright greenish-yellow (BGY) fluorescence in cotton fibers and the occurrence of *Aspergillus flavus* in the fluorescing spots. On laboratory-inoculated cotton, fungi isolated from fluorescing fibers produced a BGY-emitting substance similar to that extracted from field-contaminated cotton.

Reports from various laboratories have established a correlation between the presence of BGY fluorescence in cotton fiber and aflatoxin in the seed (6,8,9).

[1]Agricultural Research Service, U.S. Department of Agriculture. Mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

Limited *A. flavus* contamination and 30 p.p.b. of aflatoxin were detected in nonfluorescent cotton seed (9). However, the preponderance of aflatoxin, up to 11,000 p.p.b., was associated with BGY-emitting seed. These observations have served as a basis for development of a qualitative aflatoxin test through detection of the BGY emission under ultraviolet (UV) light (6). Techniques have also been devised for removal of fluorescent seed as a method for reducing aflatoxin levels (10).

Investigation of the origin of the BGY-fluorescing material has demonstrated that *A. flavus* strains isolated from fluorescent cotton fibers produced kojic acid, which was converted to the fluorescing substance by plant tissue peroxidase (7). Although various *Aspergillus* species are known to produce kojic acid, Parrish et al. (11) found that synthesis of aflatoxin was restricted to kojic acid-producing strains of *A. flavus* and *A. parasiticus*. However, a requisite simultaneous synthesis of kojic acid and aflatoxin appears unlikely, since it has been shown that in certain instances *A. flavus* will produce aflatoxin without detectable formation of kojic acid (12).

Experiments by Marsh and his colleagues (7) demonstrated that BGY fluorescence could be produced experimentally in fruits, leaf sheath, silks, and culms of corn artificially injured before *A. flavus* inoculation.

Following preliminary observations of a UV-induced fluorescence associated with aflatoxin-contaminated white corn, Shotwell et al. (13) suggested that a screening procedure based on this fluorescent emission might be developed. These authors reported that damaged kernels and fragments selected from white corn samples containing aflatoxin routinely exhibited the glowing fluorescence. Of 18 such corn fragments, 17 contained high levels of aflatoxin B_1 (284 to 101,000 p.p.b.). No toxin was detected in kernels or fragments that did not exhibit the characteristic fluorescence. Cursory examination of damaged kernels under UV light demonstrated that the BGY emission was generally observed in the germ region of the grain.

Our study was intended to provide information on: 1) the region of BGY emission within kernels of white corn naturally contaminated with aflatoxin; 2) the location of *A. flavus* infection within the kernels; and 3) the possible relationship between BGY fluorescence and the mold profile.

MATERIALS AND METHODS

A 7-bu. lot of aflatoxin-contaminated white corn (U.S. Sample Grade) was obtained from a commercial grain dealer. One 50-g. sample from each bushel was assayed for aflatoxin (14). The mean level of aflatoxin was 1,080 p.p.b. with an 80% confidence interval of 661 to 1,559 p.p.b. Equal portions of corn from each bushel were mixed, and a 50-g. sample of the blend was divided by hand on the basis of kernel condition and the presence or absence of external BGY fluorescence under UV light (13). Four fractions were obtained: A) Nonfluorescing whole undamaged kernels; B) fluorescing whole kernels with obvious fissures in the integument over the germ; C) nonfluorescing whole damaged kernels and fragments; and D) fluorescing fragments.

To detect possible internal fluorescence in whole kernels of fractions A and C, a 100-g. sample of each was coarse ground. Aflatoxin assays were carried out on one 50-g. sample of each fraction. The fraction composition of the 50-g. blend sample and the aflatoxin content of each fraction are shown in Table I. An aflatoxin-free

TABLE I. CORN FRACTIONS AND THEIR AFLATOXIN CONTENT

Fraction	Description	% of 50-g. Sample	Aflatoxin Content[a] p.p.b.
A	Nonfluorescing whole undamaged kernels[b]	70	<20
B	Fluorescing whole kernels with cracks over germ	5	7,500
C	Nonfluorescing whole damaged kernels and fragments[b]	22	3,000
D	Fluorescing fragments	3	3,800

[a]Assayed according to the AOAC method (14); lower detection limit 20 p.p.b.
[b]When coarse ground, fraction A contained no bright greenish-yellow fluorescent particles; fraction C contained a limited number (0.8% by weight).

high-quality white corn sample (U.S. Grade No. 1) from a grain dealer in Decatur, Ill., was used as the control.

