# MYCOTOXINS IN HOT SPOTS IN GRAINS. I. AFLATOXIN AND ZEARALENONE OCCURRENCE IN STORED CORN[1]

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## ABSTRACT

A hot spot that developed *Aspergillus flavus* growth in a bin of corn in central Illinois during warm weather has been investigated for mycotoxins. High levels of aflatoxin (1,000–1,700 ppb) were detected in samples collected near the center of the hot spot that was defined by visible *A. flavus* sporulation. The location of the hot spot relative to an open window indicated the moisture necessary for mold growth, and aflatoxin formation could have come from rains blown through the window. Corn collected at all depths from seven locations and probe samples taken under the hot spot were assayed for aflatoxin and zearalenone. Aflatoxin was not detected in samples collected furthest from the window. Zearalenone was detected in some of the samples collected, but it was not confined to any one part of the bin. The corn had been in the field an unusually long time before harvest because of cold, wet weather. Individual kernels selected from locations likely to contain aflatoxin and zearalenone were assayed for mycotoxins. Even in kernels connected with *A. flavus* mycelia, aflatoxin-free kernels occurred adjacent to highly contaminated kernels. Of 140 kernels analyzed for both aflatoxin and zearalenone, 16 contained aflatoxin (260–38,000 ppb $B_1 + B_2$) and 12 had zearalenone (9,000–1,700,000 ppb). In no kernel were both aflatoxin and zearalenone detected.

Surveys indicated low incidences and levels of aflatoxin in corn collected from commercial channels in the Midwest (1,2,3). However, corn stored on farms in the Midwest under certain circumstances is vulnerable to mold invasion and subsequent aflatoxin formation. Development of hot spots in bins of stored grain—pockets where molds grow, generating heat—has been observed many times (4). In one such incident, brought to our attention in June 1973, a greenish area appeared on the surface of some yellow corn stored in a rectangular bin in a wooden shed on a central Illinois farm. The corn was from crop year 1972 and had not been harvested until January 1973 because of unfavorable weather conditions. It was dried artificially to below 15% moisture after harvest.

The first sample collected from the center of the greenish area contained 1,600 ppb aflatoxin $B_1$ and 150 ppb $B_2$, and one kernel covered with *Aspergillus flavus* mycelia had over 200,000 ppb $B_1$. Later we detected zearalenone in the corn sample from the bin. This corn presented an unusual opportunity to study the formation and occurrence of a mycotoxin in a hot spot in stored corn because the owner was willing to cooperate. We are now reporting the results of our investigation.

## MATERIALS AND METHODS

### Storage Bin

The bin was in a wooden building and was 6.5 × 7 ft. The depth of corn varied from 7 in. at the front to 20 in. at the back of the bin. On the east wall side of the

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bin was an open window. The wooden building is pictured showing the open window in Fig. 1 and the inside of the building in Fig. 2. The bin is depicted with collection points and amounts of aflatoxin and zearalenone detected in samples taken at each point in Figs. 3 and 4.

**Collection of Samples**

On July 12 and August 27, 1973, samples were taken at different places in the bin. At designated points A, B, C, and D, samples (50–100 g) were collected from the bottom, middle, and top of the corn. Samples were also taken from the bottom and middle of the corn at E, near the center of the obviously moldy area. Surface samples were collected at F and G. A grain probe was inserted from front to back of the bin under the moldy spot about 8 in. from the floor. Sections of the probe were collected separately (P 1-12). A clump of kernels (25–30 g) covered with *A. flavus* growth was taken from the center of the hot spot. On July 12, three test tubes of kernels were collected to be analyzed individually. On August 27, surface samples (30–60 g) were taken at H and I.

**Bright Greenish-Yellow (BGY) Fluorescent Inspection (5, 6)**

The samples (25–100 g) and individual kernels (whole and cracked) were inspected for BGY fluorescence under high-intensity ultraviolet light (365 nm) with a Blak Ray lamp (Model B, 100-A).

