Effects of Storage History and Hybrid on Carbon Dioxide Production by Rewetted Shelled Corn

BRADLEY P. MARKS,2 RICHARD L. STROSHINE,2 and LARRY D. DUNKLE3

ABSTRACT

Carbon dioxide evolution tests were conducted with samples of fresh corn and samples of corn previously stored in bins for either four months or five years. Storage history affected the carbon dioxide evolution. Statistically significant differences in carbon dioxide evolution rate between hybrids P3377 and FRB73×Mo17 at harvest were still significant after low-moisture (13.2–13.4%) storage for 56 months. Analysis of covariance was used to analyze the initial respiration rates (first 72 hr) of the test results. This statistical evaluation revealed differences in carbon dioxide evolution in one-fourth the time required by previous methods, such as time to reach 0.5% dry matter loss. The initial slope of the respiration rate curves varied with length of previous bin storage, and therefore, gave an indication of potential for future spoilage.

In a comprehensive study of U.S. grain quality, the U.S. Office of Technology Assessment reported a major problem in the grain industry: no rapid test is available to determine the stage of deterioration or the remaining storage life of grain (U.S. Congress 1989). Several researchers (Multon 1988, Harburgh 1990, Sauer et al 1992) have advocated such a test. Previous research in storability testing was focused on measuring mold populations during accelerated deterioration tests (Welty et al 1963, Christensen 1971, Perez et al 1982). This type of testing is laborious and typically requires more than a week to complete.

Carbon dioxide evolution testing has been used by numerous researchers to quantify deterioration of freshly harvested corn (Saul and Steele 1966, Steele et al 1969, Seitz et al 1982, Friday et al 1989). These studies measured carbon dioxide evolution from samples for 10–20 days and used the time to reach 0.5% dry matter loss (DML) as a standard measure of deterioration. A careful examination of data published by Seitz et al (1982) and Friday et al (1989) suggests that differences in storability might be detected in three days, or less, by using the rate of carbon dioxide evolution as a predictor of long-term fungal growth. This would reduce the time required to evaluate large numbers of samples and would produce an estimate of deterioration potential within three days.

This research is the preliminary phase of a larger research program aimed at: 1) identifying factors that reflect the future spoilage risk of shelled corn, and 2) developing a rapid method for evaluating storability.

This study had two specific objectives. The first was to use carbon dioxide evolution testing of rewetted shelled corn to examine the interaction and effects of long-term previous storage conditions and hybrid. The second was to evaluate the use of initial slopes of respiration rate curves to predict storage mold potential.

MATERIALS AND METHODS

Harvest, Drying, and Bin Storage

The corn used in this study was grown at the Purdue University Throckmorton farm, near West Lafayette, IN, during the summers of 1986 and 1991. It was harvested with an axial-flow combine and transported to the Agricultural Engineering machinery shed, where it was cleaned with a no. 4 mesh, rotating-screen cleaner with square holes 5.45 mm (0.214 in.) on the side. Within 3–6 hr, the corn was loaded into 0.81-m³ (23 bu) drying bins (Friday et al 1990); the bins were 0.56 m (1.8 ft) in diameter and 3.7 m (12 ft) deep.

The corn was dried with ambient air (≈1.3 m³/t/min or 1.2 cfm/bu) within 70 days and stored in the same bins. In this study, two bins were loaded in 1986 and two in 1991. Table I lists the harvest dates, harvest moistures, and drying time for the four lots used in the study.

After the drying was completed, the corn was stored in the bins, without aeration, for up to 59 months. The bins were located inside an unheated pole-construction building. Periodically during drying and subsequent storage, samples were drawn from each bin at 0.61 m (2 ft) depth intervals. Sampling at several locations in the bins allowed withdrawal from depths where a given hybrid had been stored at the desired moisture. The withdrawn samples were stored at 3°C for ≈72 hr and then divided into subsamples, using a Precision divider, for various tests and analyses.

