GROWTH OF FUNGI IN SORGHUM GRAIN STORED AT MOISTURE CONTENTS OF 18-21%

by 1264

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INTRODUCTION

"Grain sorghums give trouble in storage for two primary reasons: (1) they contain too much moisture when threshed in the fall to keep safely in storage, and (2) they are commonly stored in unventilated bins which do not permit them to dry out." This statement is as true in 1969 as it was 30 years ago when made by F. C. Fenton of Kansas State University (9). Improved technology has compounded the problems even though satisfactory methods for drying freshly harvested sorghum grain have been developed.

Farmers have tended to harvest sorghum at higher moistures because combining losses are lower and early harvesting gets the crop out when prospects for favorable weather are best (4). Producers rely on post-harvest artificial drying, drying with unheated air, cooling, or chemical fungicides to put the grain in good storage condition. The result is often large quantities of warm, moist grain waiting for treatment. This grain is vunerable to rapid mold invasion and the accompanying mustiness, heating, and loss of quality.

Growth conditions divide the fungi infecting grains into two distinct groups -- storage fungi requiring as little as 70% RH for development and field fungi which do not grow below 90% RH (7). Sorghum, like other grains, is hygroscopic and tends to reach and maintain a moisture content in equilibrium with the surrounding air. The relative humidity of the air in the interstitial spaces varies with the temperature (9). No investigators have determined equilibrium moisture curves for sorghum above 20% moisture, and there are no comparisons of equilibrium moisture curves for different kinds of sorghums or for mature and immature sorghum seeds. It seems likely these would differ from the curves determined for Blackhull kafir by Fenton in 1939 and still given in the Agricultural Engineers Yearbook (2).

Reducing moisture content to the safe storage level of 12-13%, lowering temperature to about 40 F, or a combination of lower moisture and temperature produces conditions unfavorable, or at least less favorable, for mold growth. The methods devised for post-harvest handling of shelled corn are based on this premise. Studies with corn have established the maximum safe holding times at stated moisture contents and temperatures (24), and these guidelines are applicable to sorghum with certain qualifications. The sorghum plant remains green until killed by frost; so moisture is available for developing seeds up to harvest time. The same head may have fully developed seed and immature seed at harvest. Seeds of considerably different moisture may be in each bulk, and seeds of the highest moisture content must be considered when determining safe holding periods.

There are no reported investigations of safe storage periods for sorghum with over 18% moisture content although sorghum is harvested in that moisture range in some years. In one reported study from Texas, Person et al (18) determined the maximum cooling time without fungus damage from harvesting temperatures of 85-95 F for sorghum of 16-18% moisture content is 7 days; for moisture content of 14-16%, 20 days.

Aeration has become an established practice for maintaining quality of sorghum in commercial facilities after it has been dried to a safe moisture content (15), and is being more widely used in on-farm storage. Unheated air is effective in cooling freshly harvested grain and for maintaining uniform temperature and moisture in a grain bulk. Such ventilation will lower the moisture content, but drying is quite slow. Depending on the weather, drying often requires several weeks, and grain may deteriorate before a safe moisture content is reached.

Sorghum seeds have a high infestation of field fungi. The predominant genera found are Alternaria, Chaetomium, Cladosporium, Curvularia, Fusarium, and Helminthosporium, with Alternaria by far the most common followed by Fusarium (13, 21). Alternaria often occurs in 100% of seeds as it does in wheat and barley (6). The seeds become infected prior to harvest probably just before seeds reach maturity. Entry by two routes has been proposed: via the stalk and seed attachment from a systemic infection of the plant or by penetration of the immature caryopsis by mycelium from spores deposited on the seeds (11). It is generally accepted that the loss in grain quality due to field fungi occurs before harvest or at least before the grain is dried below 20-22% moisture content. Field fungi may live for years in dry grain, but die fairly rapidly at moisture contents in equilibrium with relative humidities of about 70% and above. This is about 11% moisture in sorghum. Alternaria counts have been used as an assay to judge whether grain is going out of condition, since a decreased percentage of invasion indicates grain has been stored above a safe moisture content.

In addition to reducing the nutritive quality, lowering germination, altering flavor, and affecting processing quality, fungi growing in stored grains may produce mycotoxins injurious to man and domestic animals. Two genera of field fungi commonly found in sorghum, Alternaria and Fusarium, are known to produce toxic metabolites in feedstuffs. The conditions of toxin production by these fungi under natural conditions are not clearly defined. Isolates of fungi from feedstuffs suspected of containing toxins are grown on sterile substrates and then assayed by feeding to test animals (6).

Fusarium species are known to produce a toxin lethal to man (F. tricinctum), a toxin causing liver lesions (F. roseum), a toxin, F-2, producing an estrogenic syndrome in swine (F. roseum) and toxins lethal to rats and turkey poults (F. roseum and F. moniliforme). A period of low temperature is required for toxin production by F. tricinctum and for F-2 production by F. roseum under laboratory conditions, but low temperature may not be required by all Fusarium species which produce toxins (6). Stored sorghum in the Midwest is subjected to periods of low temperature as well as moderate to high temperature.

Screening tests for toxic fungi in suspected corn, feeds, and food conducted at the University of Minnesota showed 88% of 60 isolates of Alternaria were toxic to rats, 75% of 87 Fusarium isolates were lethal to rats and turkey poults, and 46% of 41 Cladosporium isolates produced lethal toxins (6). The extent of mycotoxin-caused syndromes in poultry and domestic animals cannot be estimated with present knowledge.

Although the relationships of moisture content, temperature, and time in storage to invasion by storage fungi has not been as extensively studied in sorghum grain as in wheat and corn, Lopez and Christensen have reported on the invasion of sub-species of the Aspergillus glaucus group in sorghum stored with moisture content up to 15-16% (14). Texas workers demonstrated no change in either field or storage fungi after more than 6 months when sorghum averaging 18% moisture content at harvest was stored in bins and aerated with "conditioned" air at 45 F. The relative humidity was held at 73% for 3 months and then lowered to 65%. Some drying occurred and final moisture content was 15.6% (18).

The behavior of fungi in sorghum at over 20% moisture content has not been studied. It is of importance because of the need to define safe periods for holding freshly harvested sorghum grain to preserve quality before drying and cooling. High moisture conditions may occur at later times in sorghum storage. Spring temperatures produce an increase in relative humidity in sorghum stored at above 11% moisture content and maintained satisfactorily through cold weather. Moisture shifts, leaking bins, and the practice of reconstituting sorghum for cattle feeding (17) may also provide conditions favorable for growth of fungi.

This paper presents a study of growth of selected fungi in sorghum of 18-21% moisture content under controlled conditions. Particular emphasis is placed on field fungi and their individual performances alone and in combination.

MATERIALS AND METHODS

Sorghum. A sample of farm-stored sorghum was used to observe the effects of high moisture and time in storage on growth of field fungi. The grain was taken from a wooden storage containing approximately 500 bushels of sorghum. Moisture content was 13.5%, germination 49%. The grain had been stored about 7 months, and no storage fungi were detected in surface disinfected seeds.

RS610 sorghum from the 1967 crop grown in Nebraska for seed was used in experiments on fungus invasion. Moisture content was 8-9%, germination was 97-99%; less than 1% of seeds were originally invaded by storage fungi.

Preparation of samples for storage. Treatments used prior to moisture adjustment are given for each experiment. The moisture content of 500 g lots of sorghum contained in sterile 2 qt mason jars was adjusted by addition of sterile distilled water. Jars were kept at room temperature and shaken frequently during the next several hours to aid water uptake. When free water was absorbed, jars were placed overnight at 15 C to equilibrate moisture. For each sample, 30-35 g of seed were aseptically dispensed in a Microvoid sterile air hood into sterile, screw-capped, 4 oz prescription bottles. After inoculation bottles were stored with caps loosened in incubators at 15 or 25 C and relative humidity of not less than 95%.

