Storage of High-Moisture Corn: Fungal Growth and Dry Matter Loss

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ABSTRACT

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Carbon dioxide, ergosterol, and aflatoxin were monitored to investigate the relationship between dry matter loss (DML) and fungal growth during storage of 150–200-g samples of rewetted and freshly harvested corn under controlled conditions at 22–27% moisture. Corn that was surface sterilized with hypochlorite solution and had little or no internal fungi lost more than 0.5% dry matter without significant fungal growth during storage. Any samples that were invaded by fungi during storage showed relatively little change in respiratory rate compared to the change in fungal invasion indicated by ergosterol and aflatoxin contents. Results suggested that respiration of grain itself was a major contributor to DML. The amount of fungal invasion when storage time was equivalent to 0.5% DML varied considerably depending on the amount and type of kernel damage and on whether or not *Aspergillus flavus* inoculum was added. *A. flavus* growth during storage was sufficient, especially in damaged corn, to produce aflatoxin before loss of 0.5% dry matter. Results indicate that current recommendations based on DML for temporary storage of high-moisture corn should be used cautiously when conditions are favorable for fungal growth.

Dry matter loss (DML) has been used by various researchers since 1966 as an index of corn deterioration, based on research of Saul and Steele (1966) and Steele et al (1969). These authors concluded that corn quality, evaluated as percentage of mold-damaged kernels, could drop below grade 2 if DML exceeded 0.5% during storage and/or drying. The relationships they developed relating DML to corn deterioration are currently used in many computerized simulations of corn drying and in the development of recommendations for safe, temporary storage of high-moisture corn (Brooker et al 1974, Morey et al 1979, Thompson 1972, USDA 1969). Those relationships were established by using standard grading procedures, based primarily on visual inspection, to estimate deterioration during storage. Amount of fungal growth in grains cannot be adequately judged visually.

The relative contributions of fungal and seed respiration to total respiration of grain has been argued for many years (Milner and Geddes 1954, Pomeranz 1974). We report a study of the relationship between DML, respiratory rates, and fungal growth, as measured by ergosterol, during storage of high-moisture corn. We also studied the effects of physical kernel damage and initial fungal inoculum (*Aspergillus flavus*) on DML, respiratory rates, and fungal growth. Effect of mechanical damage on DML had been considered previously (Kalbasi-Ashtari et al 1979, Steele et al 1969), but initial fungal inoculum had not.

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MATERIALS AND METHODS

Corn Samples

Hand-harvested, yellow dent corn grown near St. Paul, MN, in 1966 was used for the storage test involving fungus-free, surface-sterilized grain. The corn had been kept dry (10–13% moisture content) and frozen for 14 years, had a germination of >90%, and had no detectable internal fungi as determined by plating on agar media.

Open-pollinated white dent corn was hand harvested from the Kansas State University agronomy farm near Manhattan, KS, in 1976. The corn was kept dry under refrigeration and had germination of >90%. Seventy-eight percent of the kernels were internally invaded with *Fusarium moniliforme*, *Cephalosporium acremonium*, and *Penicillium* spp. This corn was used for the storage test with surface-sterilized corn having internal mold and for all tests involving rewetted white corn.

Freshly harvested, yellow dent corn was harvested in 1979 by machine from a field near Wamego, KS, and by hand from the Kansas State University agronomy farm. Storage tests were started immediately after the grain was harvested.

Surface Sterilization

Corn samples were surface sterilized by washing seeds with 5% sodium hypochlorite (Clorox), pH 10.6, for 1 min followed by two sterile water rinses. This process raised the moisture content by about 3 percentage points. The sample was placed in a sterile gas washing bottle, and additional sterile water was added to obtain the desired moisture content.

