Effect of Physical Damage to Corn Kernels on the Development of *Penicillium* species and *Aspergillus glaucus* in Storage

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ABSTRACT

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Three tests were used to evaluate the relationship between resistance to storage molds and damage to corn kernels of different genotypes. Seed germination, visible mold, number of fungal propagules, and ergosterol content were similar in undamaged and crown-damaged kernels inoculated with three species of the *Aspergillus glaucus* group. Cutting the pericarp over the embryo and, to a lesser extent, removing the pedicel significantly increased mold development and reduced germination, but the genotypes were affected differently. Kernels of two susceptible and two resistant genotypes inoculated with three *Penicillium* species maintained their differential responses whether undamaged or damaged with an abgerminal cut. Excising the pedicel, cutting the pericarp over the embryo, or puncturing the embryo, respectively, resulted in increasing amounts of propagule development by *Penicillium* and decreased seed germination. Despite the enhancement of mold development by seed damage, the resistant genotypes were more resistant than the susceptible genotypes. This differential response was confirmed in a second test with *Penicillium* spp. in which the pedicel was excised or the hilum was pierced. The rankings of stored ground kernels, as determined by numbers of propagules of *Penicillium*, differed from the rankings of stored whole kernels.

Additional key words: *Aspergillus amstelodami, A. repens, A. ruber, Penicillium brevi-compactum, P. cyclopium, P. viridicatum*, and *Zea mays*.

Results of previous studies (2,7) show differences in resistance to storage molds in dent corn as measured by mold growth and loss in seed germination. Therefore, genetic improvement of the storability of corn through improved resistance to storage molds appears promising but must eventually be evaluated with kernels that have been damaged by commercial harvesting and handling. The rate of deterioration is influenced by the extent and type of kernel damage (12,13). The purpose of these studies was to simulate some of the physical damage to corn kernels that we and others (1,3,5) have encountered in combine harvesting and to assess the effects of specific kinds of damage on mold development in genotypes with differing resistance. In testing the effects of different kinds of kernel damage we also hoped to identify specific barriers to fungal invasion and development.

MATERIALS AND METHODS

Test fungi. One isolate each of *Aspergillus amstelodami*, *A. repens*, and *A. ruber* (species of the *A. glaucus* group) were used for the first test. In the second and third tests *Penicillium brevi-compactum, P. cyclopium*, and *P. viridicatum* were used. Aqueous spore suspensions were applied by atomizer at a concentration that resulted in 1,000 spores per gram of kernels. Prior to the addition of the spore suspensions, calculated amounts of water were added which, combined with the water in the inoculum, gave a final moisture content of 16.5% for the test with *A. glaucus* and 19% for *Penicillium*. After addition of the inoculum and supplementary water, the kernels were allowed to equilibrate for several days at 4 C.

Corn genotypes. In the first test, we used three genotypes that differed to some degree in their resistance to storage molds. In the second and third tests, we selected four genotypes from a test of 19 corn belt genotypes that clearly differed in their reactions to storage molds. The two that were the most susceptible were H95 and VFA (Visual Flint A), the latter from a cross between Colombian flints and dent corn hybrids selected for visual flint characteristics by Paul Crane, and two that were the most resistant, B73 × Mo17 and Dekalb XL67. Corn seeds used in these tests were grown at the Purdue Agronomy Farm. Ears were hand harvested from plants grown from the seeds of these crosses or inbred, dried at 40 C to 10–12% moisture content (MC), hand shelled, and stored at 4 C prior to testing.

Seed damage treatments. The treatments for seeds exposed to *Aspergillus glaucus* were: undamaged, one corner of the crown cut with a razor blade to expose a portion of the floury endosperm, the tip cap (pedicel) removed with forceps, and the pericarp cut over the germ parallel to the germ axis without injury to the germ. The seed damage treatments in the first test with *Penicillium* were: undamaged, tip cap removed, pericarp cut on the nongerm (abgerminal) side parallel to the germ axis but not reaching the dent end or the tip cap, pericarp cut over the germ, and germ lightly punctured in the middle of the embryonic axis with a dissecting needle. The second test with *Penicillium* included: undamaged, tip cap removed, tip cap removed and the hilar layer pierced twice with a size 00 insect pin, and corn ground in a Quaker City Mill, Model 4E, adjusted to the finest setting.