Incubators satisfactorily maintained the initial moisture content. Fenton (9) showed that as moisture content increases to 20%, the vapor pressure of sorghum approaches that of saturated water vapor at that temperature, and further increases in moisture content of grain beyond 20% have relatively little effect on vapor pressure. At 60 F the vapor pressure at 100% relative humidity is 25.5 p.s.i. and that of sorghum with 18% moisture content is 24 p.s.i. At 77 F, saturated water vapor has a vapor pressure of 46 p.s.i. and sorghum with 18% moisture content 43 p.s.i. Grain gains or loses moisture because of differences between vapor pressure of the grain and the surrounding air, and small differences allow little change in moisture content.

Moisture contents. The two-stage, air-oven method specified by the U. S. Department of Agriculture (23) was compared with that of Hart *et al* (10) for sorghum employing a 10 g sample heated at 130 C for 18 hrs. Replication was good by either method, usually within 0.1%. Variation was always within the 0.2% limit specified by the U.S.D.A.

Agreement of moisture content values determined by the 2 methods was within 0.1% for samples of low and high moisture contents with the one-stage method giving the higher figure. Because of inherent sources of error and time consuming factors in the 2-stage determination, the 18 hr method was considered superior and was used for most tests.

Germination. One hundred to 400 seeds were incubated at room temperature on moist paper towels enclosed in sealed aluminum foil folders. Counts were made at 3 and 6 days. Any sprouted seed was considered germinated.

Fungi used for inoculum. Isolations of commonly encountered fungi *Aspergillus repens*, *Alternaria*, *Fusarium*, *Mucor*, and *Rhizopus* were made from sorghum samples. Cultures were maintained on potato dextrose agar and malt agar with 1% sodium chloride. Storage samples were inoculated with mycelium and spores transferred with a bacteriological needle. Bottles were shaken thoroughly.

Mold index. At each sampling time stored bottles were examined and scored for moldiness. A visual mold index (MI) was established: 0 - seeds free flowing, 1 - some seeds sticking together, 2 - seeds clumped but easily broken up, mold visible on careful examination, 3 - seeds clumped, moldy, and 4 - seeds caked, very moldy. Fungus growth giving a mold index of 1 or 2 in experimental storages would easily escape notice in a grain bulk especially with mechanized handling.

Agar. Difco potato dextrose agar with 4% sodium chloride was used for plating farm-stored sorghum. Rose bengal, 100 ppm, was added to restrict vegetative growth and facilitate counting when the grain was inoculated with *Rhizopus*.

For experiments with RS610 sorghum, Difco malt agar with 4% sodium chloride and 200 ppm Tergitol NPX (a nonionic detergent distributed by Union Carbide Corp.) was used. Tergitol was effective in reducing vegetative growth without preventing sporulation and has the advantage over rose bengal of being colorless.

Seeds invaded by fungi. After visual observation of the storage condition, each sample was mixed thoroughly by shaking, and about 100 seeds were removed aseptically. Kernels were shaken for 1 min in 2% sodium hypochlorite (NaOCl), rinsed in sterile distilled water, placed on agar -- 25 seeds per plate, and incubated 5-10 days at room temperature. Plates were examined microscopically and fungi identified. Seeds also were plated without hypochlorite treatment. Invasion is reported as percent of seeds infected based on observation of 25 to 100 seeds. Fifty surface-disinfected seeds and 25 non-surface-disinfected seeds from each sample were examined at intervals during experimental storage. The effectiveness of seed disinfection treatments is based on 100-seed samples. Initial mold invasion in sorghum sources was determined by examining 100 seeds.

EXPERIMENTAL RESULTS AND DISCUSSION

Field Fungi in Naturally Infected and Autoclaved Sorghum Stored at High Moisture Content

The potential for growth of field fungi in a lot of farm-stored sorghum was studied by adjusting the moisture contents of four 500 g lots to 18, 20, 22, and 24%, storing sample bottles at each level at 15 and 25 C, and examining grain for fungi. Before moisture adjustment 92% of the seeds were internally infected with Alternaria, 10% with Cladosporium, 4% with Fusarium, and seeds also had Penicillium, Mucor, and Rhizopus on their surfaces. Concurrently two storage experiments were conducted under the same conditions using the same sorghum source. For one study a 4 oz bottle at each moisture content was inoculated with Mucor; another group of four bottles was inoculated with Rhizopus, and four bottles were inoculated with Fusarium isolate "A" and four isolate "B". For the second series, 500 g lots in 2 qt mason jars were autoclaved 1 hr at 15 lb pressure and 120 C on 2 sucessive days. After sterilization jars were kept at room temperature and shaken at intervals for a day before moisture adjustment. Similar groups of 4 bottles were each inoculated with Alternaria, Fusarium isolate "A", Fusarium isolate "B", Mucor or Rhizopus. Two sets of 4 bottles each were double inoculated with Alternaria and Fusarium isolate "A" or isolate "B".

The percentage of infected seeds and mold index for naturally infected sorghum stored at high moistures are shown in Tables 1 and 2. The nearly 100% initial infection by Alternaria made it difficult to follow the succession of fungi developing in storage at high moisture. Mold growth increased, indicated by the mold index, with increased moisture, temperature, and time.

At both temperatures grain stored at 18% moisture content was not noticeable moldy after 35 days. After 49 days the sample stored at 18% moisture

Table 1. Effect of high moisture and time on fungi in sorghum at 15 C

Days Stored	% Moisture	Mold Index	Percent surface disinfected seeds yielding: Alt.	Clado.	Fus.	Mucor	Pen.
0[a]	13	0	94	10	4	0	0[b]
4	18	0	88	20	32	0	0
	20	0	92	12	16	4	0
	22	0	96	20	20	0	0
	24	0	100	24	16	0	0
12	18	0	100	0	24	0	0
	20	1	92	12	24	0	4
	22	1	92	8	12	0	0
	24	1	100	4	8	8	0
20	18	1	92	12	8	0	0
	20	2	92	12	0	0	0
	22	1	100	4	4	0	0
	24	3	96	16	0	4	0
35	18	1	100	4	8	0	0
	20	2	100	4	4	0	16
	22[c]	4	100	0	16	0	32
	24[d]	4	44	0	12	0	4
49	18	2	100	28	4	0	0
	20	3	100	4	12	0	36
	22	4	100	4	24	0	40
	24[e]	4	80	0	32	0	0

[a] Seven months in farm storage; day stored indicates subsequent controlled storage.

[b] Non-surface disinfected seeds also had *Penicillium* (11%), *Mucor* (2%), *Rhizopus* (2%).

[c] 12% *Tricothecium roseum*.

[d] 88% *T. roseum*.

[e] 44% *T. roseum*.

Table 2. Effect of high moisture and time on fungi in sorghum at 25 C

Days Stored	% Moisture	Mold Index	Percent surface disinfected seeds yielding: Alt.	Clado.	Fus.	Mucor	Pen.
0[a]	13	0	94	10	4	0	0[b]
4	18	0	100	12	16	0	0
	20	0	100	24	36	0	0
	22	0	100	0	16	0	0
	24	0	100	4	24	0	0
12	18	1	96	12	16	0	0
	20	1	100	8	24	0	0
	22	2	100	0	32	4	4
	24	3	96	8	64	12	0
20	18	2	96	0	0	0	8
	20	3	64	4	24	4	16
	22[c]	3	56	4	60	4	8
	24[d]	4	56	2	80	2	4
35	18	2	100	0	4	0	4
	20	3	100	0	36	0	22
	22[e]	4	72	0	40	4	16
	24	4	12	0	72	0	0

[a] Seven months in farm storage; day stored indicates subsequent controlled storage.

[b] Non-surface disinfected seeds also had *Penicillium* (11%), *Mucor* (2%), *Rhizopus* (2%).

[c] 12% *Trichothecium roseum*.

[d] 16% *T. roseum*.

