Fungal Growth and Dry Matter Loss During Bin Storage of High-Moisture Corn[1]

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ABSTRACT

Carbon dioxide, ergosterol, aflatoxin, percent of kernels with fungi, moisture contents, and temperatures were monitored during storage of freshly-harvested, high-moisture (22.9–25.6%) corn in 1978 and 1979. The corn was stored in two 100-bu bins and aerated from the bottom at 0.17–0.66 m^3/min/t. Fungal invasion was highest at the top and lowest at the bottom of the bins. The tests showed that 1) fungal invasion and aflatoxin content could be unacceptable before the grain lost 0.5% dry matter, and 2) the initial amount of inoculum, particularly *Aspergillus flavus*, on the grain significantly affected the extent of fungal invasion during storage. Standard visual inspection procedures did not adequately reveal the fungal contamination in samples of the corn when surface mold was removed by agitation in a stream of air.

Because corn is commonly harvested at high moisture content, knowing how much time can be taken to dry the grain before it deteriorates excessively is necessary. Saul and Lind (1958), Saul and Steele (1966), and Steele et al (1969) investigated carbon dioxide production and accompanying dry matter loss (DML) as an index of corn deterioration. Saul and Steele (1966) concluded that 0.5% DML could be reached before deterioration became visible enough to cause market grade to drop below No. 2. The 0.5% guideline and the data of Steele et al (1969) concerning dependence of DML on moisture, temperature, and damage have been used in recommendations given to farmers (U.S. Department of Agriculture 1969) and in computer simulations of corn drying (Bakker-Arkema et al 1977, Morey et al 1979, Thompson 1972).

Although fungal growth is the main cause of deterioration of high-moisture corn, the relationship between fungal growth and DML has not been established. Respiration of both the grain itself and the fungi in the grain contribute to DML. The contribution to DML from fungi is usually small at the start of storage, but as the fungi grow, DML increases at a rate dependent on moisture, temperature, amount and type of kernel damage, and the amount and type of fungal inoculum on the grain. The relative importance of these factors was studied with small-scale laboratory experiments (Seitz et al 1982).

In 100-bu bin tests conducted in 1978 and 1979, we monitored fungal growth by ergosterol, aflatoxin, and whole-seed plating assays and DML by measuring the carbon dioxide concentration in the effluent air. Ergosterol is a measure of total fungal growth (Seitz et al 1979). Effects of initial fungal inoculum level on fungal growth, aflatoxin content, and dry matter loss were studied in the 1979 test.

MATERIALS AND METHODS

Yellow corn at 22–26% moisture content was harvested by conventional combine near Wamego, KS, on October 2, 1978, and September 18, 1979. Within 3–4 hr after harvest, the corn was placed in two 100-bu bins inside an unheated building at the U.S. Grain Marketing Research Laboratory in Manhattan, KS. In 1978 a rotary cleaner (Gilmore Tatge, Clay Center, KS; model 41-10010 with a screen having 5-mm square openings) was used to remove the fine material from the grain as each bin was filled. In 1979, the cleaner was not used, and fungal inoculum was added to the grain in one bin. The inoculum was a mixture of *Aspergillus flavus, A. niger, Penicillium brevi-compactum*, and *P. citrinum* grown on wheat kernels. About 1 L of the wheat kernels was evenly distributed into the corn as it was augered into the bin.

Each bin was 2 m (6 ft) in diameter and had a drying floor (24% open area) mounted 20 cm (8 in.) above the bin base. Grain height at the start of each test was about 1.5 m (4.5 ft). A plywood cover with an airtight seal was mounted on the top of each bin. A single air outlet hole 10 cm (4.0 in.) in diameter was located in the center of the bin cover.

Grain temperatures were monitored with copper-constantan thermocouples connected to an Easterline-Angus, multipoint strip chart recorder. In 1978, thermocouples were located at 5, 10, 15, 30, 46, and 61 cm from the bottom and at the surface of the grain. Three thermocouples were at the 61-cm (24-in.) level, one at the center and two on opposite sides. At each of the other levels, one thermocouple was placed near the center of the bin. In 1979, thermocouples were placed near the center of the bin at 5, 10, 15, 30, 46, 61, 76, 107, 117, and 127 cm from the bottom and at the grain surface.

