The Effects of Heat Treatment and Inoculum Concentration on Growth and Sporulation of *Penicillium* spp. on Corn Genotypes in Storage

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This research was supported in part by the Andersons Research Fund of the Ohio State Development Fund.
We thank Sherry Fulk Bringman and Bryan Maloney for technical assistance and Richard Stroshine and Paul Crane for their generous help.
Accepted for publication 8 June 1989 (submitted for electronic processing).

ABSTRACT

Yao, B., and Tuite, J. 1989. The effects of heat treatment and inoculum concentration on growth and sporulation of *Penicillium* spp. on corn genotypes in storage. Phytopathology 79:1101-1104.

The effects of heat treatment and inoculum concentration of *Penicillium brevi-compactum*, *P. cyclopium*, and *P. viridicatum* on storability of corn kernels were determined in separate tests for 10 genotypes of dent corn and a visual flint (VF) selection. Hand-shelled corn grown during 1983–1985 was used. Kernels of B73 × Mo17 and Dekalb XL67, resistant, and H95 very susceptible and VF, moderately susceptible to storage Penicillia were heated at 80 C for 20 min, then inoculated with 2×10^3 spores/g and stored at 88% RH and 14 C for 49 days. Host reaction was determined by a visible mold rating on individual kernels and number of propagules isolated after dilution on a modified potato-dextrose agar. Molding was substantially increased by the heat treatment as measured by propagules and less so by visible mold for all four genotypes. Increase was greater for the resistant hybrids but the disease rankings among the genotypes remained. The heat-treated resistant hybrids did not support significantly more sporulation of *Penicillium* than the unheated, susceptible genotypes. In three tests, a total of 11 corn genotypes were inoculated with 2×10^3 to 10^4 or 2×10^3 to 10^5 spores of *Penicillium* spp. per gram of corn and stored at 13–14 C and 88% RH. Increasing the inoculum to either 10^4 or 10^5 spores/g generally had no significant effect on relative resistance as measured by propagules and visible mold, although there was an increase in propagules with an increase in the amount of inoculum, particularly when 2×10^4 or 10^5 spores/g was used. There appeared to be a more defined separation of hybrids with inoculum at 2×10^4 and 10^5 than at 2×10^3 spores/g.

Additional keywords: maize, storage molds.

Resistance to storage fungi has been demonstrated in corn (2,3,6) and appears useful in controlling fungal deterioration. The practical significance of resistance, however, may be less if resistance to mold development has no concomitant inhibitory effect on mycotoxin production, or is highly compromised by commercial practices such as high temperature drying and combine harvesting.

Physical damage, although it increases mold growth considerably, does not eliminate differences between resistant and susceptible genotypes (6). This paper explores two other aspects of commercial storage that influence mold development: 1) the effect of high drying temperatures that exceeds temperature recommendations, and 2) exposure to high amounts of inoculum that can occur when spoiled and sound corn are blended.

MATERIALS AND METHODS

Corn genotypes. Nine hybrids and one inbred H95, of dent corn (*Zea mays* L.) commonly grown in the Midwest and a visual flint (VF) selection were used. The VF was from a subpopulation selected on the basis of flint-type appearance from a population developed by crossing a corn belt composite with a tropical flint. The VFs tested in succeeding years were different lines but were from the same subpopulation. The 1983 and 1984 corn were grown in randomized blocks at the Purdue Agronomy Farm (W. Lafayette, IN), the 1985 corn was grown in nonrandomized strips, with random samples taken. The corn was hand harvested, dried at 40 C to 11–13% M.C., hand-shelled, butt and tip kernels discarded, and stored at 4 C before use.

Test fungi. Three common storage *Penicillium* spp. were used, *P. brevicompactum* Dierckx, *P. cyclopium* Westling, and *P. viridicatum* Westling revived from lyophilized cultures. They were grown on PDA for 7–10 days at 21–23 C and suspended in deionized water with two drops of Tween 20 per liter. Spore concentration was determined with a hemacytometer and adjusted to the desired concentration.

