Aspergillus flavus and Aflatoxin in Preharvest Corn From Indiana in 1971 and 1972[1]

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ABSTRACT

No *Aspergillus flavus* infection was detected in 156 samples of preharvest sound corn from Indiana in 1971 and only 0.08% (all from southern Indiana) from 369 samples in 1972. Samples were routinely treated with 5% NaClO. Incidence of *A. flavus* infection increased slightly in selected samples surface disinfected with 1% NaClO for 30 sec. and cultured at 35°C., but no aflatoxin was detected in these infected samples. *A. flavus* averaged 0.6 and 1.6% in physically damaged corn in 1971 and 1972, respectively. *A. flavus* and aflatoxin detected in 8 of 163 combine-harvested corn samples may have developed after harvest. A bright greenish-yellow fluorescence (BGYF), similar in appearance to the BGYF reported in *A. flavus*-infected corn, was noted on uninfected kernels and cobs free of aflatoxin.

performed with the motor running so that the no-load friction in the mixing head gears was tared from the measurements by the amplifier zero adjustment.

A 15-tooth sprocket (A, Fig. 2) was mounted on the input shaft to the mixing head with an electromagnetic pulse generator (B) (Model 3025; Electro Products
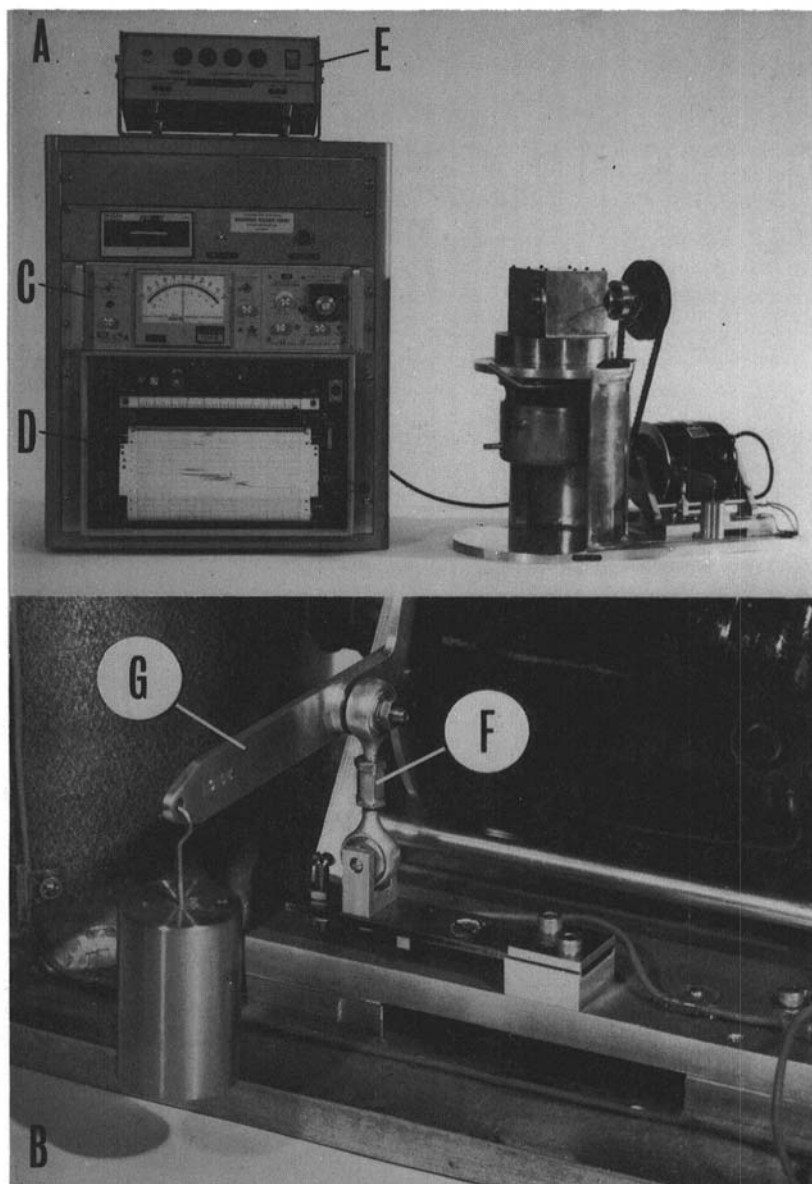

Fig. 1. A) The converted mixer; B) the transducer installation; C) strain gage amplifier; D) strip-chart recorder; E) digital tachometer; F) link; G) lever attached to motor casing.

