Growth of Fungi in Sorghum Grain Stored at High Moisture Contents

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ABSTRACT

Sorghum grain moistened to 18, 20, 22, and 24% moisture content (MC) was stored 7 weeks. At 25 C, the percentage of seeds yielding Fusarium increased at 20-24% MC; Penicillium grew at 18-24% MC; Trichothecium grew at 22-24% MC; but Alternaria decreased with increasing moisture content and time.

In sorghum harvested at 25% and 29% MC and stored 10 weeks at 15 and 25 C, Alternaria increased for a few days, then decreased. Fusarium grew more rapidly at 29% MC than at 25% MC. When the sorghum was stored at 15 C, other principal fungi were Verticillium, Scopulariopsis, and Trichothecium at 25% MC, and Verticillium, Mucor, and yeasts at

29% MC. At 25 C, the other principle fungi were *Penicillium* at 25% MC and *Aspergillus flavus* at 29% MC.

Viable, fungus-free sorghum grain was obtained from seed treated with captan, stored for about a year, and the fungicide washed off with 70% ethanol and water. Isolates of *Mucor*, *Alternaria*, *Aspergillus repens*, and *Fusarium moniliforme* were able to invade sorghum at 23% MC and 15 C, with *F. moniliforme* as the best competitor. Rate of invasion was similar in naturally infected and fungus-free viable grain. Phytopathology 61:767-772.

Studies of fungal deterioration in stored grain over the past 20 years have been concerned largely with fungi requiring minimums of 70-85% relative humidity (RH) for growth. Most grains when harvested are naturally infected with a variety of fungi. These "field" fungi generally require relative humidities in excess of 90% for growth, so they do not interfere with experiments on the more xerophytic "storage" fungi (4).

Factors affecting the growth of fungi in stored sorghum (Sorghum vulgare Pers.) have not been studied extensively. Lopez & Christensen (7) reported invasion by species of the Aspergillus glaucus group in sorghum stored with up to 15-16% moisture content (MC). Recently, Christensen (3) reported on fungi isolated from sorghum grain stored at 17.5% MC. Texas workers (8) demonstrated no change in either field or storage fungi in sorghum harvested at 18% MC and 30-32 C, provided the grain was cooled to 7 C within 7 days. There are no studies of the behavior of fungi in sorghum with moisture contents above 18%.

Sorghum seeds have a high natural infection of field fungi. Species of Alternaria often occur in 100% of seeds, and Fusarium, Chaetomium, Cladosporium, Curvularia, and Helminthosporium are frequently isolated (9). While those fungi usually are not a problem in stored sorghum, present farm practices, such as highmoisture harvesting and reconstituting grain for cattle feed, create conditions under which field fungi might grow and cause deterioration. Three genera of field fungi found in sorghum, Alternaria, Fusarium, and Cladosporium, are known to produce toxic metabolites in feedstuffs (4).

We studied growth of fungi in sorghum with moisture contents raised to 18, 20, 22, and 24% before storage and in sorghum harvested and stored at 25% and 29% MC. When we found that some fungi failed to

invade seeds, even though adequate moisture and inoculum were available, we reduced or eliminated the natural seed microflora, so that the growth potential of individual fungi could be observed.

MATERIALS AND METHODS.—Two lots of high-moisture sorghum were combine-harvested from a field of Pioneer 845 1 week apart in late September and early October. The first lot had 29% moisture, 65% germination, and numerous green or uncolored grains, and contained considerable dockage. The second lot had 25% MC, 73% germination, uniformly colored grains, and relatively little harvesting damage. To eliminate much of the leaf and stem debris and fine material, grain passing through a 12/64-inch round-holed sieve but not through a 0.064- × 0.375-inch sieve was used. Within 3 hr of harvest, three lots of approximately 5 lb. each were placed in plastic-bag-lined cans with three ½-inch holes in the lids. The cans were kept at an average room temperature of 25 C. A fourth lot was stored at 15 C.

