Genotypic Differences in Reaction of Stored Corn Kernels to Attack by Selected *Aspergillus* and *Penicillium* spp.

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ABSTRACT

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Hand-harvested and hand-shelled kernels of corn inbreds and hybrids commonly grown in the midwest were evaluated in storage for their reactions to invasion by *Aspergillus* species from the taxonomic groups of *A. glaucus* and *A. flavus*, and by *Penicillium* species. Genotypes were separately inoculated with each group of fungi and stored at three temperatures (26, 30, and 12 C) and relative humidities (85, 91, and 88%), respectively. Percentage of kernel infection, seed germination, and visible mold, number of fungal propagules, and ergosterol concentration were the criteria used to detect resistance and susceptibility. The estimation of visible mold on the kernels was an easy and generally effective way to screen for resistant and susceptible genotypes. The number of propagules and the concentration of ergosterol were useful quantitations of the amount of fungi in and on the corn kernels. Linear correlations between visible mold, fungal propagules, and ergosterol were generally high. Because kernels were rapidly infected and usually retained high germination, kernel infection and seed germination were inadequate for detecting resistance to mold development, although seed germination and number of propagules in the test with *A. flavus* exhibited a highly inverse relationship. Overall, the genotypes responded similarly to attack by all three fungal groups. Consistently high and low levels of susceptibility to growth and sporulation of the fungi were exhibited by kernels of H95 and B73 × Mo17, respectively. Variations occurred with some of the intermediate genotypes. Some genotypes, including H95 and B73 × Mo17, were also consistent in reactions to attack by *Penicillium* over two crop years. Thus, it appears that inherent and relatively consistent differences in susceptibility to the main groups of storage fungi exist among corn genotypes.

Fungi are important causes of deterioration in stored, shelled corn (*Zea mays* L.). They can discolor kernels, decrease seed germination, produce mycotoxins and offensive odors, and decrease dry matter (1). Lowering the moisture content is the most common way to control fungal growth in stored corn. Drying grain rapidly at high temperatures, however, is not always possible or desirable. Therefore, genotypes resistant to fungal invasion and proliferation would be valuable because slow drying and alternate storage systems could be used.

Differences in preharvest resistance of corn kernels to invasion by *Diplodia maydis* (2,5), *Fusarium moniliforme* (3,13,17) and other ear-rotting fungi (5,16), and to *Aspergillus flavus* and aflatoxin formation (6,7,18) have been reported.

In contrast to the large number of studies with preharvest corn, there have been relatively few studies of resistance of stored corn to fungal attack. Moreno-Martinez and Christensen (8) and Moreno-Martinez et al (9) reported that corn genotypes differed in germination after storage and that they believed these differences were associated with variations in susceptibility to fungal invasion.

In our study, we evaluated inbreds and hybrids commonly grown in the corn belt for reactions to three groups of storage fungi that develop optimally in corn at different moistures and temperatures. In addition, we tested these genotypes against *Penicillium* spp. for two crop years to determine if the host reactions were consistent. We also assessed the value of visible mold, kernel infection, number of fungal propagules, ergosterol, and seed germination as indicators of fungal invasion and growth.

MATERIALS AND METHODS

Inbreds and hybrids (eight in 1979 and nine in 1980) adapted to the corn belt were grown at the Purdue University Agronomy Farm, West Lafayette, IN. Inbreds were grown in semi-isolation to permit sib or self-pollination. Corn was hand harvested at 26–30% moisture content (MC) and the ears were dried to 12–14% MC with forced air at 40 C. Ears were hand-shelled and kept at 1–2 C for 2–4 mo in polyethylene bags.

Corn kernels were inoculated in separate tests with water suspensions of a mixture of spores of *A. amstelodami, A. repens*, and *A. ruber*; a mixture of *A. flavus* and *A. parasiticus*; and a mixture of *P. brevi-compactum, P. cyclopium*, and *P. viridicatum*. Genotypes grown in 1979 were tested with all fungal groups, whereas the 1980 crop was tested with *Penicillium* spp. only.

Concentrations of inocula were determined with a hemocytometer. A spore suspension (with enough water to raise the moisture content of the corn to desired levels) was atomized on the kernels to give 1,500–2,000 conidia per species per gram of kernels. Inoculated kernels were stored at 1–2 C with occasional shaking for several days before the beginning of the test.