Laboratories Inc., Chicago). For each rotation of the shaft 15 pulses were produced and applied to a solid state digital tachometer (E, Fig. 1A) (Model 2705; Electronic Research Co., Kansas) to directly indicate the rotational speed of the mixing pins.

INSTRUMENT PERFORMANCE

The relationship between mixing speed and speed control dial setting was determined and found to be linear within ±1% (Fig. 3). A mixing speed could be selected at the dial, and the exact speed set by only minor dial adjustments. Under no-load conditions the mixing pin speed was found to vary with time by a maximum of ±0.6 r.p.m. over the operating range (Table I). When mixing a standard sample (200 g. hard wheat flour + 130 cc. water), the maximum variation during a test ranged from ±1.0 to ±3.4 r.p.m. depending on the speed selected (Table I). The greatest change occurred when mixing at the highest test speed (130 r.p.m.). However, because the digital tachometer could be read to within 0.1 r.p.m., it was easy to readjust the mixing speed and keep it constant throughout a test. Since mixing torque is proportional to mixing speed, this adjustment is critical for samples where accurate results are needed.

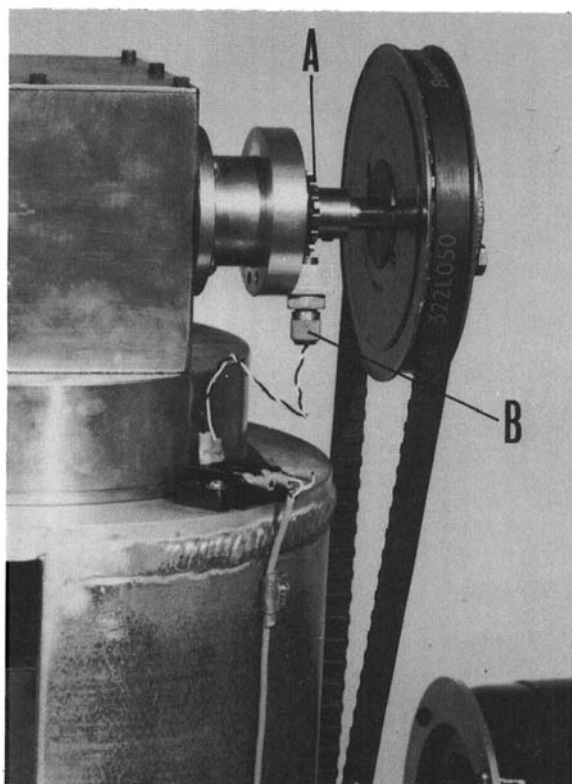It was found possible to calibrate the dynamometer so that full-scale pen

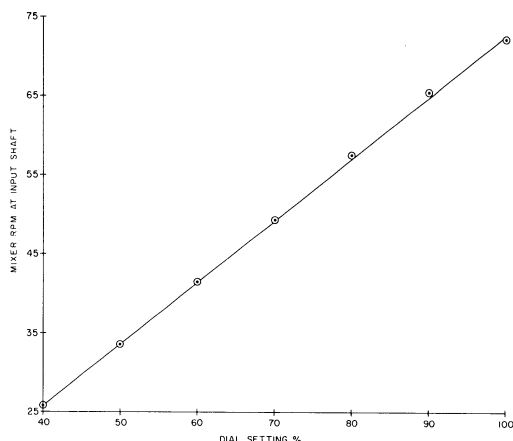Fig. 2. Mixing speed detector. A) 15-tooth sprocket; B) pulse generator.