Storage procedures. Samples of each treatment in triplicate were placed in perforated open plastic containers on screen racks in a closed 38-L aquarium tank. The containers were distributed randomly in the tank by using a list of random numbers and rerandomized after the first sampling. Air, conditioned to a relative humidity of 85% and 26 C for the test with *A. glaucus* and to 88% and 13–14 C for *Penicillium*, was passed through the tank as described by Cantone et al (2). The corn kernels were sampled twice for mold evaluations and seed germination; kernel moistures were determined once by the one-stage, whole-grain, air-oven method, at 103 C for 72 hr.

Evaluation of samples. Mold determinations were as follows: 50 kernels of each replicate were examined for visible sporulation.

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Sporulation was scored for each kernel, light = 1, moderate = 2, and heavy = 3 giving a weighted score. The numbers of propagules were determined by culturing dilutions of 20 g of kernels or 10 g of ground corn that were blended for 1 min in 0.1% water agar. Each dilution was added to two petri dishes and cooled molten agar was added. Malt salt agar containing 6% NaCl (MS) was used to isolate *A. glaucus* and potato-dextrose agar containing 30 ppm chlortetracycline and 200 ppm Tergitol NPX (Union Carbide) PDTC, was used for the isolation of *Penicillium*. Ergosterol was determined by the method of Seitz et al (11) for the test with *A. glaucus*. Germination of 50 surface-disinfested (1% NaClO for 1 min) kernels on moistened filter paper was determined after 7 days at 22–24.C. A one-way analysis of variance was calculated at $P = 0.05$ by using Duncan's multiple range test. Linear correlations (r) of the means were calculated at $P = 0.05$.

RESULTS

Tests with *Aspergillus glaucus*. The three genotypes used in this test were similar in their reactions to kernel damage and the development of *A. glaucus* (Table 1). Kernels of A632 cut over the germ had significantly less visible mold, numbers of propagules, and ergosterol than those of the other entries. This inbred, however, was more susceptible to visible mold development than hybrid B73 × Mo17 in the undamaged and crown damage treatments. Cutting the pericarp over the germ had the most adverse effect on all genotypes as measured by all criteria except for kernel infection. As shown previously (2), this technique does not readily distinguish between the reactions of genotypes to storage molds when stored at relative humidities above 85%, unless the grain is sampled early in the storage period. Numbers of propagules and visible mold were highly correlated ($r = 0.93$). Ergosterol, although correlated with numbers of propagules and visible mold ($r = 0.97$ and 0.96, respectively), did not reveal as many significant differences between treatments as did the other mold criteria. Removal of the tip cap accelerated molding and seed germination loss in two of the three genotypes but not to the extent caused by damage to the pericarp over the germ. Crown-damaged and undamaged kernels reacted similarly.

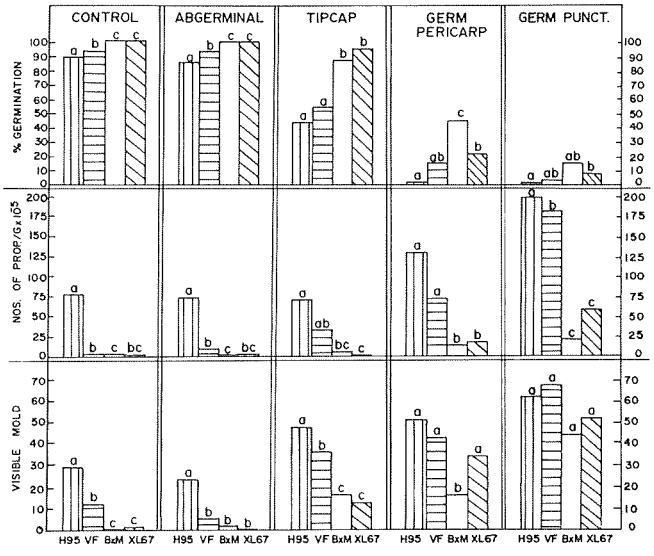

Fig. 1. First test with *Penicillium*. Visible mold and numbers of fungal propagules developed on, and germination of, seeds of four corn genotypes variously damaged and stored for 35 days at 13–14 C and 88% RH. Values within each treatment followed by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

First test with *Penicillium*. Visible mold ratings (Fig. 1) after 35 days of storage indicate significant differences between the two pairs of genotypes. H95 and VFA had more visible mold than B73 × Mo17 and XL67. An abgerminal cut had little or no effect on visible mold development compared to undamaged corn. Removal of the tip cap increased visible sporulation on seeds of three of the four genotypes as much as cutting the pericarp over the germ. The greatest amount of visible sporulation in all four genotypes was caused by puncturing the germ; for two genotypes, the difference was significant compared to those in which the pericarp had been cut over the germ. Numbers of propagules that developed were highly correlated ($r = 0.83$) with the visible mold estimates. Sporulation of the penicillia was restricted most by B73 × Mo17 and to a lesser degree by XL67 than by the susceptible genotypes. These differences were maintained in most of the seed damage treatments. An abgerminal cut had little effect on propagule development compared to that on undamaged seeds. Molding, whether determined visually or by dilution, was higher (but not always significantly different) when the tip cap was removed compared to that resulting from an abgerminal cut. Cutting the germ pericarp or puncturing the germ resulted in similar amounts of heavy sporulation.