The temperature and relative humidity of the air entering and leaving the corn were continuously measured. A hygrothermograph recorder was placed in an instrument box between the fan and the bin plenum so that only air entering the bin would influence the recorder readings. A hygrothermograph recorder was also placed in a partially enclosed instrument box on the bin cover in the air stream leaving the bin.

To measure CO_2 evolved from the grain, a low airflow rate was necessary to keep the CO_2 concentration in the effluent air significantly above ambient. In 1978 a 0.12 kW fan was used to maintain an airflow of 0.66 m^3/min/t (0.60 ft^3/min/bu) for the first 2.7 days, then adjusted to 0.24 m^3/min/t (0.22 ft^3/min/bu) for the remainder of the test. In 1979 a 0.016 kW fan was used to maintain an airflow of 0.17 m^3/min/t (0.15 ft^3/min/bu) throughout the test except for 5.3 hr during the ninth day when a 0.12 kW fan was installed to provide an airflow of 1.79 m^3/min/t (1.61 ft^3/min/bu) to prevent severe overheating. Airflow rate was determined with a 10-cm (4.0-in.) vane anemometer to measure the velocity of the air passing through the exit hole in the bin cover.

Carbon dioxide concentration in the effluent air was measured as follows. A peristaltic pump was used to draw air from the air space at the top of the bin into a sampling bulb (134 ml). With another peristaltic pump, the air sample was circulated through the sample cell of a carbon dioxide analyzer (Beckman model 865). The system was calibrated by filling the sample bulb with calibration standards obtained from Matheson Gas Products. The average ambient carbon dioxide concentration, obtained by sampling the air drawn into the bins several times during each test, was subtracted from the concentration in the effluent air to determine the concentration increase produced by the grain. Respiratory rate (R), defined as milligrams of CO_2 per kilogram of dry matter per hour, was

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calculated as follows:

$$R = \frac{(C - A) \cdot F \cdot D}{M}$$

where

C = concentration, by volume, of CO_2 in the effluent air, ie, milliliters of CO_2 per milliliters of air

A = concentration, by volume, of CO_2 in ambient, inlet air

F = airflow, in milliliters per hour

D = CO_2 density, in milligrams per milliliter. A value of 1.75 was considered appropriate for most of the temperature and pressure conditions involved in these experiments.

M = kilograms of dry matter at the beginning of storage.

By using a computer, we calculated percent DML vs time curves by integrating the respiratory rate vs the time data. Storage time was divided into intervals representing amounts of time between CO_2 concentration measurements. The amount of CO_2 during a time interval was computed by using the respiratory rate at the end of the interval. Total CO_2 produced during a storage period was then the sum of the interval amounts produced in that period. Loss of about 15 g of CO_2 per kilogram of grain dry matter is equivalent to 1% DML (Steele et al 1969). Thus, total grams of CO_2 evolved per kilogram of dry matter was divided by 15 to obtain percent DML.

Grain samples were taken from the bins with a partitioned sampling probe (Burroughs Equipment Co., Evanston, IL). Grain was captured in 10 cells. We emptied the probe so that six samples were collected in plastic bags representing grain heights centered on 14, 28, 48, 75, 103, and 130 cm. After portions were taken for moisture assay, the two lower samples were usually combined into one sample representing a grain height of 21 cm.

The probe was pushed into the grain through portholes in the bin cover. Airtight covers closed the ports when samples were not being taken. Through the 40th day of the 1978 test and the 16th day of the 1979 test, probe samples were taken from the center (air-outlet) port and four ports equidistant from each other and midway between the center and the wall. Those five probe samples were combined, keeping cells separate as described above. After the 40th day of the 1978 test and the 16th day of the 1979 test, the same procedure was followed except that four "offset" ports, instead of the regular ports, were probed. The offset ports were between the regular ports and about 12 in. from the bin wall.

Immediately after probe samples were removed from the bins, 20–50-g subsamples were oven dried at 103°C for 72 hr to determine moisture content. The remainder of the sample was spread thin and allowed to air-dry. After 100 kernels were taken for the whole-seed plating assay, the sample was ground in preparation for ergosterol and aflatoxin assays.

