

Effect of Hybrid and Physical Damage on Mold Development and Carbon Dioxide Production During Storage of High-Moisture Shelled Corn¹

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ABSTRACT

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Carbon dioxide (CO₂) evolution was measured during storage of shelled corn at 26°C and 20.5% mc. The effects of hybrid and physical damage on mold development were determined. Hybrids were selected as either susceptible or resistant to storage mold on the basis of laboratory tests. Number of propagules, percent seeds infected, visible mold, and percent mold damage were the criteria used to evaluate mold growth. Results of the CO₂ evolution tests showed significant differences in storability

due to hybrid. CO₂ production and associated measures of mold growth for hybrids FRB73 × Mo17 and FR35 × FR20 were significantly lower than for hybrids DF20 × DF12 and Pioneer 3377. For all hybrids except DF20 × DF12, CO₂ was produced at a slower rate than that predicted by Saul's curves. The corn dropped in grade to U.S. no. 4 at 0.5% dry matter loss.

Carbon dioxide production is used extensively to estimate corn deterioration (Saul and Lind 1958, Saul and Steele 1966, Fawole 1969, Seitz et al 1982). Allowable storage time of shelled corn is based on dry matter loss (DML) as predicted by an equation (Steele et al 1969) and is a function of temperature, moisture, and mechanical damage. Steele et al (1969) proposed 0.5% DML as an index of deterioration of shelled corn because they believed that exceeding it could cause a loss of grade. Significant hybrid differences in resistance to storage mold have been demonstrated (Moreno and Christensen 1971, Cantone, et al 1983, Tuite et al 1985). Although invasion by fungi is affected by the amount and kind of damage, differences among corn genotypes persist (Tuite et al 1985). Yao (1987) detected differences among corn genotypes in resistance to three species of *Penicillium* as measured by visible mold and number of propagules. He inoculated kernels killed by heat at 105 spores/g. Tuite and Stroshine (*unpublished*) found that hybrid FRB73 × Mo17 was consistently resistant to *Penicillium* spp. over three crop years, suggesting that resistance is stable for certain hybrids. The nature of these differences in resistance has not been identified.

Thompson (1972) developed a simulation model to predict changes in moisture, temperature, and grain condition during high-moisture corn drying that used CO₂ production as a measure of deterioration. He used the equation of Steele et al (1969) to calculate the amount of CO₂ produced at the "standard" conditions of 15.5°C (59.9°F), 25% mc, and 30% mechanical damage:

$$Y = 1.3 [\exp(0.006t) - 1] + 0.015t \quad (1)$$

where Y = grams of CO₂ produced per kilogram of dry matter and t = time in hours. The time required to produce a given amount of CO₂ under conditions other than the standard was predicted from the expression (Steele et al 1969):

$$T = T_r \times M_r \times M_m \times M_d \quad (2)$$

where T = estimated time in hours to produce a given amount of CO₂; T_r = time to produce the CO₂ at 15.5°C, 25% mc, and 30% mechanical damage; and M_r , M_m , and M_d = multipliers for temperature, moisture, and damage calculated from equations reported by Steele et al (1969) and Thompson (1972).

Thompson's model and the criterion of 0.5% DML have been used extensively in low-temperature grain drying to predict corn deterioration and allowable storage time. To date, investigators have assumed no differences in allowable storage time due to hybrid. However, hybrid differences in susceptibility to storage mold suggest that hybrid has a significant effect on CO₂ production, and this effect should be quantified.

Our objectives were: 1) to determine CO₂ production of several hybrids previously determined to differ in susceptibility to storage mold, 2) to compare CO₂ production with other measures of deterioration, and 3) to compare these measurements with Saul and Steele's prediction of allowable storage time. A more detailed description of the study is given by Friday (1987).

MATERIALS AND METHODS

Carbon dioxide production during storage of mold resistant and mold susceptible hybrids was measured by an absorption technique similar to that used by Steele and Saul (1968) and Fernandez et al (1985). The system consisted of the following steps: CO₂ removal, humidification, sample storage and aeration, water absorption, and CO₂ absorption. Each experiment included six separate CO₂ absorption systems that allowed three replicates of two treatments. The experiment was conducted in a temperature controlled room at 26°C. Ammonium phosphate was used to maintain corn at 20.5% mc (91.0% rh at 25°C).