Inoculated corn from each genotype was placed in triplicate (160–280 g) in perforated (side and bottom) containers with open tops. These containers were placed randomly on wire racks in covered 38-L glass tanks. Corn inoculated with *A. glaucus* was stored at 26 C and 85% RH (equilibrium moisture content [EMC] of ~16.5%). Relative humidity was maintained by bubbling air successively through separate containers of deionized water, a saturated $NH_4H_2PO_4$ solution (92% RH), a glycerol-water solution (GWS) with a refractive index (RI) of 1.3895, and then into a GWS of the same RI in the bottom of the glass tanks. In the test with *A. flavus*, corn was stored at 30 C and 91% RH (~18% EMC) with a saturated KNO_3 solution (91% RH) and a GWS of 1.3711 RI. Corn inoculated with *Penicillium* was stored at 12 C and 88% RH (~19% EMC) with a saturated $NH_4H_2PO_4$ solution and a GWS of 1.3798 RI.

Kernels from each replicate were sampled twice in 1979 and thrice in 1980. One hundred kernels were examined with an ×3 illuminated lens to determine visibly molded kernels. Fifty kernels

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were surface disinfected with 5% NaOCl (Timesaver or Clorox bleach brand) for 1 min in a flask or petri dish and rinsed twice with sterile deionized water. Kernels were then plated (10 kernels per plate) on 6% malt salt agar (the test with *A. glaucus*), potato-dextrose agar with Tergitol NPX (100 ppm) and chlortetracycline (30 ppm) (the test with *A. flavus*) or Czapek Solution agar with 1% corn steep, Tergitol NPX (100 ppm), and chlortetracycline (30 ppm) (the test with *Penicillium*), incubated at 22–24 C for 7–14 days, and the number of infected kernels and fungal species determined. Refer to Tuite (15) for additional media details. Germination (emergence of radicle or shoot) of 50 surface-disinfected kernels was determined after 7 days at 22–24 C on either 1.5% water agar or moist filter paper. To determine the number of fungal propagules, 20 or 25 g of corn kernels was comminuted for 1 min in a Waring blender that contained 500 ml of 0.1% sterile water agar, serial dilutions were made, and 1 ml from a dilution was delivered to each of three or four petri dishes. Cooled (~50 C) molten media (20–30 ml) was added to each petri dish, and these were incubated at 22–24 C for 7–14 days. The 1980 crop inoculated with *Penicillium* was analyzed for ergosterol by the method of Seitz et al (12). Moisture contents were determined by drying two 5- to 8-g samples of corn at 103 C for 72 hr. All data were analyzed by Duncan's multiple range test ($P = 0.05$). Linear correlations (r) between variables were also determined.

RESULTS

A. glaucus. Invasion of most genotypes by *A. glaucus* was rapid, as measured by visible mold (Fig. 1A). H95, Mo17, A632, and H99 × B68 had the highest, whereas B73 × Mo17 had the lowest percentage of kernels with visible growth at 9 days. The unexpected decrease in visible mold at 16 days may have been caused by extensive handling of the kernels during sampling. Nevertheless, H95 and B73 × Mo17, respectively, remained the most susceptible and resistant as measured by visible molding. Visible sporulation was confined to the tip cap (pedicel region) of every genotype except H95. The latter was more extensively colonized, with sporulation often on the crown.

The numbers of fungal propagules produced on each genotype were relatively low at 9 days (Fig. 1B). H95, the most susceptible genotype, supported a 15-fold increase in propagules between 9 and 16 days. Many genotypes exhibited large increases in fungal propagules in contrast to the decrease in visible mold from 9 to 16 days. Conidial fructifications were either disrupted by handling and the spores were distributed over the kernel surface, or sporulation, undetected by visual inspection, occurred within the kernel. B73 × Mo17 and Mo17 × H100 supported the least sporulation by *A. glaucus*. The number of fungal propagules and the percent visible mold were significantly correlated at 9 and 16 days ($r = 0.79$ and 0.83, respectively).

Kernel infection and seed germination data gave little information about resistance to *A. glaucus*. B73 × Mo17 and B73 were the only genotypes to have <90% infection (82 and 87%, respectively) at 9 days, but at 16 days all genotypes had ≥90% infection. High seed germination in most genotypes (83–99%) at 16 days reflected little damage to the embryos despite heavy fungal sporulation.

A. ruber was prevalent from plated kernels. This species infected 50% more kernels than both *A. amstelodami* and *A. repens* combined. *A. amstelodami* sporulated more than *A. repens* and *A. ruber* at 9 days, whereas all three species had similar numbers of propagules at 16 days.