Measuring Carbon Dioxide Evolution

A complete description of the carbon dioxide measurement system is given by Marks (1992). The test apparatus was a modification of the one used by Friday et al (1989). The system included an air-conditioning section, an air manifold, flowmeters, corn storage columns (capable of holding ≈1 kg of corn each), valves and switches to direct effluent air, an infrared gas analyzer, and a computer-based data acquisition system. The entire system was located in a controlled-temperature room set at 26°C. A 30% potassium hydroxide solution was used to remove the ambient carbon dioxide from the inlet air. This was done so that an inlet stream of air with zero concentration of carbon dioxide could be compared with a reference gas of known concentration for calibration of the gas analyzer. The inlet air was conditioned with water and salt solutions to maintain ≈93% rh. This is approximately the equilibrium relative humidity of corn at 20.5% moisture content (mc). The chemical absorption technique used by Friday et al (1989) to measure the carbon dioxide evolved was replaced by an infrared gas analyzer technique. Flow sensors measured the airflow rate into each sample tube. Airflow was continuous and maintained at ≈250 ml/min (equivalent to 0.50 m³/t/min or 0.45 cfm/bu). The gas analyzer measured the difference between inlet and outlet carbon dioxide concentration. The flow rate and gas analyzer output were recorded by the data acquisition system at 1-min intervals. The rate of carbon dioxide production was calculated by multiplying the airflow rate by the increase in carbon dioxide concentration and applying appropriate

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Harvest Date, Harvest Moisture, and Low-Temperature Drying Time for the Four Lots of Corn Used in the Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>Hybrid</td>
</tr>
<tr>
<td>1986</td>
<td>FRB73×Mo17</td>
</tr>
<tr>
<td>1986</td>
<td>P3377</td>
</tr>
<tr>
<td>1991</td>
<td>FR25×FR20</td>
</tr>
<tr>
<td>1991</td>
<td>P3377</td>
</tr>
</tbody>
</table>

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656 CEREAL CHEMISTRY
unit conversions.

In the tests of freshly harvested corn, the samples were stored at \(-10^\circ\text{C}\) in double polyethylene bags for four months at harvest moisture. Fernandez et al. (1985) showed that this was the best of several alternative methods for preserving corn for this type of testing. Before testing, the corn was thawed at 3\(^\circ\text{C}\) for \(\approx 48\) hr and dried to 19.5\% mc in a 30\(^\circ\text{C}\) forced-air drying chamber. A spore suspension of equal parts *Penicillium brevicompactum*, *P. cyclopium*, and *P. viridicatum* was added at a rate of 500 conidia per gram of wet corn. Inoculation of the samples ensured the presence of *Penicillium*, which are responsible for much of the shelled corn deterioration during storage in the midwestern United States. These organisms typically grow well at \(>20\%\) mc. The amount of water used in the spore suspension was calculated to bring the sample to 20.5\% mc. The corn was allowed to equilibrate at 3\(^\circ\text{C}\) for 48 hr. The bags of corn were agitated several times during this period. Three 500-g replicates (each placed in a separate column) were tested for each sample. Results are reported as mean values of the three replicates.

Moisture content of corn previously stored in bins was determined after sampling. The dry (<14.5\% mc) samples were stored at 3\(^\circ\text{C}\) in double polyethylene bags. Five to six days before a test, water was added to bring the samples to 18.5\% mc. The samples were allowed to equilibrate at 3\(^\circ\text{C}\) for two to three days. Then the samples were wetted to \(\approx 20.5\%\) mc, inoculated, and equilibrated using the same procedure as described above.

Other Measurements

Each of the samples used in the carbon dioxide evolution tests was subjected to a battery of other laboratory tests. Moisture contents were determined by the whole-kernel air oven method (ASAE 1988) and reported on a wet (fresh weight) basis. Physical damage of samples was evaluated by two procedures. First, a weight percent of damaged kernels was determined by manually separating out all kernels that had any visible damage. Second, the procedure of Chowdhury and Bucchele (1976) was used to determine a weighted damage index. Numbers of viable fungal propagules were determined by serial dilution tests of unsterilized seeds (Stroshine et al. 1984, Tuite et al. 1985). For germination tests, 50 kernels per sample were evaluated using the method described by Tuite et al. (1985).

