Mold Growth and Carbon Dioxide Production During Storage of High-Moisture Corn[1]

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ABSTRACT

Freshly harvested and preserved samples of combine-harvested corn, approximately 22 and 19% m.c. (moisture content, wet basis), were stored in the laboratory at 26° C. Carbon dioxide (CO_2) production, fungal propagules, kernel infection, visible molds, and kernel germination were measured. CO_2 production of freshly harvested corn at 22% m.c. was within 2% of that predicted from Saul and Steele's equations, and values for fresh corn at 19% m.c. varied from predicted values by 5–30%. Other indices correlated with CO_2 production included number of *Aspergillus* and *Penicillium* spp. propagules ($r = 0.92$), percent kernels infected with *Aspergillus* and *Penicillium* spp. ($r = 0.85$), and germination decrease ($r = 0.84$). *Aspergillus* and *Penicillium* spp. were the prevalent storage molds. When 0.5% dry matter loss occurred, there were approximately 9×10^5 propagules per gram in the 22% m.c. sample and 5×10^6 propagules per gram in the 19% m.c. sample. Methods used to preserve samples for subsequent testing included storage at 3° C, −10° C, and −29° C, and drying and rewetting. Freezing of 22% moisture corn at −10° C gave the best agreement with tests on freshly harvested corn. Samples stored moist at 3° C for 70 days or −29° C for 23 days produced substantially more CO_2 than freshly harvested corn. The CO_2 evolution was best described by a second order polynomial in time.

Low-temperature drying systems are often designed to dry grain before a specified amount of dry matter loss (DML) occurs. The storage criterion of DML was first adopted by Saul and Steele (1966). They stored corn in bins holding 5 bu and determined that kernel damage would remain at grade 2 if no more than 0.5% DML occurred. DML can be estimated from the cumulated CO_2 production by respiring seed and the microflora (Steele et al 1969). A formula for CO_2 production developed by Steele et al (1969) and modified by Saul (1970) and Thompson (1972) has been used routinely in computer simulation models for design and evaluation of grain drying and storage systems (Pierce and Thompson 1979, Harner et al 1981, Keener et al 1981, Brooker and Duggal 1982). In only one of the studies by Saul (Saul and Lind 1958), was CO_2 production tested for its correlation with measures of fungal activity, and only the mold counts at the completion of the experiments were compared.

Factors that affect CO_2 evolution by both respiring seeds and fungi include moisture content, temperature, ratio of carbon dioxide to oxygen in the interseed air, and length and conditions of previous storage (Milner and Geddes 1945, Milner et al 1947, Hummel et al 1954). Thompson (1972) gave the following equation relating CO_2 production to time of storage at the "standard" conditions of 15.5° C (60° F), 25% moisture, and 30% mechanical damage:

$$Y = 1.3\,[\exp(0.006\,T) - 1] + 0.015\,T,$$

where Y = grams CO_2 produced per kilogram dry matter, and T = time in hours. The time required for a given amount of CO_2 production under conditions other than standard can be predicted from the expression:

$$T = T_R \times M_T \times M_M \times M_D;$$

where T = estimated time to produce a given amount of CO_2 in hours; T_R = time to produce the CO_2 at 15.5° C (60° F), 25% moisture, and 30% mechanical damage; and M_T, M_M, and M_D = multipliers for temperature, moisture, and damage, respectively. The multipliers can be calculated from equations reported by Steele (1967).

Several recent studies suggest that Saul and Steele's experiments should be evaluated in more detail. Seitz et al (1982a,b) measured aflatoxin production and found unacceptable levels of the toxin prior to 0.5% DML. Studies by Cantone et al (1983) demonstrate that corn genotypes can affect rate of mold growth. The studies described here relate carbon dioxide production to measures and/or effects of mold growth—visible molds, percent kernel infection, number of propagules, and decrease in seed germination. CO_2 production is compared to that predicted by Saul and Steele's formula. As freshly harvested corn is readily available in the Midwest for only a few weeks, the number of tests which can be performed in a given year is severely restricted. Therefore, methods of preserving samples after harvest and before testing were also evaluated.

MATERIALS AND METHODS

Storage Apparatus and Procedures

Carbon dioxide production was measured by chemical absorption (Fig. 1). This method is inexpensive, easy to use, and reliable. It is similar to the method used by Steele (1967), with the exception of the temperature control device and the shape of the storage container. It consists of three sections which have the following functions: 1) air humidification and CO_2 removal, 2) sample storage and aeration, and 3) CO_2 absorption.

The system was placed in a controlled temperature room set at 26° C. The first section, used for air humidification and CO_2 removal, consisted of two Fisher-Milligan gas washing bottles in series followed by a Drechsel gas washing bottle. CO_2 was removed from the entering air by bubbling it through a potassium hydroxide solution (30% KOH by weight). Next, the air was bubbled through water and then through a saturated salt solution. This gave the air the appropriate relative humidity needed to maintain the corn at the proper moisture content.

The storage section consisted of a Plexiglas tube, 3.8 cm in diameter and 122 cm long, with a wire screen forming a plenum at the inlet end. Four storage containers (i.e., four replicates) were used, and each contained 1 kg of corn. Airflow rates were adjusted to maintain CO_2 concentration in the exhaust airstream at or below 0.03%. The initial flow rate was based on respiratory rates in preliminary tests. Airflow rates ranged from 350 ml/min (0.35 m^3/min-ton or 0.346 cfm/bu) to as high as 500 ml/min (0.50 m^3/min-ton or 0.495 cfm/bu). Calculations showed that, at the airflow rate used, the heat released by respiration would increase the air temperature less than 0.5° C. Therefore, the grain temperature remained reasonably constant so that mold growth was not accelerated.