[e] 44% *T. roseum*.

content and 25 C was very moldy while grain stored at the same moisture content and 15 C appeared in good condition. There was little change in mold flora during the storage period.

Sorghum stored at 15 C and 20% moisture content was not obviously moldy after 1 month, but 16% of the seeds were invaded by Penicillium. In 20 days, storage at 25 C produced moldy looking sorghum with 16% invaded by Penicillium and 24% by Fusarium. The sorghum of 22% moisture content was clean-looking after 20 days at 15 C and had a mold index of 2 in 12 days at 25 C. At 24% moisture content sorghum was moldy after 12 days at 25 C. It was in apparently good condition after 12 days at 15 C but was moldy 8 days later. After 20 days Fusarium had invaded more than half the seeds at 22 and 24% moisture content stored at 25 C.

Samples stored at 22 and 24% moisture content were invaded by Trichothecium roseum. At 15 C T. roseum was present after 35 days, and at 25 C the mold was found after 20 days of storage. T. roseum produces an antibiotic metabolite with fungicidal properties (3). Reduction in Alternaria and Fusarium counts and failure of Penicillium and Mucor to continue invasion of the sorghum may have been due to fungicidal activity rather than natural dying out of field fungi. The infection by T. roseum made it impossible to determine the optimal development of field fungi in the naturally infected sorghum.

As a group the field fungi require 90 to 100% relative humidity for development and sporulation. The relative humidity in equilibrium with sorghum grain of 20% moisture content at 40 F is 90%; at 70 F, sorghum of 18% moisture content is in equilibrium with 90% relative humidity (2). The minimum moisture condition for growth of the naturally infecting fungi may have been met in all samples except the one stored at 18% moisture content and 15 C where grain remained in good condition.

No "safe periods" -- times above which mold growth causes enough loss in quality to bring about a lowering of grade -- for storage of sorghum over 18% moisture content have been established. Texas workers (18) found the maximum allowable time for cooling sorghum to 40 F from an initial temperature of 85-95 F before loss in quality was 7 days. A series of experiments on storage of moist corn showed that when storage air temperature is 75 F, freshly harvested corn at 25% moisture content has a 4.5 day safe period, corn with 20% moisture content a 12.1 day safe period. At 60 F safe periods lengthen to 9.6 days for corn at 25% moisture content and 27 days for corn containing 20% moisture. If a visual mold index above 1 is used as an indication of quality loss, the safe periods for storage of high-moisture sorghum appear at least equal to those for corn at 25 C (Table 2), but a little shorter at 15 C (Table 1). The U. S. Department of Agriculture's "Guidelines for mold control in high moisture corn " (24) points out that storage at recommended conditions of temperature, moisture, and time does not guarantee the absence of mycotoxins.

Field fungi inoculated into high-moisture sorghum did not establish infection in the naturally infected grain. Mucor contaminated the surface of 8 to 32% of seeds in all treatments in 4 days; after 12 days, minimal (4 to 8%) invasion occurred in both storages at 24% moisture content. No Mucor was found at later sampling times. Rhizopus was detected on non-surface disinfected sorghum containing 24% moisture at 15 and 25 C after 4 days but was not found again.

Both Fusarium isolates were able to invade naturally infected sorghum in all storages, except for Fusarium isolate "B" in grain at 18% moisture content at 15 C and at 24% moisture content at 25 C; percent invasion was generally high after 4 days of storage (Table 3). There was an abrupt decrease in percentage of invaded seeds at the next sampling period. The drop between 4

Table 3. Effect of high moisture, temperature, and time on invasion of sorghum by isolates of Fusarium

Temp. °C	Days Stored	18% moisture nsd[a]	18% moisture sd[b]	20% moisture nsd	20% moisture sd	22% moisture nsd	22% moisture sd	24% moisture nsd	24% moisture sd
		Fusarium isolate "A" + initial infection[c]							
15	4	52	36	68	52	60	28	56	32
	12		0		8		12		8
	20		4		4		16		12
	35		12		4		32		72
25	4	84	72	84	80	88	76	92	48
	12		36		56		48		40
	20		0		8		40		52
		Fusarium isolate "B" + initial infection							
15	4	32	4	56	60	80	64	76	60
	12		16		8		16		0
	20		28		0		16		0
	40		8		4		20		8
25	4	80	72	84	96	100	88	92	20
	12		8		32		36		28
	20		8		32		28		24

[a] Not surface disinfected.
[b] Surface disinfected.
[c] 4% Fusarium in surface disinfected sorghum; 11% in non-surface disinfected.

and 12 days is not explained. It is unlikely that the high levels of Fusarium found on the fourth day were due to inadequate surface disinfection, for samples had low mold indices and were not difficult to wash. Treatment of seeds for 1 min in 2% NaOCl proved adequate for removing surface contamination of samples throughout the study.

Fusarium counts in the naturally infected sorghum at 22 and 24% moisture content stored 35 days at 15 C were higher when Fusarium "A" was inoculated than when no inoculation was made. At 25 C after 20 days storage, counts were lower in inoculated samples. The percent invasion by Fusarium did not increase at 15 C after 35 days or at 25 C after 20 days when Fusarium isolate "B" was inoculated into naturally infected sorghum. Mold indices were comparable to those in naturally infected sorghum storages.

Sterilized sorghum was used to determine conditions for development of common field fungi. Autoclaving alters chemical composition and physical properties of seeds, but growth responses of fungi indicate the relative humidity of interstitial spaces in equilibrium with moisture content of sorghum seeds is similar for autoclaved and viable sorghum at least up to 20% moisture content. Equilibrium moisture curves are not given for 15 or 25 C in the Agricultural Engineers Yearbook (2); so relative humidity values were interpolated from the temperature curves given.

In the 18% moisture sorghum no kernels were invaded at 15 C where relative humidity was about 88% though Mucor and Rhizopus spores were present on seed surfaces. Invasion was negligible after 12 days in 20% grain at 15 C at about 91% RH. After 20 days Fusarium isolate "A" had invaded 88% of seeds, Fusarium "B" 82%, Alternaria 24%, Rhizopus 8%, and Mucor 0% (Table 4).

Fusarium isolates developed in sterilized sorghum at 18% moisture content and 25 C when relative humidity was about 90%; Fusarium isolate "A" invaded more

Table 4. Percentage of autoclaved sorghum invaded by field fungi at 15 C

Days Stored	18% moisture nsd[a]	18% moisture sd[b]	20% moisture nsd	20% moisture sd	22% moisture nsd	22% moisture sd	24% moisture nsd	24% moisture sd
				Alternaria				
4	0	0	0	0	0	0	8	4
12	0	0	0	0	8	0	92	58
20	0	0	58	24	92	58		100
				Fusarium isolate "A"				
4	0	0	8	0	32	8	60	20
12	8	0	16	4	96	88	100	96
20	0	0	100	88		84		100
40	0	0		100				
				Fusarium isolate "B"				
4	0	0	0	0	0	0		
12	0	0	14	0	100	36		
20	2	0	100	82		96		
40	0	0		100				
				Mucor				
12	40	0	44	4	76	8	100	56
20	16	0	24	0		12		64
				Rhizopus				
4	20	0	8	0	0	0	+[c]	16
12	4	0	44	0	4	0		52
20	0	0	96	8		52		56
50	8	0	100	84		96		

[a] Not surface disinfected.
[b] Surface disinfected.
[c] Plate covered with mycelium.

seeds at 20 days than *Fusarium* isolate "B". *Alternaria*, *Mucor*, *Rhizopus* did not grow. When moisture content was raised to 20%, invasion was general in about 12 days (Table 5).

Panasenko (16) listed minimal and optimal humidity requirements for conidial development of some field fungi: *Alternaria tenuis*, 90 and 98%, *Mucor racemous* 95 and 98-99%, *Rhizopus stolonifer* 96 and 98-99%. Growth of *Alternaria* after 20 days on grain at 20% moisture content and 15 C was greater than that of either *Mucor* or *Rhizopus* suggesting minimal requirement for sporulation of the *Alternaria* isolate used is between 91 and 95%.