Ergosterol content was determined by Larry Seitz at the U.S. Grain Marketing Research Laboratory in Manhattan, KS. High-pressure liquid chromatography procedures (Seitz et al. 1979), with slight modification, were used to measure the total ergosterol content in the kernels. Ergosterol is a sterol component of fungi evidencing previous mold growth or currently viable fungi.

Statistical Methods

Data were analyzed with statistical analysis software (SAS 1989). Mean times to reach 0.5\% DML for the carbon dioxide evolution tests previously conducted by Friday (1987) were reevaluated using the general linear model with Tukey's comparison of means (SAS 1988). Simple linear regression was used to estimate initial slopes (0–3 days) of the respiration rates in all tests. Analysis of covariance (ANCOVA) was used to evaluate the significance of the relevant treatment (e.g., hybrid, storage moisture, etc.) in each test. This was done with the general linear model process, using a model statement to include the continuous variable of time and the appropriate categorical variable (e.g., hybrid). For a comparison of two hybrids, the model for the estimated respiration rate is:

\[
E(R) = \beta_0 + \alpha_i + \beta_1 t + (\beta_{i1} - \beta_0)t; \quad (i = 1, 2)
\]

where \(\beta_0\) is a constant, \(\alpha_i\) is the treatment effect (i.e., the offset of the intercept for the linear model for each different hybrid), and \(t\) is the time variable. The index \(i\) denotes the treatment number. The parameter \(\beta_{i1}\) is the slope for a particular treatment \(i\), and \(\beta_1\) is the mean value of all the \(\beta_{i1}\). This is a first-order model with categorical-continuous interaction. If the interaction between the categorical variable (hybrid) and the continuous variable (time) is not statistically significant, then the difference in slopes is not significant.

For the hybrid comparisons of fresh corn from 1986 and 1991, the carbon dioxide evolution data were transformed. This eliminated the complicating effects of damage variations in the 1986 samples and moisture variations in the 1991 samples. It was assumed that the damage multiplier of Stroshine and Yang (1990) accurately represents the effect of damage on carbon dioxide evolution. It was also assumed that the moisture multiplier of Steele (1967), reported by Thompson (1972), accurately represents the effect of moisture. For each point in the carbon dioxide evolution data, \(t\) was divided by the appropriate multiplier \(M_t\) (moisture or damage) to give a transformed time:

\[
I_{\text{transformed}} = t / M_t
\]

This effectively transformed the data to the reference value of the relevant parameter (moisture or damage).

RESULTS AND DISCUSSION

Sample History

In the fall of 1986, the corn was evaluated as part of a low-temperature drying study (Friday 1987). The Pioneer brand hybrid (P3377) and the Illinois Foundation Research hybrid (FRB73xMo17) had damage indices of 20.9 and 19.8, respectively. The two bins filled in 1991 contained P3377 with a damage index of 19.8 and FR35xFR20 with a damage index of 19.5. During low-temperature drying without stirring, a gradient in moisture content develops within a bin. From the perspective of long-term storage, a single bin has corn with a spectrum of time-moisture history. Moisture contents and mold levels in the bins during drying in 1986 and 1991 are given in Friday (1987) and Marks (1992), respectively.

Effect of Storage History, 1986

In the first carbon dioxide evolution test, two samples of hybrid FRB73xMo17 from the 1986 harvest were evaluated. The corn had been stored in the bins for 59 months. Samples, removed with a probe from 1.83 m (6 ft) and 2.44 m (8 ft) above the bin floor, had been stored in the bin at 12.2 and 14.0\% mc, respectively. Because low-temperature drying had been used in the bins, a moisture gradient existed from top to bottom of each bin during drying and storage. Therefore, the two samples had been stored for a long period at low, but different, moisture contents.