A. flavus. An environment of 91% RH and 30 C favored invasion by *A. flavus* and *A. parasiticus*. H95 was extremely susceptible as measured by visible sporulation at 7 and 23 days (Fig. 2A). Sporulation was evident at the pedicel and frequently on the rest of the kernel. All other genotypes had significantly less visible mold, especially B73 × Mo17, which had the smallest percentage of visible sporulation by *A. flavus*.

H95, the most susceptible entry at 7 days, and B73 had significantly higher numbers of propagules (Fig. 2B). B73, however, had few visibly molded kernels. Considerable shifts in ranking occurred between 7 and 23 days as Mo17 had a large increase in fungal propagules. H95 had fewer fungal propagules than expected from the visible mold ratings despite the release of "green clouds" of spores when replicates of H95 were sampled. B73 × Mo17 and Mo17 × H100 were resistant as reflected by the low numbers of propagules at 7 and 23 days. The correlation between number of propagules and visible mold decreased significantly with time ($r = 0.81$ and 0.51 at 7 and 23 days, respectively).

Early in storage (7 days) genotypes could be separated on the basis of kernel infection. B73 × Mo17 and B73 were invaded the least by *A. flavus* (37 and 54%, respectively), whereas H95, Mo17, and A632 × H95 had 96% or greater infection. At 23 days infection exceeded 93% for every genotype.

Seed germination at 23 days exhibited a high inverse correlation with numbers of fungal propagules ($r = -0.91$), but was not significantly correlated with visible mold ($r = -0.41$). Mo17, B73, and A632 had <54% germination, whereas Mo17 × H100 and B73 × Mo17 germinated above 92%. Kernels of H95 germinated 73%.

A. flavus invaded slightly more kernels and produced many more spores than *A. parasiticus* at both 7 and 23 days. *A. glaucus*, although uninoculated, infected up to one third of the kernels of many genotypes at 23 days, but produced few propagules.

***Penicillium,* 1979.** Evaluations of visible mold among genotypes differed widely (Fig. 3A). H95 was the most susceptible at both

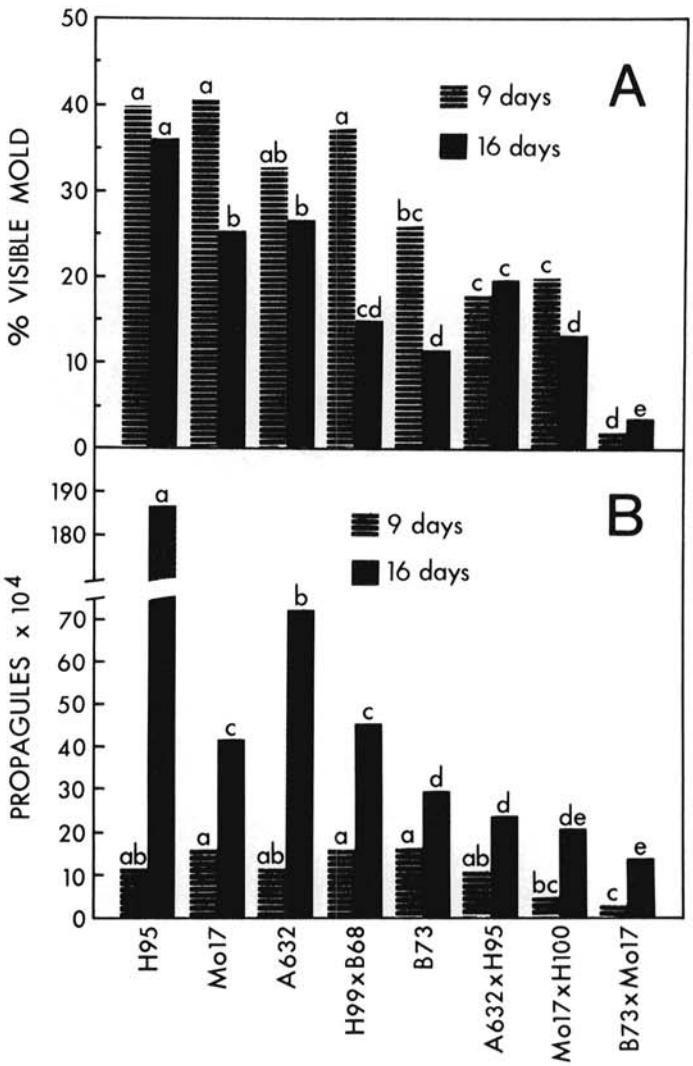

Fig. 1. Percentage visible mold and number of propagules from corn inoculated with *Aspergillus amstelodami, A. repens,* and *A. ruber* and stored at 85% RH and 26 C for 9 and 16 days. **A,** percentage visible mold; **B,** number of fungal propagules per gram of seed (×10⁴). At each time period, bars with different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

samplings. Sporulation on kernels of all genotypes was restricted to the pedicel at 18 days, but at 28 days spores and mycelium covered much of the kernel of H95. The genotypes B73 and B73 × Mo17 were relatively resistant with very light sporulation on the pedicel.