Airflow rates were monitored using Matheson acrylic purge

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flow meters (0.25–25.0 standard cubic feet of air per hour, model 7262) located at the grain column inlet and the U-tube outlet. Fernandez et al (1985) had placed the flowmeters before the CO₂ removal section. Flowmeters were calibrated with a Gilmont no. 12 flowmeter. Airflow rate was initially 350 ml/min but was increased to 500 ml/min when respiration rate increased. The system was checked for air leaks using the Gilmont flowmeter at 12-hr intervals. A third U-tube containing CO₂ absorbent was connected to the system periodically to confirm that all CO₂ was being absorbed. Reliability of this system was verified by Fernandez et al (1985).

Two separate tests (1986A and 1986B) matched a mold resistant and a susceptible hybrid that were similar in physical damage. The percentage by weight of damaged kernels was determined using the method of Steele (1967). Damaged kernels in a 100-g sample were determined without staining or magnification. In test 1986A, FR35 × FR20 harvested at 600 rpm with 21.6% mechanical damage was matched with Pioneer hybrid 3377 (P3377) at the lowest mechanical damage level available, 27.4%. In test 1986B, FRB73 × Mo17 harvested at 600 rpm had 24.7% mechanical damage, and was matched with DF20 × DF12 harvested at 500 rpm having 25.7% damage. They were stored at 2°C for several days and then transferred to -10°C. Fernandez et al (1985) found that preservation at -10°C gave results that agreed most closely with tests on freshly harvested samples. After several weeks, samples were thawed and sieved with a 4.76-mm (12/64-in.) round hole sieve, dried to 19.5% mc at 40°C, and returned to -10°C. Five days before the test, samples were thawed and inoculated with a spore suspension containing equal amounts of *Penicillium brevicompactum*, *P. cyclopium*, and *P. viridicatum* to give 500 conidia per gram of corn. (Steele did not inoculate.) Samples were equilibrated at 2°C for four to five days. Corn moisture was approximately 20.5% after addition of the spore suspension. Three samples of each hybrid, each weighing 1.1 to 1.2 kg, were used.

Carbon dioxide production was measured at 12-hr intervals. Mold analysis samples weighing 155 g were taken from each column when DML of the three replicates of a given hybrid averaged 0.25, 0.5, and 1.0%. Samples were obtained by emptying the column of corn into a plastic bag, gently mixing the contents, and then removing a 130-g subsample. The remaining corn was returned to the Plexiglas tube, and the system was reconnected. In determinations of the number of propagules (NP), two 20-g subsamples were run in an attempt to reduce variability. When 0.5% DML occurred for one hybrid, the other hybrid was also sampled to compare mold development. Samples of 230–240 g were removed when 0.5% and 1.0% DML occurred and graded for percent mold damage by a licensed commercial grain inspector (Titus Grain Inspection Service, Lafayette, IN).

Laboratory Analysis of Mold Growth

Grain moisture content was determined using the air-oven method (ASAE 1986) and reported on a wet weight basis. Percent kernels infected by fungi were determined after surface disinfection in 1% NaClO (Clorox brand) for 1 min. For NP determinations, 50 kernels each were plated on potato dextrose agar with 100 ppm Tergitol NPX (Union Carbide) and 30 ppm chlortetracycline, and malt salt agar with 6.0% NaCl. NP reflects internal and external sporulation. Twenty-gram samples were blended with 500 ml of 0.1% water agar in a Waring Blendor for 1 min. Successive 10-fold dilutions were plated in molten potato dextrose and malt salt agar. A visible mold score (VM) was determined by inspecting 50 kernels with 11× magnification for fungal sporulation and blue eye. Sporulation was recorded for each kernel as light (1), moderate (2), or heavy (3), and the weighted scores were totaled. Germination was determined on 50 seeds submerged in 1% NaClO for 1 min and germinated on filter paper. Germination, defined as any visible sprouting, was measured after six to seven days.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) was

used for statistical analysis. Linear correlations were determined using regression analysis. The data collected were analyzed by one-way analysis of variance (ANOVA) (Nie et al 1975), and the means were compared by Duncan's multiple range test ($P=0.05$). Multiple comparisons were made by comparing means over all times.

RESULTS AND DISCUSSION

The average values of CO₂ produced for test 1986A comparing P3377 with FR35 × FR20 (Figure 1) show a statistically significant difference. The 0.5% DML level occurred at 275 and 361 hr for P3377 (mold susceptible) and FR35 × FR20 (mold resistant), respectively. This compares with Steele's predictions of 222 and 258 hr at the same moisture, damage, and temperature conditions.