Sorghum for studies with artificially moistened grain was taken from a 500-bushel wooden bin where it had been stored 7 months. Moisture content was 13.5% and germination 29%; no storage fungi were detected in surface-disinfected seeds. The moisture contents of four 500-g lots of sorghum, contained in sterile 2-qt mason jars, were adjusted to 18, 20, 22, and 24%. Four equal lots were autoclaved in mason jars for 1 hr at 15 psi on 2 successive days; then MC was raised. When free water was absorbed, jars were placed overnight at 15 C to equilibrate moisture. For each sample, 30-35 g of seed was aseptically dispensed into a sterile, screw-capped, 4-oz prescription bottle. Inoculations were made as required with spores and mycelium from fungi previously isolated from commercial sorghum. Bottles were stored with caps loosened in incubators at 15 and 25 C and above 70% RH. All samples were prepared in duplicate.

Attempts to eliminate fungi from seeds by washing in NaOCl, ethanol, and commercial disinfectants damaged the seeds and did not completely eliminate the fungi. Some lots of fungicide-treated seed which had been stored several months were found to be viable and free of fungi, and were readily invaded by fungi after the fungicide was removed. Sorghum (cultivar RS-610) from a single field, some with a captan-malathion treatment, the rest without, was used to compare mold growth in fungus-free and naturally infected sorghum grain. When received, moisture content was 8-9%; germination was 93% in the untreated and 88% in the treated seed. The untreated grain contained 33% Alternaria-invaded seeds; less than 1% were invaded by storage fungi. No fungi were detected in the treated grain after removing the fungicide by stirring the grain for 5 min in a volume of 70% ethanol equal to twice the volume of grain, followed by 5 min in running water, 5 min in 70% ethanol, and 5 min in running water. Two 500-g lots of fungicide-treated seed and two 500-g lots of naturally infected (untreated) seed were washed with alcohol and water as described, then washed in 2% NaOCl for 1 min and rinsed in sterile distilled water. The seeds were drained on sterile paper towels and put into sterile 2-qt mason jars. Two additional 500-g lots of untreated sorghum were autoclaved. The moisture content was adjusted so that one jar of each lot had 19% and the other 23% MC. Sorghum was dispensed into 4-oz bottles, inoculated, and stored at 15 C for 4 weeks.

Captan was applied as a slurry to untreated seed grade RS-610 sorghum to determine how quickly such treatment kills fungi in the seeds. Treatment rate was 1 oz/bu in grain of 15% MC and 3 oz/bu at 9% MC. The grain was stored in closed containers at room temperature and sampled at intervals. Captan was removed as described above before testing seeds.

Before removing grain for testing, the appearance of the grain was noted, and the containers were shaken to mix the grain. Fungi were enumerated by plating 25-50 seeds without surface-disinfection and 50-100 seeds disinfected by shaking for 1 min in 2% NaOCI and rinsing in sterile water. Seeds were incubated 5-10 days at room temperature on malt agar containing 4% NaCl and 200 ppm Tergitol (Union Carbide Corp., N.Y.). Fungi in the genus Aspergillus were identified to group species. Moisture content was determined by heating 10 g of whole sorghum at 130 C for 18 hr (5). Germinability was tested by placing 100-400 seeds on wet paper towels in aluminum foil folders and incubating at room temperature for 6 days. Any sprouted seed was considered germinated. Equilibrium relative humidities of sorghum grain at the moisture contents and temperatures used were estimated from values given in the Agricultural Engineers Yearbook (1).

RESULTS.—Fungi in sorghum harvested at 25% and 29% MC.—Alternaria, Cladosporium, and Fusarium were the three predominant genera found in surface-disinfected sorghum seeds immediately after harvest.

Of these three fungi, Fusarium was the only one to grow significantly during storage. Alternaria increased during the first few days, then decreased; Cladosporium gradually disappeared. Fungi most frequently isolated during storage at 15 C and room temperature are shown in Table 1. Also isolated from 5-10% of seeds at one or more sampling times were the following: Aspergillus niger, A. terreus, Circinella, Helminthosporium, and Rhizopus. Present, but less numerous, were A. candidus, A. clavatus, A. glaucus, Choanephora, Nigrospora, Phoma, Thermomyces, and Trichoderma. Few seeds were invaded by bacteria during the experimental storage period.