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The third section absorbed the CO_2 produced. The exit air passed through a drying column filled with 6-16 mesh indicating silica gel. This air entered a U-tube filled with magnesium perchlorate ($Mg[ClO_4]_2$, anhydrous, reagent grade) and then passed through two U-tubes filled with ascarite (a combination of asbestos particles and sodium hydroxide called Ascarite II, manufactured by Thomas Co., Philadelphia, PA). Since the absorption of CO_2 was accompanied by the release of water, which could wet the ascarite and block air flow, the outlet arm of each tube was filled with a 2.5-cm layer of $Mg(ClO_4)_2$ which absorbed water vapor. CO_2 production was determined by periodically weighing the U-tubes containing ascarite.

The reliability of the CO_2 absorption method was tested by injecting into the system a CO_2-air mixture certified by the supplier to contain 0.52% CO_2 (± 0.01%) in air into the system. The expected weight increase of the ascarite was calculated on the basis of CO_2 concentration and flow rate. The calculated increase ranged from 90 to 99.2% of the actual weight increase and averaged 98.7% for the four reliability tests.

Prestorage Conditions

The samples tested were from Beck 60X dent corn, combine-harvested at 21.8% m.c. at the Purdue Agronomy Farm in mid-November 1981. After harvest the sample was blended, and subsamples were taken for damage determination. The amount of fine material was determined by sieving a 1-kg sample on a 4.76-mm (12/64 in.) round-hole sieve. Mechanical damage was determined on four subsamples using the method of Saul and Steele (1966) and Steele (1967). Damaged kernels were separated from sound kernels and weighed. Percent damage was the percentage of total sample weight which was fines, chipped kernels, or kernels with cracks in the seed coat.

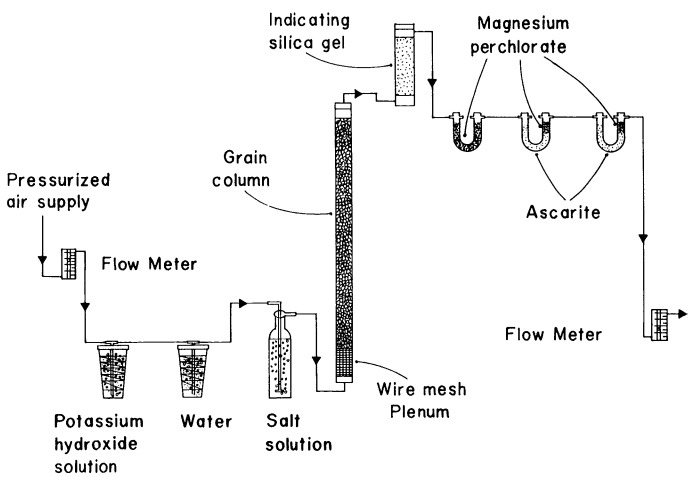

Fig. 1. Schematic of apparatus used to measure carbon dioxide production in samples of shelled corn.

The blended sample was divided into subsamples, which were stored and conditioned as summarized in Table I. The first subsample (recently harvested, 22% m.c.) was tested immediately. Another was dried to 19% m.c. and stored at 3° C for approximately 15 days before testing (recently harvested, 19% m.c.). The remaining samples were stored undried at 3° C (prestored at 3° C), stored wet at −10° C (prestored at −10° C), or dried to 14.7% m.c., stored at 3° C and rewetted (dried and rewetted to 22% m.c.). Room temperature air was used to dry the samples to 19% and 14.5% m.c. Before testing, frozen samples were thawed. Dried samples were rewetted and, to allow for equilibration, were stored overnight at 3° C.

Two additional prestorage treatments were used to study the effects of freezing at lower temperatures and freezing followed by drying and rewetting. After 100 days at −10° C and 22% m.c., a sample was transferred to −29° C for 23 days, removed from storage, dried to 15.6%, and stored at 3° C. After seven more days, the samples were rewetted to 22% m.c., stored overnight at 3° C, and tested for CO_2 evolution and fungal growth.

Mold, Moisture, and Germination Determination

Moisture content, percentage of infected kernels, number of propagules per gram of corn, and germination were determined periodically. In tests with 22% and 19% m.c. corn, 225-g samples were removed at 4- and 8-day intervals, respectively. Moisture was determined using the 72-hr whole-kernel air oven method (standard S-352, ASAE 1980). Infected kernels were determined by plating surface-disinfected kernels. Two hundred kernels were shaken for 1 min in 250-ml flasks containing 1% NaClO (Clorox bleach). One hundred kernels were placed on each of two media: 1) potato-dextrose agar with 100 ppm ethyloxylated nonylphenol (Tergitol NPX) and 30 ppm chlortetracycline (PDTC), and 2) malt-salt agar (6% NaCl). After seven days at 25° C, infected kernels were counted. Fungal and bacterial propagules were determined as follows. Dilutions were made by blending 25 g of corn in 500 ml of 0.1% sterile water agar for 1 min in a Waring Blendor. Successive dilutions (5 ml in 45 ml of 0.1% water agar) were made, and 1 ml of the appropriate dilutions was cultured on plates of the malt-salt and potato-dextrose agar media. Numbers of fungal colonies on the dilution plates were counted after seven to 10 days at 25° C. Aerobic bacteria were determined by culturing on nutrient agar containing 30 ppm cycloheximide. In addition, 100 surface-disinfected kernels were placed on wet filter paper in petri dishes and germination determined after seven days at 25° C. Visible mold