Mold evaluation. Sporulation of *Penicillium* on the corn kernels was measured by a visible mold rating and numbers of propagules.

Visible mold was determined for 50 or 100 seed at ×10 magnification. Sporulation on the seed was rated as light = 1, moderate = 2, and heavy = 3, except for two tests with heavier amounts of inoculum where the ratings was weighted as trace = 1, light = 2, moderate = 3, heavy = 4, and very heavy = 5. The numbers of propagules were determined using 20 g of corn. The corn was blended for 1 min in 0.1% water agar, diluted, and added to cooled molten potato-dextrose agar containing 100 ppm of Tergitol NPX (Union Carbide) and 30 ppm of chlortetracycline added before pouring. Colony counts were made after 4 days.

Heat treatment. Kernels harvested from B73 × Mo17, DK XL67, H95, and VF grown in 1983 were used for the test in July 1984. The moisture was adjusted to 17% wet basis and stored 24 hr at 4 C. Moisture content was determined by the official whole seed oven method (10–15 g, 103 C for 3 days) and is given on the wet weight basis. For each genotype 360 g of corn was placed in Seal-n-Save polyester bags (Sears) and heated at 80 C for 20 min. After cooling, the corn was inoculated with a water suspension of spores of the three Penicillia to give 2×10^3 spores/g and about 19% M.C. The corn was divided into three 120-g lots and placed in perforated open-top plastic containers that were randomly distributed on a screen support in a 17-L aquarium tank (2). Air was conditioned to 88% RH and bubbled through aquarium bubblers placed in a glycerol solution of R.I. of 1.3798 (1). The R.I. was checked weekly with a refractometer and adjusted when necessary. The corn was stored at 13–14 C for 19 days.

Effect of inoculum concentration. Eight genotypes were used in the first two tests. Corn was adjusted to 17% M.C. by addition of water and stored at 4 C for several days then brought to 18.5% M.C. with a spore suspension of the *Penicillium* spp. used in

the heat treatment tests. The inoculum was adjusted to 2×10^3, 5×10^3, and 10^4 spores/g. In a third test, seven genotypes were used and the inoculum was 2×10^3, 2×10^4, and 10^5 spores/g. Four replicates of 120 g each were used for all three tests and the corn stored at 88% RH and 13–14 C as described.

Statistical analysis was performed using the SAS program (SAS Institute, Cary, NC) for one-way analysis and the mean separation with SNK with $P = 0.05$. Linear correlations (Pearsons) were calculated using DataDesk Professional, a Macintosh statistical program (Odessa Corp).

RESULTS

Effect of heat treatment. The heat treatment (80 C, 20 min) killed all seeds and fungi, as determined by plating on PDTC. Sporulation of *Penicillium* was increased substantially by the heat treatment after storage (Fig. 1A and B). Propagules, although fairly well correlated with visible mold ($r = 0.84$ for 29 days and 0.67 for 49 days' storage), indicated a greater increase in sporulation than did visible mold as a result of the heat treatment. The resistant genotypes, B73 × Mo17 and DK XL67 that were heat treated, had 18–23 times the propagules, respectively, as compared to the unheated controls after 49 days. The magnitude of increase for propagules for the susceptible genotypes was much less, ranging from no significant increase for H95 to about five times for VF. However, the heated resistant genotypes had lower numbers of propagules at both sample times and visible mold at 29 days than the heated susceptible genotypes. H95 was always significantly different from the resistant genotypes for both criteria at both samplings. Importantly, the resistant heat-treated genotypes supported the same amount or less of *Penicillium* as judged by propagules and visible mold than the susceptible unheated genotypes.