At 15 C and 25% MC, Fusarium, Trichothecium, and Verticillium were the dominant fungi (Table 1). At the end of 2 months, the grain was very moldy, but not solidly caked. Sorghum at 15 C and 29% MC was caked after 2 months; Fusarium, Mucor, Verticillium, and yeasts were the principal organisms involved.

At room temperature, Fusarium and Penicillium dominated in the 25% MC sorghum. At 29% MC, Fusarium and A. flavus dominated during the first 2-3 weeks, then declined. Schizophyllum, usually known as a wood-rotting fungus, grew at both 25% and 29% MC. The invasion by A. flavus in the grain stored at 29% MC and room temperature was rather irregular. Percentages of seeds invaded in the three replicate storages were 36, 42, and 40 after 8 days; 79, 5, and 13 after 3 weeks; and 2, 2, and 1 after 5 weeks. In each replicate, a period of rapid growth was followed by a sharp decline. The fluctuation in number of seeds yielding A. flavus did not occur at the same time in all replicates. At 3 weeks, two had declined considerably, and one had not. No other organism varied this widely among replicates at any sampling time.

Fungi in artificially moistened sorghum stored at 18-24% MC.—Before moisture adjustment, 94% of the seeds were internally infected with Alternaria, 10% with Cladosporium, and 4% with Fusarium; Penicilium, Mucor, and Rhizopus were present as surface contaminants. Fungi most frequently found in surface-disinfected seeds during storage are shown in Table 2. At one or more sampling intervals the following were also identified from surface-disinfected seeds: Aspergillus candidus, A. flavus, A. glaucus, A. nidulans, Cephalosporium, Circinella, Epicoccum, Helminthosporium, Mucor, Phoma, Rhizopus, Thermomyces, and Trichoderma. Bacterial colonies were noted occasionally.

When stored at 15 C, sorghum with 18% MC had only a small amount of visible fungal growth on seeds in 7 weeks. At 20% MC, the grain did not look moldy in 5 weeks, although *Penicillium* had invaded 16% of the kernels, but in 7 weeks the grain was visibly moldy. The sorghum stored at 22% MC appeared clean at 3 weeks, then rapidly deteriorated. Grain with 24% MC was in good condition at 12 days, but not at 3 weeks. *Penicillium* became the dominant organism at 20 and 22% MC; *Fusarium* and *Trichothecium* grew best at the highest moisture contents.

At 25 C, sorghum with 18% MC looked fairly good at 5 weeks, but was caked at 7 weeks (Table 2). Grain

25 C at 15 C and TABLE 1. Relation of moisture content and time to fungal populations in sorghum grain harvested at high moisture contents and stored

| | | | | | | | Percent | tages of su | Percentages of surface-disinfected seeds yielding | nfected se | eds yieldi | ng | | | | | |
|------------|--------|-------|-----------|------|---------|----------|---------|-------------|---|------------|------------|----------|------|----------|------|-------|------|
| | | | | Clan | Clado- | 1 | | ; | | Tricho- | ro- | | | £ | | Vonst | + |
| Moisture | Days | Alter | naria | cods | sporium | Fusarium | rinm | Mucor | 200 | thecium | um | A. Havus | 2113 | Fenculum | nun | 2 | 120 |
| content, % | stored | 15 C | 15 C 25 C | 15 C | 25 C | 15 C | 25 C | 15 C | 25 C | 15 C | 25 C | 15 C | 25 C | 15 C | 25 C | 15 C | 25 C |
| 25 | 0 | | 11 | | 30 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 11. | | 86 | | 33 | 15 | 22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| | 0 | | 93 | 20 | 26 | 30 | 43 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| | 36 | 10 | 20 | 9 | 1 | 64 | 39 | 0 | 0 | 63 | 0 | 0 | 0 | 0 | 94 | 0 | 0 |
| | 50a | | 2 | | 0 | | 55 | | 0 | | 0 | | 0 | | 23 | | 0 |
| | 64b | 2 | | 0 | | 94 | | 0 | | 33 | | 0 | | 0 | | 0 | |
| 20 | С | | 82 | | 22 | N | w | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| ì | , w | | 86 | 24 | 17 | 32 | 47 | 7 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 0 |
| | 6 | | 86 | | 9 | 58 | 81 | 2 | 0 | 0 | 0 | 0 | 39 | 0 | П | 0 | 0 |
| | 21 | | 0 | 4 | 0 | 87 | т | 0 | 0 | 0 | 0 | 0 | 32 | 0 | 0 | 0 | 8 |
| | 36c | | 0 | 0 | 0 | 16 | 8 | 1 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 10 | w |
| | 72d | | | 0 | | 38 | | 27 | | 0 | | 0 | | 0 | | 56 | |
| | | | | | | | | | | | | | | | | | |