Fig. 3. Mixing speed vs. motor speed control dial setting.

TABLE I. MIXING SPEED CONTROL

Nominal Outer Pin Speed r.p.m.	Speed Changes No-Load r.p.m.	Speed Changes during Mixing[1] r.p.m.	Maximum Torque[1] cm.-g.
56	±0.6	±1.3	6,400
94	±0.4	±1.0	8,200
130	±0.4	±3.4	10,200

[1]200 g. hard wheat flour + 130 cc. water.

TABLE II. LINEARITY AND HYSTERISIS OF RELATIONSHIP BETWEEN TORQUE AND CHART READING SHOWN AS MAXIMUM DEVIATION FROM A STRAIGHT LINE. MEASURED WITH MIXER RUNNING

Full-Scale Torque cm.-g.	Maximum Deviation %
2,000	±0.5
4,000	±2.5
10,000	±2.0
20,000	±0.5
30,000	±0.5

deflection was produced for torques ranging from 2,000 to 30,000 cm.-g. Application of calibration weights in increments (10), with the motor running, showed that the relationship between pen deflection and torque was linear within a maximum error of ±2.5% (Table II). The errors increased as the dynamometer sensitivity was increased because the friction in the bearings supporting the motor became greater relative to the applied torque.

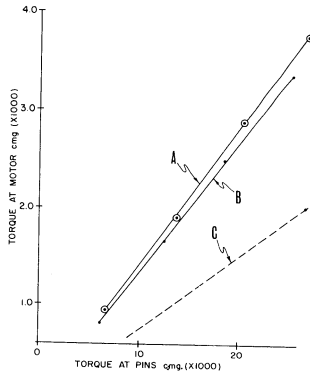To operate the recording mixer, the speed was selected and the dynamometer

Fig. 4. Illustration of the effect of mixing speed on the efficiency of the mixer mechanism. A) 130 r.p.m.; B) 70 r.p.m.; C) theoretical friction-free slope determined from the mechanical advantage relationship between motor and mixing pins (13.33:1).
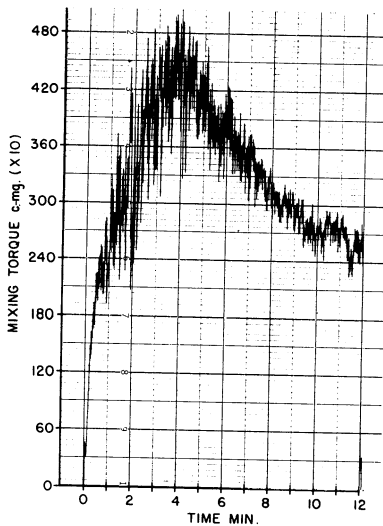

Fig. 5. Typical record obtained in mixing hard wheat flour at 130 r.p.m.

calibrated with the mixer running at this speed. Thus, zero on the recorder scale represented the torque required to drive the mixing mechanism under no load at the selected speed, thus eliminating this error from the measurement. The efficiency of the mechanism, however, varied with mixing speed and the torque transmitted through the gears and bearings. This was evaluated by measuring the torque applied to the mechanism by the motor at selected torques applied to the mixing pins using a previously described technique (9). This technique was improved by using wire rope and larger spools to apply torque to the mixing pins eliminating the correction factor previously required to allow for rope stretch.

Figure 4 shows the relationship between torque at the motor and torque at the

Preharvest invasion of dent corn by *Aspergillus flavus* Link was reported by Taubenhaus (1) in 1920 in Texas. Later reports indicated that *A. flavus* rarely invaded corn kernels in the field in the Corn Belt (2,3,4,5). However, most of the corn examined was free of obvious physical damage. Doupnik (6) reported increased incidence of *A. flavus* in Georgia corn attacked by *Helminthosporium maydis* (Nisik and Miyake). Shotwell et al. (7) and Watson and Yahl (8) indicated a low incidence of aflatoxin in stored corn from the Midwest, but recently corn from the South showed a much higher incidence of *A. flavus* infection and aflatoxin contamination (9). According to W. R. Wichser[2], invasion of corn kernels by *A. flavus* in the field with concomitant production of aflatoxin is important, particularly in southern United States.