Measures of mold are shown in Figures 2–5. Analysis of variance was performed at the 275 and 361-hr sampling times. Table I summarizes the statistical analyses. The NP was greater on P3377 than on FR35 × FR20 throughout the test (Fig. 2). However, the difference in NP was statistically significant only after 361 hr. The percent seeds infected with *Penicillium* spp. was greater for P3377 than for FR35 × FR20, and the difference was statistically significant for both sampling times (Table I and Fig. 3). Also at both sampling times, greater than 90% of the P3377 kernels were infected compared with 60% infected for FR35 × FR20. For both hybrids, greater than 80% of the kernels were infected with *Fusarium moniliforme*, and FR35 × FR20 had slightly greater NP than P3377. P3377 also had higher VM (Fig. 4), and the difference was statistically significant for both times. The lower seed germination (Fig. 5) for P3377 was statistically significant only after 361 hr. Carbon dioxide production was highly correlated with NP and VM ($r = 0.85$

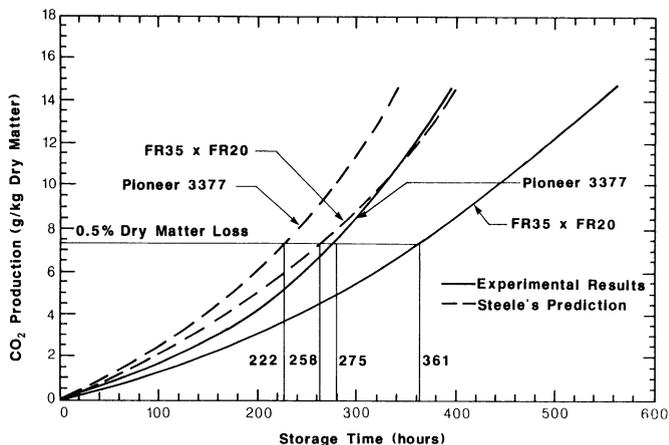


Fig. 1. Test 1986A: CO₂ produced by P3377 and FR35 × FR20.

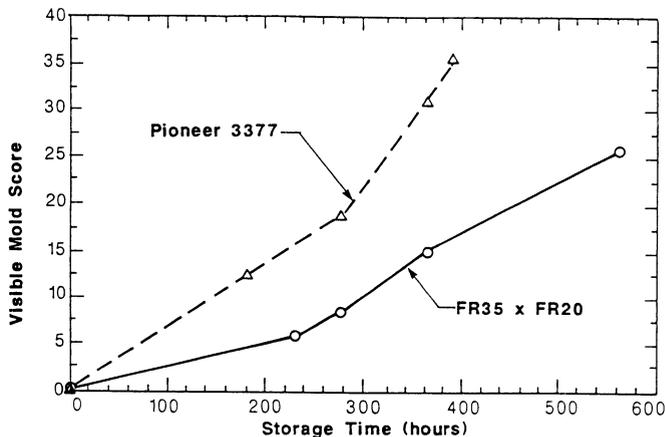


Fig. 2. Test 1986A: Number of fungal propagules of *Penicillium* spp. in FR35 × FR20 and P3377.

and 0.91, respectively). Carbon dioxide production, NP, and VM were correlated negatively with seed germination ($r = -0.89$, -0.86 , and -0.84 , respectively).

Saul and Steele (1966) anticipated that corn would have 5–7% mold damage when 0.5% DML occurs. However, mold damage on the samples graded by an inspection service exceeded their

prediction. At 0.5% DML, FR35 × FR20 and P3377 had, respectively, mold damage of 8.6% (grade no. 4) and 13.6% (grade no. 5). There are several possible explanations why mold damage exceeded that reported by Saul and Steele. At each sampling time, the corn in each tube was mixed and then a sample was removed for mold analysis. Mixing would distribute mold spores over a greater percentage of the kernels. Also, the use of inoculum (500 spores/g) could have increased mold activity and CO₂ production. Fernandez et al (1985) tested uninoculated corn that had been stored at -23.3°C for 90–95 days before testing. They found low mold values at 0.5% DML where NP ranged from 0.4 million to 9.2 million/g, percent kernels infected with *Penicillium* and *Aspergillus* were between 23 and 52%, and VM scores were between 23 and 28. In Fernandez's study, competition between *Aspergillus* spp. and *Penicillium* spp. may have lessened total mold growth. In this study, NP at 0.5% DML ranged from 50 to 73 million and kernel infection from 60 to 90%. It appears that addition of inoculum increased mold activity without significantly increasing CO₂ production. Nevertheless, there were significant differences between mold resistant and susceptible hybrids in CO₂ production as well as several of the other mold indexes.