a Also 12% Schizophyllum.
b Also 17% Scopulariopsis, 43% Verticillium.
c At 25 C, also 16% Schizophyllum.
d Also 39% Verticillium.

was moldy within 20 days at 20 and 22% MC and within 12 days at 24% MC. The same fungi predominated at both temperatures, although they developed at lower moisture contents and/or in less time at 25 C than at 15 C.

To study the potential of selected fungi to grow at high moisture contents, sorghum adjusted to 18, 20, 22, and 24% MC was inoculated with either Mucor sp., Rhizopus sp., Fusarium moniliforme, or F. tricinctum The inoculated fungi generally were unable to become established. Fusarium isolates invaded sorghum to some extent under most of the conditions tested, but populations then declined. Other fungi which were naturally present in the seeds grew and dominated over the fungi that were added artificially. Inoculum of the various isolates could be recovered from seed surfaces at least during the 1st week of storage, but internal infections could not be demonstrated.

Sterile sorghum was used to determine moisture and temperature requirements for development by several fungi without competition from natural seed microflora. Autoclaved sorghum with 18, 20, 22, and 24% MC was inoculated with isolates of each of the following: Alternaria, F. moniliforme, F. tricinctum, Mucor, Rhizopus, Alternaria plus F. moniliforme, or Alternaria plus F. tricinctum. Autoclaved grain was invaded more rapidly at any given moisture content than nonautoclaved grain; however, fungi did not grow at lower moisture contents in autoclaved sorghum than in natural sorghum.

In autoclaved samples stored at 15 C and 18% MC (88% RH), no fungi invaded in 50 days, although viable Mucor, Rhizopus, and Fusarium were detected on seed surfaces. At 15 C and 20% MC (91% RH), invasion was negligible after 12 days, but after 20 days F. moniliforme had invaded 88% of seeds, F. tricinctum 82%, Alternaria 24%, Rhizopus 8%, and Mucor 0%. The RH in the interstitial spaces of sorghum with MC over 20% at 15 C exceeds 91%, and invasion times were correspondingly shorter as the RH increased. At 22% MC, all fungi invaded the grain; F. moniliforme grew fastest and Mucor the slowest.

Sorghum at 25 C and 18% MC has an equilibrium RH of 90%. Under these conditions, Fusarium isolates established infection after 20 days, but Alternaria, Mucor, and Rhizopus did not infect the seeds. All isolates grew readily at 25 C when moisture contents were 20% and above (RH above 95%).

When Alternaria and F. tricinctum were inoculated together, the highest percentage of autoclaved sorghum invaded by Alternaria was 32 at 25 C and 22% MC after 8 days. Fusarium tricinctum invaded 88% of the same seeds. Alternaria failed to grow well in competition with F. tricinctum, even at moisture contents which allowed Alternaria to grow rapidly when inoculated alone. In competition with F. moniliforme in autoclaved sorghum, Alternaria did not establish infection under any conditions tested.

Invasion of fungus-free and naturally infected sorghum.—When sorghum was treated with captan in the laboratory, the internal fungi gradually died. Infection was reduced from an initial 45% to 5-12% in 3 months.