The number of fungal propagules produced on the genotypes (Fig. 3B) generally agreed with the percentage of visibly molded kernels (correlation coefficients of 0.75 and 0.82 at 18 and 28 days, respectively). Propagules produced on each genotype increased substantially with time, and H95 was again most susceptible. B73 × Mo17 and Mo17 × H100 had considerably fewer propagules. The inbred B73 supported large numbers of propagules at 18 and 28 days. This was unexpected because B73 had little visible fungal growth suggesting that the fungi sporulated internally.

There were no significant differences in susceptibility as measured by kernel infection or seed germination. Kernel infection ranged from 90 to 100% at 18 days, and seed germination was >95% at 28 days.

***Penicillium*, 1980.** There were significant differences in visible mold among the genotypes (Fig. 4A). H95 had a high percentage of severely molded kernels at 85 days. B73 × Mo17, A632, and A632 × H95 had little if any visible sporulation at any time.

In dilution isolations, H95 had the largest number, whereas B73 × Mo17 had the lowest number of propagules (Fig. 4B). Propagule numbers correlated very well with the percentage of moldy kernels at 23, 53, and 85 days ($r = 0.90$ to 0.98).

Ergosterol levels correlated very well with number of propagules ($r = 0.96$) and also with visible mold ($r \geq 0.92$) at each sampling. H95 was highly susceptible, as were H100 and Mo17, all with large amounts of fungal biomass as indicated by ergosterol (Fig. 5). B73 × Mo17 contained the lowest amount of ergosterol, but the values were not statistically different at 23 and 85 days.

Differences in kernel infection among genotypes were detected only up to 53 days of storage (Table 1). Five of nine genotypes had less than 80% infection at 23 days, but at 53 days only B73 × Mo17 had less than 80% of its kernels infected with *Penicillium* spp.

Seed germination (Table 1) revealed few differences at 53 days as seeds of most genotypes germinated over 91%. H100 and Mo17 were highly susceptible to fungal attack if judged by low seed germination. At 85 days, only four genotypes (A632, A632 × H95, H95 × B73, and B73 × Mo17) germinated >85%. H95 maintained high germination at 85 days despite considerable fungal development. Seed germination correlated poorly with visible mold, number of propagules, and ergosterol ($r = -0.22$ to -0.60).