Damage Treatments

Nonspecific damage similar to damage that might occur during

normal handling of corn was produced by using a grain accelerator (breakage tester) as described by Miller et al (1979). Kernels (white corn) were accelerated to a velocity of about 30 m/sec. A 4.8-mm (12/64-in.) round-hole sieve was used to remove fines and broken kernels. From the grain that remained on the sieve, we selected kernels with no damage visible to the unaided eye and kernels that were cracked or chipped but still mainly whole.

Several specific types of damage were inflicted with a razor blade on samples of white corn kernels. The kernels of another sample were given a tiny puncture in the pericarp over the germ with the aid of a sharp dissecting needle and a microscope; the damage was hardly visible with the naked eye.

The first damaged sample tested contained 57% damaged kernels by weight, so all subsequent tests with damaged grain, including those with a specific type of damage inflicted, were arbitrarily adjusted to the same percentage of damaged kernels.

Inoculating with *A. flavus*

An *A. flavus* strain isolated from corn and known to produce aflatoxin B_1 and B_2 was the inoculum. A liquid spore suspension was used to inoculate freshly harvested corn. For convenience, all other samples were inoculated by adding one wheat kernel that had spores on its surface.

The spore suspension was prepared by using sterile water containing Tween 20 (one drop per 250 ml) to wash spores from a potato dextrose agar slant on which *A. flavus* had grown for seven days. The suspension was diluted to 25–30% of transmittance at 640 nm, equivalent to about 10^6 spores per milliliter. Two milliliters of this suspension was added to 250 g of corn, which was then shaken vigorously to disperse the spores.

To prepare moldy wheat kernels, wheat containing 30% moisture was autoclaved for 30 min at 121° C. The wheat was inoculated with *A. flavus*, incubated for seven days at 25° C, and then air dried for 48 hr. After the corn sample (150–200 g) had been adjusted to proper moisture over a 4–5-hr period, one infected wheat kernel was added and the sample shaken vigorously to disperse the spores. CO_2 monitoring began immediately after inoculation. The wheat kernel was removed at the end of the test before the sample was ground for ergosterol and aflatoxin assays.

Analytical

The system used to monitor carbon dioxide evolution from 150–200-g corn samples is shown in Fig. 1. Airflow rate, held constant by a flow controller (model 202-3(3)-2, Condyne Instruments, LaCanada, CA), was 30–60 ml/min, depending on the respiratory rate of the sample. A soap bubble meter was used to measure flow rate after the air had passed through the sample cell of the infrared monitor. The flow meter near the flow controller was only for quick visual monitoring of flow rate. Before entering the sample container, the air was bubbled through saturated potassium hydroxide solution to remove carbon dioxide and then through distilled water at the same temperature as the sample so that the air would approach water saturation. After leaving the sample container and before entering a 10-cm gas cell in a Perkin-Elmer model 467 infrared spectrophotometer, the air was dried with silica gel (6–16 mesh, indicating) and magnesium perchlorate (anhydrous, reagent grade).

The spectrophotometer was set at a wavelength corresponding to carbon dioxide absorption at 2,330 cm^{-1}. Transmittance output was recorded on a strip chart recorder. The system was calibrated by using standards from Matheson Gas Products. Calibration data was also obtained at intervals by diverting the airstream through a sampling bulb just ahead of the 10-cm cell for measurement of CO_2 concentration with a Beckman model 865 analyzer.

Gas washing bottles (500 ml) were used to contain the corn, distilled water, and potassium hydroxide; bottles for the liquids were equipped with fritted cylinder gas dispersion tubes. Corn sample and distilled water containers were placed in a water bath fitted with an insulated lid that controlled temperature within ±0.1° C of the set point.