Before testing, an attempt was made to estimate the DML that had occurred during storage of these two samples. The prediction equations of Steele (1967) and Thompson (1972) were used, along with the multipliers of Steele (1967) and the damage multiplier of Stroshine and Yang (1990). Average temperatures from historical weather data were used. These were based on the dry bulb and wet bulb temperatures during 1986 drying and an average ambient temperature for the entire storage period. A temperature estimate of 10.6\(^{\circ}\text{C}\) was used for the drying period; 11.2\(^{\circ}\text{C}\) was used for the storage period. For each sample, the drying period was divided into two portions. An average moisture content for each portion, based on the data of Friday (1987), was estimated. For days 0–25, a moisture content of 19.5\% was used. For days 26–70, estimates of 16.0 and 17.5\% mc were used for the corn stored at 12.2 and 14.0\% mc, respectively. The 12.2\% mc is below the validated range of Steele's moisture multiplier. If the lowest validated moisture content (13\%) were used, the calculated storage moisture multiplier would be 147.1, compared to an extrapolated value of 345.5 for 12.2\% mc. It was assumed that the correct moisture multiplier falls between these limits.

The multipliers were applied, and equivalent reference storage times (Thompson 1972) were calculated. For the corn stored at 14.0\% mc, the calculated DML during drying was 0.24%. For the combined drying and storage periods, the estimated DML was 1.24\%. For the corn stored at 12.2\% mc, the total calculated DML was in the range of 0.25–0.39\%. These rough estimates
show that some dry matter (perhaps a significant amount in the 14.0% mc corn) was lost before the 1991 sampling and testing. The estimated DML in the corn stored at 14.0% mc was three to five times greater than the estimated DML in the corn stored at 12.2% mc.

Table II lists physical damage level, damage index, initial ergosterol content, initial test moisture, and final test moisture of each sample. The visual damage determination was done by different evaluators in 1986 and 1991, which could account for the large discrepancy between 1986 and 1991 samples. Ergosterol results indicate that previous fungal invasion in the 14.0% mc stored corn was approximately five times that of the 12.2% mc stored corn. This is consistent with the DML estimates.

Respiration rates of the 1986 fresh corn (Friday 1987) are included in Figure 1 with data for the two samples stored for 59 months. The respiration rate of the 14.0% mc stored corn increased sharply during the initial hours of the test and then reached a maximum. The respiration rate of the 12.2% mc stored corn remained low initially but then also increased sharply. As the test continued, the respiration rate of the 12.2% mc stored corn approached a maximum that was greater than the maximum rate for the 14.0% mc stored corn. If the test of fresh corn had been continued longer, its respiration rate may have also approached a maximum.

Numbers of fungal propagules were evaluated at the end of the test. After 690 hr of testing, the 14.0% mc stored corn had $60 \times 10^6$ ($s = 36 \times 10^6$) propagules of *Penicillium* spp. per gram of corn. The 12.2% mc stored corn had $>220 \times 10^6$ propagules per gram. (The standard deviation of the 12.2% sample was not calculated because plate counts were too large for an accurate count). In fresh corn, after 426 hr, there were 532 $\times 10^6$ ($s = 200 \times 10^6$) propagules of *Penicillium* spp. per gram of corn.

The fresh corn had more propagules of *Penicillium* spp. than did the 12.2% mc stored corn. The 12.2% mc stored corn, in turn, had more propagules of *Penicillium* spp. than did the 14.0% mc stored corn. The data are consistent with the fact that the 14.0% mc stored corn reached a maximum respiration rate early in the test. The respiration rate of the fresh corn, in contrast, continued to increase at the end of the test. At the end of the test, *Aspergillus glaucus* propagules were detected in both of the stored corn samples. The 12.2% mc sample had $20 \times 10^6$/g, and the 14.0% mc sample had $5.0 \times 10^6$/g. The standard deviations were $6.6 \times 10^6$/g and $5.0 \times 10^6$/g, respectively.