TABLE 2. Relation of moisture content and time to fungal populations in artificially moistened sorghum grain stored at 15 and 25 C

| | | | | Percer | tages of s | urface-dis | infected se | eds yieldi | ng | | |
|------------|---------------------------|-------------------------------|-------------------------|--------------------------|--------------------|---------------------------|----------------------|-------------------------|--------------------|-------------------------|--------------------|
| Moisture | Days | Alte | rnaria | Clados | porium | Fuse | arium | Penic | illium | Tricho | thecium |
| content, % | stored | 15 C | 25 C | 15 C | 25 C | 15 C | 25 C | 15 C | 25 C | 15 C | 25 C |
| 13 | 0 | 94a | 94 | 10 | 10 | 4 | 4 | 0 | 0 | 0 | 0 |
| 18 | 4 12 20 35 49 | 88 100 92 100 100 | 100 96 96 100 | 20 0 12 4 28 | 12 12 0 0 | 32 24 8 8 | 16 16 0 4 | 0 0 0 0 | 0 0 8 4 | 0 0 0 0 | 0 0 0 |
| 20 | 4 12 20 35 49 | 92 92 92 100 100 | 100 100 64 100 | 12 12 12 4 4 | 24 8 4 0 | 16 24 0 4 12 | 36 24 24 36 | 0 4 0 16 36 | 0 0 16 22 | 0 0 0 0 | 0 0 0 |
| 22 | 4 12 20 35 49 | 96 92 100 100 | 100 100 56 72 | 20 8 4 0 4 | 0 0 4 0 | 20 12 4 16 24 | 16 32 60 40 | 0 0 0 32 40 | 0 4 8 16 | 0 0 0 12 | 0 0 12 44 |
| 24 | 4 12 20 35 49 | 100 100 96 44 80 | 100 96 56 12 | 24 4 16 0 0 | 4 8 2 0 | 16 8 0 12 32 | 24 64 80 72 | 0 0 0 4 0 | 0 0 4 0 | 0 0 0 88 44 | 0 0 16 0 |

a Nonsurface-disinfected seeds also contaminated with Penicillium (11%), Mucor (2%), and Rhizopus (2%).

After 15 months, no fungi were detected in the seeds treated with 3 oz of captan/bu; 2% of seeds were still infected in the lot treated with 1 oz/bu.

Table 3 shows results of inoculating 3 lots of 23% MC sorghum with Alternaria, Mucor, F. moniliforme, and A. repens (member of A. glaucus group) individually. Duplicate sample bottles were similar with respect to the percentage of inoculated seeds invaded by these fungi, but there were differences between pairs in natural infection by Alternaria, Fusarium, and A. glaucus. The percentages given for nonsurface-disinfected seeds contaminated by fungi indicate survival of the inoculum. Fungus-free and autoclaved sorghum which was not inoculated remained free of fungi throughout the experiment.

When already present as a natural infection, Alternaria continued growth and invasion in grain at 23% MC and 15 C. The fungus invaded 80% of the autoclaved sorghum in 2 weeks, but required 4 weeks to initiate invasion in viable, fungus-free seeds. Invasion of autoclaved sorghum by Mucor in 2 weeks was equal to that of Alternaria, but only a few Mucor-invaded seeds were sticking together, while Alternaria invasion produced moldy grain that was becoming caked. Mucor had invaded only 4% of nonautoclaved seeds in 4 weeks. During 4 weeks of storage, F. moniliforme invaded 96-98% of nonautoclaved sorghum with 23% MC. In the first 3 weeks, invasion of fungus-free samples was negligible, although seeds were loosely clumped. The naturally infected sorghum samples had similar clumping of the seeds, and 76% were invaded. Although the rapid development of A. repens in autoclaved sorghum indicates that growth conditions at 23% MC and 15 C are favorable, A. repens invaded only 60-64% of nonautoclaved sorghum in 4 weeks.