One complicating factor is the higher level of mechanical damage in P3377 compared with FR35 × FR20 (27.4 versus 21.6%). Using equations 1 and 2, the change in the time required to reach 0.5% DML attributable to the difference in damage can

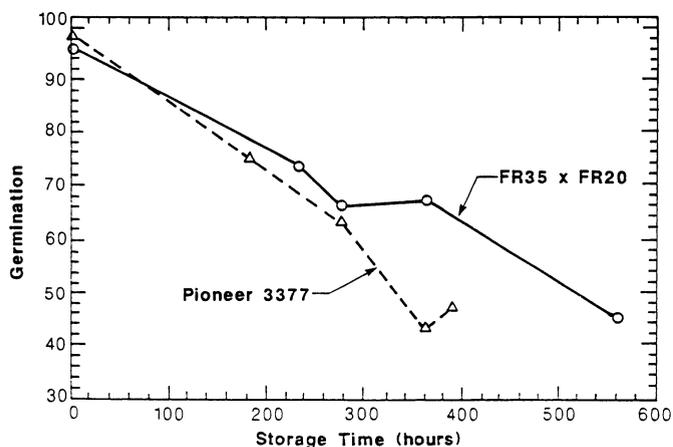


Fig. 3. Test 1986A: Percent seeds infected with *Penicillium* spp. in FR35 × FR20 and P3377.

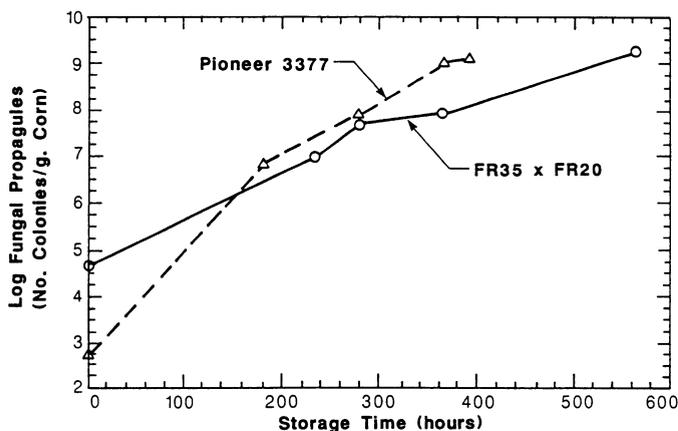


Fig. 4. Test 1986A: Visible mold scores of FR35 × FR20 and P3377.

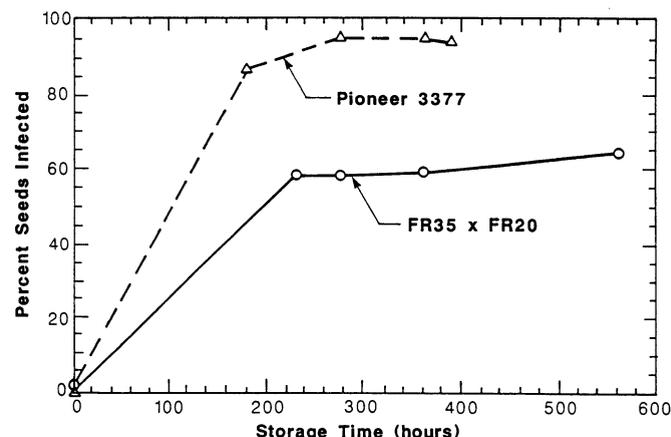


Fig. 5. Test 1986A: Percent seed germination of FR35 × FR20 and P3377.