TABLE I
Storage and Conditioning of Samples Used in the Respiration Tests

Test	Treatment	Moisture Content During the Test (% m.c., w.b.)[a]	Conditions During Prestorage: Moisture Content (%, w.b.)	Temperature (°C)	Time (days)
Recently harvested 22%	Harvested at 22% m.c., tested	22.0	...	...	0
Recently harvested 19%	Harvested at 22% m.c., dried to 19%, tested	19.2	19.0	3	15
Prestored at 3° C	Harvested at 22% m.c., prestored at 3° C, tested	22.1	21.8	3	70
Dried and rewetted	Harvested at 22% m.c., dried to 14.7%, prestored at 3° C, rewetted and tested	22.2	14.7	3	80
Prestored at −10° C	Harvested at 22% m.c., frozen at −10° C, thawed, tested	22.0	21.8	−10	95
Prestored at −29° C	Harvested at 22% m.c., frozen at −10° C, then moved to −29° C, thawed, tested	21.9	21.8	−10	100
			21.8	−29	30
Frozen, dried, rewetted	Harvested at 22% m.c., frozen at −10° C then moved to −29° C, thawed, dried to 14.6% m.c., rewetted, tested	21.9	21.8	−10	100
			21.8	−29	23
			14.6	3	7

[a]This was the average of the moisture content of the four samples taken at the beginning, during, and at the end of each test. For example, the moisture contents in the test of recently harvested, 22% corn were as follows: 21.8% at the beginning, 21.8% after the first sampling, 21.9% after the second sampling, and 22.6% at completion. The average of these four values is 22.0%. m.c. = Moisture content, w.b. = wet basis.

was determined by inspecting the germs of 100 kernels for blue eye and other discolorations with a 3× illuminated lens. The extent of moldiness was rated on the basis of the fraction of the germ which was visibly molded: light (< 1/4), medium (> 1/4 and < 1/2), and heavy (> 1/2). Factor weights of 1, 2, and 3 were assigned to each category, respectively. Mold rating was defined as the sum of the number of kernels in each category multiplied by their weighting factors.

Statistical Analysis of the Data

CO_2 production at five and 10 days was compared using the Statistical Package for the Social Sciences (SPSS) subroutine ONEWAY, a model for one-way analysis of variance with one fixed effect (Nie et al 1975). For comparison of tests on recently harvested corn at 22 and 19% m.c., the fixed effect was assumed to be moisture content of the corn. For the four tests at 22% m.c. (Table I), the fixed effect was the method of preservation of the sample before testing. The analysis was performed using a range function, the Student Newman-Keuls procedure. The five- and 10-day values of CO_2 production, number of fungal propagules, percentage of kernels internally infected, visible mold rating, and germination decrease were compared.

RESULTS

The corn harvested for the tests had the following characteristics: 22% m.c. (wet basis), 0.4% fine material (passed through a 12/64-in. round-hole sieve), and 15.5% chipped, cracked, or broken kernels. At the time of testing, moisture of prestored grain varied from the harvest moisture by 0.1–0.5%. Corn moisture varied by less than 0.8% during the storage tests. These variations were equal to or less than the typical variations of 0.4–1.0% reported by Steele (1967).

CO_2 Production

CO_2 production was normalized using the dry matter in the corn, averaged over the replicates for each test, and plotted against time (Fig. 2). Standard deviations of average interpolated 10-day values are indicated with bars. As reported by Steele (1967) and Fawole (1969), CO_2 production versus time is nonlinear. The effect of a 3% moisture difference was substantial. The fresh corn at 22% m.c. produced CO_2 four times faster than the 19% m.c. corn. Statistical analysis of CO_2 production at five and 10 days gave differences significant at the 0.05 level.

After 10 days, the corn frozen at −10° C and the sample dried and rewetted before testing had produced, respectively, 10% and 23% more CO_2 than the recently harvested corn. CO_2 production by the sample prestored at 3° C for 70 days was the highest, probably because *Penicillium* grew abundantly during prestorage. Differences in CO_2 production at five days were statistically significant at the 0.05 level. As indicated in Figure 2, the 10-day CO_2 production for tests on 22% m.c. shelled corn prestored at 3° C, or dried and rewetted, was significantly higher than that of the 22% m.c. recently harvested corn, but CO_2 production for the 22% corn prestored frozen at −10° C was not. There was no significant difference between the dried and rewetted sample and the sample prestored at −10° C; the ranges shown (Fig. 2) overlap. More detailed results are given in Fernandez (1982) and Fernandez et al (1982).

Microbial Growth

The predominant microorganisms in the samples isolated by the dilution technique are listed in Table II. *Penicillium* spp. were the most common storage molds, with more than 10^6 propagules per gram isolated in all five tests. *Aspergillus wentii,* ordinarily a rare storage mold, and members of the *A. glaucus* group were the next most common. The latter two were more frequent at the lower moisture content of 19%. Yeast numbers increased in most tests. Because *Cephalosporium acremonium* tended to disappear with time, it probably did not grow during storage. Bacterial numbers were erratic and usually decreased with time. However, all fungi were not effectively inhibited by the fungal antibiotic, cycloheximide, and bacteria may have been suppressed in the isolation medium used. Rewetting or freezing of the grain resulted in more colonies of *A. glaucus, A. wentii,* and yeasts. Freshly harvested grain had the greatest variety of microorganisms.

Average numbers of *Aspergillus* and *Penicillium* propagules and percent of infected kernels for each test are plotted against time in Figures 3 and 4. Standard deviations for interpolated 10-day values are shown with bars. Storage mold propagules (1.5×10^4 to 5×10^6 per gram of corn) significantly increased in all tests of 22% m.c. corn after only five days of storage. A comparison of number of propagules and kernel infection for tests on recently harvested corn at 22% and 19% m.c. showed a significant difference for numbers of propagules at 10 days but none at five days. The large coefficients of variation for the number of propagules in the tests at 22% and 19% m.c. (63 and 71%, respectively) made it difficult to detect differences. Kernel infections in the two tests were not statistically significant after five or 10 days. In general, the plating technique did not reveal differences among treatments as did the dilution technique.