Effect of inoculum concentration. Sporulation of *Penicillium* spp. was measured by visible mold and propagules increased slightly if at all with an inoculum increase from 2×10 to 10^4

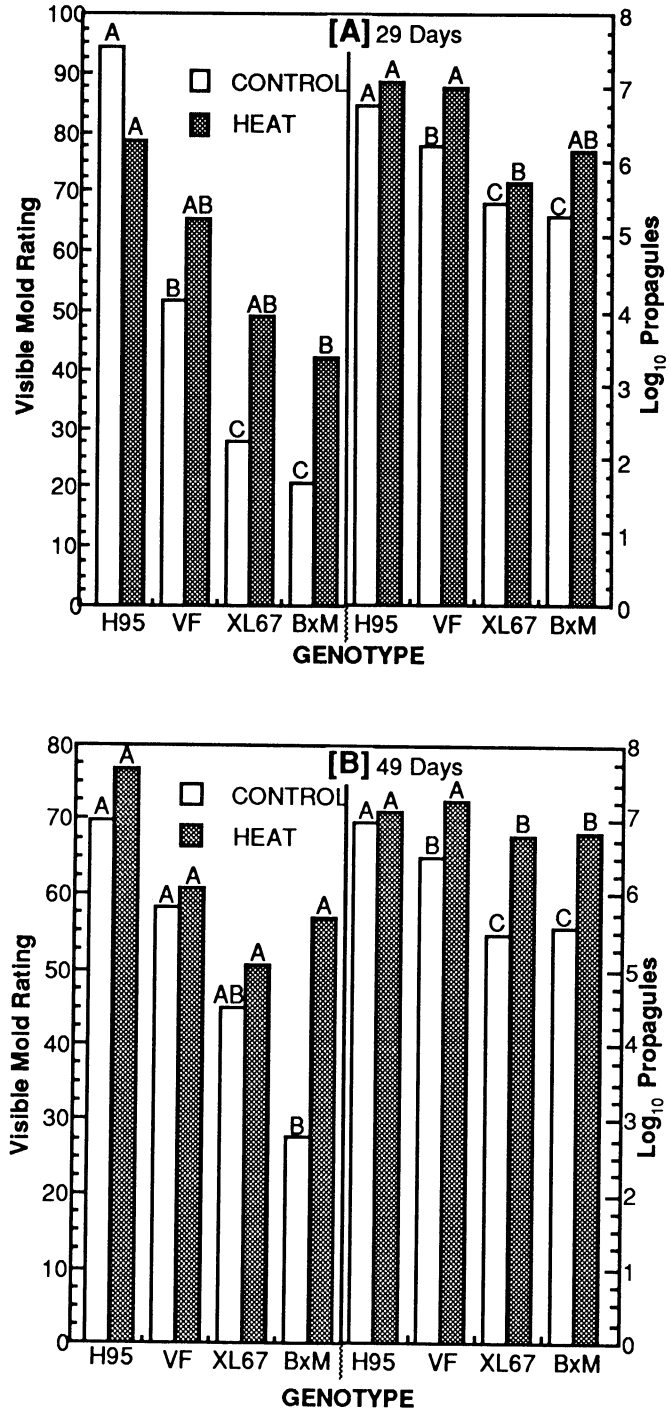

Fig. 1. Visible mold rating and number of propagules of four genotypes (H95, visual flint, Dekalb XL67, B73 × Mo17) of heated and unheated corn inoculated with *Penicillium* spp. and stored at 13–14 C and 88% RH for 29 and 49 days. Different letters indicate significant differences among genotypes at each inoculum concentration (SNK Test, $P = 0.05$).