The extensive molding that occurred on undamaged kernels of H95 appeared to be associated with a modest loss in seed germination (Fig. 1), but the controls of the other genotypes did not lose viability after 35 days. Damage to the abgerminal pericarp did not decrease seed germination but removal of the tip cap substantially reduced seed germination for H95 and VFA; B73 × Mo17 was affected to a smaller degree and there was no effect on XL67. Direct injury (germ puncture) or exposure of the germ by a cut in the pericarp substantially reduced germination of all four genotypes, the former to a greater degree. The resistant genotypes, B73 × Mo17 and XL67, suffered substantial but proportionally

TABLE 1. Visible mold, propagules of *Aspergillus glaucus*, ergosterol, kernel infection, and seed germination of seeds of three corn genotypes that had been damaged and stored at 85% RH and 26 C for 17 days

Genotype	(Damage)	Visible mold (%)	Numbers of prop./G × 10^4	Ergosterol (μg/g)	Kernel infection (%)	Seed germination (%)
Mo17 × H100	Undamaged	13.0 cd[a]	20.25 b	0.36 cd	93.0 b	99.0 a
	Crown damage	11.5 d	17.45 d	0.34 cd	93.0 b	97.0 a
	Tip cap removed	21.0 b	14.00 ab	0.32 cd	99.0 ab	82.0 b
	Germ pericarp cut	100.0 a	124.50 e	1.49 a	100.0 a	1.0 e
A632	Undamaged	12.6 ab	12.57 ab	0.30 cd	95.3 ab	97.3 a
	Crown damage	11.3 ab	11.30 ab	0.39 cd	96.0 ab	97.3 a
	Tip cap removed	18.2 b	18.17 b	0.36 cd	98.0 ab	75.3 c
	Germ pericarp cut	12.6 ab	76.47 c	1.08 b	99.3 ab	6.0 d
B73 × Mo17	Undamaged	0.3 e	3.73 a	0.22 d	82.7 c	98.0 a
	Crown damage	0.7 e	5.27 a	0.31 cd	86.0 c	100.0 a
	Tip cap removed	16.7 bc	16.33 b	0.49 c	96.7 ab	76.0 c
	Germ pericarp cut	97.7 a	103.67 d	1.57 a	98.0 ab	4.0 de

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

smaller losses in seed germination than the susceptible genotypes. Visible mold and numbers of propagules showed a high negative correlation with germination ($r = -0.86$ and -0.72, respectively).

After 51 days, differences in resistance of the two groups of genotypes to sporulation by *Penicillium* were maintained in the three less drastic damage treatments: undamaged kernel, abgerminal cut, and tip cap removal (Fig. 2). Cutting the pericarp over the germ or germ puncture enhanced sporulation compared to undamaged. This effect was particularly noticeable in the resistant genotypes. As a result, there was less difference in visible mold score between the genotypes in these treatments. Removal of the tip cap increased molding of all genotypes but did not obscure the relative ranks of the two groups.

Numbers of propagules of *Penicillium* spp. and the visible mold criterion differentiated the treatments similarly but the former distinguished the resistant from the susceptible genotype to a greater degree. Visible mold and numbers of propagules were highly correlated ($r = 0.89$). Propagule increase resulting from kernal damage treatments is shown in Table 2. The relatively larger increases for the resistant genotypes are explained by the fact that numbers of propagules on undamaged kernels of the resistant genotypes were low and those on the susceptibles were high.

Seed germination (Fig. 2) of the controls for the resistant genotypes remained relatively higher than that of the controls of the susceptible genotypes. Removal of the tip cap reduced seed germination but was not as adverse as cutting the pericarp over the germ or puncturing the germ. These damages alone were not a factor as damaged kernels germinated well at the start of the test. Visible mold and numbers of propagules were correlated negatively with seed germination ($r = -0.90$ and -0.82, respectively).