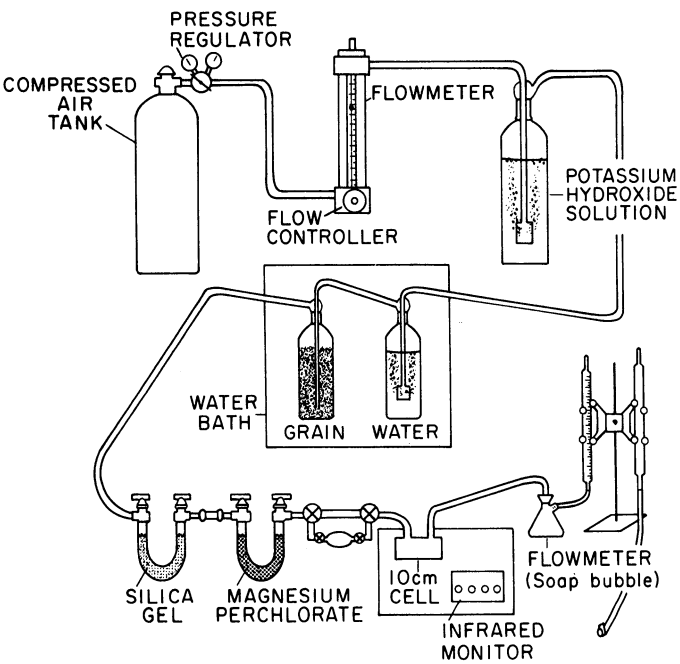

Fig. 1. System for monitoring carbon dioxide production from grain stored at constant moisture and temperature.

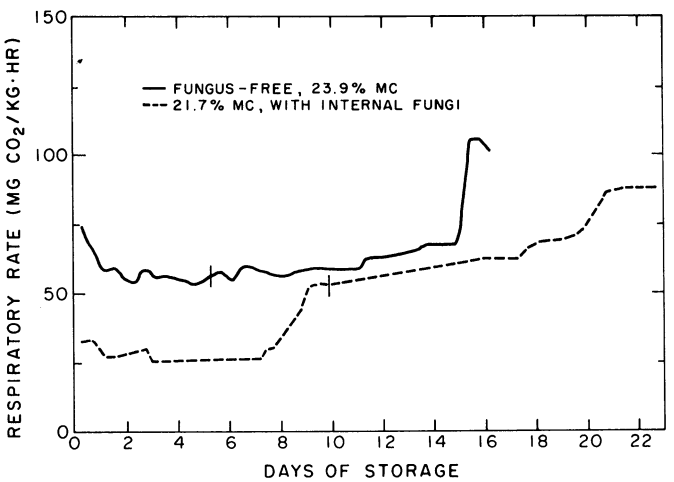

Fig. 2. Respiratory rates of rewetted, surface-sterilized corn stored at 29.4° C (85° F). Short vertical lines denote when dry matter loss was 0.5%. MC = moisture content.

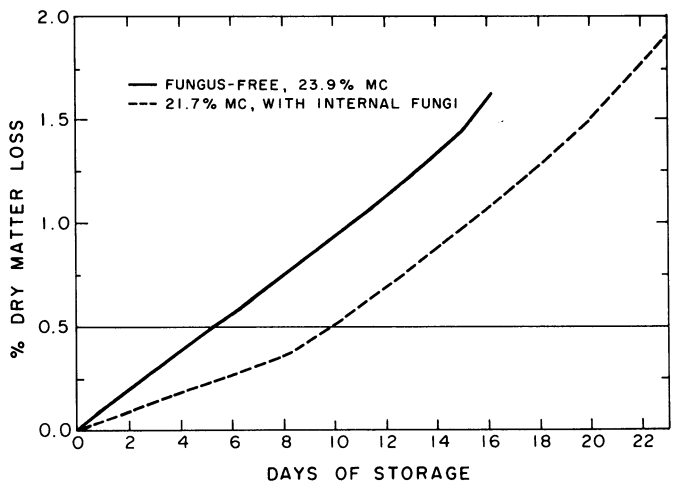

Fig. 3. Dry matter loss from rewetted, surface-sterilized corn stored at 29.4° C. MC = moisture content.