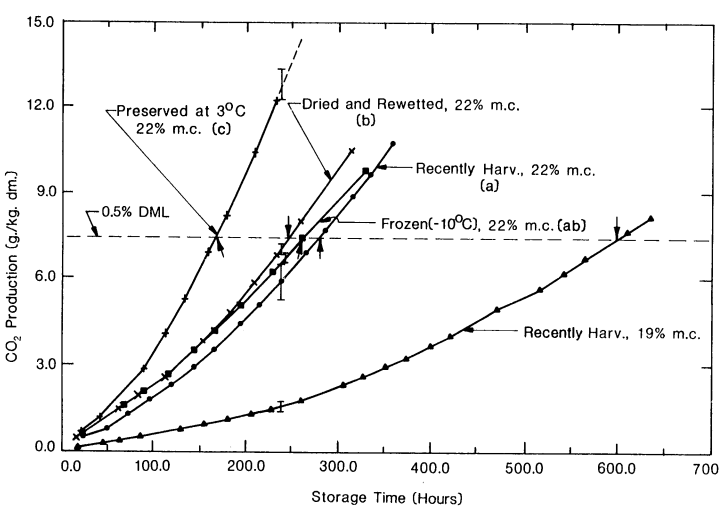

Fig. 2. Curves showing carbon dioxide evolution from corn samples during storage tests. Vertical lines represent range of ± one standard deviation from the mean. Letters in parentheses indicate statistical significance. Differences between 10-day means of the 22% moisture content corn with different letters are statistically significant at the 0.05 significance level.

TABLE II
The Predominant Microorganisms Isolated by the Dilution Technique During Storage Tests on Different Corn Treatments Before Testing

Pretest Treatment	Storage Moisture	Predominant Microorganisms[a]
Recently harvested	22%	*Penicillium* (10^6), bacteria[b] (10^6), yeast (10^5), *C. acremonium*[b] (10^5), *A. wentii* (10^4), *A. flavus* (10^4)
Recently harvested	19%	*Penicillium* (10^6), *A. wentii* (10^6), *A. glaucus* (10^5), *C. acremonium*[b] (10^4), yeast (10^4), bacteria[c] (10^6)
Prestored at 3° C	22%	*Penicillium* (10^6), bacteria (10^6), *A. wentii* (10^4)
Dried and rewetted	22%	*Penicillium* (10^6), bacteria[b] (10^6), yeast (10^5), *A. wentii* (10^5), *A. glaucus* (10^4)
Prestored at −10° C	22%	*Penicillium* (10^6), yeast (10^5), *A. wentii* (10^5), *C. acremonium*[b] (10^5), *A. glaucus* (10^4), bacteria[c] (10^6)

[a] Number in parenthesis gives maximum numbers of colony-forming units isolated per gram.
[b] Probably represents survival rather than an increase.
[c] Bacteria numbers decreased as the test proceeded.

Differences in number of propagules and kernels infected after five and 10 days were statistically significant for corn previously stored at 3° C compared to other prestorage treatments. Both number of propagules and kernel infection were high before the storage test, because there was a substantial amount of fungal growth during prestorage at 3° C. Differences in numbers of propagules and kernel infection were not statistically significant in tests on recently harvested, dried and rewetted, and prestored at −10° C samples. Coefficients of variation for these tests ranged from 7 to 50%, and most were 20% or more. This made differences more difficult to detect.

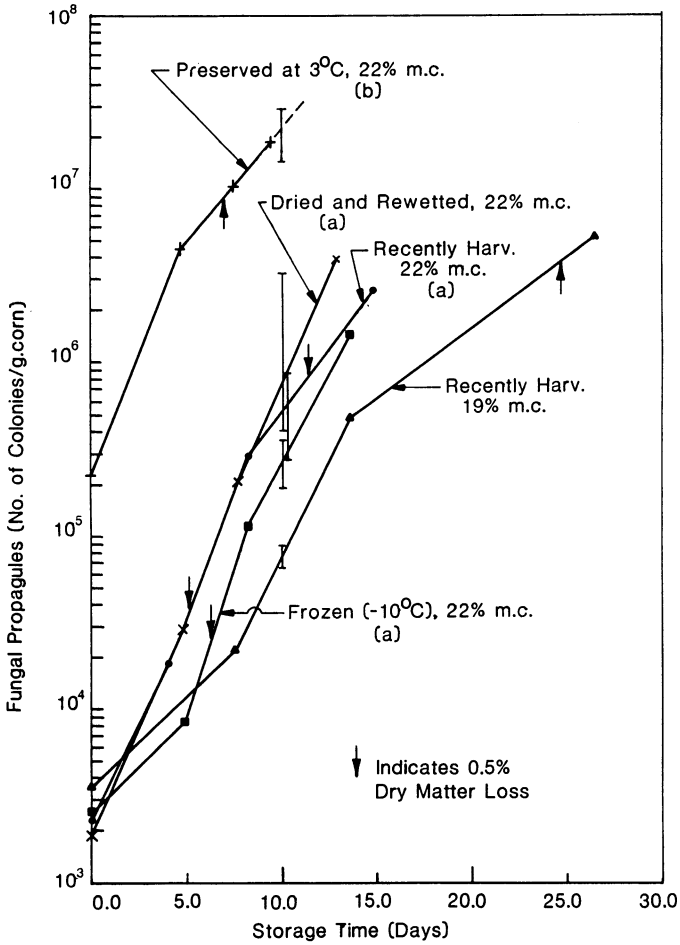

Fig. 3. Number of propagules of storage fungi found in subsamples taken during storage tests. Vertical lines represent range of ± one standard deviation from the mean. Letters in parentheses indicate statistical signficiance. Differences between 10-day means at the 22% moisture content with different letters are statistically significant at the 0.05 significance level.