Ergosterol content of the corn was determined as previously described (Seitz et al 1979). Aflatoxin assays were by the method of Seitz and Mohr (1977) except for the following modifications. A thin-layer chromatographic plate was streaked with the entire concentrated extract. After the plate was developed and dry, it was placed under a longwave ultraviolet lamp just long enough to mark the aflatoxin band. The aflatoxin band was scraped into a small fritted glass filtering funnel and aflatoxins were eluted from the silica with chloroform/acetone (9:1, v/v). The eluant, which was collected in a two-dram vial, was evaporated to dryness over a steam bath with a stream of nitrogen directed into the vial. The residue was dissolved in 0.2–8.0 ml of methanol depending on the expected aflatoxin concentration. A 10–20-μl portion of the sample was injected into a high-pressure liquid chromatograph that consisted of a Varian Associates model 4200 pumping system, a Schoeffel model 770 variable wavelength detector, a Hewlett Packard 3385A chromatography automation system, and a Waters Associates μ-bondapak-C_{18} column. The mobile phase was methanol/water/acetonitrile (50:35:15, v/v) with a flow of 1.67 ml/min. The detector was set at 365 nm. Average aflatoxin B_1

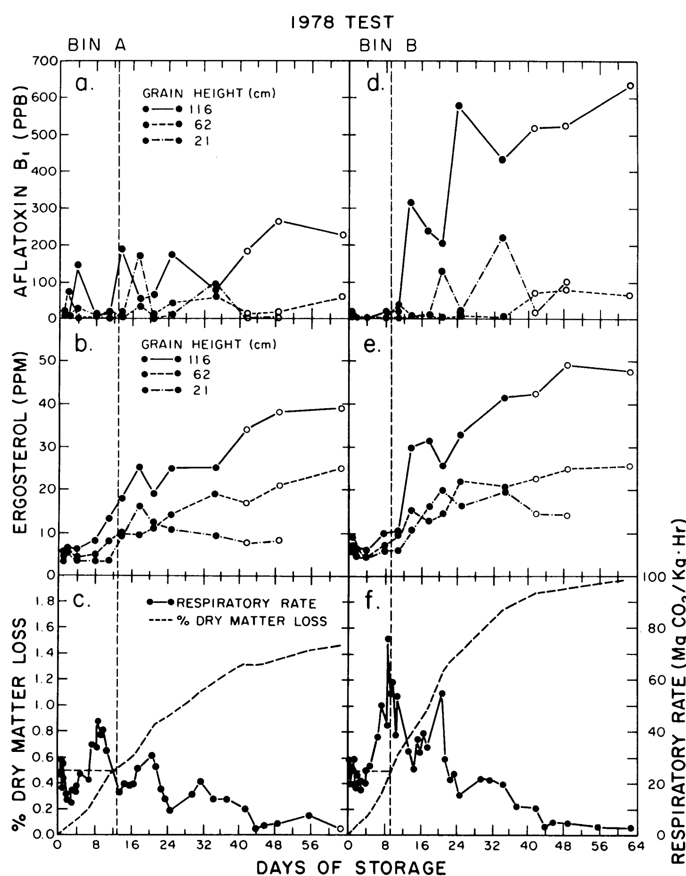

Fig. 1. Aflatoxin (**a** and **d**) and ergosterol contents (**b** and **e**), and respiratory rates and dry matter loss (**c** and **f**) during storage of 1978 corn in two 100-bu bins. Vertical dashed lines indicate times at which average dry matter loss was 0.5%. • = samples from regular ports, o = samples from offset ports.