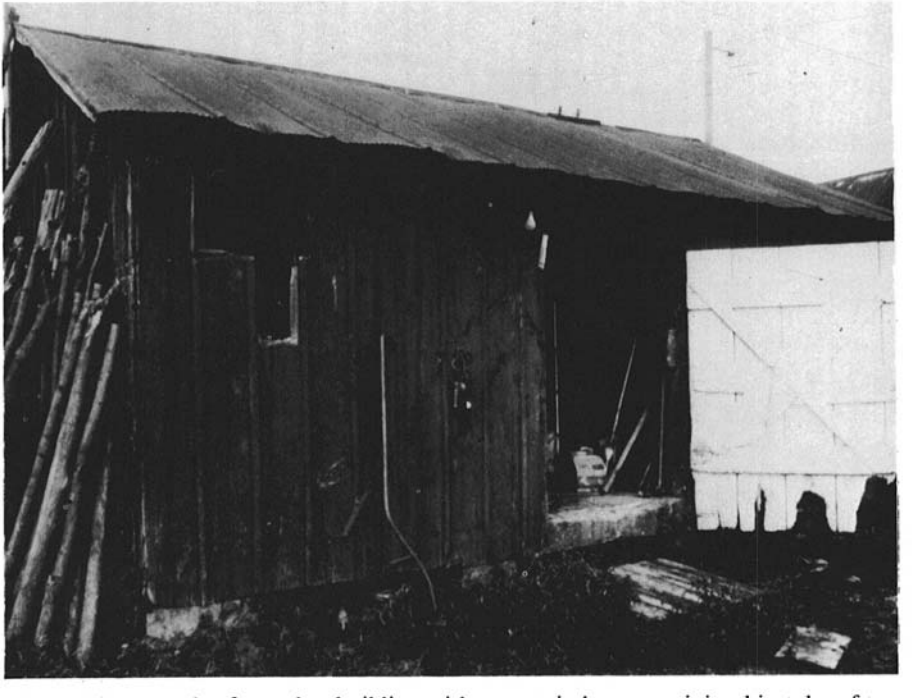

Fig. 1. Photograph of wooden building with open window containing bin taken from southeast.

**Aflatoxin and Zearalenone Analysis**

Samples (25–100 g) were simultaneously ground and extracted in a Waring Blendor to be assayed for aflatoxin and zearalenone by the multitoxin assay developed by Eppley (7) and by procedures approved in Official First Action by the Association of Official Analytical Chemists (8). Amounts of aflatoxin in partially purified extracts were determined on thin-layer chromatography (tlc) plates coated with 0.5 mm Absorbosil-1. Plates were developed with water:acetone:chloroform (1.5:12:88, v/v/v), and aflatoxin-fluorescent zones were measured densitometrically. The identity of $B_1$ was confirmed in representative samples by the formation of the water adduct (9). Determination of zearalenone was made on tlc plates with glacial acetic acid:benzene (1:9, v/v), and amounts on plates were determined visually by comparison with standards. The presence of zearalenone was confirmed by gas chromatography of representative samples (10).

Individual kernels were cracked with pliers and extracted 48 hr in 2–3 ml chloroform and a few drops of water in small vials. After a second extraction, combined extracts were evaporated on a steam bath under nitrogen. Residues were dissolved in 0.1 ml acetonitrile:benzene (2:98, v/v) for tlc. Residues from

Fig. 2. Photograph of inside of building, bin, and open window taken from the north.

extracts of the more damaged or molded kernels required 0.25 ml acetonitrile:benzene. Individual adjacent kernels were either collected separately or selected for assay from larger samples. When an individual kernel was part of a larger sample, its contribution of aflatoxin or zearalenone was added to the assay of the larger samples.

**Air Exposure Plates for Molds**

Petri plates of yeast extract agar (11) were exposed 2 min at various locations in the bin and around the wooden building containing the bin. After 3 days' incubation at 28°C, the number of mold colonies were counted and identified.