The higher initial ergosterol level in the 14.0% mc stored corn indicated a higher level of cumulative fungal activity before testing. This previous fungal development may have left residual secondary metabolites at levels sufficient to inhibit mold growth later. Subsequently, in the carbon dioxide evolution test, the corn with the greatest previous DML showed the earliest increase in initial respiration rate. However, the previous fungal growth, indicated by the ergosterol level, may have had a limiting effect on subsequent growth of fungi during the test.

The first 72 hr of data from the tests were compared by ANCOVA. The estimated slopes of the respiration rate curves for 48 and 72 hr of testing are given in Table III. There was no statistically significant difference in the slopes of the 14.0 and 12.2% mc stored corn for the first 72 hr. However, the difference in the slopes calculated for the first 48 hr was statistically significant ($P < 0.001$). The two comparisons gave different results, because the peak for the 14.0% mc stored corn occurred early, near 48 hr. The difference in slopes between the 12.2% mc stored corn and the fresh corn from 1986 was statistically significant ($P < 0.10$) after 72 hr. In summary, differences among the evolution rates of the samples were statistically significant after 48 hr for one comparison and after 72 hr for the other.

**Effect of Storage History, 1991**

A similar carbon dioxide evolution test was conducted with hybrid P3377. The fresh corn sample had 20.1% physical damage and a damage index of 22.1. The 13.2% mc stored corn sample (stored for four months at low moisture in small storage bins) had 15.8% physical damage and a damage index of 19.8. Initial and final test moisture were 20.5 and 20.8% for the fresh sample and 20.7 and 21.2% for the stored sample.

As observed in the tests of corn stored in 1986, the respiration rate of previously stored corn increased more rapidly earlier in the test (Fig. 2). The leveling of the curve from the stored sample

<table>
<thead>
<tr>
<th>Sample</th>
<th>Physical Damage (%)</th>
<th>DI*</th>
<th>Ergosterol (µg/g)</th>
<th>Estimated Previous DML (%)</th>
<th>Accelerated Storage Test (mc, % wb)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td>Fresh, 1986</td>
<td>24.7</td>
<td>19.8</td>
<td>NA*</td>
<td>0</td>
<td>20.6</td>
</tr>
<tr>
<td>Stored, 12.2% mc</td>
<td>11.8</td>
<td>17.1</td>
<td>1.22</td>
<td>0.25–0.39</td>
<td>20.5</td>
</tr>
<tr>
<td>Stored, 14.0% mc</td>
<td>15.0</td>
<td>19.4</td>
<td>6.32</td>
<td>1.24</td>
<td>20.5</td>
</tr>
</tbody>
</table>

*Damage index (Chowdhury and Burele 1976).
D1 Dry matter loss.
NA Not available.

![Graph](image_url)