Visible Mold

Mold ratings (Fig. 4) reflected differences among tests. For a given storage time, the corn that had little or no prestorage (recently harvested 22 and 19% m.c.) had lower visible mold ratings than the prestorage treatments. Differences in mold rating between the two recently harvested samples were statistically significant at 10 days, but not at five days. After five days, the visible mold ratings for the samples freshly harvested (22% m.c.), prestored at 3° C, dried and rewetted, and prestored at −10° C were 0, 15, 13, and 6, respectively. The sequential range test at the 0.05 significance level using the mold ratings distinguished three groups, with the prestored at 3° C and the dried and rewetted samples in the same group, and the freshly harvested and prestored at −10° C samples in separate groups. However, at the end of 10 days only the corn prestored at 3° C showed a significant difference in mold rating compared to the other treatments.

These results suggest that visible molding of the germ does not always reflect the level of fungal activity, because in two tests there was significant CO_2 production and sporulation before fungi were visible on the germ. For example, the recently harvested corn at 22 and 19% m.c. had low visible mold ratings, but after eight days CO_2 production was 4.2 and 1.1 g/kg dry matter, and number of propagules had increased 15 and 4 times, respectively. On the other hand, mold soon became visible in the tests with corn prestored at 3° C, dried and rewetted, or prestored at −10° C. Thus the visible mold rating seemed less sensitive to the initial stages of the mold growth when fresh corn was tested. This was probably the result of lower sporulation of the *A. glaucus* and *A. wentii* in fresh corn. Based on our results, it would be unsatisfactory to use a visual rating of the germ to measure the extent of fungal growth in freshly harvested corn. The results could possibly be improved if the germ were cut lengthwise and examined with a dissecting microscope, and if mold development on the remainder of the kernel were measured. It would also be desirable to examine the kernels before they were removed from the test chamber, because handling disrupts surface molds.

Germination

Germination decreased considerably in all samples, but more slowly for the kernels of lower moisture (Fig. 4). There was no germination of samples prestored at −29° C (not shown in Fig. 4).

TABLE III
Regression Analysis of Relationships Between Carbon Dioxide Production, Number of Fungal Propagules, Percent Infected Kernels, and Percent Decrease in Germination

Variables		Correlation Coefficients for Functional Form	
Y	X	$Y = a + bX$	$Y = ab^X$
No. of fungal propagules			
Total	CO_2 production	.759	.816[a]
Penicillium and *Aspergillus*	CO_2 production	.821	.924[a]
Kernel infection			
Total	CO_2 production	.787[b]	.785[b]
Penicillium and *Aspergillus*	CO_2 production	.854[b]	.760[b]
Germination decrease	CO_2 production	.804	.838[a]
Visible mold rating	CO_2 production	.748[b]	.659[b]
No. of fungal propagules			
Total	Germination decrease	.697	.740[a]
Penicillium and *Aspergillus*	Germination decrease	.710	.897[a]
Visible mold rating	Germination decrease	.571	.564[a]
Kernel infection			
Total	Germination decrease	.632[a]	.639
Penicillium and *Aspergillus*	Germination decrease	.806[b]	.780[b]

[a] Based upon an examination of the plot and residuals, this is the preferred relationship.
[b] One form could not be clearly distinguished as better.

With the exception of these samples, germination at the time of 0.5% DML was similar, ranging from 55 to 60%. Freezing at −10° C or prestorage at 3° C for 70 days gave initial seed germinations of 81 and 77%, respectively, but recently harvested or rewetted corn had an 87% initial germination. A comparison of decrease in seed germination shows that these two treatments had smaller losses. Statistical analysis of the decrease in germination showed significant differences as a result of the 3% difference in moisture. However, statistically significant differences among the tests run at 22% moisture at five days were no longer significant at 10 days.

Effect of Prestorage Treatment

Prestorage at 3° C dramatically increased CO_2 production. Freezing at −10° C gave results similar to those of recently harvested corn. Drying and rewetting gave slightly greater CO_2 production than freezing at −10° C, but the difference was not statistically significant. Samples prestored at −29° C did not germinate and produced more CO_2 than freshly harvested samples. After five and 10 days, DML for prestorage at −29° C was, respectively, 4.3 and 10.2 g/kg dry matter, approximately equal to the values for corn prestored at 3° C. The five-day visible mold ratings for samples prestored at −29° C were greater than ratings for samples prestored at 3° C, and the difference was statistically significant for prestorage at −29° C combined with drying and rewetting. Furthermore, for this prestorage treatment, kernel infection at five days was statistically significant as compared to infection of samples prestored at −10° C or −29° C (without drying and rewetting), or dried and rewetted. There were substantial differences in fungal propagules, but these were not statistically significant because standard deviations were large.

Differences were more apparent after 10 days of storage. For the sample prestored at −29° C and subsequently dried and rewetted, the 10-day values were 13.2 kg/g dry matter for CO_2 production, 36% visual mold rating, 83% kernel infection, and 5.2×10^6 *Penicillium* and *Aspergillus* spp. propagules per gram of corn. These values were greater than those for corn prestored at −10° C or −29° C without drying and rewetting, or dried and rewetted corn. CO_2 production and kernel infection were higher than for corn prestored at 3° C. The CO_2 production of the corn prestored at −29° C without drying and rewetting was equal to that of corn prestored at 3° C. Propagules and visible mold ratings were also very high.