Dual inoculations of F. moniliforme with Alternaria. Mucor, or A. repens were made to observe competition of two organisms in autoclaved and fungus-free sorghum seeds and to observe the effect of established Alternaria infection on invasion by other fungi. Fusarium moniliforme invaded more autoclaved and fungusfree sorghum at 23% MC and 15 C in 1 month than did A. repens, but when the two fungi were inoculated into grain with a natural Alternaria infection, F. moniliforme did not appear to grow as well as A. repens (Table 4). Mucor invaded a high percentage of autoclaved seeds as a single infection (Table 3), but the maximum infection when inoculated along with F. moniliforme under the same conditions was 16%. When Alternaria and F. moniliforme were inoculated into autoclaved sorghum at 23% MC, rapid growth of F. moniliforme during the 1st week prevented detection of Alternaria. However, in fungus-free seeds, F. moniliforme invasion was slower than in autoclaved seeds, and Alternaria was found in a few seeds after 3 weeks. Fusarium moniliforme became the predominant organism when inoculated into sorghum with 33% Alternaria infection, although Alternaria invasion increased to 60% during the first 3 weeks of storage. After 4 weeks, the percentages of seeds infected with F. moniliforme and Alternaria were 98 and 22, respectively.

At 19% MC and 15 C with dual and single fungus inoculations, the only significant invasion in 4 weeks was by A. repens in autoclaved grain. Viable surface inoculum of A. repens and Mucor was detected throughout the storage period on autoclaved, naturally infected and fungus-free grain. Fusarium was present on autoclaved grain throughout the period, but was infrequent on naturally infected and fungus-free seeds. Alternaria was present on all the autoclaved grain, and was infre-

quent on the fungus-free grain. Surface contamination and internal infection by *Alternaria* in naturally infected grain did not change during 4 weeks.

DISCUSSION.—The length of time sorghum can be safely stored at moisture contents above 18% could not be determined from available literature. Experiments with freshly harvested corn (10) showed that at 24 C, corn can be safely stored for only 4.5 days at 25% MC and 12 days at 20% MC. At 15 C, the safe storage

periods were 9.6 days at 25% MC and 27 days at 20% MC. Our data and observations indicate that safe storage periods for sorghum are similar to those for corn at 24 C, but a little shorter at 15 C.

The succession of fungi in high-moisture grain was marked, particularly at 25 C. Under such conditions, a toxin-producing fungus could grow and produce its toxin, then die. If the grain were then tested for known toxin-producing fungi, the fungus might be absent or

TABLE 3. Growth of four selected fungi in sorghum grain following inoculation and storage at 23% moisture content and 15 C

| | | | Percent | ages of seeds yie | lding inoculated | d fungus | |
|-------------------------|----------|----------|--------------|-------------------|------------------|-----------------|--------------------------------|
| | Days | Autoclas | ved seeds | Fungus- | free seeds | Naturall see | y infected eds ^a |
| Fungi | stored | NSDb | SDc | NSD | SD | NSD | SD |
| Alternaria | | | | | ' | | |
| | 3 | 36 | | 12 | | 52 | |
| | 3 7 | 96 | 4 | 12 8 | | 56 | |
| | | 205000 | 80 | 12 | 0 | 100 | 48 |
| | 14 21 | | 100 | 36 | 0 | | 52d |
| | 28 | | | 96 | 0 4 | | 76d |
| Mucor | | | | | | | |
| TH. 11.5.5.1. | 3 | 40 | | 20 | | 60 | |
| | 3 7 | 100 | 20 | 48 | | 76 | 0 |
| | 14 | 100 | 88 | 84 | 0 | 72 | 0 |
| | 14 21 | | 96 | 84 | 0 | | 0 |
| | 28 | 100 | 90 | | 0 0 4 | 80 | 4 |
| Fusarium moniliforme | | | | | | | |
| | 3 | 96 | | 4 | | 8 | |
| | 3 7 | 100 | 96 | 0 | | 32 | 0 |
| | 14 21 | | 100 | 0 8 | 0 | 84 | 4 |
| | 21 | | | 100 | 16 | | 76 98 |
| | 28 | | | | 96 | | 98 |
| Aspergillus repens | | | | | | | |
| repens | 3 | 100 | | 8 | | 76 | |
| | 3 7 | 100 | 96 | 16 | | 52 | 0 |
| | 14 | | 100 | 72 | 0 | 100 | 0 52 52 |
| | 21 | | 175.77.57.57 | 100 | 20 | 70.70 M | 52 |
| | 28 | | | 22.2 | 60 | | 64 |

a 33% Alternaria, <1%, A. glaucus, and <1% Fusarium before storage; no additional inoculation of Alternaria.