Second test with *Penicillium*. *Whole and damaged kernels.* Undamaged and damaged kernels of B73 × Mo17 and XL67 were more resistant to proliferation of *Penicillium* than were those of H95 or VFA, as measured by numbers of propagules and visible mold after 35 and 51 days (Figs. 3 and 4). H95, the most susceptible to molding, also suffered the greatest losses in seed germination with all treatments. Removal of the tip cap or piercing of the hilar layer caused a greater increase in molding in the resistant than in the susceptible group. The susceptible group, however, still supported the most molding. Removal of the tip cap greatly increased losses in seed germination in three genotypes but the seed germination of XL67 remained almost the same as in the undamaged control. Piercing the hilar layer caused the largest losses in seed germination, ranging from 60 to 85% after 51 days storage. Losses of undamaged kernels ranged from 5 to 25%, and 7 to 62% when the tip cap was removed.

Ground corn. We isolated significantly different numbers of propagules of *Penicillium* from the different genotypes that were ground and stored for 20 and 27 days (Table 3). Rankings, as determined by the number of colonies of *Penicillium* spp. isolated, were consistent for both samplings. The rankings of the ground samples, however, differed from the rankings of the intact-kernel counterparts that were run concurrently. For example, H95, consistently the most susceptible to molding in all kernel tests, produced the lowest number of propagules when ground, and XL67, the most resistant in kernel tests, was second in numbers of mold propagules produced when stored ground.

TABLE 2. The proportion of propagules of *Penicillium* in damaged compared to undamaged corn after 35 and 51 days at 88% RH and 13–14 C

	Abgerminal cut		Tip cap (removed)		Germ pericarp cut		Germ punctured	
Genotype	35	51	35	51	35	51	35	51
H95	1.0	4.0	1.0	5.5	1.8	7.1	2.7	30.2
VFA	3.0	2.2	9.4	4.4	22.9	21.1	57.0	40.1
B73 × Mo17	1.3	2.6	19.0	49.6	40.0	151.3	79.0	258.3
XL67	0.3	1.1	1.1	4.7	12.1	64.1	53.0	99.2

DISCUSSION

All three tests showed that certain kinds of damage to the corn kernel greatly increased susceptibility to molding in storage, but differences in resistance among genotypes were maintained to some degree depending on the type of damage and length of storage. Our results confirm earlier reports (9,12) that damage to the germ has the greatest effect on mold development. In addition, access to the germ by cutting the pericarp over the germ or removal of the tip cap enhanced molding, but not as much as a puncture of the germ in the embryonic axis or piercing the hilar layer into the scutellum. Removal of the tip cap exposes the interior face of the pericarp. According to Tsuruta et al (15) there is an opening between the germ and the pericarp which may be a common route of invasion by storage fungi. It is generally believed that the hilar layer is resistant to fungal penetration (4,6) which is supported by the dramatic loss in seed germination which we obtained by injuring the hilar layer. Removal of a portion of the crown or an abgerminal cut of the pericarp had a minor effect on mold sporulation. Severe injuries had a disproportionate effect on increase of molding of the resistant genotypes, but the difference between the two groups was usually maintained. Therefore, despite the effects of injuries to the pericarp over the germ and loss of the tip cap, development of hybrids resistant to storage molds holds promise in decreasing storage mold losses.

TABLE 3. Numbers of propagules of *Penicillium* in seeds of four genotypes of corn inoculated with *Penicillium* spp., ground, and stored at 88% RH for 20 and 27 days

	Propagules/g ($\times 10^{-3}$)	
Genotype	Day 20	Day 27
H95	2.14[y] a[z]	9.24 a
VFA	76.78 c	351.13 c
B73 × Mo17	3.86 a	18.72 a
XL67	12.25 b	69.67 b

[y] Average of four subsamples of three replicates each.
[z] Data transformed to log base 10 and analyzed by Duncan's multiple range test. Values followed by different letters are significantly different ($P = 0.05$).