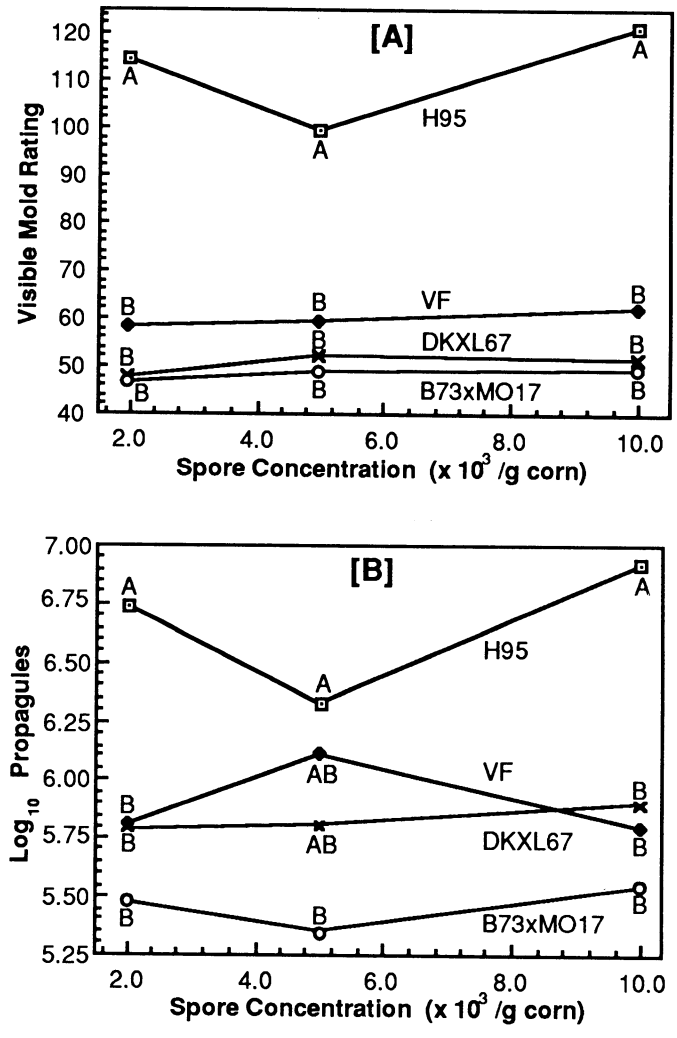

Fig. 2. A, Visible mold rating and, **B,** log number of propagules of four genotypes of corn inoculated with 2×10^3, 5×10^3, or 10^4 spores/g of *Penicillium* spp. and stored at 13–14 C and 88% RH for 28 days. Different letters indicate significant differences among genotypes at each inoculum concentration (SNK test, $P = 0.05$).

spores/g (Fig. 2A and B), and ANOVA indicated no significant effect of inoculum concentration as measured by either mold criterion. The two criteria were well correlated, $r = 0.84$. H95, the most susceptible genotype, had the highest visible mold and propagules at all inoculum concentrations. VF, which was a different selection from a later year than the VF used in the heat treatment test, reacted somewhat differently. It had a higher visible mold and propagules than the resistant hybrids but was not significantly different from them. All selections of VF have never been as susceptible as H95 in previous tests, but they were more susceptible than most hybrids (6). Although reactions appeared relatively stable in the range of 2×10^3 to 10^4 spores for the four genotypes, the need to use more genotypes was indicated.

Seven additional hybrids, and the highly susceptible inbred H95 and the resistant B73 × Mo17, were included to further evaluate the effect of the same three inoculum levels. The goal was to increase precision of visual mold by using more mold categories and a different weighted scoring.

There was an increase in visible mold but not propagules with increased inoculum (Fig. 3A and B), but ANOVA found no significant effect of inoculum concentration. Visual mold and propagules were highly correlated at all three inoculum levels, $r = 0.88$, 0.87, and 0.87 at 2×10^3, 5×10^3, and 10^4 spores/g, respectively. Also of interest was the finding that the hybrid DK T1100 was significantly more susceptible than the four other hybrids at all inoculum levels, and B73 × Mo17 continued to be highly resistant.

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TABLE 1. Analysis of variance of log number of *Penicillium* propagules from seven corn genotypes inoculated with *Penicillium* spp. of various concentrations and stored at 13–14 C and 88% RH for 4 wk

		Mean square (Genotype)						
Source	df	H95	DF20HTX12HT	P3707	LH74XLH123	B73XMo17	DK656	FR20X35
Treatment	2	0.005	0.123[a]	0.323	0.498	0.012	0.117*	0.154
Error	9	0.014	0.006	0.094	0.097	0.044	0.009	0.058

[a] * F significant at $P = 0.05$.