In summary, prestorage treatment can have a substantial effect on mold growth. Storage of 22% m.c. corn well below freezing (< −29° C) makes the corn more susceptible to molding, and prestorage at 3° C permits mold growth. Apparently, drying and rewetting subsequent to freezing at −29° C further increases susceptibility. Therefore, such techniques should not be used for prestorage of shelled corn. Drying and rewetting enhanced CO_2 production only slightly, whereas freezing at −10° C had a minimal effect.

The various methods of evaluating mold growth and grain deterioration showed different levels of variability. Coefficients of variation were determined for the tests on 22% m.c. corn (freshly harvested, prestored at 3° C and −10° C, and dried and rewetted). The averages were CO_2 production, 5.7%; total number of propagules, 33.2%; kernel infection, 16.6%; visible mold, 24.8%; and germination decrease, 5.6%. CO_2 production and germination gave the most consistent results, and numbers of propagules varied most.

Regression Analysis

To investigate relationships among measures of mold growth, regression analysis was performed on CO_2 production versus number of fungal propagules, percent kernels infected, and visible mold. Relationships of mold growth measures (i.e., visible mold, kernel infection, and number of propagules) to seed germination loss were also determined by regression analysis. Data used were from the three tests that gave similar results: 22% m.c. corn recently harvested, corn dried and rewetted to 22% m.c., or corn prestored at −10° C and 22% m.c. The four replicates and three sampling times for the three tests gave 36 data points for analysis.

Table III gives the correlation coefficients for linear and exponential models. Models in which the X and Y variables were interchanged were also tested, but none gave better correlations. The exponential model best described the relationship between number of propagules and either CO_2 production or decrease in germination. Correlations were higher when only *Penicillium* and *Aspergillus* propagules were counted, because many of the yeast, bacteria, and other fungus species appeared to be dying out as the experiment progressed. It was not clear which model best described the relationship between kernel infection and CO_2 production; however, the linear model appeared to be slightly preferable for kernel infection versus germination decrease. As with numbers of propagules, the correlation coefficient was higher when only

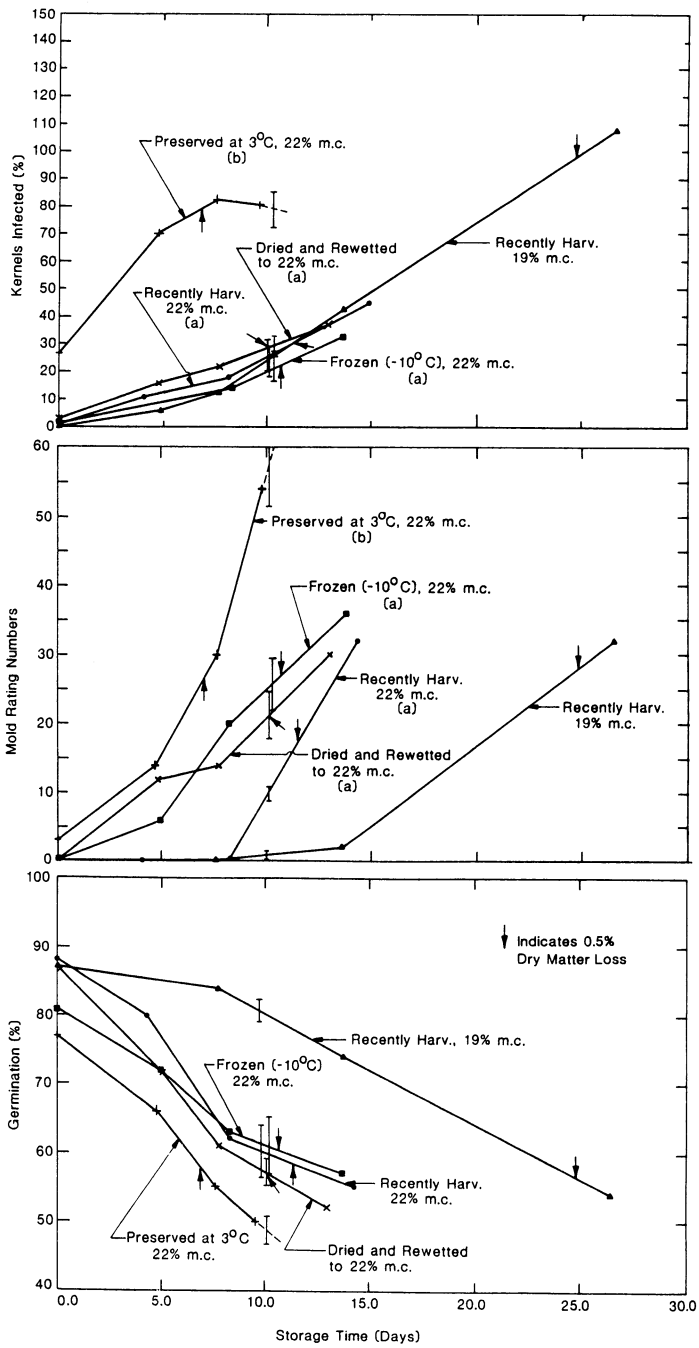

Fig. 4. Kernel infection by storage fungi, mold rating, and germination for samples variously treated. Vertical lines represent range of ± one standard deviation from the mean. Letters in parentheses indicate statistical signficiance. Differences between 10-day means of the 22% moisture content corn with different letters are statistically signficiant at the 0.05 signficance level.