TABLE I
Comparison of Moisture Content, CO₂ Production, Number of Propagules, Percent Kernels Infected, Visible Mold Score, and Germination^a

Hybrid	Combine Cylinder Speed (rpm)	Mechanical Damage (%)	Moisture Content (% wb)	CO ₂ Production (g/kg DM)	Nos. of Propagules <i>Penicillium</i> spp. (log ₁₀)	Percent Kernels Infected		Visible Mold Score	Germination (%)
						<i>Penicillium</i> spp.	<i>Fusarium moniliforme</i>		
Test 1986A									
Pioneer 3377 (0.5% dry matter loss at 275 hr)									
FR35 × FR20 ^b	600	21.6	20.6 a ^c	4.75 a	7.53 a	58.0 a	92 a	8.3 a	66.0 a
Pioneer 3377	500	27.4	20.8 b	7.35 b	7.83 a	95.3 b	86 a	18.7 b	63.3 a
FR35 × FR20 (0.5% dry matter loss at 361 hr)									
FR35 × FR20 ^b	600	21.6	20.6 a	7.35 a	7.92 a	58.7 a	92.0 a	15.0 a	67.3 a
Pioneer 3377	500	27.4	20.6 a	12.56 b	8.98 b	95.3 b	91.3 a	31.0 a	43.3 b
Test 1986B									
DF20 × DF12 (0.5% dry matter loss at 233 hr)									
FRB73 × Mo17 ^b	600	24.7	20.4 a ^b	4.51 a	6.66 a	59.3 a	75.3 a	11.7 a	62.0 a
DF20 × DF12	500	25.7	20.6 b	7.37 b	7.61 a	88.7 b	84.0 b	28.3 b	48.7 a
FRB73 × Mo17 (0.5% dry matter loss at 302 hr)									
FRB73 × Mo17 ^b	600	24.7	20.4 a	7.35 a	7.06 a	64.7 a	80.7 a	14.3 a	36.7 a
DF20 × DF12	500	25.7	20.6 a	13.37 b	8.40 b	93.3 b	86.7 a	39.0 b	22.7 b

^aNumbers represent averages of three replicates.

^bThese hybrids were relatively resistant to storage mold.

^cNumbers followed by different letters have differences that are statistically significant according to Duncan's multiple range test at the 0.05 significance level.

be calculated as 36 hr. The observed difference in safe storage time was 86 hr. Therefore a portion of the increase in safe storage time was apparently due to varietal differences in mold resistance.

Carbon dioxide production for test 1986B comparing DF20 × DF12, a susceptible hybrid, with FRB73 × Mo17, a mold resistant hybrid, is shown in Figure 6. The 0.5% DML level occurred at 233 hr for DF20 × DF12 and 302 hr for FRB73 × Mo17. Predictions from equation 1, after correcting for damage, moisture and temperature, were 233 and 251 hr, respectively. Note that CO₂ production for DF20 × DF12 started out below Steele's prediction (equations 1 and 2) but later exceeded it (Fig. 6). The two curves cross near 0.5% DML. Moisture content was uniform with differences between the hybrids equal to or less than 0.2%. According to the CO₂ production criterion, DF20 × DF12 was more susceptible to mold invasion than FRB73 × Mo17.

Analysis of variance was performed at the 233- and 302-hr sampling times (Table I). NP on DF20 × DF12 was higher than on FRB73 × Mo17. The difference was statistically significant at 302 hr but not at 233 hr. The percent kernels infected with *Penicillium* spp. was significantly greater for DF20 × DF12 at both 233 and 302 hr. The percent kernels infected by *F. moniliforme* was significantly lower for FRB73 × Mo17 (Table I) even though the initial kernel infection for this species was higher for FRB73 × Mo17 (58%) than for DF20 × DF12 (48%). In test 1986A, the hybrid resistant to *Penicillium* spp. was infected to a greater extent with *F. moniliforme* or other storage fungi (Table I). A small number of FRB73 × Mo17 kernels were infected with *Aspergillus glaucus* and *A. flavus* (less than 6%), whereas none of the DF20 × DF12 kernels were infected. Therefore, competition of other fungi probably does not account for the difference in *Penicillium* spp. growth between these two hybrids.

The higher VM for DF20 × DF12 was statistically significant at both sampling times (Table I). Throughout the test, FRB73 × Mo17 had a greater germination than DF20 × DF12 even though FRB73 × Mo17 had a lower initial germination. However, the difference was not statistically significant at either sampling time (Table I). Carbon dioxide production was highly correlated with NP and VM ($r = 0.82$ and 0.81 , respectively). Carbon dioxide production, NP, and VM were correlated negatively with germination ($r = -0.89$, -0.76 , and -0.76 , respectively).

As in the comparison of P3377 and FR35 × FR20, the samples graded by a licensed inspector showed greater mold damage at 0.5% DML than reported by Saul and Steele (1966). FRB73 × Mo17 and DF20 × DF12 had mold damage of 11.0 (grade no. 5) and 16.2% (sample grade), respectively.