Respiratory rate (R = milligrams of CO_2 per kilogram of dry matter per hour) was calculated from the following relationship:

$$R = \frac{C \cdot F \cdot D}{M}$$

where C = concentration by volume of CO_2 in the airstream, ie, milliliters of CO_2 per milliliter of air; F = flow rate of air in milliliters per hour; D = density of CO_2 in milligrams per milliliter (a value of 1.75 was appropriate for these experiments); and M = kilograms of dry matter at the start of storage.

DML was calculated by integration of respiratory rates with respect to time. Storage time was divided into intervals ranging from less than an hour to several hours, depending on the rate of change in respiratory rate. Amount of CO_2 evolved during an interval was calculated from the respiratory rate at the end of the interval. Total amount of CO_2 produced during a storage period was then determined by summing the interval amounts in that period. The loss of about 15 g of CO_2 per kilogram of grain dry matter is equivalent to 1% DML (Steele et al 1969). Thus, grams of CO_2 evolved per kilogram of dry matter was divided by 15 to obtain percent DML.

At the end of storage, the corn was air dried, ground, and assayed for ergosterol (Seitz et al 1979) and aflatoxin. The method of Seitz and Mohr (1977) was modified to assay for aflatoxin. The thin-layer chromatography 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 fritted glass filtering funnel, and aflatoxins were eluted from the silica with chloroform:acetone (9:1, v/v). The eluant was evaporated to dryness in a two-dram vial over a steam bath with nitrogen. The residue was dissolved in 0.2–8.0 ml of methanol:water:acetonitrile (50:35:15, v/v), 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 recovery by this method was 80%; therefore, results were multiplied by 1.25 to correct for aflatoxin loss. Only aflatoxin B_1 was quantitated. Aflatoxin B_2 was usually present, but its concentration was small compared to the concentration of B_1. Aflatoxin G_1 and G_2 were not present.

For the whole-seed plating assay, kernels were shaken for 1 min in 5% sodium hypochlorite (Clorox), pH 10.6, followed by a 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). Fungi growing from kernels were identified after five days at 25° C.

Moisture content was determined by drying whole corn for 72 hr at 103° C in a forced-air oven.

RESULTS AND DISCUSSION

Respiratory rates and percent DML from two surface-sterilized corn samples stored under conditions favorable to fungal growth are shown in Figs. 2 and 3, respectively. Ergosterol and aflatoxin contents of those samples at the beginning and end of storage are listed in Table I. The sample labeled "fungus-free" was truly that at the start of storage because ergosterol was undetectable and no fungi grew from the seeds when plated on several different agar media. No fungi were visible on the corn during storage until day 15, when we noticed a young mycelial colony about 1 in. in diameter. On day 16, we ended the test because *A. flavus* was sporulating throughout the flask; its growth, however, was still fairly sparse. Apparently, the corn was contaminated on day 10 when we opened the flask to remove samples for plating and moisture assays. Of 10 kernels plated on day 10, one had *A. flavus* on the surface after incubation. We believe the small amount of ergosterol and aflatoxin found in the corn after storage (Table I) was produced by the *A. flavus* growth toward the end of the storage period.

The surface-sterilized corn with internal fungi (Figs. 2 and 3, Table I) contained *Fusarium moniliforme*, *Cephalosporium acremonium*, and *Penicillium* spp. at the beginning of storage. On day 6, a small sample was taken from the flask and plated; *Fusarium* or *Penicillium* grew from every kernel. Slight discoloration of some kernel tips was noticed on day 3, and fungi were discernible on day 6. Sparse mycelial projections on the kernel surfaces were noted on day 8. However, the corn changed little in appearance during the remainder of storage. Whether the fairly abrupt change in respiratory rate between days 7 and 9 was caused by fungal growth is not known.