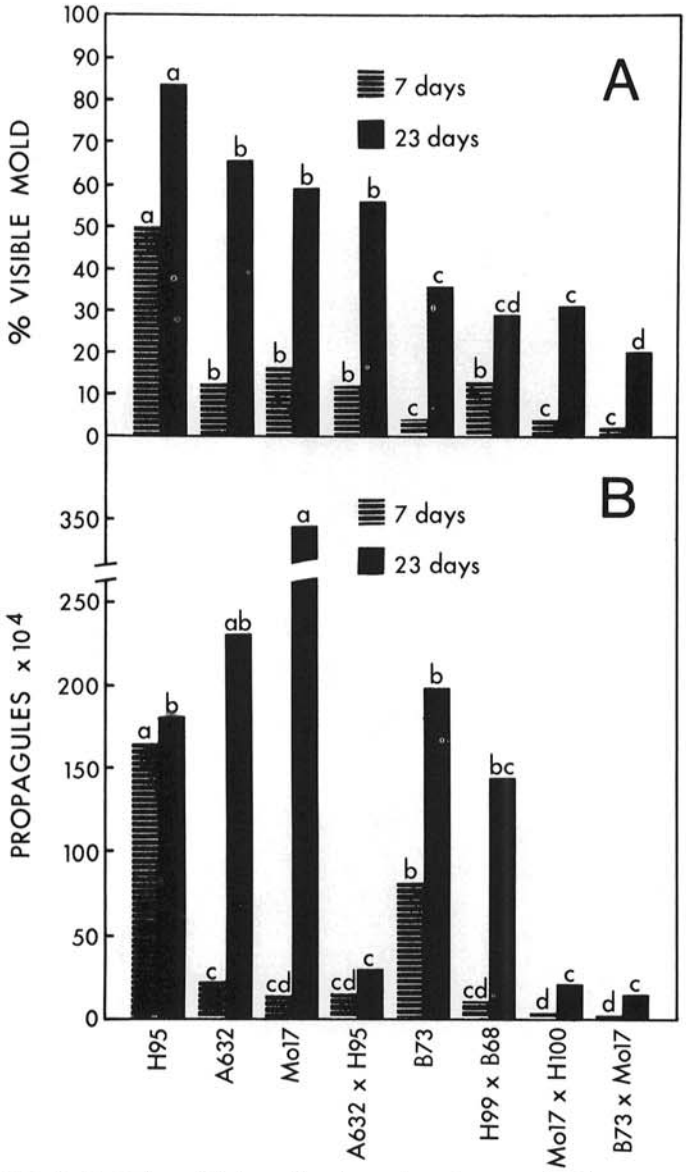

Fig. 2. Percentage visible mold and number of propagules from corn inoculated with *Aspergillus flavus* and *A. parasiticus* and stored at 91% RH and 30 C for 7 and 23 days. **A,** percentage visible mold; **B,** number of fungal propagules per gram of seed ($\times 10^4$). At each time period, bars with different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

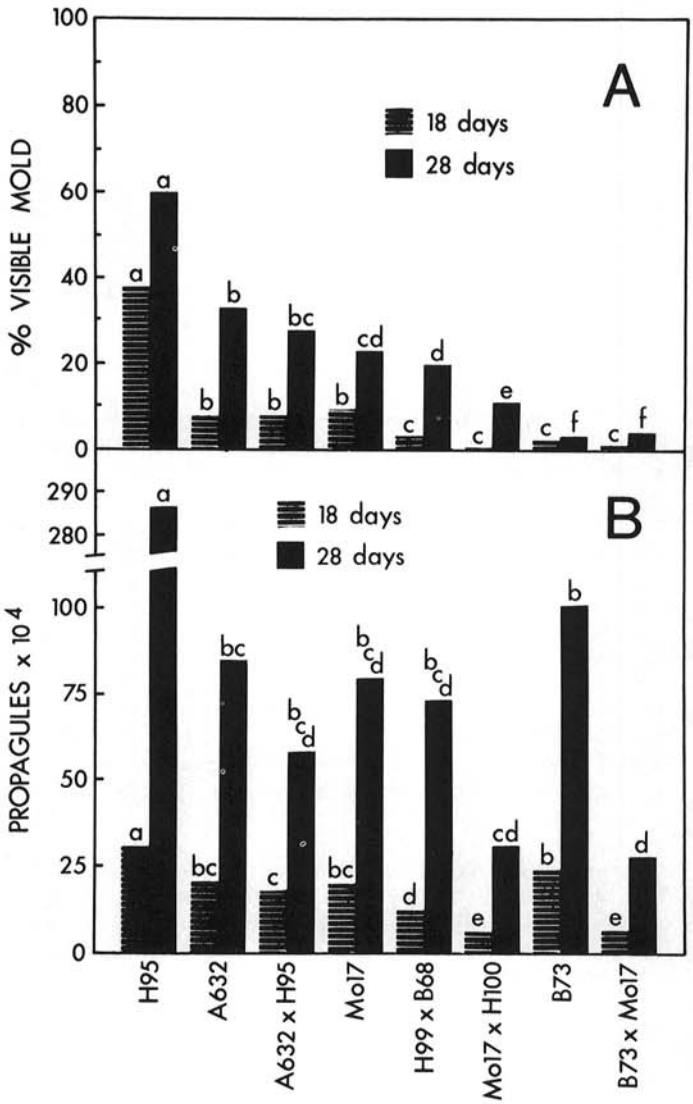

Fig. 3. Percentage visible mold and number of propagules from corn (1979) inoculated with three species of *Penicillium* and stored at 88% RH and 12 C for 18 and 28 days. **A,** percentage visible mold; **B,** number of fungal propagules per gram of seed ($\times 10^4$). At each time period, bars with different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

A much better curve fit, however, was obtained with a second-order polynomial equation ($Y = a + bX + cX^2$). Significant correlation coefficients were obtained with visible mold ($R = -0.76$ and -0.92 at 53 and 85 days, respectively) and ergosterol ($R = -0.74$ and -0.79 at 53 and 85 days, respectively), but correlations were less significant with number of propagules ($R = -0.51$ and -0.69 at 53 and 85 days, respectively).