## RESULTS AND DISCUSSION

Levels of aflatoxin and zearalenone at different places in the bin at the time of

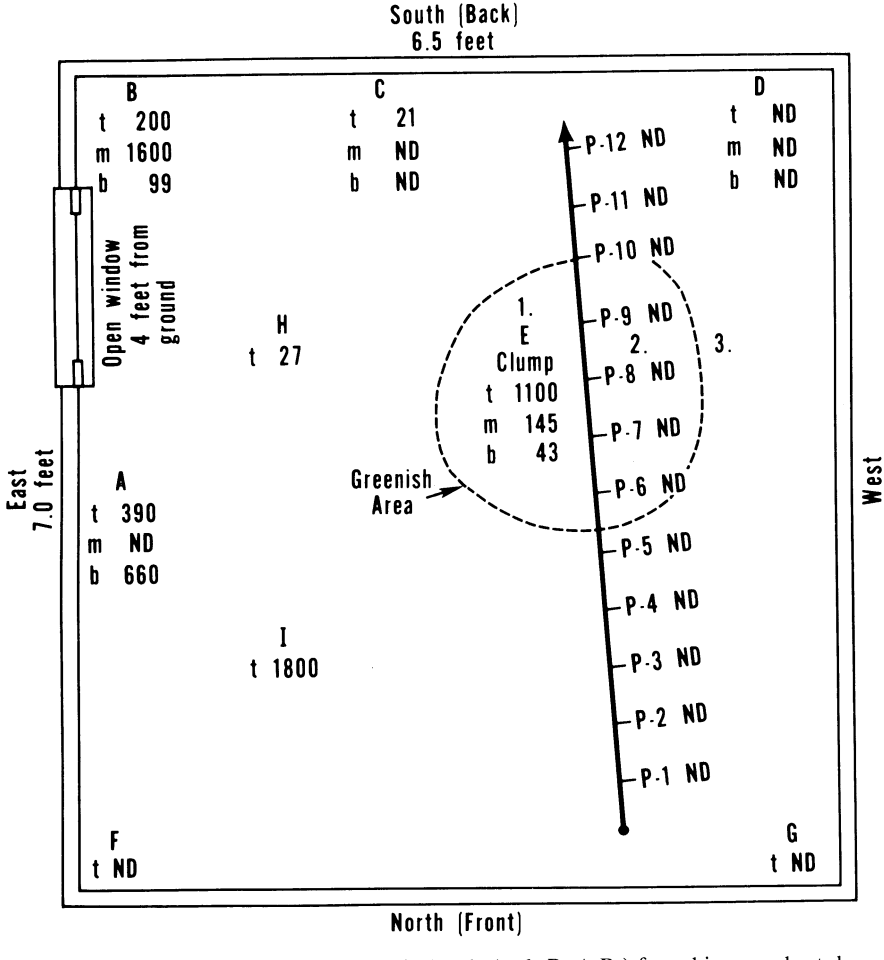


Fig. 3. Diagram of bin showing aflatoxin levels (ppb $B_1 + B_2$) found in samples taken at different locations on August 27, 1973. East and south were outside walls of building.

the two collections (July 12 and August 27, 1973) are shown in Table I. Higher concentrations (1,100–1,400 ppb) of aflatoxin tended to be in the clumps collected from the center of the hot spot. High levels (355–660 ppb) of aflatoxin were found in some samples collected near the open window, but very little toxin was detected in samples taken from parts of the bin furthest from the window (D,F,G) (Fig. 3). No aflatoxin was found in probe samples (P 1-12) collected below the hot spot. Aflatoxin was found in the same parts of the bin 6 weeks (August 27, 1973) after the first collection, but levels tended to be higher. The first samples taken in the corner near the window contained 0, 50, and 0 ppb total aflatoxin; on the second collection from the same spot, samples contained 200, 1,600, and 100 ppb aflatoxin. Samples taken at the surface between the hot spot and the window (H,I) during the second visit contained aflatoxin. Aflatoxins $G_1$ and $G_2$ were not detected in any of the samples. Aflatoxin-positive samples had at