Kernels or fragments representative of each fraction (A, B, C, and D) were surface-sterilized with 1% sodium hypochlorite (NaOCl) for 1 min., washed twice with sterile water, and placed on ME agar (malt extract, 30 g. and agar, 15 g. per liter). Petri plates were incubated at 28°C. and examined under a stereoscopic microscope after 2 or 3 and 5 days. Fungi developing on each kernel and, in one trial, their location on kernels from fractions A and B were recorded.

Representative strains of the most commonly occurring fungi were isolated. On the basis of head color, size, and shape, members of the *A. flavus* group were observed to fall into four categories. One representative from each category was tested for aflatoxin and kojic acid production. Fermentations were carried out in 2.8-liter Fernbach flasks containing 150 g. of aflatoxin-free white corn prepared according to the method of Kurtzman and Ciegler (15). Each flask was inoculated with 1.0 ml. of a suspension (about 10^7) prepared by washing conidia from a 2-week-old Czapek's agar ($NaNO_3$, 3.0 g.; K_2HPO_4, 1.0 g.; $MgSO_4 \cdot 7H_2O$, 0.5 g.; KCl, 0.5 g.; $FeSO_4 \cdot 7H_2O$, 0.01 g.; sucrose, 30 g.; agar, 15 g. per liter distilled H_2O) slant with distilled H_2O containing 0.005% Triton X-100. Flasks were incubated at 28°C. on a rotary shaker at 200 r.p.m. for 7 days. Moldy corn was steeped in chloroform for 24 hr. and the extract filtered through cheesecloth. Filtrates were assayed for aflatoxin according to AOAC's Official Methods of Analysis (14).

The chloroform-treated corn was further extracted with 300 ml. of distilled water; kojic acid in the aqueous extract was determined by thin-layer chromatography on 0.2-mm. Polygram Sil N-HR plates (Brinkmann Instruments) developed in benzene:methanol:acetic acid (45:10:4, v./v./v.) and sprayed with 1% $FeCl_3$ solution.

Color photographs (Plates I and II) were taken with a Nikon FTN camera with bellows and Mikro-Nikkor f3.5 lens. An Ednalite 2A filter was used at all times. Illumination was provided by a Meteor SP-GH electronic flash unit at 2 ft. for "daylight" pictures and at 2 in. with a No. 19057 Blak-Ray UV filter (365 nm.) for fluorescent pictures. High speed Ektachrome, processed at ASA 400, gave good representation of the colors observed.

RESULTS

Internal Fluorescence and Fungi

In split or sectioned kernels (Plate I, Figs. 1-6), fluorescence is observed in a narrow region outlining the germ and appears to be concentrated either in the

epithelial cells of the scutellum (Fig. A) and the compressed inner cells of the floury endosperm or in the thin film of hyaline material between the epithelial cells and the endosperm (16). BGY fluorescence in broken kernels and fragments (Plate I, Figs. 7–10), as it is observed in the UV screening procedure, appears diffuse and its location is not clearly defined. The fluorescence is visible on the face of whole kernels immediately above the germ (Plate II, Figs. 11 and 12) which is covered only by the pericarp and a single layer of aleurone cells. When cut kernels are placed on wet filter paper for 24 to 36 hr., fluorescence diffuses into the floury endosperm as well as into the underlying filter paper.