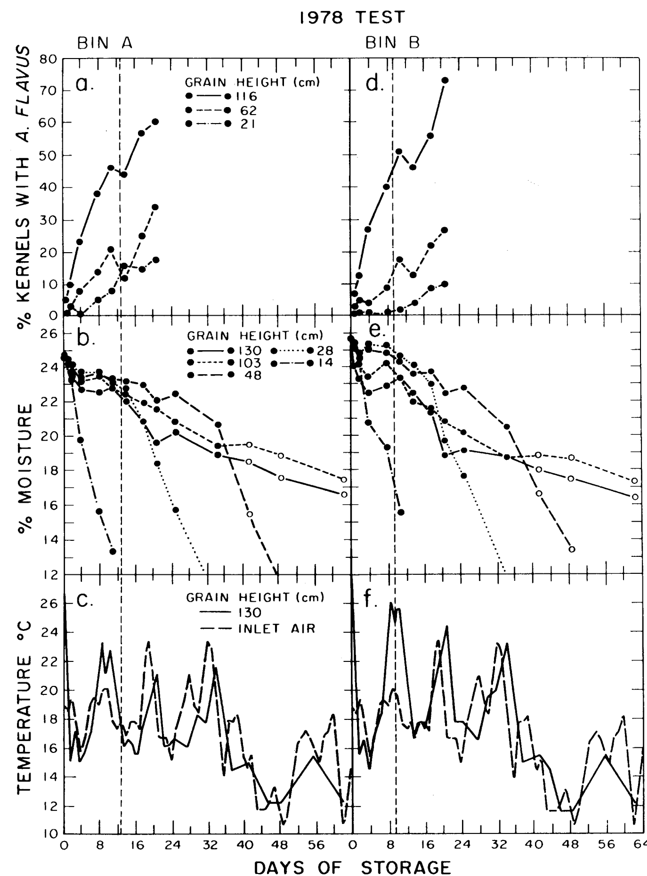

Fig. 2. *Aspergillus flavus* invasion (**a** and **d**), moisture content (**b** and **e**), and temperatures (**c** and **f**) during storage of 1978 corn in two 100-bu bins. Vertical dashed lines indicate times at which average dry matter loss was 0.5%. • = samples from regular ports, o = samples from offset ports.

TABLE I
Fungi in Surface-Disinfected Corn Kernels from Different Portions of Two Bins after 4, 8, and 16 Days of Storage in 1978 Tests[a]

		Bin A,[b] Percent Kernels with					Bin B,[c] Percent Kernels with				
		Aspergillus					*Aspergillus*				
Days Stored	Grain Height (cm)	*flavus*	*niger*	*Rhizopus*	*Fusarium* and *Cephalosporium*	*Alternaria*	*flavus*	*niger*	*Rhizopus*	*Fusarium* and *Cephalosporium*	*Alternaria*
0	...	2	3	2	29	4	2	3	2	29	4
4	0–34	1	1	1	34	22	1	2	4	34	33
	34–89	8	3	6	35	36	4	4	5	29	43
	89–144	23	18	7	33	20	28	15	5	32	35
8	0–34	5	9	4	36	35	1	1	10	31	59
	34–89	14	13	7	45	42	9	15	10	43	43
	89–144	37	32	12	29	21	39	33	9	34	21
16	0–34	15	29	10	54	58	6	12	17	58	46
	34–89	18	26	11	53	39	18	16	16	52	44
	89–144	50	45	13	54	19	45	40	12	56	19

[a] Other fungi, present in less than 5% of the kernels, included *Penicillium, Mucor, Cladosporium*, and *Nigrospora*.
[b] Moisture content, 24.7%.
[c] Moisture content, 25.6%.

recovery by this method was 80%, so results were multiplied by 1.25 to correct for aflatoxin loss during the assay. Only aflatoxin B_1 was quantified. Aflatoxin B_2 usually was present but its concentration was low compared with the concentration of B_1. Aflatoxins G_1 and G_2 were not present.

The whole-seed plating assay was performed as follows. Kernels were surface-disinfected with 5% sodium hypochlorite (Chlorox), pH 10.6, followed by one sterile water rinse. Fifty or 100 kernels were placed on malt agar containing 4% NaCl and 200 ppm Tergitol NPX (Union Carbide Co., New York, NY). Petri plates were incubated five days at 25°C, and then fungi growing from kernels were identified.

RESULTS AND DISCUSSION

Although the two bins in the 1978 test were filled at the same time with cleaned corn from a single truckload, the initial moisture content of the corn was 24.7% in bin A and 25.6% in bin B. The corn in bin A, which had the lower moisture content, deteriorated less than the corn in bin B (Figs. 1 and 2). Whether the moisture content difference was the sole cause of the difference in deterioration is not known. Average DML reached 0.5% in 12.9 and 9.3 days, respectively, in bins A and B. Maximum carbon dioxide production and grain temperature were attained on the ninth day of the test in both bins, but the maximums were lower in bin A than in bin B. Ergosterol (Fig. 1b and e) and aflatoxin contents (Fig. 1a and d), particularly in the upper portions of the bins, were less in bin A than in bin B. Percentages of seeds with *A. flavus* and other fungi (Fig. 2a and d; Table I), however, were fairly similar at corresponding grain heights in the two bins.