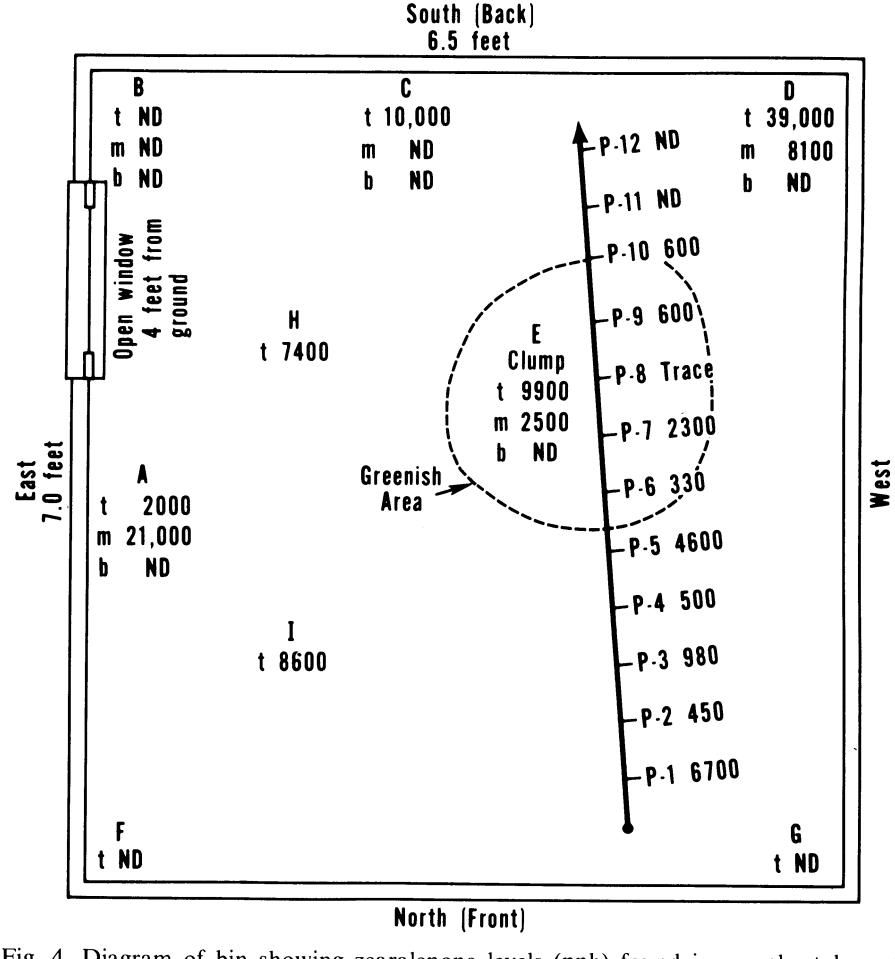


Fig. 4. Diagram of bin showing zearalenone levels (ppb) found in samples taken at different locations on August 27, 1973.

TABLE I
Mycotoxins (ppb) in Bin of Corn Containing "Hot Spot"

| Location (Refer to Figs. 3 and 4) | July 12, 1973 Aflatoxin $B_1$ | July 12, 1973 Aflatoxin $B_2$ | July 12, 1973 Zearalenone | August 27, 1973 Aflatoxin $B_1$ | August 27, 1973 Aflatoxin $B_2$ | August 27, 1973 Zearalenone |
|---|---|---|---|---|---|---|
| A | | | | | | |
| Top | ND[a] | | 92,000 | 390[b] | | 2,000 |
| Middle | 350 | 30 | 1,100 | ND | | 21,000 |
| Bottom | 5 | | ND | 600 | 60 | ND |
| B | | | | | | |
| Top | ND | | ND | 160 | 36 | ND |
| Middle | 42 | 5 | 4,400 | 1,300 | 260 | ND |
| Bottom | ND | | ND | 91 | 8 | ND |
| C | | | | | | |
| Top | ND | | 7,200 | 20 | 1 | 10,000 |
| Middle | ND | | ND | ND | | ND |
| Bottom | ND | | ND | ND | | ND |
| D | | | | | | |
| Top | ND | | 10,000 | ND | | 39,000 |
| Middle | ND | | 1,800 | ND | | 8,100 |
| Bottom | ND | | 1,600 | ND | | ND |
| Clump in middle of spot[c] | 1,300 | 150 | NA[d] | 990 | 90 | 9,900 |
| E | | | | | | |
| Middle | ND | | 4,100 | 120 | 25 | 2,500 |
| Bottom | 160 | 30 | ND | 43 | | ND |
| F | | | | | | |
| Surface | ND | | ND | ND | | ND |
| G | | | | | | |
| Surface | ND | | ND | ND | | ND |
| H | | | | | | |
| Surface | NC[e] | | NC | 27 | | 7,400 |
| I | | | | | | |
| Surface | NC | | NC | 1,400 | 420 | 8,600 |