The fact that respiratory rate changed relatively little during storage of the surface-sterilized samples suggests that grain respiration was the major, and fungal respiration the minor, contributor to DML. Fungi are not likely to have contributed to any of the first 0.5% DML from the "fungus-free" sample. Respiratory rate of the "fungus-free" sample remained constant until the last day, when fungi were visible. In the other sample, respiratory rate increased about threefold by the end of the test. If the respiration was mainly from fungi, its rate should have increased considerably during storage because the extent of fungal invasion indicated by ergosterol content increased 30–42 times (Table I). Essentially constant respiratory rates during extended

TABLE I
Ergosterol and Aflatoxin Content of Corn Stored at 29.4° C

Sample	Inoculum Added	Days Stored	Moisture Content (%)		Ergosterol (ppm)		Aflatoxin B_1 (ppb)	
			Initial	Final	Initial	Final	Initial	Final
Rewetted, surface sterilized								
Fungus-free	None	16.0	···	23.9	<0.02	1.1	0	10
With internal fungi	None	23.0	···	21.7	0.59	25.0	0	0
Rewetted								
57% Damage[a]	*Aspergillus flavus*	6.5	···	24.6	0.59	42.0	0	25,000
	None	6.7	···	24.0	0.59	24.0	0	2,400[b]
Undamaged	*A. flavus*	6.8	···	23.0	0.59	30.0	0	4,100
	None	7.1	···	23.3	0.59	5.0	0	86[b]
Freshly harvested								
By machine	*A. flavus*	11.0	24.9	27.1	2.9	75.0	0	4,400
	None	11.0	24.9	26.1	2.9	44.0	0	420[b]
By hand	*A. flavus*	18.0	24.2	26.4	0.17	50.0	0	3,700
	None	18.0	24.2	25.8	0.17	21.0	0	2,700[b]

[a] Kernels were damaged by a grain accelerator (Miller et al 1979).
[b] From *A. flavus* growing on the corn as a result of "natural" contamination.

storage of soybeans and wheat was also considered indicative of purely seed respiration (Hummel et al 1954, Milner and Geddes 1946).

The time at which fungal growth became sufficient to change the rate of carbon dioxide evolution from the grain is more obvious in a plot of respiratory rates than of DML. Certainly, the change in carbon dioxide production at the end of the "fungus-free" storage test is more noticeable in Fig. 2 than in Fig. 3.

Amount of damage and *A. flavus* inoculum greatly influenced respiratory rate (Fig. 4) and extent of fungal invasion as indicated by ergosterol and aflatoxin contents (Table I) during storage of high-moisture white corn at 29.4° C (85° F). Respiratory rates from the damaged samples were higher initially and, after day 2, increased much more than those of the undamaged samples. At the end of storage, both inoculated samples had higher respiratory rates and ergosterol and aflatoxin contents than the corresponding controls (Table I). The undamaged samples showed remarkably little increase in respiratory rate during storage (Fig. 4) considering the ergosterol and aflatoxin contents at the end of storage (Table I). With all four samples, respiratory rate changed much less during storage than did extent of fungal invasion as indicated by ergosterol and aflatoxin contents.

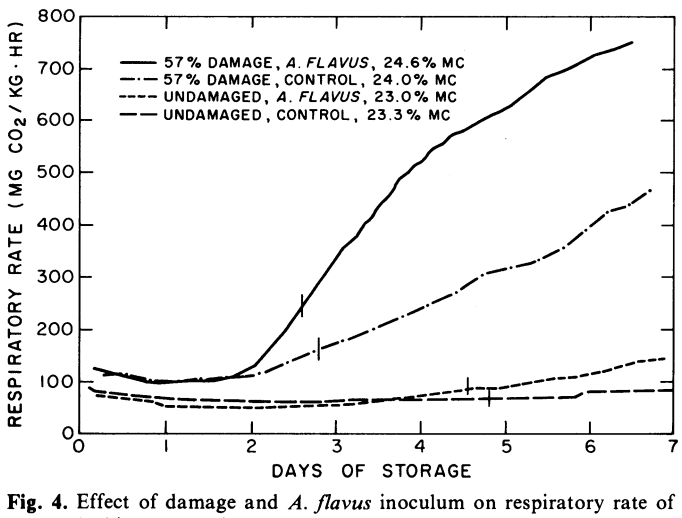

Fig. 4. Effect of damage and *A. flavus* inoculum on respiratory rate of rewetted white corn during storage at 29.4° C. Damaged samples consisted of mostly whole kernels, of which 57% by weight had various types of damage caused by a grain accelerator. Short vertical lines denote when dry matter loss was 0.5%. MC = moisture content.