The organisms chosen for these last two tests were three common penicillia found in stored corn. In the 1979 crop test, *P. brevi-compactum* was isolated more frequently from plated kernels and dilution plates than *P. cyclopium* and *P. viridicatum*. In 1980, *P. brevi-compactum* and *P. cyclopium* were more frequently isolated from plated kernels, whereas *P. brevi-compactum* and *P. viridicatum* were more frequent from dilution plates.

Genotypes were rated from 1 to 7 (with 1 the most resistant and 7 the most susceptible) based on the amount of visible mold and propagules. Table 2 summarizes the reactions of each genotype to invasion by the three fungal groups. H95 and B73 × Mo17 were consistently the most susceptible and the most resistant genotypes, respectively. The remaining genotypes reacted consistently as measured by visible growth and sporulation except for the inbred B73. In each test, B73 supported significantly greater numbers of propagules than expected from the visible mold ratings, suggesting that sporulation inside the kernel was undetected by visual examination. Reactions of most genotypes to invasion by *Penicillium* species over two crop years (1979 and 1980) were fairly consistent (Table 3). Again, H95 and B73 × Mo17 were the most susceptible and the most resistant, respectively, to visible mold and production of fungal propagules in both years. A few of the remaining genotypes, however, were not as consistent. Mo17 × H100 appeared to support more visible molding and number of propagules in 1980 than in 1979. The opposite reaction was observed with A632 as it had less fungal growth and sporulation in 1980 than in 1979. A632 × H95 also was more resistant to visible mold in 1980 than in 1979.

DISCUSSION

Resistance to storage fungi would considerably improve the storability of corn kernels. Important to the study of resistance are the methods used to estimate fungal growth. Visual estimates of fungal sporulation were valuable in assessing fungal invasion. Conidia produced by *Aspergillus* and *Penicillium* both within and on the corn kernel appear to reflect growth of these fungi and thus,

TABLE 1. Percentage of kernel infection and percentage of seed germination of corn genotypes inoculated with three species of *Penicillium* and stored at 88% RH and 12 C for 23, 53, and 85 days in 1980

Corn genotype	Kernel infection (%)[a]		Seed germination (%)[a]	
	23 days	53 days	53 days	85 days
H95	99 a	99 a	93 a	77 b
H100	95 a	96 abcd	45 c	28 d
Mo17	92 ab	98 ab	62 b	29 d
H95 × B73	91 abc	98 ab	100 a	90 a
A632 × H95	77 abc	91 de	97 a	93 a
B73	65 abc	87 e	91 a	61 c
Mo17 × H100	59 bc	92 cde	95 a	78 b
A632	55 c	86 e	97 a	93 a
B73 × Mo17	59 c	76 f	99 a	87 ab

[a]In each column, values followed by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