[a]ND = Not detected, sensitivity limit = 1–3 ppb $B_1$; 50 ppb zearalenone.
[b]One kernel in 26 g accounts for 98% of this activity.
[c]The sample that was collected at this point when the hot spot was first noticed had 1,600 ppb $B_1$ and 150 ppb $B_2$.
[d]NA = Not analyzed.
[e]NC = Not collected.

**TABLE II**
**Zearalenone[a] in Probe Sample Portions[b] from Bin**

| Sample | Zearalenone (ppb) | |
|---|---|---|
| | July 12, 1973 | August 27, 1973 |
| P-1 | 2,400 | 6,700 |
| P-2 | ND | 450 |
| P-3 | 90 | 980 |
| P-4 | 280 | 500 |
| P-5 | 380 | 4,600 |
| P-6 | 90 | 330 |
| P-7 | 150 | 2,300 |
| P-8 | 100 | Slight trace |
| P-9 | ND | 600 |
| P-10 | ND | 600 |
| P-11 | NC[c] | ND |
| P-12 | NC | ND |

[a]Aflatoxin was not detected (ND) in any probe sample.
[b]Probe was run through corn under "hot spot" 8 in. parallel from floor. Depth of grain varied from 7 to 20 in. See Fig. 4.
[c]NC = Not collected.

**TABLE III**
**Total Aflatoxin ($B_1 + B_2$) (ppb) and Zearalenone ($F_2$) (ppb) in Kernels Connected by Mold or Adjacent to Each Other (in Physical Sequence as Posted)**

| | July 12, 1973[a] | | | August 27, 1973 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | A | | | | D | |
| | Tube 1[b] $B_1 + B_2$ | Tube 2 $B_1 + B_2$ | Clump[b] $B_1 + B_2$ | Clump[b,c] $B_1 + B_2$ | Top[b] $B_1 + B_2$ | Top[b] $F_2$ | Middle[b,d] $F_2$ | Bottom[b,c] $B_1 + B_2$ | Top[d] $F_2$ | Middle[d] $F_2$ |
| 1 | ND | ND | 23,000 | ND | ND | ND | ND | 1,200 | ND | ND |
| 2 | 1,800 | ND | 860 | ND | ND | ND | ND | ND | ND | ND |
| 3 | 980 | ND | ND | ND | ND | ND | ND | 16,000 | ND | ND |
| 4 | ND | ND | 150 | ND | ND | ND | ND | 22,000 | ND | ND |
| 5 | 11,000 | ND | 4,400 | 22,000 | ND | ND | ND | 32,000 | ND | ND |
| 6 | ND | 6,440 | 6,100 | 160 | 38,000 | ND | ND | ND | 94,000 | ND |
| 7 | 1,600 | ND | 2,900 | ND | ND | ND | 68,000 | ND | ND | 9,400 |
| 8 | ND | ND | ND | 3,200 | ND | ND | ND | ND | ND | ND |
| 9 | ND | ND | 190 | ND | ND | ND | ND | 860 | ND | ND |
| 10 | 1,700 | ND | | ND | ND | ND | ND | 260 | ND | ND |
| 11 | ND | ND | | 3,800 | ND | ND | ND | ND | ND | ND |
| 12 | ND | ND | | ND | ND | ND | ND | ND | ND | ND |
| 13 | ND | | | ND | ND | ND | ND | ND | ND | 170,000 |
| 14 | | | | 14,000 | ND | ND | ND | ND | 35,000 | ND |
| 15 | | | | 170 | ND | ND | ND | ND | 37,000 | ND |
| 16 | | | | ND | ND | ND | ND | 2,100 | 1,700,000 | ND |
| 17 | | | | ND | ND | ND | ND | ND | ND | ND |
| 18 | | | | 430 | ND | 37,000 | ND | ND | 530,000 | ND |
| 19 | | | | ND | ND | ND | ND | ND | ND | ND |
| 20 | | | | 2,600 | ND | ND | ND | ND | ND | ND |