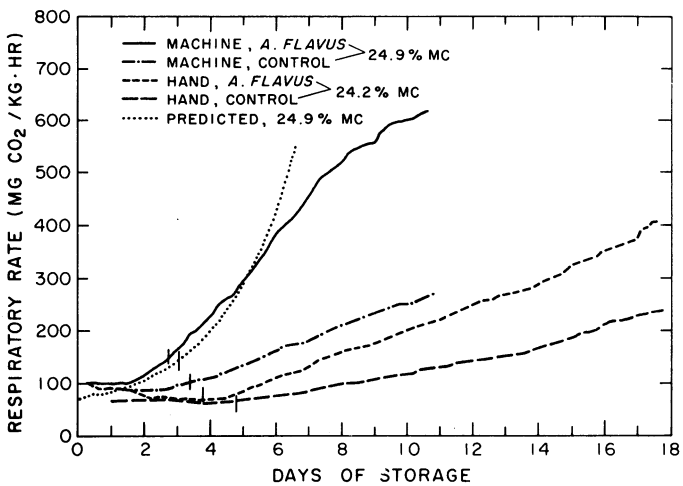

Fig. 5. Effect of harvesting method and *A. flavus* inoculum on respiratory rate of freshly harvested yellow corn during storage at 29.4° C. Predicted curve representing machine-harvested corn was calculated from relationships of Steele et al (1969). Short vertical lines denote when dry matter loss was 0.5%. MC = moisture content.

Addition of *A. flavus* inoculum and damage from machine harvesting significantly enhanced respiratory rates, DML, fungal growth, and aflatoxin production during storage of freshly harvested yellow corn (Fig. 5 and Table I). Bin storage (100 bu) tests with this corn also demonstrated that the addition of fungal inoculum increased fungal growth and DML during storage (Seitz et al 1982). Also shown in Fig. 5 is a predicted respiratory rate curve calculated by taking the derivative of Steele's relationship (Steele et al 1969) relating total carbon dioxide to storage time. The predicted curve represents machine-harvested corn with typical 30% damage and 24.9% moisture content. The amount of damage in the corn we stored was not measured, but it appeared to be lower than that for typical machine-harvested corn. Storage fungi were found in a low percentage of surface-sterilized kernels before storage, so the initial amount of natural fungal inoculum was probably low.

The effect of different types of kernel damage on respiratory rates during storage of rewetted white corn is shown in Fig. 6. Ergosterol and aflatoxin contents after storage of those samples are listed in Table II. Initial respiratory rates were highest in the sample consisting of broken kernels and fine material (BKF) and in the sample with whole kernels severely cut through the germ. The respiratory rate data clearly show that fungal growth started much earlier and was faster and more extensive in the BKF sample than in whole-kernel samples. Also, ergosterol content was highest in the

TABLE II
Ergosterol and Aflatoxin Contents of White Corn with Different Types of Damage When Inoculated with *A. flavus* and Stored at 29.4° C

Sample	Days Stored	Final Moisture Content (%)	Ergosterol (ppm)	Aflatoxin (ppb)
100% Brokens and fines[a]	2.9	23.5	78.1	8,440
57% of kernels damaged				
Germ cut[b]	7.7	23.7	46.6	19,100
Germ puncture, slight[c]	7.1	24.1	48.2	17,900
Backside cut[d]	6.5	24.1	25.5	15,100
Dent end cut[e]	8.3	23.8	14.5	13,300
Undamaged[f]	7.7	23.7	8.5	1,900