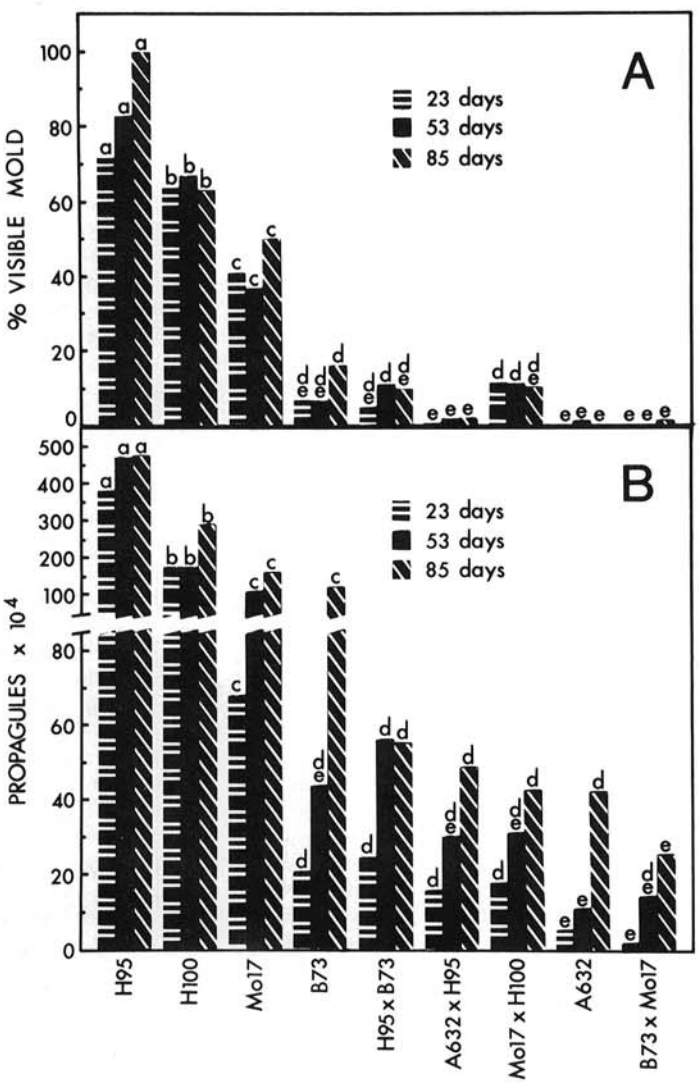

Fig. 4. Percentage visible mold and number of propagules from corn (1980) inoculated with three species of *Penicillium* and stored at 88% RH and 12 C for 23, 53, and 85 days. **A,** percentage visible mold; **B,** number of fungal propagules per gram of seed (×10⁴). At each time period, bars with different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

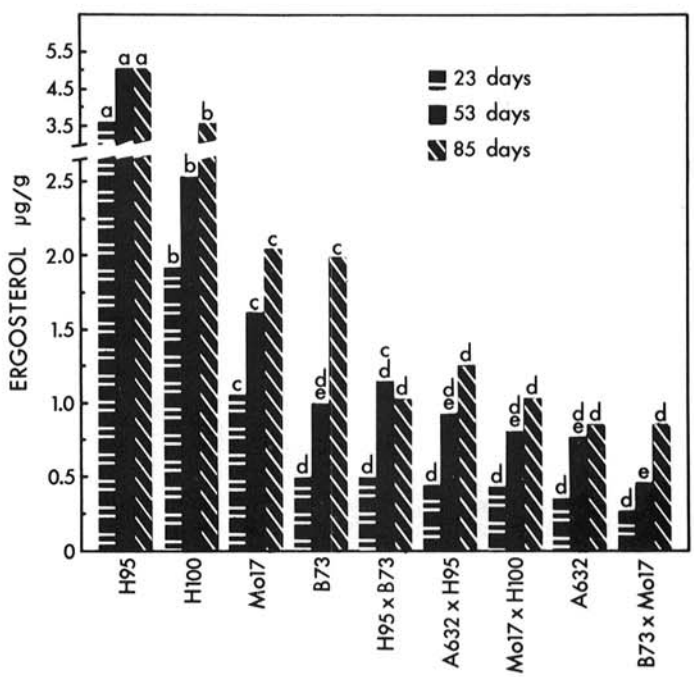

Fig. 5. Ergosterol (micrograms per gram of seed) from corn kernels (1980) inoculated with three species of *Penicillium* and stored at 88% RH and 12 C for 23, 53, and 85 days. At each time period, bars with different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

TABLE 2. Disease ratings[a] of corn genotypes inoculated with *Aspergillus glaucus* (*A gl*), *A. flavus* (*A fl*), and *Penicillium* (*Pen*) species

Corn genotype	Visible mold						No. of Propagules					
	A gl		*A fl*		*Pen*		*A gl*		*A fl*		*Pen*	
	9 days	16 days	7 days	23 days	18 days	28 days	9 days	16 days	7 days	23 days	18 days	28 days
B73 × Mo17	1	1	1	1	2	2	1	1	1	1	2	1
Mo17 × H100	3	3	2	2	1	3	2	2	2	2	1	2
B73	4	2	3	3	3	1	7	4	6	5	6	6
A632 × H95	2	4	4	4	5	5	3	3	4	3	3	3
Mo17	7	5	6	5	6	4	6	5	3	7	4	4
A632	5	6	5	6	4	6	4	6	5	6	5	5
H95	6	7	7	7	7	7	5	7	7	4	7	7

[a]Genotypes were ranked from 1 to 7 (with 1 as the most resistant and 7 as the most susceptible genotype) based on the values for visible mold and number of propagules.

TABLE 3. Disease ratings[a] of corn genotypes inoculated with *Penicillium*

Corn genotype	Visible mold					Propagules (no.)				
	1979		1980			1979		1980		
	18 days	28 days	23 days	53 days	85 days	18 days	28 days	23 days	53 days	85 days
B73 × Mo17	2	2	1	1	1	2	1	1	1	1
Mo17 × H100	1	3	5	5	4	1	2	4	4	3
B73	3	1	4	4	5	6	6	5	5	5
A632 × H95	5	5	3	3	3	3	3	3	3	4
Mo17	6	4	6	6	6	4	4	6	6	6
A632	4	6	2	2	2	5	5	2	2	2
H95	7	7	7	7	7	7	7	7	7	7

[a]Genotypes were ranked from 1 to 7 (with 1 as the most resistant and 7 as the most susceptible genotype) based on the values for visible mold and number of propagules.

is a measure of fungal invasion. Conidia are agents of survival and dissemination and are probably important in epidemiology in commercial storage. Sporulation is also important in commerce when spore production and the associated musty odor are sufficient to decrease grade.