[a]Not assayed for zearalenone.
[b]Connected with mold.
[c]No zearalenone detected in kernels; sensitivity, 0.25 g kernel = 2,000–4,000 ppb.
[d]No aflatoxin detected in kernels; sensitivity 0.25 g kernel 20–40 ppb.

least one BGY particle in them. Of 20 negative samples, four had some BGY fluorescence.

Zearalenone did not appear to be so localized in the bin as aflatoxin (Table I and Fig. 4). It was not detected in any sample taken from the bottom of the bin or at F and G. Levels of 1,100–92,000 ppb zearalenone were determined. There was no dramatic change in levels between the two collections, but the corn appeared to be in very poor condition on August 27 and on September 11, 1973, when the bin was unloaded. Much mold was present and the corn had a musty odor. Some samples had high levels of both aflatoxin and zearalenone. One, for example, had 1,100 ppb aflatoxin and 9,900 ppb zearalenone.

Column chromatography by the Eppley procedure (7) of extracts from samples with extensive mold damage presented problems. Zearalenone is normally eluted in the first fraction from the column and aflatoxin in the second; but with extracts of very moldy corn, part of the aflatoxin appeared in the zearalenone fraction. Fortunately, the two toxins are easily differentiated by tlc.

Zearalenone was detected in the sections of the probe sample taken under the hot spot through the bin 8 in. from the floor (Table II). Levels of zearalenone were 90–2,400 ppb on the first collection and 330–6,700 ppb on the second.

Results of the analysis of individual kernels for aflatoxin and zearalenone are summarized in Table III. The two groups of kernels (tube 1 and from clump) collected July 12 from the center of the hot spot were connected by *A. flavus* mycelia. Assays of adjacent kernels are posted in physical sequence. Aflatoxin was detected (980–11,000 ppb $B_1 + B_2$) in only five kernels in tube 1; none was found in eight kernels even though they were held together by mycelia. Highly contaminated kernels were often adjacent to aflatoxin-free kernels. Of the nine kernels taken from the clump, two were negative and seven had 150–23,000 ppb total aflatoxin. Only one positive kernel was detected in tube 2, containing 12 kernels and collected in the hot spot but further from the window than kernels in tube 1. No positives were detected in tube 3 kernels collected still further from the window and taken from corn without visible mycelia. Kernels collected during the first visit were assayed for aflatoxin only. When the larger samples of corn taken at the same time were analyzed by the multitoxin assay, zearalenone was detected. We then analyzed the individual kernels collected during the second visit for zearalenone as well as for aflatoxin.

From the samples collected August 27, 1973, 20 individual kernels were taken that were likely to have aflatoxin (clump and A) or zearalenone (D). As noted in Table III, some of the kernels were connected with mold. Once again a highly contaminated kernel would be surrounded by kernels in which neither toxin was detected. No aflatoxin was found in kernels collected at D. Of 140 kernels assayed for both aflatoxin and zearalenone, 16 had aflatoxin (260–38,000 ppb $B_1 + B_2$) and 12 had zearalenone (9,000–1,700,000 ppb). In no kernel were both mycotoxins detected together. The sensitivity limits in analyzing a 0.25-g kernel are 20–40 ppb aflatoxin and 2,000–4,000 ppb zearalenone; a number of kernels containing lesser amounts of either toxin could be missed.