Unexpectedly, the NP and VM for DF20 × DF12 were lower in the sample taken at 312 hr compared with the sample at 301 hr. The sampling procedure disrupts fruiting heads and displaces spores to walls of storage and sampling containers. The opposite phenomenon occurred for the percent seeds infected with

Penicillium and *F. moniliforme*, which showed a small increase over the same sampling period. This suggests that the observed differences in NP represent sample and handling variation. According to Steele's prediction (equations 1 and 2), the differences in damage would cause DF20 × DF12 to reach 0.5% DML 18 hr before FRB73 × Mo17. The observed difference was 69 hr. As in the comparison of P3377 and FR35 × FR20, the increased storage time can be attributed to variety.

The respiration rates (grams of CO₂ per kilogram dry matter per hour) for the two sets of tests are shown in Figure 7. During the earlier stages of mold growth, there was usually a temporary decrease in respiration immediately after sampling. The dip was more obvious for mold susceptible hybrids. Sampling may reduce CO₂ production by disturbing the fungi. This may also explain some of the discrepancy between these results and Steele's prediction.

Results of the measures of mold growth at 0.5% DML for both tests are presented in Table II. The two mold resistant hybrids, FR35 × FR20 and FRB73 × Mo17, had lower *Penicillium* spp. kernel infection, percent mold damage, and VM than the two susceptible hybrids, P3377 and DF20 × DF12. Among all hybrids, NP ranged from 18.5 million to 93.8 million per gram. Fernandez et al (1985) observed lower NP at 0.5% DML, ranging from only 0.4 million to 9.2 million per gram. However, in that study *Aspergillus wentii* was frequently dominant. It was not found in our study. *A. wentii* has large fruiting heads but appears to produce fewer spores than *Penicillium* spp. In this study, percent kernels infected with *Penicillium* spp. varied widely, from 59 to 95%. Percent mold damage also varied widely among hybrids, from 8.6 to 16.2%. These results indicate that different levels and kinds of fungal growth can be obtained at 0.5% DML. Because moisture, prestorage conditions, and test

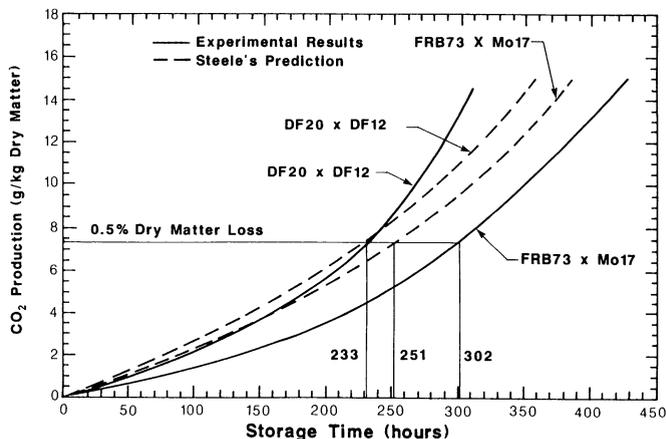


Fig. 6. Test 1986B: CO₂ produced by FRB73 × Mo17 and DF20 × DF12.