In general, correlations between visible mold, number of propagules, and ergosterol concentrations were significant. Thus, visual estimates of fungal growth could be a fast and effective method to initially screen genotypes. More refined techniques such as measurement of the number of propagules and ergosterol could then be used to verify resistant genotypes.

Adequate inoculum and optimum conditions for fungal growth in our storage tests allowed for early and uniform kernel infection. Consequently, kernel infection ≥90% occurred early in most of the tests. This prevented discrimination between resistant and susceptible genotypes and resulted in poor correlations with visible mold, number of propagules, ergosterol, and seed germination. Much of the infection probably occurred in the pericarp and the pedicel and not in the embryo because seed germinations remained high. Plating was valuable in determining incipient growth and distinguishing differences among corn genotypes inoculated with *A. flavus*. Presumably, earlier sampling in the other tests also would have revealed differences among genotypes.

A significant loss in seed germination of corn stored at conditions favorable for fungal growth is often caused by fungal invasion. Because mold-free controls were not included in this study, it is uncertain whether or not the small decreases in germination were caused by fungal invasion. Qasem and Christensen (10), however, demonstrated that aspergilli decreased the germination of corn in storage. Generally, seed germination remained high in our tests and was poorly correlated with mold evaluations, except in corn inoculated with *A. flavus*. The low seed germination of many of the genotypes at 23 days was probably associated with the aggressiveness of *A. flavus* and the adverse storage temperature. *A. glaucus* and *Penicillium* spp. did not cause rapid and large decreases in seed germination, apparently because penetration and invasion of the embryo was insignificant. Perhaps with longer storage germination would reflect the additional fungal invasion. The high germination of H95 was surprising because this inbred usually supported the largest amount of fungal growth and sporulation. This finding supports the idea that fungal invasion was largely limited to the pedicel and the outer layers of the kernel in many genotypes.

Corn genotypes differ in reactions to invasion by fungi in storage as shown by Moreno-Martinez and Christensen (8), and Moreno-Martinez et al (9). They used seed germinability as the primary criterion of resistance of kernels to fungi. Substantial differences in germination were observed among the genotypes, and these differences generally agreed with the amount of visible growth by the fungi. They suggested that the differences in germination among genotypes were due to intrinsic characteristics of the kernels.

In our study, visible fungal growth and sporulation were the primary criteria used to measure resistance and susceptibility. None of the genotypes tested were immune to fungal attack, but significant and rather consistent differences in reaction to the three major groups of storage fungi in three environments were found. Differences among hybrids, however, were not as great as between inbreds and hybrids. The inbreds were generally more susceptible than the hybrids.

Overall, the genotypes reacted similarly to attack by *A. glaucus, A. flavus*, and *Penicillium* species. The relatively high and low levels of susceptibility to fungal attack exhibited by H95 and B73 × Mo17, respectively, remained constant. Variations in ranking occurred with intermediate genotypes, especially A632 and A632 × H95. Most genotypes, especially H95 and B73 × Mo17, reacted consistently to attack by *Penicillium* in two crop years. Therefore, it appears that corn genotypes have inherent and relatively consistent differences in susceptibility to attack by storage fungi.

Entry into undamaged corn kernels by various fungi has been studied by several researchers (2,4,11,14). Qasem and Christensen (11) observed that if the pericarp of the kernel remained unbroken, penetration of the fungi was probably through the pedicel. Tsuruta et al (14) found invasion via the pedicel and in some instances directly through the pericarp over the embryo. Johann (2) observed that field infections by *Diplodia maydis* usually occurred at the proximal end of the kernel. Koehler (4) also reported infection via the pedicel in mature, undamaged kernels. Access into the kernel in any other way was rare. Since differences in fungal growth and sporulation among the genotypes were visually detected at the pedicel, resistance may be located here.

The application of resistance to fungal growth in storage appears promising, but needs to be assessed by: testing diverse genotypes to obtain greater resistance, further determining the stability of resistance by growing corn at different locations and crop years, testing the effect of harvest damage and high-temperature drying on kernel resistance, identifying the resistant factor(s), and transferring resistant characteristics of inbreds to hybrids.

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