**TABLE III**

Estimated Slopes of the Rate Curves from Carbon Dioxide Evolution Tests

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Year</th>
<th>Moisture (% wb)</th>
<th>Storage (months)</th>
<th>Estimated Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRB72×Mo7</td>
<td>1986</td>
<td>NS*</td>
<td>0</td>
<td>$-26.9 (17.3)$ $-11.0 (8.8)$</td>
</tr>
<tr>
<td>FRB73×Mo7</td>
<td>1986</td>
<td>12.2</td>
<td>59</td>
<td>$-124 (35.0)$ $121 (34.6)$</td>
</tr>
<tr>
<td>FRB73×Mo7</td>
<td>1986</td>
<td>14.0</td>
<td>59</td>
<td>$198 (41.5)$ $92.6 (24.2)$</td>
</tr>
<tr>
<td>P3377</td>
<td>1991</td>
<td>NS</td>
<td>0</td>
<td>NA*             $75.0 (14.2)$</td>
</tr>
<tr>
<td>P3377</td>
<td>1991</td>
<td>13.0</td>
<td>4</td>
<td>323 (79.7) $503 (53.9)$</td>
</tr>
<tr>
<td>FRB73×Mo7</td>
<td>1986</td>
<td>13.4</td>
<td>56</td>
<td>$69.0 (44.0)$ $142 (25.2)$</td>
</tr>
<tr>
<td>FR35×FR20</td>
<td>1991</td>
<td>13.2</td>
<td>4</td>
<td>$221 (78.8)$ $289 (44.4)$</td>
</tr>
<tr>
<td>P3377</td>
<td>1991</td>
<td>13.0</td>
<td>4*</td>
<td>$323 (79.7)$ $503 (53.9)$</td>
</tr>
<tr>
<td>DF20×DF12</td>
<td>1986</td>
<td>NS</td>
<td>0</td>
<td>$71.8 (11.2)$ $76.9 (7.3)$</td>
</tr>
<tr>
<td>P3377</td>
<td>1986</td>
<td>NS</td>
<td>0</td>
<td>$-11.4 (14.8)$ $22.6 (10.3)$</td>
</tr>
<tr>
<td>FRB73×Mo7</td>
<td>1986</td>
<td>NS</td>
<td>0</td>
<td>$-36.4 (16.2)$ $-6.0 (10.2)$</td>
</tr>
<tr>
<td>FR35×FR20</td>
<td>1986</td>
<td>NS</td>
<td>0</td>
<td>$-98.2 (16.6)$ $-46.0 (9.3)$</td>
</tr>
</tbody>
</table>

*Standard deviations are given in parentheses.
*Not stored in bins; tested immediately after harvest.
NA Not available, due to diskette error.
*Same sample listed above for 1991.
was less dramatic in the 1991 comparison, possibly because the sample had been stored in the bin for only four months. If secondary metabolites were responsible for the leveling effect, then fewer compounds would be present after four months than after four years. ANCOVA of the initial 72 hr of these two curves revealed a statistically significant difference at $P < 0.0001$. The initial slopes ($t < 72$ hr) of the rate curves are included in Table III. The von Sieboldian rate curve of the sample that had previously lost some dry matter due to deterioration in drying and storage had a significantly greater initial slope. Similar results were obtained for hybrid FR35×FR20.

The levels of fungal propagules in each sample at the start and end of the tests are listed in Table IV. Some propagules of *Aspergillus* spp. were observed in both samples at the end of the test. Numbers of propagules of both *Penicillium* spp. and *Aspergillus flavus* increased during testing. The higher respiration rates observed for the stored corn may have been partly due to a significantly higher initial inoculum of *Penicillus*. However, the phenomenon of the sharp increase in initial respiration rate could also be due to the previous deterioration during drying and storage.

**Effect of Hybrid, 1986**

P3377 and FRB73×Mo17 hybrids were sampled and tested after 56 months of bin storage at 13.2 and 13.4% MC, respectively. Reevaluation of the data of Friday (1987) showed that these two hybrids were significantly different ($P < 0.10$) in storability at harvest, as evaluated by time to reach 0.5% DML. The P3377 sample was removed from 0.61 m (2 ft) above the bin floor, and the FRB73×Mo17 sample was removed from 1.83 m (6 ft). Both samples were rewetted to 20.5% MC for the carbon dioxide evolution tests. At the end of the tests, P3377 and FRB73×Mo17 had moisture contents of 21.2 and 21.0%, respectively.

Figure 3 is a plot of the carbon dioxide evolution rates of the two samples tested in the accelerated storage test. As in previous tests, the rate increased rapidly early in the test until it reached a maximum. The respiration rate of the P3377 sample increased more rapidly. A comparison of the first 72 hr revealed a significant difference between the two hybrids ($P < 0.001$). The estimated slopes ($t < 72$ hr) of the rate curves are included in Table III. In 1986, as evaluated by carbon dioxide evolution, the freshly harvested P3377 was more susceptible to deterioration than was the freshly harvested FRB73×Mo17. Therefore, after long-term bin storage, we expected a similar ranking. The shapes of the rate curves for the previously stored corn were different from the rate curves of fresh corn. Nevertheless, even after 56 months of bin storage, the P3377 was more susceptible to deterioration than the FRB73×Mo17 was, as measured

by initial (0–72 hr) respiration rates in an accelerated deterioration test.