When *Alternaria* and *Fusarium* were used together as the inoculum, the percentage of sterilized sorghum invaded by *Alternaria* was low, never exceeding 32% (Table 6). As a single infection *Alternaria* invaded 100% of seeds at 25 C and 24% moisture content after 8 days of storage and all seeds of 20 and 22% moisture content by 20 days. *Fusarium* became established more rapidly; both isolates infected 76% of seeds at 20% moisture content and all seeds at 22 and 24% moisture content by the eighth day (Table 5). However, *Alternaria* had invaded more seeds in 4 days than *Fusarium* isolate "B", and *Alternaria* was better able to compete with *Fusarium* isolate "B" than *Fusarium* isolate "A". Christensen showed that wheat already infected by *A. glaucus* was not infected by *A. ochraceus* and suggested *A. glaucus* may become so well established that other fungi with higher moisture requirements are excluded (5). Species of *Fusarium* produce antibiotic substances (3) which may be important in restricting other fungi in storage. *Trichothecium roseum* appeared to have a similar inhibitory effect on other fungi in naturally infected sorghum (Tables 1 and 2).

When kernels were invaded already, it was difficult to establish another infection even by a species having a lower moisture requirement as demonstrated by attempts to infect 100% naturally infected sorghum with isolates of *Fusarium*.

Table 5. Percentage of autoclaved sorghum invaded by field fungi at 25 C

Days Stored	18% moisture nsd[a]	18% moisture sd[b]	20% moisture nsd	20% moisture sd	22% moisture nsd	22% moisture sd	24% moisture nsd	24% moisture sd
				Alternaria				
4	8	0	52	0	96	4	100	56
8	4	0	60	4	88	56	100	100
20		0	100	100		100		100
46						100		100
				Fusarium isolate "A"				
4	0	0	20	8	88	32	100	96
8	4	0	100	76	100	100		
12	0	0	100	84	100	100		100
20	82	32		92		100		100
46		78						
				Fusarium isolate "B"				
4	0	0	4	0		0	60	20
8	0	4	100	76	100	100	100	100
12	0	0	100	84	100	88		100
20	28	4		96		92		100
46		88						
				Mucor				
8	60	0	52	0	88	44	100	84
20	0	0	92	40		80		68
46				64		84		
				Rhizopus				
4	4	0	44	0	+[c]	+	+	+
12	8	0	100	52	100	76		48
20	0	0		40		44		72
40				16				88

[a] Not surface disinfected.
[b] Surface disinfected.
[c] Plate covered with mycelium.

Table 6. Percentage of autoclaved sorghum invaded by *Fusarium* and *Alternaria*

Temp. C	Days Stored	18% moisture nsd[a] F	Al	18% sd[b] F	Al	20% moisture nsd F	Al	20% sd F	Al	22% moisture nsd F	Al	22% sd F	Al	24% moisture nsd F	Al	24% sd F	Al
								Fusarium isolate "A"									
15	4	0	0	0	0	0	0	0	0	0	0	0	0	44	12	8	4
	12	2	0	0	0	34	0	2	0	100	0	88	0	100	0	98	0
	20	6	0	0	0	100	0	74	0			100	8			100	0
25	4	0	0	0	0	24	8	0	4	56	4	76	0	100	0	92	0
	8	12	0	0	0	100	8	88	0	100	0	96	0	100	0	100	0
	12	20	0	0	0			72	8			100	0			100	0
	20	88	0	76	0	100	0	100	0			100	0			100	0
	40			100	0			100	0								
								Fusarium isolate "B"									
15	4	4	0	0	0	8	0	0	0	12	0	0	0	72	24	12	4
	12	4	0	0	0	2	10	0	0	100	0	68	8	100	40	100	16
	20	0	0	0	0	100	0	100	0			100	4			100	4
25	4	0	0	0	0	20	0	4	0	96	8	40	20	100	28	100	16
	8	0	0	0	0	80	12	16	8	100	44	88	32	100	12	100	12
	12			0	0	100	28	64	0			52	12			100	0
	20	32	0	8	0			88	12			94	4			100	2
	40	100	0	84	0			100	0								

[a] Not surface disinfected.
[b] Surface disinfected.

The chief natural infection was by Alternaria which had a higher moisture requirement in sterilized sorghum than the Fusarium isolates. Mucor and Rhizopus, with 95% and 96% minimal relative humidity requirements for conidial development, were unable to establish internal infections in moist sorghum from natural surface contamination (Tables 1 and 2) and were excluded when inoculated into naturally infected grain. Both invaded sterilized sorghum (Table 4 and 5).

Infection of seeds by Fusarium inoculated into autoclaved sorghum increased with time in storage at 15 and 25 C. The percentage of seeds invaded by Fusarium in naturally infected sorghum increased at 25 C in 35 days (Table 2) but not at 15 C in the same length of time (Table 1). Responses of Fusarium isolates inoculated into naturally infected grain were variable; highest invasion was 72% by Fusarium "A" in grain of 24% moisture content at 15 C (Table 3).

Whether exclusion of field fungi from naturally infected sorghum is due to the presence of established infection or to properties of intact, living seeds cannot be determined from these experiments. Germinability is used as an indication of grain condition (6) and viable grain has better storability than non-viable suggesting that living seeds are less vulnerable to fungal invasion. Since fungal invasion is more rapid in autoclaved than in non-autoclaved grain, autoclaved sorghum is unsuitable for studying safe storage times at high moistures.

Seed Disinfection

Surface disinfected wheat and corn have proved satisfactory for experimental storage at initial grain moistures up to 16% (20). Because field fungi require a relative humidity in excess of 90% for development and sporulation, their presence in the seeds did not interfere with studies on storage fungi.

The problems encountered in the preceeding experiment utilizing farm-stored sorghum pointed out the need for viable sorghum free of field fungi for experimental storage at high moistures.

Sorghum obtained from a local elevator was treated as shown in Table 7 with various combinations of NaOCl and 70% ethyl alcohol. Volatile disinfecting agents were used to avoid residues. One hundred-gram lots were placed in a beaker with the alcohol or NaOCl, stirred with a magnetic stirrer, and rinsed with sterile distilled water after each treatment.

Table 7. Effects of disinfection treatments applied to commercial sorghum

No.	Treatments 70% EtOH	3% NaOCl	Seeds yielding fungi	Germination
	min	min	%	%
1	0	1 (2%)	100	87
2	3	3	100	79
3	3	20	80	74
4	3	30	62	71
5	5	30	56	71
6	10	30	36	71
7	3	20 (6%)	56	63

No treatment sufficiently reduced *Alternaria* infection to warrant its use in preparing commercial sorghum for a storage experiment. Treatment 6 bleached seeds and cracked many; milder treatments caused less damage. Whole seeds were selected for the germination test and for plating; seed damage caused by treatments was not determined.

A shorter series of disinfection treatments was conducted using No. 2 yellow corn having 72% *Fusarium* and 6% each of *Penicillium* and *A. flavus* in surface disinfected kernels. All treatments eliminated *Penicillium* and *A. flavus* and reduced *Fusarium* to 8-10%; germination remained 98-99%. Kernel damage was not apparent.

Sorghum seed is routinely treated with fungicide-insecticide combinations to control insects and such diseases as seed rots, seedling blights and smut. Fungus-free seedlings are easily grown under laboratory conditions from treated seed, and some seed lots produce nearly 100% sterile seedlings (J. W. Klink, personal communication).

To test the possibility of using treated seed for storage experiments, the captan-malathion was removed from a 250 g lot of RS610 sorghum by stirring 5 min in a volume of 70% ethyl alcohol equal to twice the volume of the sorghum sample followed by a 5 min wash in running tap water and the sequence repeated. Seeds were spread on sterile paper towels in the hood to remove excess water. No fungal growth was detected upon plating 200 washed seeds. Germination was 88% before washing and 73% after. Washing with 95% alcohol reduced germination to 26%.