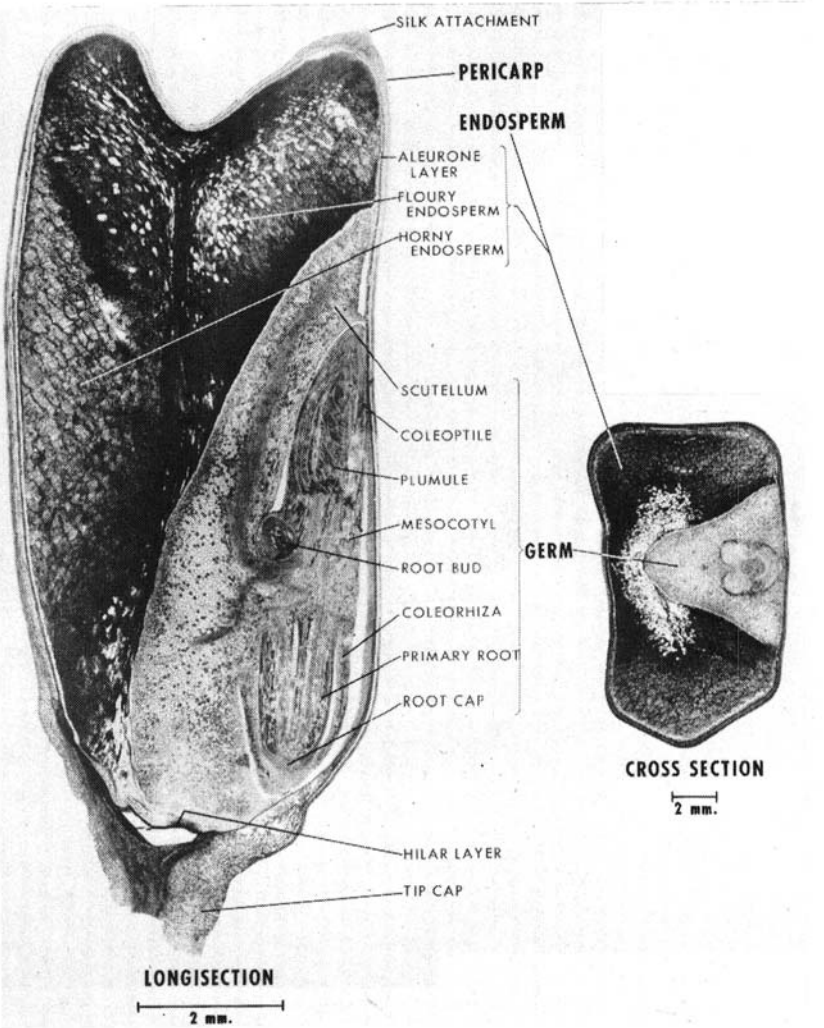


Fig. A. Sectioned corn showing the parts of a kernel and their relationship to each other. (left) A median longitudinal section, cut perpendicular to the face; (right) a transverse section through the mesocotyl. (Composite photomicrograph by Roland W. Haines.)

In split and sectioned whole kernels that fluoresced externally, massive infection by *A. flavus* and sporulation of the fungus was observed within the germ (Plate II, Fig. 16). Externally nonfluorescing kernels failed to show obvious development of *A. flavus*, but small masses of fungal mycelium were frequently observed at the outer edges of and external to the hilar layer or in the air space between the upper and lower hilar layers. Isolations from these mycelial aggregates yielded pure cultures of *A. flavus*. In addition, nonfluorescing kernels occasionally exhibited a fine nonsporulating mycelium covering the outer surface of the embryonic axis and the vascular tissues of the scutellum. Plumules excised from these embryos, as well as those removed from kernels with mycelial clumps between the hilar layer and the tip cap, have also yielded pure *A. flavus* (Plate II, Fig. 17).

Fungi from Plated Kernels and Fragments

As anticipated from previous studies on fungi associated with stored corn, a wide but almost predictable variety of fungi was encountered: *Aspergillus flavus, A. niger, A. chevalieri, A. amstelodami, A. tamarii,* and *A. ochraceus; Penicillium oxalicum, P. variabile, P. funiculosum, P. citrinum, P. steckii,* and *P. cinnamopurpureum; Fusarium* and *Rhizopus*. Other genera (*Trichoderma, Mucor, Alternaria,* and *Syncephalastrum*) were observed rarely and are not believed to represent internal infection.