TABLE 4. Growth of Fusarium moniliforme (Fus.) and Aspergillus repens (Asp.) inoculated together in sorghum grain at 19 and 23% moisture content and 15 C

| | | Percentages of surface-disinfected seeds yielding fungi | | | | | | | | | | |
|----------------|---------|---|-------------|-------------|------|-------------------|----------|--|--|--|--|--|
| D | Autocla | ved seeds | Fungus | -free seeds | Nat | urally infected s | eedsa | | | | | |
| Days stored | Fus. | Asp. | Fus. | Asp. | Fus. | Asp. | Alt. | | | | | |
| | | | 19% moistur | e content | | | | | | | | |
| 7 | 0 | 20 | | | | | | | | | | |
| 14 | 0 | 84 | 0 | 0 | 0 | 0 | 32 | | | | | |
| 21 | 0 | 82 | 0 | 0 | 0 | 0 | 26 | | | | | |
| 28 | 0 | 100 | 0 | 0 | 0 | 4 | 26 40 | | | | | |
| | | | 23% moistur | e content | | | | | | | | |
| 7 | 24 | 80 | | | | | | | | | | |
| 14 | 60 | 68 | 0 | 0 | 4 | 60 | 44 | | | | | |
| 21 | 92 | 60 | 56 | 8 | 4 | 56 | 28 | | | | | |
| 28 | 100 | 32 | 92 | 12 | 18 | 54 | 32 | | | | | |

a 33% Alternaria (Alt.), < 1% A. glaucus, and < 1% Fusarium before storage; no additional inoculation of Alternaria.

b NSD = Not surface-disinfected before plating.

^c SD = Surface-disinfected before plating.

d 20% A. glaucus.

nearly so, while its toxin might still be present. Christensen & Kaufman (4) indicated that this had occurred with Fusarium. Also, a material need not be overgrown by a fungus to contain measurable amounts of toxin. Some strains of A. flavus, for example, can produce aflatoxin when moisture and temperature are high enough to allow vigorous growth for only a few days.

Invasion by fungi into high-moisture-harvested sorghum appears to be similar to invasion of artificially moistened sorghum. Trichothecium, for example, was detected after the same storage time in artificially moistened and naturally moist grain.

Several problems interfere with studies on the abilities of certain species of fungi to grow in high moisture grain. We encountered considerable difficulty in trying to artificially establish a particular fungus in grain which already contained fungi. The existing fungi appeared to exclude or have a competitive advantage over the inoculated fungi. Christensen (2) observed a similar situation in which grain infected with Aspergillus glaucus was not invaded by A. ochraceus even though adequate moisture was present. When a fungus is present in nearly 100% of the seeds before storage, as is often the case with Alternaria in sorghum, measurement of its growth in storage is difficult. Autoclaving the grain kills the existing flora and permits study of a single fungus without competition. However, such grain is altered chemically and physically, and is not comparable to live seeds. Fungal invasion was much more rapid in autoclaved sorghum than in viable sorghum (Tables 3, 4).

The fungicide-treated seed which we used in this study seems to offer a reasonable compromise between naturally infected grain and autoclaved grain. Growth of fungi was similar in fungus-free and normal grain, but the fungus-free grain allowed study of the growth of individual fungi or of selected combinations of fungi at different temperatures and moisture contents. Relative humidity or moisture requirements for the fungal isolates tested were lowest for A. repens, followed in order by the Fusarium species, Alternaria, Rhizopus, and Mucor.

When high moisture sorghum was inoculated with various combinations of fungi, Fusarium moniliforme was the best competitor. The apparent ability of Fusarium to dominate over other fungi has been reported in other stored seeds. In a study of interaction of fungi in peanuts, Welty & Cooper (11) reported that as Fusarium increased at moistures above 20%, A. repens, A. flavus, A. ruber, and Penicillium decreased. Koehler (6) found that after 3 months of storage, F. moniliforme, as an internal infection or when inoculated, competed well in corn above 23% MC and frequently predominated over Aspergillus and Penicillium.

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