Penicillium and *Aspergillus* were considered. The correlation between visible mold and germination decrease was relatively low, and it was slightly higher for visible mold versus CO_2 production. The two best correlations were CO_2 production with number of *Penicillium* and *Aspergillus* and CO_2 production with *Penicillium* and *Aspergillus* kernel infection. The third highest correlation was between germination decrease and CO_2 production. Additional studies are needed to confirm these relationships.

There were 140 data points in the three tests that were used to determine a regression equation for CO_2 against time. Simple linear models using CO_2 versus time and $\ln(CO_2)$ versus time were tested along with a second order polynomial in time. The residual plots of the simple linear models showed a systematic tendency, but the polynomial did not. The following polynomial had a correlation coefficient of 0.983:

$$CD = -0.03186 + 0.01788\ T + 0.00003736\ T^2;$$

where CD = CO_2 production (g/kg dry matter), and T = time (hours). (This form of the equation is an alternative to that presented in Thompson 1972.) The prediction of the model is plotted in Figure 5 along with actual data.

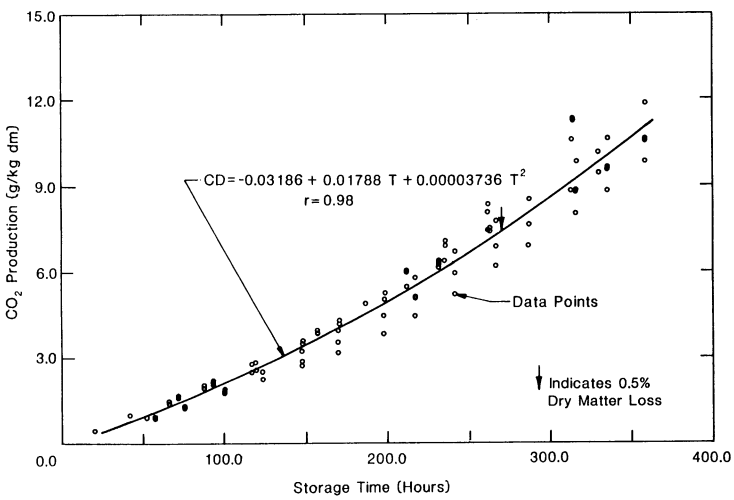

Fig. 5. Solid line is a graph of the polynomial equation describing CO_2 production by shelled corn (approximately 22.1% moisture content, 15% damage) stored at 26° C. Points represent actual data from tests with samples freshly harvested, prestored frozen at −10° C, and dried followed by rewetting to 22%.

Comparison with Steele's Predictions

In Table IV, the times required for 0.5% DML are compared to predictions of Steele's equations. For corn with 15.7% damage stored at an average 22% m.c. and 26° C, predictions were closest to results from test samples that were 1) recently harvested, 2) prestored at −10° C, or 3) rewetted. Corn at 22% m.c. prestored at 3° C produced more CO_2 than predicted, probably because molding was well advanced before the storage test started. During the entire storage period, agreement between predictions from Thompson's (1972) equation and actual CO_2 production was excellent for the 22% m.c. freshly harvested sample. Deviations for storage times between four and 14 days were less than 5%. Agreement for the 19% m.c. freshly harvested sample was not as good. Predictions of CO_2 production from the equation were 31% higher after six days of storage but only 6% higher after 26 days.

The values of different indices of mold growth (or more accurately *Aspergillus* and *Penicillium* sporulation and kernel infection), when CO_2 production is equivalent to 0.5% DML, are also summarized in Table IV. The number of fungal propagules varied widely, ranging from 4.0×10^5 to 9.2×10^6 propagules per gram. However, the variation was smaller among samples recently harvested, rewetted, or prestored at −10° C, for which it ranged from 4×10^5 to 9×10^5 propagules per gram. The 19% m.c. test was longer, and the number of fungal propagules was very high, 5×10^6 propagules per gram, when 0.5% DML was reached. During the time required for 0.5% DML, the number of propagules reached 9.2×10^6 for samples prestored at 3° C. This was 10 times the value for other prestorage treatments on 22% m.c. corn and reflects growth during prestorage. Percentage of infected kernels also varied widely among the tests, from 23 to 107% (kernel infection exceeds 100% because of double infection of *Aspergillus* and *Penicillium*). Values for the samples recently harvested, rewetted, or prestored at −10° C were more nearly equal, ranging from 23 to 31%. Infection of samples prestored at 3° C was much higher than for other prestorage treatments of 22% m.c. corn. The 19% m.c. recently harvested sample had the highest percentage of kernels infected when 0.5% DML was reached. These results suggest that by the time 0.5% DML is reached, fungal growth (as indicated by fungal propagules and percent kernels infected) can reach different levels, depending on the corn moisture content and prestorage

TABLE IV
Comparison of Steele's Predictions with Experimental Results and Comparison of CO_2 Production with Other Measures of Mold Growth

Test	Average Moisture Content (% m.c., w.b.)[a]	Time to Reach 0.5% Dry Matter Loss (DML) (days)			Total No. of *Penicillium* and *Aspergillus* Propagules/Gram Seed ($\times 10^6$)		*Penicillium* and *Aspergillus* Kernel Infection (%)		Visible Mold Rating		Germination (%)	
		This Study	Steele's Prediction	Difference, % of Steele's Prediction	Initial	At 0.5% DML	Initial	At 0.5% DML	Initial	At 0.5% DML	Initial	Decrease At 0.5% DML
Freshly harvested, 22% m.c.	22.0	11.4	11.7	2.6	.0022	0.850	1	31	0	17	88	30
Freshly harvested, 19% m.c.	19.2	24.8	23.7	4.6	.0035	5.0	2	107	0	28	87	31
Prestored at 3° C, 22% m.c.	22.1	5.0[b]	7.9	36.7	.216	9.2[c]	27	81[c]	3	27[c]	77	20[c]
Dried and rewetted to 22% m.c.	22.2	10.1	11.7	13.7	.0019	0.9	3	25	...[d]	22	87	30
Prestored at −10° C, 22% m.c.	22.0	10.7	11.7	8.5	.0025	0.4	0	23	...[d]	28	81	21