A. flavus and *A. niger* both were initially present in amounts much higher than normal for the Manhattan, KS, area.[4] After 10 days, *A. flavus* and *A. niger* each were found in 40–50% of the kernels in the tops of the bins and in about 5–10% of the kernels in the bottoms of the bins. In three weeks, the counts were about 60% in the top and 10–20% in the bottom. *Fusarium moniliforme, Cephalosporium acremonium, Alternaria alternata*, and *Rhizopus* spp. were the other principal fungi that grew in the 1978 corn (Table I).

In the 1979 test, the observed difference between the two bins in extent of deterioration (Figs. 3 and 4) clearly was caused by the fungal inoculum added to the corn in one bin. Initial moisture content of the corn was 22.9% in both bins. Average DML reached 0.5% at 3.9 days in the inoculated bin and 5.4 days in the control. For the first 9.8 days, before the larger fans were used to reduce temperatures, carbon dioxide production and grain temperatures (in the upper portion) were considerably higher in the inoculated bin than in the control. Fungal invasion was significantly higher in

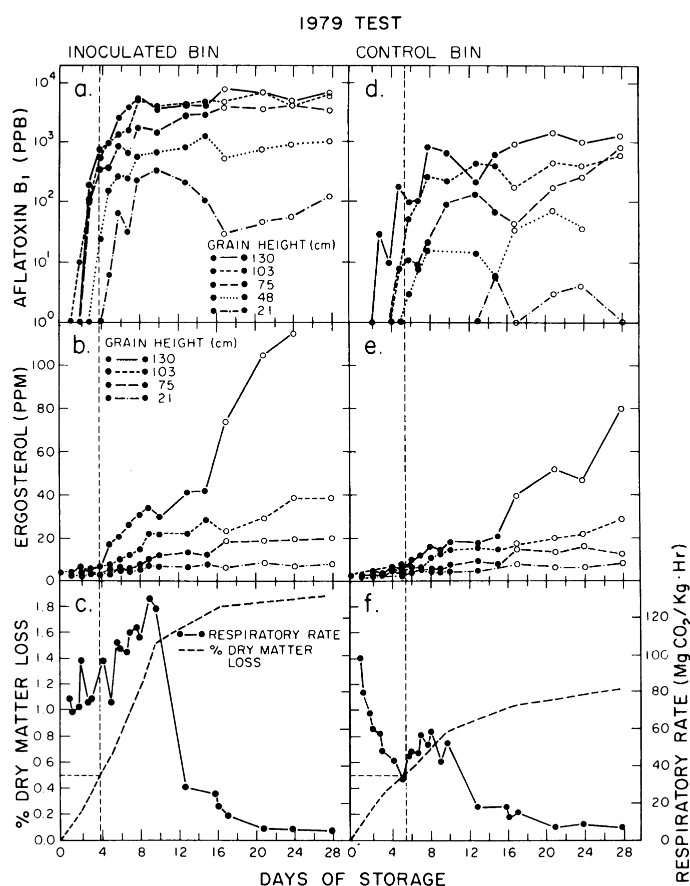

Fig. 3. Effect of added fungal inoculum on aflatoxin (**a** and **d**) and ergosterol contents (**b** and **e**), and respiratory rates and dry matter loss (**c** and **f**) during storage of 1979 corn. Vertical dashed lines indicate times at which average dry matter loss was 0.5%. Aflatoxin contents shown are measured values plus one. • = samples from regular ports, o = samples from offset ports.

the inoculated bin than in the control, as indicated by higher ergosterol contents (Fig. 3b and e), aflatoxin contents (Fig. 3a and d), and percentages of kernels with *A. flavus* (Fig. 4a and d). In the inoculum, *A. flavus* was more abundant than the other three fungi. *Penicillium* and *A. niger* inoculum apparently contributed little or nothing to the difference in fungal invasion between the two bins (Table II). *Penicillium* was much more abundant in the control bin than in the inoculated bin, especially after *A. flavus* counts became high in the inoculated bin (Table II).