Lots of 100-200 washed seeds were placed in sterile petri plates containing moistened sterile filter paper. Seeds were inoculated with either *A. glaucus*, *A. flavus*, *Alternaria*, *Mucor*, or *Rhizopus* by adding an infected sorghum kernel and shaking the dish thoroughly. The dishes were sealed in plastic bags and incubated at room temperature. Growth was visible on seed surfaces in 2-4 days. After 8 days seeds infected with *Alternaria* and *A. flavus* were surface sterilized and plated. Invasion was 100% by both organisms.

Captan was applied as a slurry to RS610 sorghum at 9% and 15% moisture content and to hand-harvested corn of 10% moisture content. The grains were stored in closed containers at room temperature and sampled at intervals. Before plating, captan was removed with 2 alternate 5-min washes in 70% ethyl alcohol and running tap water (Table 8).

Treated seeds from three 1968 crop sorghum varieties were checked 6-7 months after fungicide application. After removing the fungicide with 70% alcohol and water washes, 54, 30 and 2% Alternaria were present.

Sterilizing with fungicides requires considerable time, and some decrease in Alternaria in sorghum at 15% moisture content is perhaps due to time and not fungicide treatment (7). The internal infection rate of untreated RS610 sorghum from the same source as the captan-treated seed was 40% Alternaria, 1% Cladosporium, 1% Fusarium, and 1% Helminthosporium. The seed was subjected to disinfecting treatments shown in Table 9.

Washing sorghum grain twice in 70% alcohol and twice in water had less effect on germination than did treatment with 70% alcohol plus 3% NaOCl (Tables 7 and 9). Also the wash with NaOCl alone was as effective in reducing infection by field fungi as was that with the combination of NaOCl with 70% alcohol. Seed damage was not apparent after a 20 min NaOCl treatment, but germination was slower with 58% of seeds sprouted after 3 days compared with 90% in the seeds treated 1 min in 2% NaOCl.

Christensen and Lopez (7) stated that storage at controlled moisture content and temperature will result in death of all or nearly all field fungi without much reduction in germination percentage or seedling vigor, and suggest this may offer a simple means of ridding seeds of noxious fungi. Sorghum samples obtained from the K.S.U. Agronomy Department were surface disinfected and plated to check for infection by field fungi. One lot of Redlan variety harvested in October, 1966, and fumigated during storage, contained 6% field fungi in surface disinfected seeds in April, 1969. The seed surfaces were contaminated with Alternaria (19%), A. glaucus (21%), A. niger (10%), Fusarium (18%), Penicillum (52%), and small numbers of Cladosporium, A. versicolor, and

Table 8. Reduction of internal fungi by captan

Days Stored	Percentage surface disinfected seeds yielding: Alt.	Clado.	Fus.	Pen.
	RS610 sorghum 15% moisture content, 0.5 g captan/lb			
10	28	1	0	0
21	20	2	0	0
85	12	0	0	0
	RS610 sorghum 9% moisture content, 1.5 g captan/lb			
10	35	0	2	0
21	24	0	2	0
85	4	1	0	0
	Hand-harvested corn 10% moisture content, 0.5 g captan/lb			
10	0	0	54	5
21	0	0	52	0
85	0	0	4	0

Table 9. Effect of disinfecting treatments applied to RS610 sorghum

No.	Treatment	Treatment time	Seeds yielding Alternaria	Germination
		min	%	%
1	2% NaOCl	1	40	93
2	3% NaOCl	20	4	88
3	(a) 70% EtOH (b) running H_2O (c) repeat (a) and (b) (d) 2% NaOCl	5 5 5,5 1	33	89

Rhizopus. Germination was 95%. It is doubtful that surface disinfection would make this grain useful for storage experiments at high moisture content.

Four-year-old hand-picked corn, which yielded no fungi from surface-disinfected kernels, was used in a high-moisture storage experiment. A 500 g lot was washed with 70% alcohol and water followed by 1 min in 2% NaOCl prior to conditioning to 23.5% moisture. After 7 days at 15 C four of five bottles were contaminated with A. glaucus. When the surface flora was checked, 48% of seeds were contaminated with A. glaucus, 52% with Cladosporium, 50% with Penicillium, 23% with Alternaria, 16% with A. niger, 12% with Fusarium, 12% with Rhizopus and 8% with A. flavus. The disinfection treatment was apparently inadequate and surviving spores germinated under storage conditions. When larger quantities of grain are prepared for high moisture studies, a 20 min treatment with 3% NaOCl could be used.

Invasion of Autoclaved, Fungus-free, and Naturally Infected RS610 Sorghum at 19 and 23% Moisture by Four Common Fungi: Aspergillus repens, and Isolates of Alternaria, Fusarium, and Mucor

The percentages of autoclaved, fungus-free, and naturally infected sorghum invaded by three species of field fungi and Aspergillus repens, a common storage organism, were compared. The captan-malathion was removed from 2 500 g lots of RS610 sorghum, previously shown to be free of fungi, by washing 5 min in 70% ethyl alcohol and 5 min in running water and repeating the washes. Similar lots of untreated RS610 were washed in the alcohol-water series, and an equal amount was autoclaved for 1 hr at 15 lbs pressure and 120 C on 2 successive days.

After the appropriate treatments the moisture content of each of the three lots was adjusted so that half was at 19% moisture content and half at 23%. Fungus-free and autoclaved sorghum were each dispensed into 32 bottles, 16 of each moisture content. Washed, naturally infected grain at each moisture level was dispensed into 10 bottles. Germination was 73% for captan treated, fungus-free sorghum and 89% for naturally infected seeds. Sample bottles of the three sorghum lots at each moisture were inoculated with isolates of either A. repens, Fusarium, Mucor, or Alternaria. Alternaria was inoculated into autoclaved and fungus-free sorghum samples, but no additional inoculation was made to the naturally infected sorghum which contained 33% Alternaria and less than 1% Fusarium and A. glaucus after the alcohol-water wash treatment. Dual inoculations of Fusarium and each of the other three fungi were similarly made. Storage was at 15 C and 95-100% RH for 4 weeks.

Storage samples were duplicated. When the paired samples were checked for mold invasion, replication with inoculated fungi was good, but there were differences between pairs in natural infection by Alternaria, Fusarium, and A. glaucus.

Two samples of fungus-free and autoclaved sorghum at each moisture content were kept as controls against outside contamination. Seeds were plated without and with surface disinfection at each sampling time. No external contamination occurred.

Storage conditions of 19 and 23% moisture content of the grain and 15 C were selected because the relative humidities at the two moistures provided a sharp difference in conditions related to development of field fungi. Sorghum of 19% moisture content at 15 C is in equilibrium with 89^{+}% RH, just below the lower limit of 90% required for development of field fungi. The relative humidity of air surrounding sorghum at 23% moisture content is greater than 95% (2), providing nearly optimal conditions for conidial development of field fungi. Similar humidity conditions could be experimentally created at lower moisture contents and higher temperatures and invasion times would be reduced but the 15 C temperature is more realistic in terms of storage temperature in the late fall and early spring in the Midwest. Since insect activity is greatly reduced below 20 C (15), molds are chiefly responsible for deterioration of grain in cool storage.

Tables 10, 11, 12, and 13 present the results of inoculating four fungi individually into high moisture sorghum. The percentages given for non-surface disinfected seeds contaminated by fungi are probably only an indication of survival of the organism under these temperature and moisture conditions.

Only A. repens invaded sorghum at 19% moisture content, and invasion was limited to autoclaved sorghum (Table 13) even though the relative humidity in the other storages was well above the 75% required for conidial development of the fungus (16).