We report here the results of an extensive sampling of preharvest sound and damaged dent corn in Indiana and Kentucky for aflatoxin and fungi, particularly *A. flavus.*

MATERIALS AND METHODS

Collection of Samples

Damaged Samples. In September 1971 Bob Meyer, a Purdue entomologist, collected ear samples mostly damaged by insects from 43 counties of the southern half of Indiana. In October, ears from 40 northern counties were sampled to give a total of 156 samples.

Farmers Fields. Twelve white and 184 yellow dent corn samples were collected from 27 western counties of Indiana allocated as follows: 12 southern counties (collected Sept. 20 and 21), 7 central counties (Sept. 27, Oct. 4, and Nov. 10) and 8 northern counties (Oct. 10, 1972). Approximately 10 ears were taken randomly from standing plants.

Evansville Varietal Plots. Three-pound combined harvested samples of 43 white and 120 yellow dent corn varieties were collected from yield plots in Evansville, Ind., on Oct. 10, 1972.

Kentucky Varietal Plots. Forty-three ear samples, 25 white and 18 yellow dent corn, which included 3 varieties of white dent and 2 varieties of yellow dent were collected from yield plots at 9 locations in Kentucky in the fall of 1972.

Sample Evaluation. Within 12 hr. after harvesting, the samples were dried with forced air at 40° C. for 3 to 5 days. Ear samples were dried in mesh bags and shelled samples in paper bags. Ears or shelled corn were examined for *Aspergillus* growth and physical damage with 3X magnification. Samples were examined for fluorescence, particularly bright greenish-yellow (BGYF), by using a Chromatovue chamber (Brinkman Instrument) with long-wave ultraviolet light. Moldy (presumed to be *A. flavus*), physically damaged, or fluorescing kernels were removed. Damaged kernels were submerged in 5% NaClO for 1 min., then plated on Czapek's 1% corn-steep agar containing 100 γ per ml. Tergitol NPX and 30 γ per ml. chlortetracycline. Moldy kernels and BGYF kernels were analyzed for aflatoxins (10).

Coarse grinding of corn may enhance the detection of BGYF and associative aflatoxin contamination (11). A total of 346 samples of white and yellow dent corn, 163 from Evansville, 43 from Kentucky, and 140 from Indiana (southern counties), were coarsely ground. The ground samples were inspected for BGYF

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[2]Personal communication.

TABLE I. THE NUMBERS AND KINDS OF FUNGI FOUND IN SURFACE-DISINFECTED
DAMAGED AND UNDAMAGED KERNELS PRIOR TO HARVEST IN 1971

Fungus[1]	Damaged[2] Kernels	Undamaged[3] Kernels	Damaged Kernels	Undamaged Kernels
	% Kernels infected		% Incidence in samples	
Fusarium moniliforme Sheld.	81.6	29.8	99.3	94.8
Mucor sp.	10.4	0.04	61.3	3.2
Penicillium spp.	2.5	0.5	11.4	8.1
Aspergillus flavus group	0.03	0	0.6	0

[1]Some fungi excluded from table, *Nigrospora oryzae, Cephalosporium acremonium,* were only common in undamaged seed. Other fungi present, but rare, were *Fusarium tricinctum* (Cda.) Synd. et Hans., *Chaetomium* sp., *Gibberella zeae, Acremoniella* sp.
[2]As determined by plating 20 kernels or less from 155 samples. Kernels were surface-disinfected with 5% NaClO for 1 min.
[3]As determined by plating 100 kernels from 156 samples. Kernels were surface-disinfected with 5% NaClO for 1 min.