A. flavus, A. niger, and *Fusarium* sp. were the three most common fungi in the sample (Plate II, Fig. 13). Their incidence on each fraction is summarized in Table II. In two trials, *A. flavus* infected an average of 99% of fluorescent whole kernels (B), 100% of fluorescent fragments (D), 63% of nonfluorescent whole undamaged kernels (A), and 60% of externally nonfluorescent damaged kernels and fragments (C). No *A. flavus* was observed in the aflatoxin-free control.

Representatives of the genus *Fusarium* were observed on 63% of nonfluorescing whole kernels and 24% of nonfluorescing damaged kernels and fragments, but on only 2% of fluorescing fragments and 21% of fluorescing whole kernels. *Fusarium* was the only fungus that developed on the aflatoxin-free nonfluorescing control.

A. niger was present at 5 days on approximately 50% of the kernels or fragments of both fluorescing and nonfluorescing fractions, whether or not they contained aflatoxin, but was absent in the control. While this lack of correlation

TABLE II. INCIDENCE (%) OF PREDOMINANT FUNGI ON SAMPLE FRACTIONS AND CONTROL

Fraction	Days Incubation	Aspergillus flavus		Aspergillus niger		Fusarium	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
A	2	2		16[a]		54	
	3		50		32		58
	5	74	52	40	46	58	68
B	2	74		26[a]		22	
	3		98		52		14
	5	100		54		28	
C	3	60		50		24	
D	3	100		50		2	
Control	5	0		0		2	

[a]On some kernels conidial heads too young for positive identification; probably A. niger.

with either fluorescence or aflatoxin content suggests that this fungus is not involved in fluorescence, its role has not been determined.

Two additional organisms, of less frequent occurrence and not included in Table II, show some correlation with the presence or absence of fluorescence. Members of the *A. glaucus* group (*amstelodami* and *chevalieri*) were observed on only 20% of fluorescing whole kernels but on 32% of whole kernels that failed to fluoresce. *P. cinnamopurpureum*, on the other hand, was more common on fluorescing kernels (10%) than on nonfluorescing kernels (2%). In our studies, this *Pencillium* appeared to parasitize the conidial heads of *A. flavus* and was easily recognizable at 5 days.

Location of Fungi in Plated Intact and Whole Kernels

Kernels from fractions A and B, surface sterilized and plated (germ face up) on agar, were examined after 48 hr. incubation at 28°C. Fungi developing on the pericarp covering the germ or in fissures over the germ were recorded as "on the germ" (Plate II, Figs. 14 and 15). Fungi on any other portion of the kernel, with the exception of the tip cap, were recorded as occurring on the endosperm. *A. flavus* and *Fusarium* predominated. The sites at which they were observed are recorded in Table III.

Fungal outgrowth was observed from the tip caps in 94% of both fluorescing and nonfluorescing whole kernels. *Fusarium* developed at this site on 54% of the kernels from fraction A compared with 20% of those from fraction B but was observed only once over the germ. If fungal infection occurs through the tip cap, deeper penetration of the fluorescing kernels is indicated by the observation that 86% of such kernels developed fungal outgrowth over the germ as compared with only 20% of those that failed to fluoresce. The high incidence (68%) of *A. flavus* over the germ of kernels from fraction B correlates with both fluorescence and aflatoxin concentration in this fraction.

A. niger, one of the three most common fungi at 5 days, grew and sporulated more slowly on the kernels than either *A. flavus* or *Fusarium*. The site of infection by this species could not be determined at 2 days. Later observations were obscured by the continued development of the more rapidly growing organisms but suggested that *A. niger* occurred somewhat more frequently on the tip cap than on the germ.

A. flavus Strains Isolated from Corn

A. flavus conidial heads observed on plated kernels and fragments showed differences in color, shape, and size. This variation was frequently observed in the

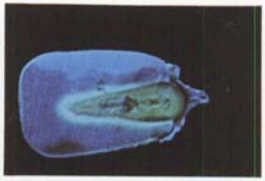
TABLE III. PERCENT INCIDENCE OF PREDOMINANT[a] FUNGI AT SPECIFIC SITES ON EXTERIOR OF KERNELS

Fungi	Tip Cap		Germ Region		Endosperm	
	Fraction A	Fraction B	Fraction A	Fraction B	Fraction A	Fraction B
Aspergillus flavus	2[b]	48	0	68	0	12
Fusarium	54	20	0	2	2	2
Total kernels showing fungal growth	94	94	20	86	10	18

[a]At 48 hr. incubation.