No *Penicillium* propagules were detected in either sample before inoculation at the start of the test. The P3377 was infected with *Aspergillus restrictus* (31,000 propagules/g), which was not detected in the FRB73×Mo17. The ergosterol contents before testing were 1.25 $\mu$g/g and 0.96 $\mu$g/g, respectively, for the FRB73×Mo17 and P3377. The difference between the ergosterol values was not statistically significant at the $\alpha = 0.10$ level. Although storage fungi were not detectable at the time of sampling, several factors could account for the ergosterol. Other types of fungi were probably present at harvest. Previous mold growth probably occurred during low-temperature drying. Also, fungi may have been present at levels below the threshold of the dilution testing method.

A comparison of the curves in Figure 3 with the data for the 1986 fresh samples of the same corn revealed that the previously stored corn had greater initial ($t < 72$ hr) respiration rate slopes than did the fresh corn (see Table III). This is consistent with the effects of storage history.

**Effect of Hybrid, 1991**

In 1991, P3377 and FR35×FR20 samples were tested for carbon dioxide evolution at harvest. Due to error in sample drying and rewetting, the FR35×FR20 was tested in the carbon dioxide procedure at $\sim 21.2%$ MC and the P3377 at $\sim 20.6%$, Therefore, for comparison, the raw data were transformed by the appropriate damage and moisture multipliers. Because the two samples were not very different in the rate of carbon dioxide production, the results have not been included in the tables or figures.

The results of tests by Friday (1987) indicated that FR35×FR20 was consistently more resistant to storage mold than was P3377. In this test, no significant difference was observed. However, 1991 was a very unusual growing and harvest season in central Indiana. Localized drought stress drastically reduced corn yields at the test farm. Corn ears were typically only half of the length expected.

<table>
<thead>
<tr>
<th>TABLE IV</th>
<th>Number of Fungal Propagules (10^6 Fungal Propagules per Gram of Corn) at the Start and End of One Carbon Dioxide Test*</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>P. saeni</th>
<th>A. flavus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh P3377</td>
<td>1.7 (1.4)</td>
<td>608 (101)</td>
</tr>
<tr>
<td>Stored P3377</td>
<td>40 (5.0)</td>
<td>1,600 (229)</td>
</tr>
</tbody>
</table>

*Standard deviations are given in parentheses.

*Stored four months after 1991 harvest.

![Fig. 2. Respiration rates of P3377 during accelerated deterioration tests with one fresh sample and one that had been previously stored at 13.0% MC for four months after the 1991 harvest. Tests conducted at 26°C and ~20.5% MC.](image1)

![Fig. 3. Respiration rates during accelerated deterioration tests of two hybrids previously stored at low moistures (P3377 at 13.2%; FRB73×Mo17 at 13.4%) for 56 months after the 1986 harvest. Tests conducted at 26°C and ~20.5% MC.](image2)
in a normal year. The FR35×FR20 had consistently lower viability going into bin storage than did the P3377. At harvest, some samples of FR35×FR20 tested below 65% germination rate. By contrast, all samples of the P3377 tested above 85% germination rate at harvest. This may have been the primary reason for the decreased mold resistance of FR35×FR20. The respiration rate curve for the P3377 was consistent with data from other years (Friday 1987). The respiration rate of the FR35×FR20 was greater than had been measured in other years. This further supports the hypothesis that the storability of the FR35×FR20 was reduced due to drought stress.