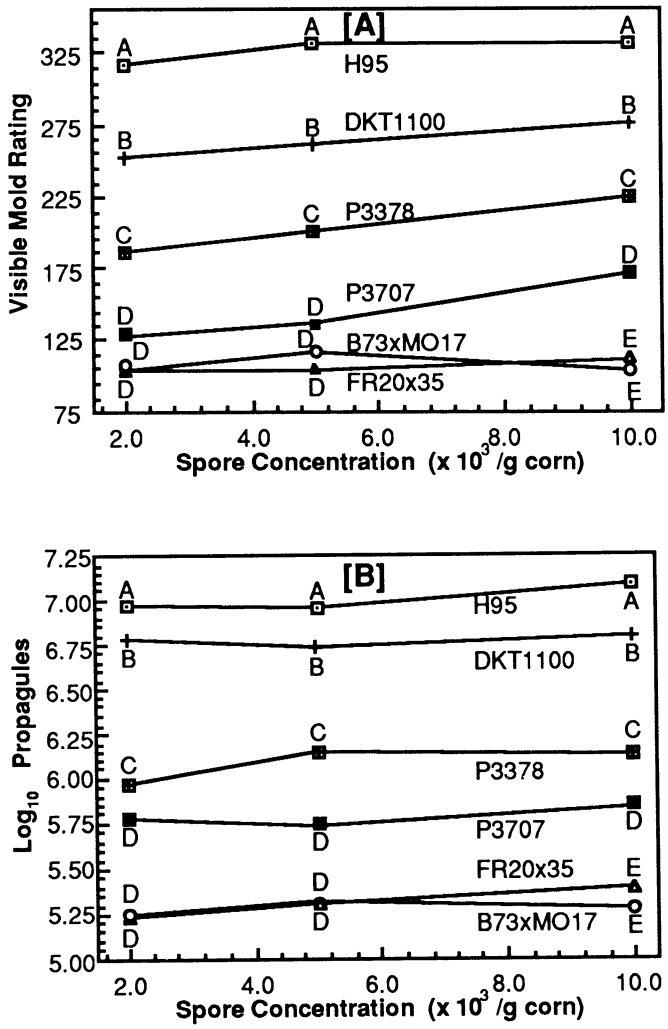

Fig. 3. A, Visible mold rating and, **B,** log number of propagules of six corn genotypes inoculated with 2×10^3, 5×10^3 or 10^4 spores/g of *Penicillium* spp. and stored at 13–14 C and 88% RH for 32 days. Different letters indicate significant differences among genotypes at each inoculum concentration (SNK test, $P = 0.05$).