A top-to-bottom fungus concentration gradient was found in all

[4] Unpublished data.

tests. Ergosterol and aflatoxin contents, and percent of kernels with *A. flavus* were consistently highest at the top and lowest in the bottom of the bins. The same gradient existed in percent of kernels with *A. niger* and *Rhizopus* in 1978 but was not consistent among other fungi in 1978 or 1979, perhaps due to competition among species (Tables I and II). Heating from fungal growth at the top of the bins, coupled with evaporative cooling in the drying zone, caused a temperature gradient in the bins early in the tests. The inoculated bin in 1979 had the most severe temperature gradient, with the temperature at the top of the bin 16–18°C above that at about 15 cm from the bin floor. High temperatures apparently enhanced drying at the top of the grain. In the 1978 tests, moisture contents were highest near the bottom just above the drying front and then gradually decreased to the top of the grain (Fig. 2b and e). In the 1979 tests, the grain at the surface was slightly wetter than grain just below the surface (Fig. 4b and e).

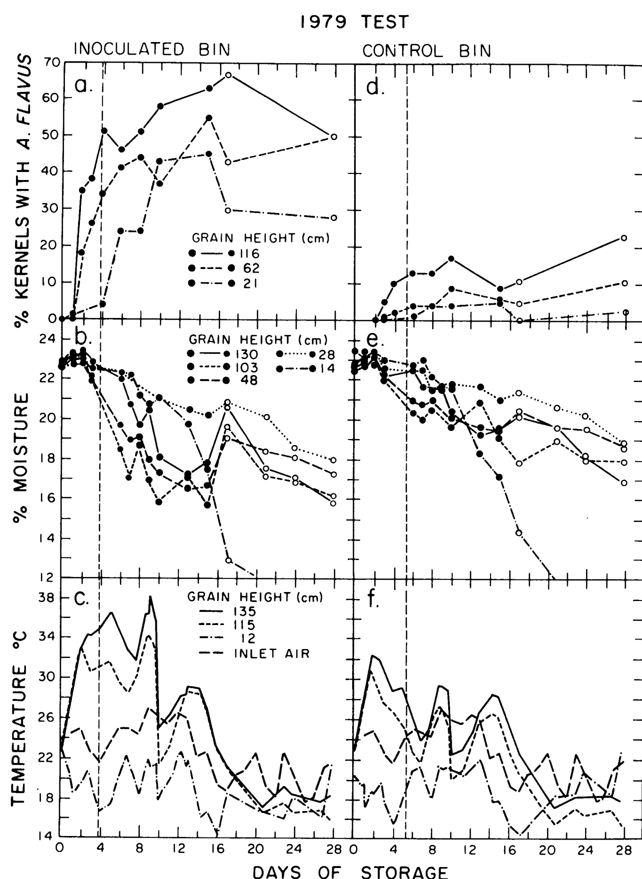

Fig. 4. Effect of added fungal inoculum on *Aspergillus flavus* invasion (**a** and **d**), moisture content (**b** and **e**), and temperatures (**c** and **f**) during storage of 1979 corn. Vertical dashed lines indicate times at which average dry matter loss was 0.5%. ● = samples from regular ports, o = samples from offset ports.

Through day 40 of the 1978 test and day 16 of the 1979 test, probe samples were taken from the regular ports in the bin cover. Later samples were removed from offset ports located between the regular ports and slightly closer to the wall. Rather abrupt changes in ergosterol, aflatoxin, moisture contents, and percent of kernels with *A. flavus*, especially in the 1979 inoculated bin (Figs. 3 and 4), corresponded to the change in sampling position. Repeated sampling through the regular ports probably mixed vertical levels and lowered resistance to air flow in the columns of grain below those ports, thus enhancing drying.

Guidelines for safe storage times based on reaching 0.5% DML assume that the corn in question has the same moisture content and temperature throughout the bulk. DML values reported in this paper represent an average for an entire bin, although temperature and moisture gradients existed through the bin. Nevertheless, consideration of the condition of the corn in our tests when average DML was at or near 0.5% is worthwhile. Invasion by *A. flavus* increased greatly before 0.5% DML in all of the tests (Figs. 2a and d, 4a and d). In 1979, aflatoxin was first detected on day 2 in the inoculated corn and on day 3 in the control corn (Fig. 3a and d). On day 3, nearly one day before 0.5% DML was attained, the corn in the upper half of the inoculated bin contained 100–190 ppb aflatoxin B_1. The upper half of the control corn contained 8–170 ppb on day 5 shortly before 0.5% DML. In 1978, a significant increase in aflatoxin content was not detected until after 0.5% DML was reached (Fig. 1a and d). The 1978 corn had kernels with high aflatoxin contents that caused substantial variation in aflatoxin results when the probe samples were analyzed. Also, lower grain temperatures contributed to lower aflatoxin production in 1978 than in 1979.