[a] m.c. = Moisture content, w.b. = wet basis.
[b] Value adjusted to take into consideration previous deterioration while this corn was stored at 3° C. Nonadjusted value was 6.9 days.
[c] Values at 6.9 days. See footnote b.
[d] Samples were not evaluated.

treatment. On the basis of tests on samples recently harvested, rewetted, or prestored at −10° C, corn with 15.7% mechanical damage stored at 26° C would reach 0.5% DML with fungal propagules (*Penicillium* and *Aspergillus*) somewhat less than 9×10^5 colonies per gram and 30% kernels infected. Corn stored at 19% m.c. had considerably more fungal colonies, 5×10^6, and 107% kernel infection when 0.5% DML was reached.

Visible mold rating at 0.5% DML was lowest for the 22% m.c. freshly harvested sample. It was highest for the 19% m.c. freshly harvested sample and the samples prestored at 3° C and −10° C. The level of visible mold growth in the germ appears to vary widely depending on prestorage treatment. Germination was less variable, as it decreased by about 30% at 0.5% DML. Decrease for the samples prestored at 3° C and −10° C was only about 20%. The germination of these samples at the beginning of the storage test, after removal from prestorage, was 11 and 7% lower, respectively, than the other samples.

DISCUSSION

The method of preserving samples before testing can affect the rate of deterioration during a storage test. Based upon this research, the preferred method is to preserve samples at −10° C. Freezing at −29° C may stimulate carbon dioxide and mold growth during subsequent testing. If preservation at −10° C is not possible, a less desirable but acceptable method is drying and rewetting. However, rewetting should be used with caution because several researchers have observed more rapid production of aflatoxin in rewetted samples as compared to freshly harvested samples (Trenk and Hartman 1970, Wilson and Jay 1975). Furthermore, Perez et al (1982) found increased fungal growth in rewetted samples stored longer than 5.5 months. Freezing at −29° C apparently stimulated mold growth, and freezing at −29° C followed by drying and rewetting further stimulated mold growth. This suggests −29° C reduces the resistance mechanisms of the kernel to fungal invasion. Drying may cause a reduction of competitive organisms (presumably bacteria and borderline storage or field fungi), thereby permitting storage molds to dominate. It is also possible that the moisture of the rewetted grain is unevenly distributed in the grain and therefore more available to the microorganisms developing at the surface.

Because Steele and Saul's (1968) results for predicting CO_2 production for shelled corn are extensively used, their applicability requires further evaluation. In view of observed varietal differences in resistance to fungal invasion and other variables, the agreement between 22% m.c. corn samples and their predictions was surprisingly good. Agreement between 19% m.c. sample results and their predictions at 0.5% DML was also good (within 6%), but predictions of CO_2 production for shorter times were not as good (31% higher at 6 days). Therefore, are these predictions less valid for lower moistures? Mechanical harvesting affects CO_2 production of apparently undamaged kernels (Kalbasi-Ashtari et al 1979). Damage caused by freezing or freezing followed by drying and rewetting stimulates mold growth and CO_2 production. Inoculum load could affect the time required to reach a given level of CO_2 production (Seitz et al 1982a). Therefore, it appears that more testing is needed to establish the effects of these variables.

The various measures of mold growth or effects of mold growth had different levels of variability. CO_2 production and germination decrease gave the lowest coefficients of variability for replications within a given prestorage treatment. This suggests that these two methods were less influenced by variations in fungal growth within the sample. A study in the Department of Botany and Plant Pathology at Purdue University by Tuite and Cantone (*unpublished*) suggested that some varieties sustain large amounts of fungal invasion without a corresponding decrease in germination. Therefore, CO_2 production may be the more desirable of the two methods.

Researchers should be cautious when using 0.5% DML as the criterion for safe storage time. For freshly harvested 22% m.c. corn or 22% m.c. corn "properly" preserved before testing, CO_2 production was correlated with *Penicillium* and *Aspergillus* propagules ($r = 0.92$), *Penicillium* and *Aspergillus* kernel infection ($r = 0.85$), and germination decrease ($r = 0.84$). Values of these measures of mold growth or, for germination decrease, the effects of mold growth, were similar when 0.5% DML was reached. However, for 22% m.c. corn prestored at 3° C or for 19% m.c freshly harvested corn, when 0.5% DML was reached the number of propagules varied by as much as a factor of 10, and kernel infection varied by a factor of 2–4. The germination decrease was less when the germination was lower at the beginning of the storage test as a result of prestorage treatment. This suggests that moisture or prestorage treatment can affect the pattern of mold growth, which could affect the magnitude of the decline in market value of the grain for 0.5% DML. Seitz et al (1982a,b) found significant amounts of aflatoxin produced before 0.5% DML was reached.

The lowest correlations for CO_2 production and germination decrease were for visible mold. The amount of mold visible on the germ was dependent on prestorage technique and moisture content during storage. These results agree with the observation of Seitz et al (1982a) that visible mold may not be adequate for detecting some kinds of mold growth, yet this is the criterion currently used for establishing official grade. It is apparent that further study of fungal growth and CO_2 production in high-moisture corn is needed.

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