Alternaria, already present as a natural infection, continued growth and invasion in grain at 23% moisture content and 15 C. The fungus invaded 80% of autoclaved sorghum in 2 weeks but required 4 weeks to initiate invasion in fungus-free seeds (Table 10).

Invasion of autoclaved sorghum at 23% moisture content by Mucor (88%) in 2 weeks was approximately equal to that of Alternaria (89%), but the mold index was 1 for Mucor and 3 for Alternaria. Species of Mucor are frequently present in high-moisture corn and sorghum at harvest and experiments at Purdue University showed Mucor grew extensively at 28% moisture with high concentrations of CO_2 in the atmosphere (22). A low mold index and high invasion percentage suggest considerable deterioration could occur before grain appeared moldy. Mucor had invaded only 1% of viable grain in 4 weeks. Growth of Alternaria was responsible for a mold index of 2 in the naturally infected grain (Table 11).

During 4 weeks of storage Fusarium invaded 96-98% of non-autoclaved sorghum with 23% moisture content stored at 15C. The percentage of invasion was the

Table 10. Percentage of sorghum invaded by Alternaria at 15 C

Days Stored	19% moisture Mold Index	nsd[a]	sd[b]	23% moisture Mold Index	nsd	sd
			Autoclaved			
3	0	4		0	36	
7	0	16	0	1	96	4
14	0	40	0	3		80
21	0	25	0	4		100
28	0	8	0	4		
			Fungus-free			
3	0	8		0	12	
7	0	4		0	8	
14	0	4	0	0	12	0
21	0	0	0	0	36	0
28	0	0	0	1	96	4
			Naturally Infected[c]			
3	0	44		0	52	
7	0	44		0	56	
14	0	28	25	1	100	48
21	0	25	40	2[d]		52
28	0	32	24	3[d]		76

[a] Not surface disinfected.
[b] Surface disinfected.
[c] 33% Alternaria, 1% Aspergillus glaucus before storage; Alternaria not inoculated.
[d] 20% A. glaucus.

Table 11. Percentage of sorghum invaded by *Mucor* at 15 C

Days Stored	19% moisture Mold Index	nsd[a]	sd[b]	23% moisture Mold Index	nsd	sd
			Autoclaved			
3	0	16		0	40	
7	0	72	0	1	100	20
14	0	76	0	1	100	88
21	0	48	0	2		96
28	0	56	0	2	100	90
			Fungus-free			
3	0	8		0	20	
7	0	40		0	48	
14	0	72	0	0	84	0
21	0	32	0	0	84	0
28	0	24	0	0		4
			Naturally Infected[c]			
3	0	44		0	60	
7	0	48		0	76	0
14	0	64	0	1	72	0
21	0	76	0	1		0
28	0	64	0	2	80	4

[a] Not surface disinfected.
[b] Surface disinfected.
[c] 33% *Alternaria* before storage.

Table 12. Percentage of sorghum invaded by Fusarium at 15 C

Days Stored	19% moisture Mold Index	nsd[a]	sd[b]	23% moisture Mold Index	nsd	sd
			Autoclaved			
3	0	52		1	96	
7	0	68	0	3	100	96
14	0	68	0	4		100
21	0	44	0	4		
28	0	36	0	4		
			Fungus-free			
3	0	0		0	4	
7	0	0	0	0	0	
14	0	0	0	1	8	0
21	0	0	0	2	100	16
28	0	0	0	3		96
			Naturally Infected[c]			
3	0	16		0	8	
7	0	4		0	32	0
14	0	8	0	1	84	4
21	0	4	4	2		76
28	0	4	0	2		98

[a] Not surface disinfected.
[b] Surface disinfected.
[c] 33% Alternaria, 1% Fusarium before storage.

Table 13. Percentage of sorghum invaded by *Aspergillus repens* at 15 C

Days Stored	19% moisture Mold Index	nsd[a]	sd[b]	23% moisture Mold Index	nsd	sd
			Autoclaved			
3	0	76		0	100	
7	0	100	4	2	100	96
14	2		100	4		100
21	3		92	4		
28	3		94	4		
			Fungus-free			
3	0	4		0	8	
7	0	8		0	16	
14	0	16	0	0	72	0
21	0	16	0	1	100	20
28	0	16	0	3		60
			Naturally Infected[c]			
3	0	40		0	76	
7	0	28		0	52	0
14	0	20	0	2	100	52
21	0	24	0	3		52
28	0	54	4	3		64

[a] Not surface disinfected.
[b] Surface disinfected.
[c] 33% *Alternaria*, 1% *A. glaucus* before storage.

highest of the 4 fungi inoculated. During the first 3 weeks invasion of fungus-free sorghum was negligible, but the mold index had increased to 2 in the fungus-free samples as well as in naturally infected sorghum samples where Alternaria was also growing (Table 12). Whether field fungi have grown on grain surfaces or have proliferated from a sub-epidermal natural infection prior to drying may be significant for maintaining nutritive value of the kernels. The relationship of toxin production to depth of seed penetration by fungi has not been explored, but residual mycotoxin activity in dry cereal products has been demonstrated (8).

Although the rapid development of A. repens in autoclaved sorghum indicates that growth conditions at 23% moisture and 15 C are in the optimum range, A. repens invaded only 60-61% of non-autoclaved sorghum in 4 weeks. A longer storage period apparently would be needed for invasion of all kernels.

Similarity of percentage of invasion by inoculated isolates of Fusarium, Mucor, and A. repens into fungus-free and naturally infected high-moisture sorghum indicates removal of the fungicide-insecticide treatment from seed grade sorghum provided a fungus-free sorghum source with properties like those of untreated seed (Tables 11, 12, and 13). Rapid invasion of autoclaved seeds must have been due to pericarp damage even though the seeds appeared intact. The pericarp is resistant to fungal invasion, and if it is unbroken, storage fungi must invade through the point of attachment (20). There is no evidence that field fungi do not invade stored grain by the same route, though the 96-98% invasion by Fusarium (Table 12) compared to 60-61% by A. repens (Table 3) suggests another factor may be involved. The 100% surface contamination by A. repens at 3 weeks indicates there was adequate inoculum available.

The percent germination of inoculated fungus-free seeds after 3 weeks at 15 C had dropped from 73% to 58, 52, 47, and 46% for storages of sorghum at 19%

moisture content inoculated with *A. repens*, *Alternaria*, *Mucor*, and *Fusarium*, respectively. When the moisture content was 23% germination was 26, 12, and 7% for grain inoculated with *Mucor*, *A. repens*, and *Fusarium*, respectively. Control values were 37% germination for grain at 19% moisture content and 22% for sorghum at 23% moisture content. The germination percentages are lower than would be expected considering the lack of invasion in grain of 19% moisture content and the short storage time. C. M. Christensen has declared germination a too sensitive indicator, in some cases, of grain storability (6). Texas workers have reported a loss of germination in fungicide-treated seeds after the second year (19) and the treated RS610 variety sorghum seed may have lost some of its viability in the same manner since it was between 1 and 2 years old when used.

Naturally infected sorghum of 19% moisture content stored 4 weeks at 15 C had a germination percentage of 81, a decrease of 8% during the storage period. When moisture content was raised to 23%, germination decreased to 41% but the stored sample contained a natural infection of 20% *A. glaucus*, which may have contributed to the loss in germination percentage. When naturally infected sorghum of 23% moisture was inoculated with *Mucor*, *A. repens*, or *Fusarium*, germination was 63, 61, and 41%, respectively. *Fusarium*, the most aggressive of the inoculated fungi, produced the greatest reduction in percentage of germination. Reduced germination due to invasion by storage fungi has been demonstrated (20), but the effects of field fungi in grain of over 18% moisture have not been reported.