[b]Percentages based on 50 kernels.


Figs. 1 and 2. Longitudinal sections parallel to face. (left) Nonfluorescent kernel; (right) fluorescent. Fig. 1, daylight; Fig. 2, UV light.

Figs. 3 and 4. Longitudinal section parallel to face of a single fluorescing kernel, enlarged. Fig. 3, daylight; Fig. 4, UV light.

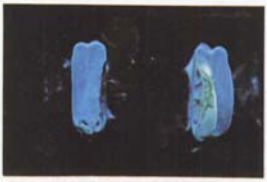
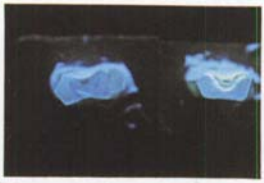
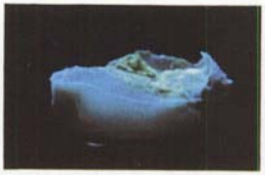



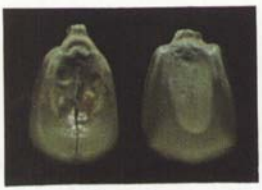
Fig. 5. Longitudinal sections perpendicular to face. (left) Nonfluorescent kernel; (right) fluorescent. UV light. Fig. 6. Cross-sections through the embryonic axis of one nonfluorescing (left) and one fluorescing (right) kernel. UV light.

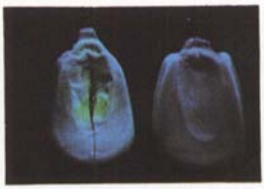



Figs. 7 and 8. Single broken fluorescing kernel enlarged. Fig. 7, daylight; Fig. 8, UV light.


Figs. 9 and 10. Single fluorescing kernel crushed. Fig. 9, daylight; Fig. 10, UV light.

Color Plate I. Location of UV light-induced BGY fluorescence in kernels of white corn. Fluorescence is seen to occur in a narrow band completely surrounding the germ.





Figs. 11 and 12. Kernels from fraction A (right) and fraction B (left). Fig. 11, daylight; Fig. 12, UV light.

Fig. 13. Fungi developed from surface-sterilized kernels after 120 hr. incubation on malt extract agar. Upper: Fraction B showing the green conidial heads of *Aspergillus flavus* on all five kernels; *A. niger* (black) on one. Lower left: Control showing no fungal growth from four of five kernels; outgrowth of *Fusarium* (pink) from one. Lower right: Fraction A, three of five kernels showing outgrowth of *Fusarium*, one of *A. flavus*, and one of *A. niger*.

Figs. 14 and 15. *A. flavus* growing from fissure in kernel incubated on moist filter paper. Fig. 14, after 24 hr. incubation; Fig. 15, after 48 hr.

Fig. 16. Enlargement of an unincubated fluorescing kernel showing massive internal infection of the germ by *A. flavus*. Fig. 17. Pure culture of *A. flavus* on a plumule excised from a nonfluorescing kernel and incubated on Czapek's agar for 24 hr.

Color Plate II. UV light-induced BGY fluorescence in unsectioned white corn and site of mold development on nonfluorescent kernels (fraction A) and fluorescent kernels (fraction B).

TABLE IV. INCIDENCE OF VARIOUS TYPES OF ASPERGILLUS FLAVUS CONIDIAL HEADS AND PRODUCTION OF AFLATOXIN AND KOJIC ACID BY REPRESENTATIVE STRAINS

Strain No. A. flavus	% Incidence[a]		Aflatoxin Production p.p.m.	Kojic Acid
	Fraction A	Fraction B		
NRRL 5518	16	67	2	+
NRRL 5519	48	69	<0.02[b]	+
NRRL 5520	26	69	1,500	+
NRRL 5521	4	13	<0.02	+

[a]Based on 50 kernels.