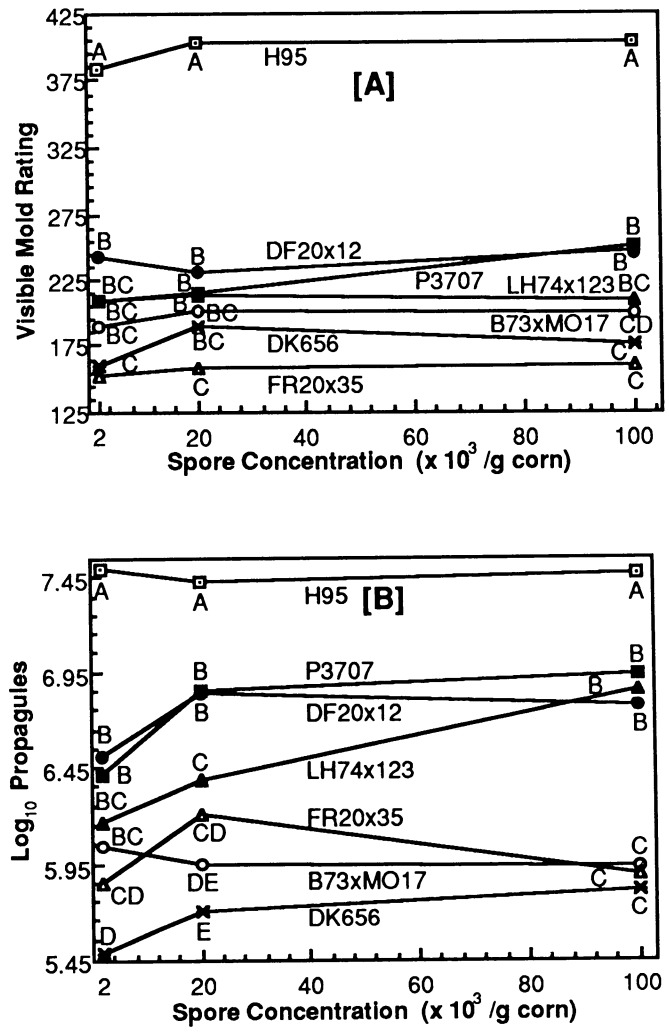

Fig. 4. A, Visible mold rating and, **B,** log number of propagules of seven genotypes of corn inoculated with 2×10^3, 2×10^4 or 10^5 spores/g of *Penicillium* spp. and stored at 13–14 C and 88% RH for 4 wk. Different letters indicate significant differences among genotypes at each inoculum concentration (SNK test, $P = 0.05$).

TABLE 2. Analysis of variance of log number of *Penicillium* propagules from seven corn genotypes inoculated with *Penicillium* spp. of various concentrations and stored at 13–14 C and 88% RH for 7 wk

Source	df	Mean square (Genotype)						
		H95	DF20HTX12HT	P3707	LH74XLH123	B73XMo17	DK656	FR20X35
Treatment	2	0.016	0.285	0.028	0.444[a]	0.046	0.316*	0.077
Error	9	0.006	0.064	0.018	0.018	0.044	0.022	0.040

[a] *F significant at $P = 0.05$.

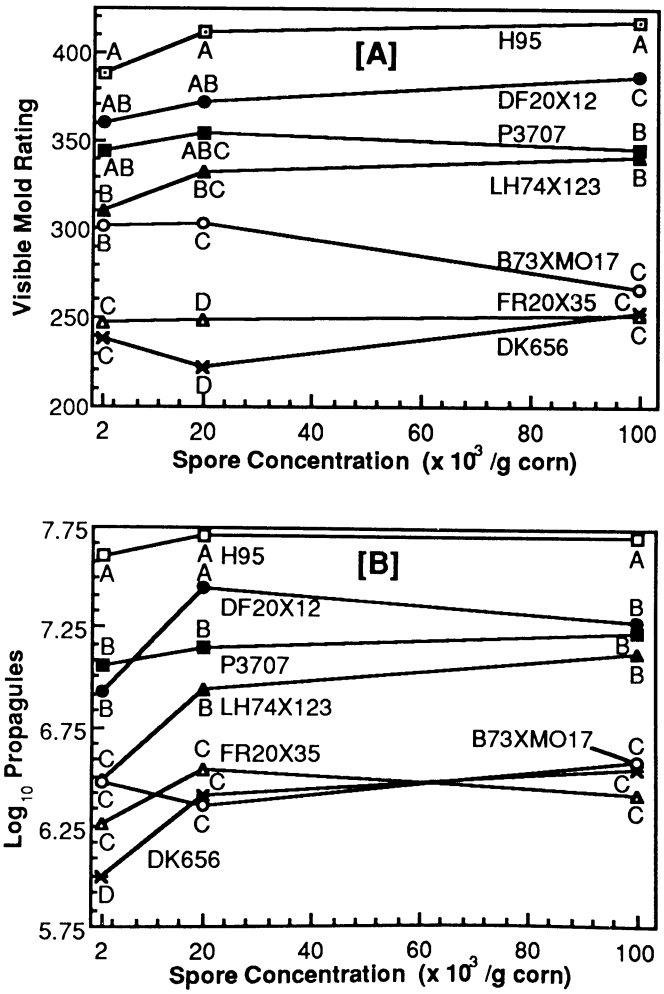

Fig. 5. A, Visible mold rating and, **B,** log number of propagules of seven genotypes of corn inoculated with 2×10^3, 2×10^4, or 10^5 spores/g of *Penicillium* spp. and stored at 13–14 C and 88% RH for 7 wk. Different letters indicate significant differences among genotypes at each inoculum concentration (SNK Test, $P = 0.05$).