[a] Determined by seiving: above a 5.84-mm (15/64-in.) sieve, 63%; through the 5.84-mm but above a 4.76-mm (12/64-in.) sieve, 15%; through the 4.76-mm sieve, 22%.
[b] A 2–3-mm cut was made lengthwise on the germ with a razor blade.
[c] With the aid of a microscope and a sharp dissecting needle, the pericarp over the germ was punctured, with little or no penetration into germ tissue.
[d] Center of the side opposite the germ was severely cut with a razor blade.
[e] Dent end of the kernel was severely cut with a razor blade.
[f] Determined by examination with a microscope.

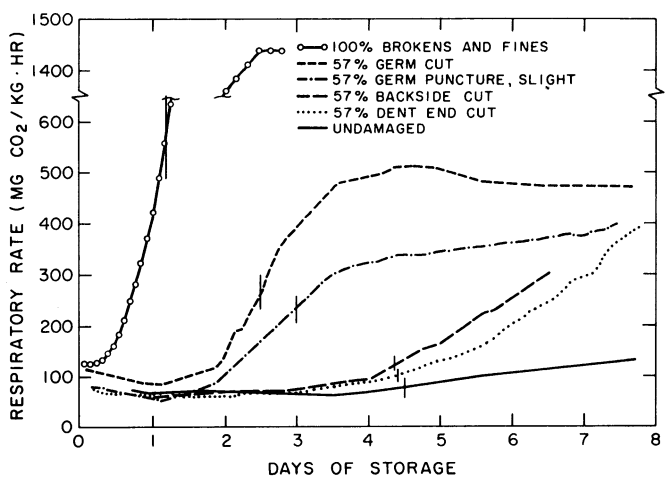

Fig. 6. Effect of damage type on respiratory rate of *A. flavus* inoculated rewetted white corn during storage at 29.4° C. Damage types and moisture contents correspond to those in Table II. Short vertical lines denote when dry matter loss was 0.5%.

BKF sample even though it was stored for the shortest time. All of the whole-kernel samples had relatively constant respiratory rates during the first two days of storage. In the germ-damaged samples, respiratory rate increased fairly sharply between days 2 and 4 and then changed relatively little. Damage to other parts of the kernel caused a more gradual increase in respiratory rate starting on about the fourth day of storage. Ergosterol contents (Table II) in the whole-kernel samples at the end of these storage tests were not as different as they might have been had the storage been stopped on day 4. These data suggest that fungi may invade germ-damaged kernels sooner and at a faster rate than kernels with other types of damage but that differences in extent of invasion may diminish as storage continues. Steele et al (1969) reported that carbon dioxide production was increased more substantially by embryo damage than by endosperm damage. Relationships of Steele et al (1969) predict that machine-harvested corn (30% damage) stored at 23.8% moisture and 29.4° C will lose 0.5% dry matter in 3.4 days and have respiratory rates closest to those for our corn with backside damage (Fig. 6).

Even a very slight amount of damage to the germ of a corn kernel significantly aids the invasion of fungi in that kernel. This was evident from the differences in respiratory rates (Fig. 6) and ergosterol and aflatoxin contents (Table II) between slightly germ-damaged and undamaged samples. To make sure that the undamaged sample was as free of damage as possible, each kernel was inspected under the microscope. Nevertheless, a few of the kernels in the undamaged sample became visibly moldy during storage. When those kernels were examined under the microscope, we could see slight damage, usually in the germ area, that was apparently missed in the prestorage inspection. The importance of invisible damage was also noted by Kalbasi-Ashtari et al (1979).