[b]Sensitivity limit of assay procedure.

sporulating structures developed from a single kernel. Isolations made from a number of heads displaying these differences yielded a series of strains which could be separated into four groups on rate of growth and size, color, and relative abundance of sclerotia but which showed no morphological differences that would warrant their exclusion from *A. flavus* (17). One representative strain from each group was retained and tested for aflatoxin and kojic acid production. Percentage occurrence of the four groups, recorded in Table IV, is based on subjective comparisons of head types displayed directly on plated corn kernels. Values should be recognized as trend indicators rather than definitive determinations. Results of tests for aflatoxin and kojic acid production by the four isolates are also included in the table. Although all four produced kojic acid, only two synthesized detectable levels of aflatoxin. Conidial heads of the types represented by both aflatoxin-producing isolates were observed on kernels of fraction A which assayed at less than 20 p.p.b. aflatoxin (Table I).

DISCUSSION

Our results confirm a positive correlation between fluorescence, *A. flavus* infection in the germ, and aflatoxin content of the kernel and thereby support the use of UV-induced BGY fluorescence as a rapid screening method for the presence of aflatoxin in white corn. However, our finding that both *A. flavus* and aflatoxin were present in fraction C (nonfluorescing whole damaged kernels and fragments) and that *A. flavus* was present in fraction A (nonfluorescing whole undamaged kernels) emphasizes the need for caution in interpretation of fluorescent screening of uncracked corn. The absence of external BGY fluorescence cannot be considered an absolute assurance of aflatoxin-free corn.

It should also be recognized that BGY fluorescence is not emitted by aflatoxin but by an unknown substance or substances that are probably produced by an interaction between *A. flavus* metabolites and enzymes in the corn (7). Heat denaturation of the enzymes during corn drying or storage could block production of BGY substances without eliminating *A. flavus*. Subsequent storage conditions could allow continued growth of the organism and aflatoxin production.

Varietal differences in the impermeability of the seed coat and the structure of the chalazal opening of cottonseed have been reported (18,19,20). Mayne et al. (21) found that impermeable cottonseed resists the growth of *A. flavus* and the

production of aflatoxin. Whether white corn has a thinner and more permeable pericarp or a chalazal region more accessible to fungal penetration than other types of corn has not been established.

The degree of maturity at harvest, picker-sheller damage, artificial drying of high-moisture immature corn, and unfavorable storage conditions all require consideration as possible factors contributing to the development of either fluorescence or aflatoxin or both. Immature corn differs from mature corn not only in moisture content, but also in protein, fat, and carbohydrate content (22). The corn's composition may determine whether fluorescence and aflatoxin are produced singly, concurrently, or not at all.

During harvesting or artificial drying, high-moisture corn kernels may be particularly prone to develop fissures in the thin part of the pericarp overlying the germ. Kernels with such pericarp damage display an enhanced level of *A. flavus* infection on the germ portion. Infection by this mold and subsequent production of aflatoxin may result from post-harvest contamination of damaged corn.

An alternative possibility is suggested by impressions gained during our investigations. Initial infection may occur in the field during early stages of ear development. *A. flavus* may be a part of the internal flora of white corn in areas where aflatoxin contamination is common. *A. flavus*, however, has been shown to be a weak invader of plant tissue (7) and field infection by this organism may be dependent on other factors, e.g., insect damage or prior invasion by other organisms.