TABLE II. MYCOFLORA OF INTACT CORN KERNELS OBTAINED PRIOR TO
HARVEST IN INDIANA AND KENTUCKY IN 1972

		% Kernels Infected[1]				
		Indiana				Kentucky
Fungus	No. of	Northern counties (34)	Central counties (32)	Southern counties (140)	Evansville (combine-harvested) (163)	(43)
Aspergillus flavus group		0	0	0.08	0.03	0
Cephalosporium acremonium Cda.		3.6	11.5	18.3	9.4	10.5
Fusarium moniliforme Sheld.		6.6	12.2	16.0	46.4	49.9
Gibberella zeae (Schw.) Petch		6.2	11.4	1.7	3.8	1.6
Nigrospora oryzae (B. and Br.) Petch		15.2	10.4	1.0	10.6	6.0
Penicillium spp.		0.6	1.7	2.1	4.8	8.2
Yeast		0	0.1	0.3	0.3	0.1
Total		32.2	47.3	39.2	75.3	76.3

[1]As determined by plating 100 kernels on PDTC after treatment with 5% NaClO for 1 min., and incubation at 22° to 24°C. for 5 to 7 days.

with a Black-ray, long-wavelength ultraviolet light (Ultraviolet Products, Inc.). BGYF particles were removed and each assayed for aflatoxin using methods of Shotwell et al. (10). In addition, 50 g. of the ground corn exhibiting BGYF was assayed for aflatoxins using methods of Pons et al. (12).

Mycoflora Determination. The remainder of the ear samples was machine-shelled and 100 kernels per sample submerged in 5% NaClO for 1 min., rinsed twice with sterile water, and plated on potato dextrose agar containing 100 γ per ml. of Tergitol NPX and 30 γ per ml. of chlortetracycline (PDTC), and incubated at about 22° to 24°C. for 7 days.

As an alternative technique, kernels from southern Indiana, Kentucky, and Evansville were surface-disinfected with 1% NaClO for 30 sec. and incubated at 35°C. on PDTC for 5 days, a modification of a procedure reported to enhance the isolation of *A. flavus* from soybean (13).

RESULTS

Mycoflora Determination

1971 Samples. The fungi commonly occurring in the damaged and undamaged kernels are given in Table I. *A. flavus* showed no significant preharvest invasion in 1971 as only one northern sample of the 156 samples yielded *A. flavus,* the amount of infection was 5.0%. There was a significantly greater amount of *Fusarium moniliforme* in damaged kernels.

1972 Samples. The number and kinds of fungi from intact corn kernels from Indiana and Kentucky treated with 5% NaClO are given in Table II. The relatively large amount of *Gibberella zeae* reflects the gibberella ear rot epidemic that occurred in 1972. *A. flavus* was found in five of the 140 samples from southern Indiana. The highest amount in any sample was 1.0% kernel invasion.

More *A. flavus* was isolated when samples from Kentucky and from southern Indiana farmers' fields and Evansville yield trials were plated following the milder disinfection treatment and incubation at 35°C. Eleven Indiana farm samples had *A. flavus* infection compared to two with 5% NaClO treatment, the highest amount observed was 8.0% of the kernels invaded. Only three of the 163 samples from Evansville were infected with *A. flavus,* the highest amount being 8.0% of the kernels. These three samples had either revealed *A. flavus* infection when plated after the 5% NaClO treatment, or had aflatoxin in individual kernels. One of the 43 Kentucky samples yielded *A. flavus* (2.0%). *A. flavus* growing from kernels disinfected with 1% NaClO seemed to originate more from the kernel surface than internally.

Damaged Kernels 1972. Kernels from Indiana and Kentucky samples assumed to be damaged by insects or birds were grouped into four lots based on geographical location of the samples, i.e., southern, central, and northern Indiana and Kentucky. An average *A. flavus* infection of 2.7% was found in samples from southern Indiana (Table III), *A. flavus* infection was not observed from other areas. The highest amount of *A. flavus* found in 1971 damaged corn in a sample was 5.0% from northern Indiana. The incidence of *A. flavus* from the state was 1.6%, which is an increase of 1.0% over that of the damaged corn from 1971 (Table I).