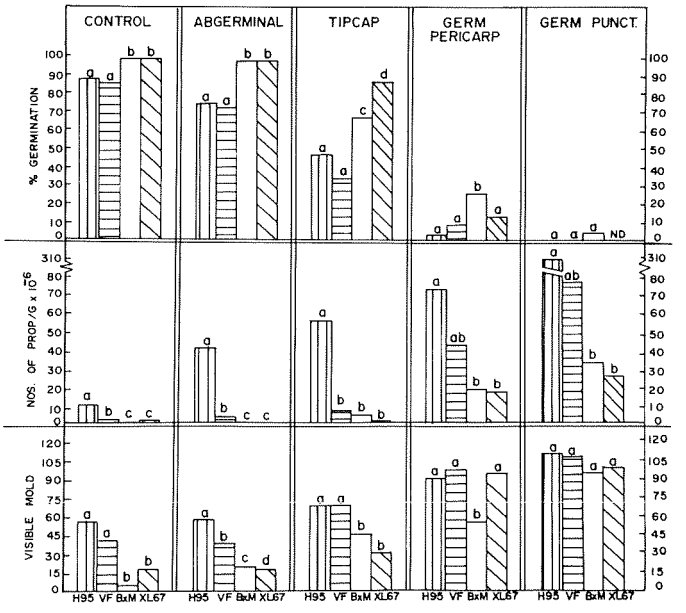

Fig. 2. First test with *Penicillium*. Visible mold and numbers of fungal propagules developed on, and germination of, seeds of four corn genotypes variously damaged and stored for 51 days at 13–14 C and 88% RH. Values within each treatment followed by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

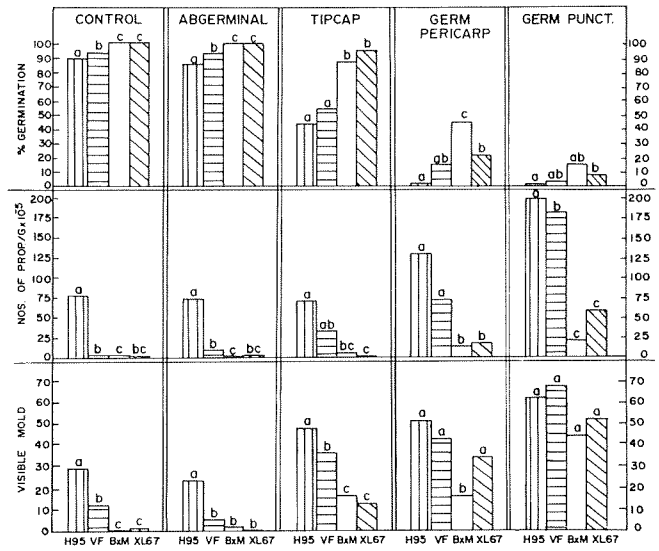

Fig. 3. Second test with *Penicillium*. Visible mold and numbers of fungal propagules developed on, and germination of, seeds of four corn genotypes variously damaged and stored for 35 days at 13–14 C and 88% RH. Values within each treatment followed by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

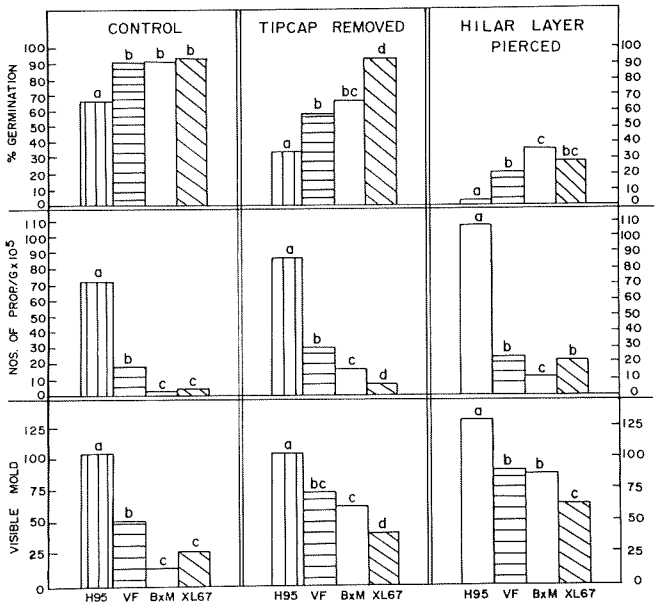

Fig. 4. Second test with *Penicillium*. Visible mold and numbers of fungal propagules developed on, and germination of, seeds of four corn genotypes variously damaged and stored for 51 days at 13–14 C and 88% RH. Values within each treatment followed by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

To be of use to farmers and the grain industry, however, improvement of storability must also include other criteria such as resistance to breakage and injury. Although a hybrid may be resistant to fungi in a laboratory storage test, it would still be undesirable for commercial storage if the kernel breaks up after artificial drying; if the pericarp, particularly near the germ, is readily broken during handling; or if the tip cap is readily detached during combine harvesting. Among the hybrids, there appears to be differences in susceptibility to combine-damage (10) and susceptibility to breakage after high temperature drying (14). Although grains with the flint character of the endosperm may be less susceptible to breakage, it did not per se confer resistance to storage molds in our tests. VFA consistently has been one of the more susceptible genotypes that we have tested. Resistance to fungi is likely to be more significant if grain is harvested at low moistures so that tip cap removal and severe kernel damage are less.