Dual inoculations of *Fusarium* with *Alternaria*, *Mucor*, or *A. repens* were made to observe competition of two organisms inoculated simultaneously into autoclaved and fungus-free sorghum and to study the effect of established *Alternaria* infection on invasion by *Fusarium* alone and combined with *Mucor* or *A. repens* (Tables 14, 15, and 16). As was found in single inoculations of

Table 14. Percentage of sorghum invaded by *Fusarium* and *Alternaria* at 15 C

Days	Autoclaved					Fungus-free					Naturally Infected[c]				
	Mold	nsd[a]		sd[b]		Mold	nsd		sd		Mold	nsd		sd	
Stored	Index	F	Al	F	Al	Index	F	Al	F	Al	Index	F	Al	F	Al
						19% Moisture									
3	0	92	0			0	8	0			0	16	28		
7	0	92	4	0	0	0	0	4			0	4	44		
14	0	64	24	0	0	0	0	4	0	0	0	8	36	0	40
21	0	68	4	0	0	0	0	0	0	0	0	4	44	4	32
28	0	52	4	0	0	0	0	0	0	0	0	4	44	0	40
						23% Moisture									
3	0	100	0			0	36	8			0	8	32		
7	3	100	0	100	4	0	20	24			0	32	68	0	28
14	4			100	0	1	20	44	0	0	1	84	72	4	52
21	4			100	0	2	84	56	0	4	2			76	60
28	4					2			92	12	2			98	22

a Not surface disinfected.
b Surface disinfected.
c 33% *Alternaria*, 1% *Fusarium* before storage; only *Fusarium* inoculated.

Table 15. Percentage of sorghum invaded by Fusarium and Mucor at 15 C

Days Stored	Autoclaved Mold Index	Autoclaved nsd[a] F	Autoclaved nsd[a] M	Autoclaved sd[b] F	Autoclaved sd[b] M	Fungus-free Mold Index	Fungus-free nsd F	Fungus-free nsd M	Fungus-free sd F	Fungus-free sd M	Naturally Infected[c] Mold Index	Naturally Infected[c] nsd F	Naturally Infected[c] nsd M	Naturally Infected[c] nsd Al	Naturally Infected[c] sd F	Naturally Infected[c] sd M	Naturally Infected[c] sd Al
19% Moisture																	
3	0	64	36			0	0	0	0	0	0	0	40	52			
7	0	44	64	0	0	0	0	4	0	0	0	4	84	32			
14	0	20	88	0	0	0	0	52	0	0	0	4	56	32	0	0	25
21	0	44	80	0	0	0	0	8	0	0	0	0	28	32	4	0	36
28	0	36	60	0	0	0	0	0	0	0	0	2	50	42	0	0	30
23% Moisture																	
3	0	100	36			0	0	12			0	0	96	32			
7	3	100	64	100	16	0	0	4			0	64	32	52			
14	4			100	4	1	44	76	0	0	2	76	28	64	8	0	28
21	4			100	12	2	100	24	44	0	2				64	0	32
28	4					3			100	0	2				88	2	40

[a] Not surface disinfected.
[b] Surface disinfected.
[c] 33% Alternaria, 1% Fusarium before storage.

Table 16. Percentage of sorghum invaded by *Fusarium* and *Aspergillus repens* at 15 C

Days	Autoclaved					Fungus-free					Naturally Infected[c]						
	Mold	nsd[a]		sd[b]		Mold	nsd		sd		Mold	nsd			sd		
Stored	Index	F	As	F	As	Index	F	As	F	As	Index	F	As	Al	F	As	Al
								19% Moisture									
3	0	36	34			0	0	4			0	0	68	32			
7	1	44	100	0	20	0	0	24			0	0	60	52			
14	1	36	100	0	84	0	0	8	0	0	0	4	80	48	0	0	32
21	3	4	100	0	82	0	0	52	0	0	0	0	64	36	0	0	26
28	3	0	100	0	100	0	0	12	0	0	0	4	74	36	0	4	40
								23% Moisture									
3	0	76	100			0	0	8			0	8	68	36			
7	3	100	96	24	80	0	8	24			0	32	36	60			
14	4			60	68	0	52	24	0	0	3	64	100	48	4	60	44
21	4			92	60	2	100	24	56	8	3				4	56	28
28	4			100	32	3			92	12	3				18	54	32

[a] Not surface disinfected.
[b] Surface disinfected.
[c] 33% *Alternaria*, 1% *Fusarium* and *A. glaucus* before storage.

sorghum at 19% moisture content, the only significant invasion was by *A. repens* in autoclaved grain. The mold index was 0 in the stored viable grain at 19% moisture content after 4 weeks at 15 C.

After *Fusarium* and *Alternaria* were inoculated into autoclaved and fungus-free sorghum at 23% moisture content, *Fusarium* invasion in 28 days was equal to that found when *Fusarium* was inoculated alone, but the invasion progressed more slowly in fungus-free seeds. *Alternaria* was unable to invade autoclaved sorghum already invaded by *Fusarium*, and the natural infection by *Alternaria*, which increased for the first 3 weeks, dropped after a high percentage of seeds were invaded by *Fusarium* (Table 14).

Mucor, inoculated with *Fusarium* into sorghum at 23% moisture content, was able to invade autoclaved sorghum along with *Fusarium* although invasion was only 12% in 4 weeks compared with 90% when *Mucor* was inoculated alone. There was no invasion by *Mucor* in fungus-free sorghum and minimal (2%) penetration of naturally infected seed. *Fusarium* invaded autoclaved and fungus-free sorghum as well in combination with *Mucor* as alone. In the naturally infected sorghum of 23% moisture content, the percentage of invasion by *Fusarium* was 10% less in 4 weeks when *Mucor* was also inoculated (Table 15).

When *A. repens* and *Fusarium* were inoculated into autoclaved sorghum of 23% moisture content, *A. repens* invaded 80% of seeds and *Fusarium* 23% after 7 days, but as single infections both invaded 96% of seeds in the same time. However, *A. repens* invasion decreased to 32% and *Fusarium* increased to 100% after 4 weeks of storage. In fungus-free seeds *Fusarium* invasion was as high in dual infection with *A. repens* as when inoculated singly, but *A. repens* had invaded only 12% of seeds after 4 weeks compared with 60% when inoculated alone. *Fusarium* invaded 18% of seeds and *A. repens* 54% after 4 weeks in the presence of natural infection by *Alternaria*. What percentage, if any, of invasion by

A. glaucus group was due to initial natural infection could not be determined although 20% invasion from natural infection was detected in one sample of 23% moisture content. *Alternaria* invasion decreased between 14 and 28 days (Table 16).

Thirty years ago Koehler (12) investigated growth limits of fungi in moist shelled corn in competition with surface borne fungi and in pure culture after surface sterilizing the grain. The fungi were studied by selecting corn carrying the fungus to be studied as an internal infection or by inoculating relatively fungus-free corn. *Fusarium moniliforme*, as an internal infection or when inoculated, competed well in corn above 23% moisture content and frequently predominated over *Aspergillus* and *Penicillium*. The observations were made after 3 months of storage. Koehler concluded that a fungus already in possession of the field may block the growth of another fungus which might have predominated if it had an equal opportunity.

After one month *Fusarium* in sorghum at 23% moisture at 15 C out-competed *Alternaria*, *Mucor*, and *A. repens* in autoclaved and fungus-free grain (Tables 14, 15, and 16). It became the predominant organism when inoculated into naturally infected sorghum with 33% *Alternaria* infection although the *Alternaria* did increase for the first 3 weeks, and also out-competed *Mucor* in naturally infected sorghum. Since the invasion by *Fusarium* and *Alternaria* after 2 weeks, as shown in Tables 14 and 15, was nearly equal, competition by *A. repens* appeared to restrict invasion by *Fusarium* when the 2 fungi were inoculated into grain with a natural *Alternaria* infection. It is possible that *Fusarium* would dominate after a longer storage period at 15 C. In a study of interaction of fungi present in peanuts, Welty and Cooper (25) reported that as *Fusarium* increased at moistures above 20%, *A. repens*, *A. flavus*, *A. ruber*, and *Penicillium* decreased. After 35 days at 25 C *Fusarium* was the predominant fungus in farm-stored sorghum of 24% moisture content when storage fungi were absent (Table 2). The possible role of antibiotic metabolites in the successful competition by *Fusarium* has been discussed.