Literature Cited

1. ANONYMOUS. FDA recalls corn meal, bread mix allegedly tainted by toxin. Southwest. Miller 50(38): 26 (1971).
2. SHOTWELL, O. L., HESSELTINE, C. W., VANDEGRAFT, E. E., and GOULDEN, M. L. Survey of corn from different regions for aflatoxin, ochratoxin, and zearalenone. Cereal Sci. Today 16: 266 (1971).
3. SHOTWELL, O. L., HESSELTINE, C. W., GOULDEN, M. L., and VANDEGRAFT, E. E. Survey of corn for aflatoxin, zearalenone, and ochratoxin. Cereal Chem. 47: 700 (1970).
4. SHOTWELL, O. L., HESSELTINE, C. W., BURMEISTER, H. R., KWOLEK, W. F., SHANNON, G. M., and HALL, H. H. Survey of cereal grains and soybeans for the presence of aflatoxin. II. Corn and soybeans. Cereal Chem. 46: 454 (1969).
5. MARSH, P. B., BOLLENBACHER, K., SAN ANTONIO, J. P., and MEROLA, G. V. Observations on certain fluorescent spots in raw cotton associated with the growth of microorganisms. Text. Res. J. 25: 1007 (1955).
6. MARSH, P. B., SIMPSON, M. E., FERRETTI, R. J., CAMPBELL, T. C., and DONOSO, J. The relation of aflatoxins in cotton seeds at harvest to fluorescence in the fiber. J. Agr. Food Chem. 17: 462 (1969).
7. MARSH, P. B., SIMPSON, M. E., FERRETTI, R. J., MEROLA, G. V., DONOSO, J., CRAIG, G. O., TRUCKSESS, M. W., and WORK, P. S. Mechanism of formation of a fluorescence in cotton fiber associated with aflatoxin in the seeds at harvest. J. Agr. Food Chem. 17: 468 (1969).
8. WHITTEN, M. E. A rapid screening method for detecting aflatoxins in cottonseed. Cotton Gin Oil Mill Press, p. 7 (Dec. 17, 1966).
9. ASHWORTH, L. J., and McMEANS, J. L. Association of *Aspergillus flavus* and aflatoxins with a greenish yellow fluorescence of cottonseed. Phytopathology 56: 1104 (1966).
10. ASHWORTH, L. J., McMEANS, J. L., PYLE, J. L., BROWN, C. M., OSGOOD, J. W., and PONTON, R. E. Aflatoxins in cottonseeds: Influence of weathering on toxin content of seeds and on a method for mechanically sorting seed lots. Phytopathology 58: 102 (1968).
11. PARRISH, F. W., WILEY, B. J., SIMMONS, E. G., and LONG, L. Production of aflatoxins

and kojic acid by species of *Aspergillus* and *Penicillium*. Appl. Microbiol. 14: 139 (1966).
12. BASAPPA, S. C., SREENIVASAMURTHY, V., and PAYSIA, H. A. B. Aflatoxin and kojic acid production by resting cells of *Aspergillus flavus* Link. J. Gen. Microbiol. 61: 81 (1970).
13. SHOTWELL, O. L., GOULDEN, M. L., and HESSELTINE, C. W. Aflatoxin contamination: Association with foreign material and characteristic fluorescence in damaged corn kernels. Cereal Chem. 49: 458 (1972).
14. ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. Official methods of analysis (11th ed.), Sect. 26, p. 429. The Association: Washington, D.C. (1970).
15. KURTZMAN, C. P., and CIEGLER, A. Mycotoxin from a blue-eye mold of corn. Appl. Microbiol. 20: 204 (1970).
16. WOLF, M. J., BUZAN, C. L., MacMASTERS, M. M., and RIST, C. E. Structure of the mature corn kernel. I. Gross anatomy and structural relationships. Cereal Chem. 29: 321 (1952).
17. RAPER, K. B., and FENNELL, D. I. The genus *Aspergillus*, 686 pp., 130 figs. Williams and Wilkins: Baltimore, Md. (1965).
18. CHRISTIANSEN, M. N., and JUSTUS, N. Prevention of field deterioration of cottonseed by an impermeable seedcoat. Crop. Sci. 3: 439 (1963).
19. CHRISTIANSEN, M. N., and MOORE, R. P. Seed coat structural differences that influence water uptake and seed quality in hard seed cotton. Agron. J. 51: 582 (1959).
20. CHRISTIANSEN, M. N., MOORE, R. P., and RHYNE, C. L. Cottonseed quality preservation by a hard seed coat characteristic which restricts internal water uptake. Agron. J. 52: 81 (1960).
21. MAYNE, R. Y., HARPER, G. A., FRANZ, A. O., Jr., LEE, L. S., and GOLDBLATT, L. A. Retardation of aflatoxin in cottonseed by impermeability of seedcoats. Crop Sci. 9: 149 (1969).
22. BRESSANI, R., and CONDE, R. Changes in the chemical composition and in the distribution of nitrogen of maize at different stages of development. Cereal Chem. 38: 76 (1961).

[Received September 8, 1972. Accepted November 30, 1972]