That various kinds of damage affected the genotypes differently suggests that resistance to storage mold attack and proliferation resides in various parts of the corn kernel, probably including the tip cap, germ, pericarp, and the hilar layer. Therefore, it seems that different genotypes have different kinds of resistance. For example, from the results of the undamaged kernels, it appears that the tip cap provides resistance for B73 × Mo17 and XL67, as little sporulation was seen on it. When the tip cap was removed, XL67 was less affected as measured by germination loss and mold proliferation than B73 × Mo17. This indicates that the hilar layer or, more likely, the interior of the pericarp and seed coat is more resistant in XL67 than in B73 × Mo17. In B73 × Mo17, the germ appears more resistant to *Penicillium* spp. than the germ of XL67 because the former decreased less in germination when the germ was exposed or penetrated. Evidence for resistance in these and other tissues is circumstantial and awaits confirmation. The proposal that resistance is located in diverse areas of the kernel is consistent with the finding that the results of storage tests with ground corn did not reflect storage behavior of whole kernels. It suggests that in vitro tests with ground corn or extracts of the kernel are unsuitable as screening tests for resistance of dent corn to storage molds. Whether or not these in vitro tests relate to inhibition of mycotoxin production in vivo (8) awaits verification.

LITERATURE CITED

1. Byg, D. M., and Hall, G. E. 1968. Corn losses and kernel damage in field shelling of corn. Trans. ASAE (Am. Soc. Agric. Eng.) 11:164-166.
2. Cantone, F. A., Tuite, J., Bauman, L. F., and Stroshine, R. 1983. Genotype differences in reaction of stored corn kernels to attack by selected *Aspergillus* and *Penicillium* spp. Phytopathology 73:1250-1255.
3. Chowdhury, M. H., and Buchele, W. F. 1978. The nature of corn kernel damage inflicted in the shelling crescent of grain combines. Trans. ASAE (Am. Soc. Agric. Eng.) 21:610-614.
4. Johann, H. 1935. Histology of the caryopsis of yellow dent corn, with reference to resistance and susceptibility to kernel rots. J. Agric. Res. 51:855-883.
5. Kline, G. L. 1973. Mechanical damage levels in shelled corn from farms. Paper 73-331. Am. Soc. Agric. Engineers, St. Joseph, MI.
6. Koehler, B. 1942. Natural mode of entrance of fungi into corn ears and some symptoms that indicate infection. J. Agric. Res. 64:421-442.
7. 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.
8. Nagarajan, V., and Bhat, R. V. 1972. Factor responsible for varietal differences in aflatoxin production in maize. J. Agric. Food Chem. 20:911-914.
9. Qasem, S. A., and Christensen, C. M. 1960. Influence of various factors on the deterioration of stored corn by fungi. Phytopathology 50:703-709.
10. Racop. E., Stroshine, R., Lien, R., and Notz, W. 1984. A comparison of three combines with respect to harvesting losses and grain damage. Paper 84-301B. Am. Soc. Agric. Engineers, St. Joseph, MI.
11. Seitz, L. M., Sauer, D. B., Burroughs, R., Mohr, H. E., and Hubbard, J. D. 1979. Ergosterol as a measure of fungal growth. Phytopathology 69:1202-1203.
12. Seitz, L. M., Sauer, D. B., and Mohr, H. E. 1982. Storage of high-moisture corn: Fungal growth and dry matter loss. Cereal Chem. 59:100-105.
13. Steele, J. L., Saul, R. A. and Hukill, W. Y. 1969. Deterioration of shelled corn as measured by carbon dioxide production. Trans. ASAE (Am. Soc. Agric. Eng.) 12:685-689.
14. Stroshine, R. L., Eman, A., Tuite, J., Cantone, F., Kirleis, A., Bauman, L. F., and Okos M. 1981. Comparison of drying rates and quality parameters for selected corn inbreds/hybrids. Paper 81-3529. Am. Soc. Agric. Engineers, St. Joseph, MI.
15. Tsuruta, O., Gohara, S., and Saito, M. 1981. Scanning electron microscopic observations of a fungal invasion of corn kernels. Trans. Mycol. Soc. Jpn. 22:121-126.