were some substitutions in hybrids because of availability, and inoculum was increased to 2×10^4 and 10^5 to compare with 2×10^3 spores/g. The higher concentrations gave a consistent increase in propagules and visible mold for four of the seven hybrids (Figs. 4 and 5) at both sampling dates. ANOVA (Tables 1 and 2) indicated a statistical increase with increased inoculum for hybrids, DK 656, LH74 × LH123, and DF20HT × DF12HT (the latter only after 4 wk). Despite the statistical increase of propagules in DK 656, the propagules and visible mold of this hybrid were consistently low. The 10^5 inoculum had no effect on merging the more resistant hybrids with the intermediate or susceptible genotypes. In fact, 10^5 inoculum density more clearly distinguished the categories of resistant, intermediate, and susceptible than the two lesser amounts of inoculum and may be useful in further screening.

DISCUSSION

Drying corn at >60 C is undesirable because of increased susceptibility to breakage and loss in milling quality. High-temperature drying also changes the hygroscopic properties of corn, which results in a higher equilibrium relative humidity (5) that enhances mold growth. Also, our tests showed that susceptibility to molding is enhanced when seeds were heated at a temperature of 80 C for 20 min. The greater increase in molding of resistant genotypes, about 20 times in propagules, as compared with more modest increases in the susceptible genotypes appears inconsistent. However, the unheated susceptible genotypes already supported significant amounts of sporulation masking the effects of heating. In addition, the heated kernels of resistant genotypes were less moldy than the heated susceptibles and the heated resistant genotypes had statistically the same or less sporulation with one exception than the unheated susceptible genotypes. It seems likely that heat-induced susceptibility of the resistant genotypes is partly a result of death of the germs, although chemical components responsible for resistance may be heat labile. Results (6) with inoculated germs in intact kernels indicated resistance operates there in storage. Additional work is needed to explore the reasons for the deleterious effects of heating and to extend the study to commercial drying regimes and additional hybrids.

It also was encouraging to find that resistance to *Penicillium* was not appreciably affected by levels of 2×10^4 or 10^5 spores/g. Levels of 10^4 spores/g probably are rarely exceeded in farm storage and these or lesser amounts would be the common exposure. Blending clean corn with molded corn or inoculum coming from localized areas of molding in a grain bulk, is to be expected, however, and may have greater impact on resistance with more aggressive organisms such as *Aspergillus flavus*. Seitz et al (4) have shown inoculation with *A. flavus* can substantially increase its growth and aflatoxin production in bin storage. In any event, inoculum of 2×10^4 or 10^5 per gram of *Penicillium* may be desirable in screening tests as it more clearly distinguished intermediate genotypes from resistant ones in one test.

LITERATURE CITED

1. American Society for Testing Materials. 1959. Maintaining constant relative humidity by means of aqueous solutions. ASTM Standards 9:1947-1951.
2. Cantone, F. A., Tuite, J., Bauman, L. F., Stroshine, R. 1983. Genotypic differences in reaction of stored corn kernels to attack by selected *Aspergillus* and *Penicillium* spp. Phytopathology 73:1250-1255.
3. Moreno-Martinez, E., and Christensen, C. M. 1971. Differences among lines and varieties of maize in susceptibility to damage by storage fungi. Phytopathology 61:1498-1500.
4. Seitz, L. M., Sauer, D. B., Mohr, H. E., and Aldis, D. F. 1982. Fungal growth and dry matter during bin storage of high-moisture corn. Cereal Chem. 59:9-14.
5. Tuite, J., and Foster, G. H. 1963. Effect of artificial drying on the hygroscopic properties of corn. Cereal Chem. 40:630-637.
6. Tuite, J., Koh-Knox, C., Stroshine, R., Cantone, R. A., and Bauman, L. F. 1985. Effect of physical damage to corn kernels on the development of *Penicillium* species and *Aspergillus glaucus* in storage. Phytopathology 75:1137-1140.