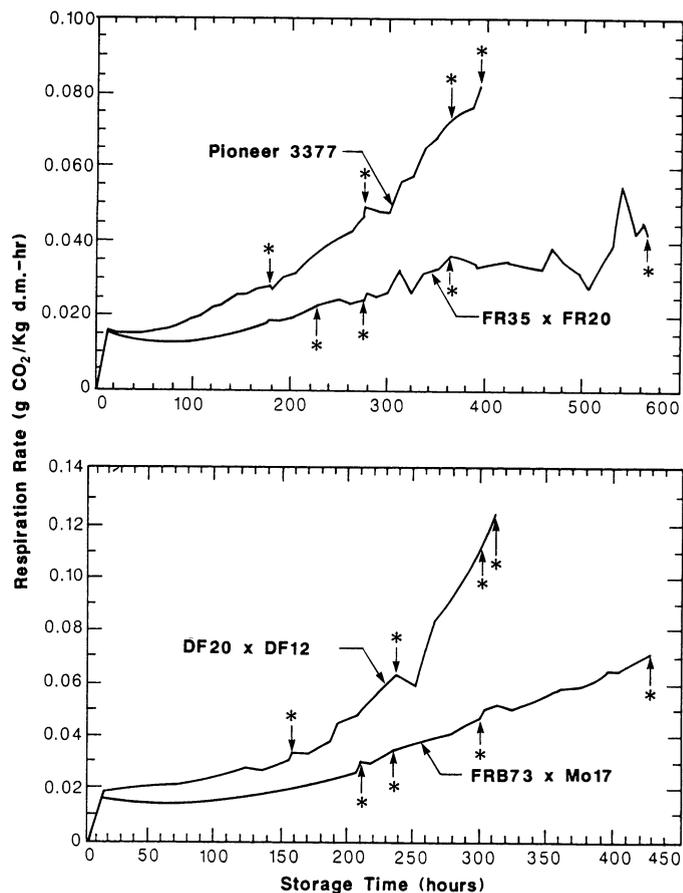


Fig. 7. Respiration rates for CO₂ tests. **Top**, Test 1986A: P3377 and FR35 × FR20. **Bottom**, Test 1986B: FRB73 × Mo17 and DF20 × DF12. Arrows with asterisks indicate sampling times.

TABLE II
Measures of Mold Growth at 0.5% Dry Matter Loss for Hybrids Used in the 1986 CO₂ Tests^a

Hybrid	Nos. of Propagules <i>Penicillium</i> spp. (log ₁₀)	Percent Kernels Infected		Visible Mold Score ^b	Percent with Blue Eye ^c	Percent Mold Damage ^d	Germination (%)
		<i>Penicillium</i> spp.	<i>Fusarium moniliforme</i>				
FR35 × FR20	7.924	59	92	15	6.0	8.6	67
Pioneer 3377	7.863	95	86	19	5.4	13.6	63
FRB73 × Mo17	7.267	65	81	14	6.0	11.0	37
DF20 × DF12	7.720	89	84	28	5.4	16.2	49

^aNumbers are averages for three replicates on each hybrid.

^b50 kernels were rated as light (L = 1), moderate (M = 2), or heavy (H = 3).

^c50 kernels were inspected for grain discoloration. This is the average for three replicates.

^dDetermined by a licensed grain inspector.

temperature were constant, differences in fungal indexes are probably a result of hybrid effects.

The two 1986 CO₂ evaluation tests showed significant differences between mold resistant and susceptible hybrids. In both tests mold developed more slowly in mold resistant FR35 × FR20 and FRB73 × Mo17 than in susceptible P3377 and DF20 × DF12. The difference was consistent for CO₂ production, NP, percent kernels infected, VM, percent mold damaged kernels (grain inspection results), and loss in seed germination. The difference can be partially explained by differences in physical damage. However, there was an additional difference in storability above that explained by damage, and it is believed that this difference was caused by the hybrid effect.

FRB73 × Mo17 and FR35 × FR20 displayed a higher level of mold resistance in all storage tests, while DF20 × DF12 and P3377 were consistently more susceptible. (Not all Pioneer hybrids are highly susceptible to storage molds because some Pioneer hybrids store well in laboratory tests.) The results of this study were consistent with those of experimental bin and laboratory studies (Friday et al 1989), and the relative resistance of hybrids also differed. It was not possible to test the greatest extremes of storage mold resistance. The hybrids shown to be most susceptible by laboratory tests of storability (such as those used by Tuite et al 1985) were short-season hybrids that matured and lost moisture rapidly while they were still in the field. This prevented comparisons in the "full scale" drying bin studies (Friday et al 1989), and it was those studies that determined the availability of samples for the CO₂ tests described here.

The results of this study point out the need for refinement of techniques for CO₂ studies. The observed decrease in respiration rate after sampling could be caused by injury to fungal hyphae and fructifications during mixing. A comparison of sampled and mixed columns with nonsampled columns would reveal such differences. Inoculation of the samples ensures that spores of *Penicillium* spp., which are common storage fungi, are uniformly present. Although the level of inoculation used was very low (500 conidia per gram), inoculation may have increased fungal development. Seitz et al (1982) demonstrated an inoculum effect, but their inoculum level was much higher (8,000 spores/g). Steele (1967) did not inoculate.

For the hybrids compared, the allowable storage time for 0.5% DML varied by at least one or two days at conditions conducive to rapid mold development. It is likely that greater differences could be achieved if a wider number of hybrids were tested and conditions were less optimal for storage molds. Furthermore, it should be possible to increase mold resistance by breeding.

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