Most of the increase in ergosterol content occurred after 0.5% DML was reached. Only bin A in 1978 (Fig. 1b) showed more than a slight increase in ergosterol content in the upper portion of the bin before 0.5% DML. The ability of the ergosterol assay to detect fungal growth early in the storage period was apparently limited by preharvest fungal invasion. Whole-seed plating and aflatoxin assays detected *A. flavus* growth before the ergosterol assay detected a significant increase in total fungal invasion. This was particularly true in the 1979 corn, which had little *A. flavus* infection and no aflatoxin before storage but some ergosterol from preharvest invasion by other fungi.

TABLE II
Fungi in Surface-Disinfected Corn Kernels from Different Portions of Two Bins of 22.9% Initial Moisture Content after 4, 8, and 16 Days of Storage in 1979 Tests[a]

Days Stored	Grain Height (cm)	Inoculated Bin, Percent Kernels with *Aspergillus flavus*	*Penicillium*	*Fusarium* and *Cephalosporium*	*Alternaria*	Control Bin, Percent Kernels with *Aspergillus flavus*	*Penicillium*	*Fusarium* and *Cephalosporium*	*Alternaria*
0	...	0	9	69	0	0	4	76	1
4	0–34	4	16	75	23	0	10	74	30
	34–89	33	6	89	16	1	11	84	35
	89–144	50	8	80	24	10	12	86	27
8	0–34	24	21	85	9	4	27	72	35
	34–89	44	21	75	7	3	50	84	20
	89–144	50	35	69	3	13	81	84	15
16	0–34	37	45	91	19	3	51	98	30
	34–89	50	33	88	9	5	54	95	24
	89–144	65	19	81	5	10	50	93	18

[a] Other fungi, present in less than 5% of the kernels, included *Aspergillus niger, A. ochraceus, Rhizopus, Trichoderma, Nigrospora, Cladosporium*, and *Mucor*.

The 1979 corn had a musty odor when the bins were sampled on day 3, well before 0.5% DML in either bin. Fungal-related odors also developed early in the 1978 tests, but the exact time they were first detected was not recorded.

None of the current models for simulation of grain drying is applicable to our storage tests. The models are designed to simulate drying grain and risk of spoilage at higher airflow rates than those we used and do not allow for heating caused by extensive mold growth. Success or failure of a simulated drying test is usually determined by whether or not calculated DML at the top of the bin reached 0.5% before the grain was dry. Our tests indicate that DML at the top of the bin would be greater than the average DML, but how much greater cannot be determined from our data.

We used relationships reported by Steele (1969) and Thompson (1972) to predict DML for the 1979 data from the tops of the bins (a layer of about 10 cm), where grain temperatures were highest. Moisture content (22.9%) was essentially constant during the first few days of storage. Temperature variations were taken into account graphically. The storage period was divided into increments. The temperature of the grain during each time increment is indicated by a horizontal bar in Fig. 5. A DML vs time (0 to >4 days) curve was calculated for each temperature. DML for each time increment was traced from the portion of the DML curve that corresponded to the temperature during that time increment. The appropriate portion of each DML curve was determined by assuming that DML at the beginning of each time increment was equal to that at the end of the preceding one. DML curve increments were traced until calculated DML reached 0.5% at about 3.7 and 4.0 days for inoculated and control bins, respectively (Fig. 5). These predicted times to 0.5% DML in the tops of the bins are only 0.2 and 1.4 days less than the corresponding observed times for average DML (Fig. 3c and f). On day 3.7, the upper three-fifths of the corn in the inoculated bin clearly had unacceptable fungal invasion and aflatoxin content. Corn in the control bin had 10–30 ppb at the top on day 4, but it had nearly 200 ppb at the top and about 10 ppb in the middle on day 5. Although extent of fungal invasion in the control corn might be considered marginal at day 4, it was certainly unacceptable one day later. Again, we emphasize that predicted DML (Fig. 5) was based on the highest temperatures in the bins.