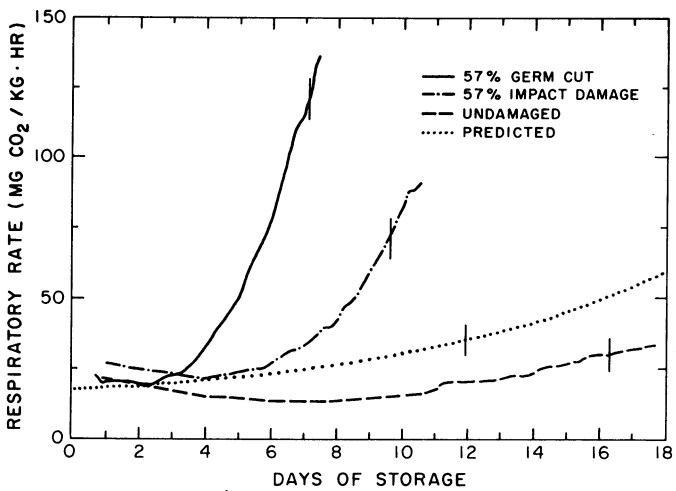

Fig. 7. Respiratory rates of rewetted (22.4% moisture) white corn with different degrees of damage stored at 18.3° C for periods equivalent to 0.6% dry matter loss. Predicted curve representing machine-harvested corn with 30% damage and 22.4% moisture content was calculated from relationships of Steele et al (1969). Short vertical lines denote when dry matter loss was 0.6%.

TABLE III
Ergosterol and Aflatoxin Content of White Corn Stored to 0.6% Dry Matter Loss

Sample[a]	Days Stored	Ergosterol (ppm) Initial	Ergosterol (ppm) Final	Aflatoxin (ppb) Initial	Aflatoxin (ppb) Final
57% Germ cut[b]	7	0.59	10.7	0	590
57% Impact damage[c]	10	0.59	8.6	0	310
Undamaged	16	0.59	3.9	0	13

[a]Each sample inoculated with *Aspergillus flavus*; storage conditions: 18.3° C and 22.4% final moisture content.
[b]Damage to 57% of sample, by weight; 2–3 mm cut made lengthwise on the germ with a razor blade.
[c]Damaged by a grain accelerator (Miller et al 1979).

At storage times corresponding to 0.5% DML in all of the above tests, respiratory rates were usually higher and increasing more rapidly for severely damaged samples than for undamaged samples. This suggested that damaged samples had considerably more fungal invasion than undamaged samples at storage times equivalent to 0.5% DML. A subsequent test showed that ergosterol and aflatoxin contents varied greatly, even though each sample was stored for an amount of time equivalent to 0.6% DML (Fig. 7, Table III). Furthermore, the test showed that aflatoxin could be produced before 0.5% DML during storage of undamaged corn at 18.3° C (65° F). The respiratory rate curve predicted from Steele's relationship for corn at 22% moisture and 18° C is shown in Fig. 7. Observed and predicted curves agree quite well at the beginning of storage and then begin to differ as fungal growth becomes a more significant factor in respiratory rates.

The following observations indicate that respiration of the grain itself is a major contributor to DML: 1) corn that had no fungal inoculum (surface-sterilized) and little or no initial internal fungal invasion had at least 0.5% DML without significant fungal growth during storage, and 2) samples that were invaded by fungi during storage showed relatively little change in respiratory rate compared to the change in extent of fungal invasion indicated by ergosterol and aflatoxin contents. The fact that the grain is respiring limits the usefulness of respiratory rate or DML measurements for detecting fungal growth in grains during storage. Even though the grain is a major contributor to DML, fungal invasion can be at a significant or unacceptable level by the time DML is about 0.5% (Fig. 7, Table III). The extent of fungal invasion in high-moisture corn after storage for a time equivalent to 0.5% DML will vary considerably depending on the amount and type of kernel damage and on the initial amount of fungal inoculum. We believe storage recommendations based on DML measurements should be used cautiously if corn suspected of having a high fungal inoculum, particularly *A. flavus*, is stored under conditions favorable for fungal growth. More information is needed for a more complete determination of how various storage conditions and types of fungi affect the relationship between fungal growth and DML.

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