After four months of bin storage, the two hybrids were sampled and tested by the carbon dioxide evolution procedure. P3377 and FR35×FR20 had bin storage moistures of 13.2 and 13.0%, respectively. The damage levels and test moistures were nearly equal. The P3377 had a higher rate of carbon dioxide production throughout most of the test. The initial ($t < 72$ hr) slopes of the rate curves are included in Table III. ANCOVA comparison for the first 72 hr of testing indicated that the difference between the samples was statistically significant ($P < 0.0025$).

Although it was anticipated that hybrid P3377 would be more susceptible to deterioration at harvest, this result was not observed, perhaps due to different effects of preharvest drought stress on the hybrids. After four months of storage, the hybrid effect may have predominated over the differences in viability at harvest.

### Reevaluation of Data

Part of the overall objective of this research was to determine whether initial ($t < 72$ hr) slopes of respiration rate curves could be used to more rapidly identify differences in storability between corn samples. Previous researchers (Steele et al 1969, Friday et al 1989) used the time required to reach 0.5% (DML) as a criterion for identifying differences in storability. This typically required 200–400 hr of testing. To further investigate whether the criterion of initial respiration rate slopes can be used to identify differences in storability, the data of Friday (1987) were reevaluated. Damage levels and harvest moistures for the samples are given in Friday (1987).

Friday (1987) made two pairwise statistical comparisons of four different hybrids, at the point of 0.5% DML for each sample. The differences between hybrids for percent of kernels infected with *Penicillium* spp. and viable mold rating were statistically significant ($P < 0.05$). Friday (1987) also compared the time required to reach 0.5% DML for each sample. Although differences in damage level between hybrids complicated this comparison, he observed a difference between samples that he attributed to hybrid. Stroshine and Yang (1990) used this difference between hybrids to estimate a hybrid multiplier.

The damage multiplier of Stroshine and Yang (1990) was used for a data transformation, as previously described, on the respiration data for all four hybrids. A plot of the transformed respiration rates is shown in Figure 4. Because all of the hybrids had less than 30% damage (the reference damage for the damage multiplier), the transformed curves were shifted left from the raw data.

Table V lists the times to reach 0.5% DML and the transformed times to reach 0.5% DML. When the Tukey multiple comparison was applied to the transformed time values, the times were significantly different ($P < 0.10$). The estimated initial ($t < 72$ hr) slopes of the transformed respiration rate curves are also listed in Table III. The ranking of the hybrids by slope values is the same as the ranking of the hybrids by the 0.5% DML criterion. This suggests that the ranking could have been achieved in less than a quarter of the testing time.

ANCOVA was used to test the statistical significance of differences between pairs of hybrids on the basis of initial ($t < 72$ hr) respiration rate slopes. Comparing P3377 with FRB73×Mo17 shows that the difference was statistically significant at a level of $P < 0.006$. For any other pairwise comparison among the four hybrids, the difference was significant at the level $P < 0.01$. This analysis also suggests that statistically significant differences between hybrids, as determined by 0.5% DML, can be detected by ANCOVA in 72 hr, or less, of testing.

### CONCLUSIONS

Previous storage history of corn affected subsequent storability, as measured by carbon dioxide evolution rates in accelerated deterioration tests. For a given hybrid, previous storage under conditions of greater greater spoilage risk (higher moisture, longer...
time) resulted in greater initial ($t < 72$ hr) slopes of respiration rate curves in laboratory tests.

Hybrid differences in storability at harvest were measurable and significant even after long-term bin storage. In 1986, hybrid P3377 had a lower storability at harvest than did hybrid FRB73×Mo17. After five years of bin storage at low-moisture, the P3377 still had a lower storability, as measured by carbon dioxide evolution testing.

A reevaluation of data from Friday (1987) indicated that, for fresh corn samples, a greater initial ($t < 72$ hr) slope of respiration rate curves corresponded to less time to reach 0.5% DML. Along with the previous two conclusions, this indicates that the initial ($t < 72$ hr) slope of respiration rate curves during accelerated storage tests relates to storability and warrants future investigation as a potentially effective means of evaluating future spoilage risk of shelled corn.

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LITERATURE CITED


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