Assessment of the amount of error in our determination of the number of days for average DML to reach 0.5% is difficult. Because DML was calculated by integration of respiratory rate with respect to time, random error in the respiratory rate data probably contributed little to DML error. We are not aware of any systematic errors that seriously affected our measurements. Conclusions from the 1979 test would not be greatly changed if average DML reached 0.5% one day earlier than what we found. Reasonable estimates of variation in our reported number of days to 0.5% DML for the 1978 and 1979 tests are about ±1 and ± 0.5 day, respectively.

Wicklow et al (1980) reported that *A. flavus* did not produce aflatoxin when it grew together with *A. niger* on sterile corn kernels. The corn we stored, especially in 1978, was naturally contaminated with *A. flavus* and *A. niger*. Both also were in the inoculum added to one bin in 1979. Our results show that aflatoxin production by *A. flavus* was not prevented, and possibly little affected, by *A. niger*. Some of the plated kernels had either *A. flavus* or *A. niger* growing from them, but many had both fungi growing from the same kernel.

At the end of the 1979 storage tests, kernels had a moldy appearance, but shaking the grain in a swift stream of air removed enough of the spores to make the grain appear considerably less moldy. Eight samples of the air-cleaned grain were graded by the Federal Grain Inspection Service for damaged kernels and type of damage. Damaged kernels, mostly classified as "blue-eye mold damage," ranged from 9.0–9.5% (grade 4) in samples from the bottoms of the bins to 11.8–17.9% (grades 5 and sample) in samples from the middles and tops of the bins. Also, the damage counts did not show a consistent middle-to-top increase and were mostly lower in samples from the inoculated bin than in those from the control. These were unexpected results considering the severe

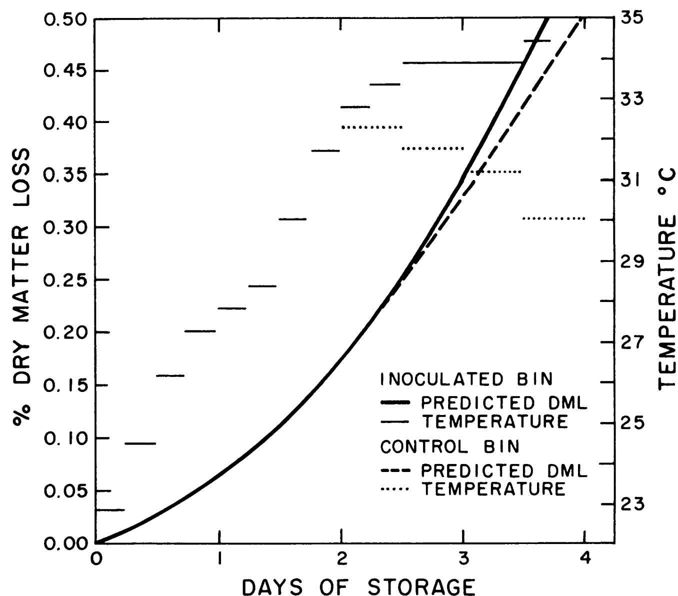

Fig. 5. Dry matter loss (DML) predicted by relationships of Steele et al (1969) and Thompson (1972) for corn in about a 10-cm layer at the tops of the bins in the 1979 tests. Horizontal bars show time increments and grain temperatures used in the DML calculations. Grain temperature in inoculated and control bins was essentially the same during the first two days of storage.

invasion indicated by ergosterol, aflatoxin, and whole-seed plating assays. Cleaning similar to our method might occur when corn is handled in an elevator, passed through a cleaner, or blended with clean grain. Visual inspection procedures may not be adequate for detecting some kinds of moldy kernels.

We concluded that storing corn at temperature and moisture conditions favorable for fungal growth could produce an unacceptable amount of fungal invasion by the time an average of 0.5% dry matter is lost. Storage at such conditions for a period equivalent to an average of 1.0% DML definitely should not be recommended. In the 1979 tests, the initial amount of fungal inoculum, particularly *A. flavus*, significantly affected the extent of invasion during storage.

Laboratory tests have also shown that the extent of fungal invasion in corn when stored to about 0.5% DML depends on amount of fungal inoculum and on amount and type of kernel damage (Seitz et al 1982). Variability in amount and type of fungal inoculum is not taken into account in the computerized models for simulation of corn drying. More tests are required to determine if fungal invasion would be significant when corn is stored to 0.5% DML under conditions considerably less favorable for mold growth than those used in this study.

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