Seeds of 19% moisture fungus-free and naturally infected sorghum inoculated with common field fungi or *A. repens* were not invaded when stored 4 weeks at 15 C. Safe storage, based on visual mold index, was between 2 and 3 weeks when grain moisture content was 23% if only field fungi were present; when *A. repens* was inoculated, the safe storage period was reduced to between 1 and 2 weeks.

SUMMARY

The moisture content of sorghum grain was adjusted to 18, 20, 22, and 24% for experimental storage at 15 and 25 C and 95% RH. Before moisture adjustment surface disinfected seeds had 94% *Alternaria* infection, 10% *Cladosporium*, and 4% *Fusarium*; *Penicillium*, *Mucor*, and *Rhizopus* were also detected on seed surfaces. The relative humidity requirement for growth of field fungi (above 90%) was met in all storages except when grain was at 18% moisture content and 15 C. Little evidence of qualitative or quantitative change in mold flora was found when surface disinfected seeds from high-moisture sorghum stored at 15 C were plated on potato dextrose agar with 4% NaCl. *Fusarium* invasion increased and infection by *Alternaria* decreased in sorghum stored at 25 C. The initially high *Alternaria* infection made it impossible to determine the potential for growth of common field fungi.

A visual mold index gave a better indication of mold growth than percentage of seeds invaded and indicated that safe storage of grain with 18% moisture content is about 6 weeks at 15 C and at 25 C about 2 weeks. At 25 C, sorghum at 20% moisture content was in good condition after 12 days of storage but not

after 20 days. At higher moisture contents sorghum was in good condition after 4 days and moldy after 12 days in storage. Lowering the temperature to 15 C increased safe storage time about 1 week for sorghum with moisture contents of 20% and higher.

Disinfection of commercial sorghum by combinations of 70% ethyl alcohol and 3% NaOCl did not eliminate Alternaria infection and reduced germinability. A 20 min wash in 3% NaOCl was as effective in reducing infection as any alcohol-NaOCl combination and lowered germination less than 5%. The treatment could be useful for preparing good quality sorghum for experiments involving grains of high moisture content since interfering surface contaminants, not completely removed by 1 min washing in 2% NaOCl, are destroyed.

A source of sorghum free of field fungi was found in fungicide-insecticide treated seed grade sorghum. After the captan-malathion was removed by washing in 70% ethyl alcohol and water, germination was high, and the seeds were susceptible to infection.

The development of isolates of Alternaria, Fusarium, Mucor and Aspergillus repens, single and in combination, was compared in autoclaved, fungus-free (captan-malathion removed), and untreated seed grade RS610 variety sorghum adjusted to 19 and 23% moisture contents, stored at 15 C and 95% RH. The natural infection of untreated, surface disinfected seeds was 33% Alternaria, and less than 1% with Fusarium or A. glaucus.

The only significant invasion of 19% moisture sorghum was A. repens in autoclaved grain. During 4 weeks of storage, Fusarium invaded 96-98% of fungus-free and naturally infected sorghum of 23% moisture content stored at 15 C; Alternaria and Mucor each invaded 1% and A. repens 60-61%. Similarity of percentage of invasion by inoculated fungi into the fungus-free and naturally infected sorghum at 23% moisture content indicated removal of the fungicide-insecticide used in treatment of seed-grade sorghum provided a fungus-free sorghum source with properties like those of untreated seed.

After 1 month Fusarium, inoculated into sorghum at 23% moisture content and stored at 15 C, out-competed simultaneous inoculations of Alternaria, Mucor, or A. repens in autoclaved and fungus-free grain. When inoculated into sorghum with 33% Alternaria infection, Fusarium became the predominant organism although Alternaria increased early in the storage period. Competition by A. repens restricted invasion by Fusarium when isolates of the two fungi were inoculated into sorghum with a natural Alternaria infection.

Seeds of fungus-free and naturally infected sorghum inoculated with three common field fungi and A. repens were not invaded when stored 4 weeks at 19% moisture content at 15 C. Safe storage at 15 C, based on visual mold index, was between 2 and 3 weeks when grain moisture content was 23% if only field fungi were present; when A. repens was inoculated, the safe period was reduced to between 1 and 2 weeks.

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GROWTH OF FUNGI IN SORGHUM GRAIN STORED AT MOISTURE CONTENTS OF 18-24%

by

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High moisture causes problems in storage of sorghum grain when the crop is combined at moisture contents above 12-13%, when storage bins are not weather proof, when spring temperatures warm storage bulks, and when sorghum is reconstituted for cattle feeding. Grain under these conditions is vulnerable to rapid invasion by molds.

Experimental storage studies of sorghum adjusted to 18, 20, 22, and 24% moisture content and stored at 15 or 25 C and 95% RH were complicated by natural infections of field fungi often occurring in nearly 100% of seeds. Species of *Alternaria*, *Fusarium*, and *Cladosporium* were common with *Alternaria* the most frequent. These organisms require relative humidities of over 90% for growth and suitable conditions were probably provided in all storages except when grain was at 18% moisture content and 15 C. A visual mold index gave a better indication of mold growth than percentage of seeds invaded and indicated that safe storage of grain with 18% moisture content is about 6 weeks at 15 C and about 2 weeks at 25 C. At 25 C sorghum at 20% moisture content was in good condition after 12 days of storage but not after 20 days. At 22 and 24% moisture contents sorghum was in good condition after 4 days and moldy after 12 days storage. Lowering the temperature to 15 C increased safe storage time about 1 week for sorghum with moisture contents of 20-24%. The percentages of seeds infected by the various fungi did not appear to change in 6 weeks at 15 C. At 25 C infection by *Fusarium* increased and *Alternaria* decreased.

A source of sorghum free of field fungi was found in fungicide-insecticide treated seed-grade sorghum. After the captan-malathion was removed by washing in 70% ethyl alcohol and water, germination was high, and the seeds were susceptible to infection.

The development of isolates of *Alternaria*, *Fusarium*, *Mucor*, and *Aspergillus repens*, singly and in combination, was compared in autoclaved, fungus-free, and untreated seed-grade RS610 variety sorghum adjusted to 19 and 23% moisture contents and stored at 15 C and 95% RH for 4 weeks. The natural infection of the untreated seeds was low, only 33% being infected by Alternaria.

Similarity of percentage of invasion by inoculated fungi in fungus-free and naturally infected sorghum at 23% moisture content indicated removal of the fungicide-insecticide used in treatment of seed-grade sorghum provided a fungus-free sorghum source with properties like those of untreated seed.

The only significant invasion of sorghum at 19% moisture content stored at 15 C was by A. repens in autoclaved grain. Autoclaved grain is unsuited for studies of storage at high moisture content; alteration of the pericarp and seed coat probably allows more rapid invasion than occurs in intact, living seeds.

After 1 month Fusarium, inoculated into sorghum at 23% moisture content at 15 C, out-competed simultaneous inoculations of Alternaria, Mucor, or A. repens in autoclaved and fungus-free grain. When inoculated into sorghum with 33% Alternaria infection, Fusarium became the predominant organism although Alternaria increased early in the storage period. Competition by A. repens restricted invasion by Fusarium during 1 month of storage when isolates of the two fungi were inoculated into sorghum with a natural Alternaria infection.

Seeds of fungus-free and naturally infected sorghum inoculated with three common field fungi and A. repens were not invaded when stored 4 weeks at 19% moisture content at 15 C. Safe storage at 15 C, based on visual mold index, was between 2 and 3 weeks when grain moisture content was 23% if only field fungi were present; when A. repens was inoculated, the safe period was reduced to between 1 and 2 weeks.