Aflatoxin Evaluations. A total of 268 kernels and several cob tips from Indiana and Kentucky ear samples were analyzed for aflatoxins. Most of the

TABLE III. PREVALENCE OF *Aspergillus flavus* IN PHYSICALLY DAMAGED
KERNELS FROM INDIANA AND KENTUCKY IN 1972

Sample Area	No. of Kernels	Percent Infected by *A. flavus*[1]
Indiana		
North	742	0
Central	445	0
South	2,424	2.7
Total	3,611	1.6
Kentucky	1,388	0

[1]As determined by plating on Czapeks, 1% corn steep agar after treatment with 5% NaClO for 1 min., incubation at 22° to 24°C. for 5 to 7 days.

kernels and all of the cob tips exhibited a "BGYF" fluorescing similar to the BGYF of *A. flavus*-infected kernels. No aflatoxins were found. However, eight combine-harvested samples from Evansville had individual kernels with aflatoxin. The amounts ranged from 7.5 to 1,100 γ per kg., with an average of 413 γ per kg.

To aid detection of BGYF, all of the 1972 samples from Indiana farms and Kentucky were ground and 16 samples (7 Indiana, 3 Kentucky, and 6 Evansville) had BGYF particles. The particles from each sample were removed, bulked, and extracted for aflatoxins (10). The weights of the bulked particles ranged between 0.2 and 0.1 g. In order to detect aflatoxin in these particles levels greater than 60 p.p.b. would be required. Fifty-gram portions, from samples in which the particles were observed, were also extracted (12). Aflatoxins were not detected in either assay procedure.

DISCUSSION

Extensive sampling of Indiana's western counties in 1972 and 83 counties in 1971 indicated that *A. flavus* was rare in preharvest corn. The greatest number of samples with *A. flavus* were from the southernmost counties of Indiana in 1972, but the incidence and amount were still very small. Increased isolation of *A. flavus* using a light surface disinfection treatment and a high incubation temperature may not be significant as reflected by the absence of aflatoxin in the *A. flavus* positive samples and the apparent lack of extensive internal kernel invasion. It may indicate that sufficient spores or mycelium were on the surface of the kernels to resist the treatment or that there was some incipient invasion in the field.

In 1971 there was only a very modest increase of *A. flavus* in the damaged corn compared to the intact corn. In 1972, however, there was an approximate twofold increase in the amount of *A. flavus* in damaged corn compared to the intact corn. The large amount of *F. moniliforme* in damaged corn in 1971 (Table II) may have acted as a barrier to *A. flavus* invasion. There may be a requirement for injury, either by insect, bird, or weather, or there may be a specific vector relationship for *A. flavus*. Taubenhaus (1) proposed an injury requirement in 1920 when he associated *A. flavus* invasion with corn earworm damage.

No sound corn from ear samples yielded aflatoxin, but eight of 163 combine-harvested corn samples from Evansville did. The shelled corn, however, was dried in paper bags which slowed the drying rate and possibly permitted some postharvest growth of *A. flavus* and production of aflatoxin. Ear samples usually dried overnight in the mesh bags, inhibiting any growth of *A. flavus*.

Some of the ground samples yielded BGYF in particles; however, no aflatoxins were detected from these samples. There were several kernels and cob tips with a fluorescence similar to the BGYF of the infected kernels. This "BGYF" was in external layers, usually at the tip of the kernel. It also occurred in the glumes or cob tips and may be identical to that reported by Fennell et al. (11) and Marsh et al. (14). The "BGYF" compound was not water-soluble, as has been reported for BGYF of infected kernels (10,11).

Field infection of corn by *A. flavus* in Indiana and possibly Kentucky appears of little consequence in regard to the production of significant levels of aflatoxin. It is possible, however, that a combination of circumstances: damage, abundant

inoculum, and an efficient vector could result in a serious field outbreak, particularly in southern Indiana. It appears that field infection may only be of importance in initiating the rapid growth of *A. flavus* in storage and concomitant production of aflatoxin. Continued field sampling will provide more information on this problem.

Acknowledgments

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