No aflatoxin was detected in 155 individual kernels; BGY fluorescence was not observed in 150 of these kernels. Of 29 aflatoxin-positive kernels, 25 had BGY fluorescence and 4 kernels were filled with an orange fluorescing powder previously noted in aflatoxin-contaminated corn.

Examination of individual kernels for mold outgrowth indicated that *A. flavus*

**TABLE IV**
**Mold Colonies on Air Exposure Plates**

| Location | Before Disturbing Bin[a] | | After Disturbing Bin | |
|---|---|---|---|---|
| | Number of colonies | Molds | Number of colonies | Molds |
| Above hot spot in bin | 80, 210, 58[b] | *Aspergillus flavus*[c]> *A. niger* | 143, 140, 225 | *A. flavus*> *A. niger* |
| Just inside door | 35, 26 | *A. flavus*> *Cladosporium, Alternaria* | 19, 83, 31 | *A. flavus*> *Cladosporium, Alternaria, Penicillium* |
| Outside open window | 160 | *Alternaria, Cladosporium, Cephalosporium* | 131 | *Alternaria, Cladosporium, Mucor, Trichoderma* |
| Near cattle rack south of building | 16,17 | *Cladosporium, Alternaria* | 19, 27 | *Cladosporium, Alternaria Cephalosporium* |
| Above D location in bin | | | 131 | *A. flavus* |
| Doorway | | | 68, 92 | *A. flavus, Cladosporium* |
| Inside west of bin, 2–3 ft from floor | | | 81 | *A. flavus,* few *A. niger* |
| Inside north of bin, 4 ft from floor, 6 ft from bin | | | 66 | *A. flavus*> *A. niger, Cladosporium* |
| Inside north of bin, 2 ft from floor, 4 ft from bin | | | 83 | *A. flavus*> *Cladosporium* |

[a]Bin was disturbed during sampling procedure.
[b]Number of colonies per plate, three plates in this instance.
[c]*A. flavus* is predominant mold.

was the predominant mold. Examination of air exposure plates after 3 days incubation at 28°C revealed colonies of *A. flavus, A. niger, Cladosporium, Alternaria, Penicillium,* and *Cephalosporium* (Table IV). *A. flavus* was the predominant organism on plates exposed inside the wooden shed. There was no indication that conidia of *A. flavus* were carried outside the building since no colonies developed on plates exposed around the exterior of the building.

## CONCLUSION

The hot spot indicated by visible *A. flavus* growth appearing in June, 1973, contained the highest levels of aflatoxin; the contamination was at the surface of the corn in the bin. The next highest levels were under the open window and at a location between the window and hot spot. Because of the position of the hot spot in the bin in relation to the open window, we postulate that rains were blown into the bin by winds from the east. With the necessary moisture and higher temperatures in June, the mold began to form the mycelia that were observed and to produce aflatoxin. Levels of aflatoxin increased in the six weeks between the first and second sample collection. Although corn has not seemed to be a high aflatoxin risk crop in the Midwest (1,2,3), these studies indicate that improper storage conditions can lead to high levels of aflatoxin in corn during warmer weather.

Zearalenone was not concentrated in one part of the bin, and none was detected in probe samples taken from the bottom of the bin or at the front of the bin. The window had been open all winter. Moisture in the form of snow, sleet, and rain could have come through the window over a period of time. The corn could have been wetted and dried several times. With the right amount of moisture, zearalenone could have been formed at the lower temperatures. As observed before for aflatoxin, analysis of individual kernels for zearalenone revealed high concentrations that could represent significant levels of toxin causing concern in a bulk sample containing several thousand kernels. The distribution of zearalenone contamination among corn kernels is similar to that observed previously in aflatoxin-containing corn (6).

The existence of toxin-free kernels adjacent to highly contaminated kernels in a group held together and presumably infected by *A. flavus* mycelia is not understood. Perhaps factors such as moisture differences, physical damage, and grain maturity are involved. This kernel difference deserves study.

### Acknowledgment

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