Fungal Populations in U.S. Farm-Stored Grain and Their Relationship to Moisture, Storage Time, Regions, and Insect Infestation

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ABSTRACT

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One hundred surface-disinfected kernels were plated from each of 2,557 samples taken from farm bins in 27 states in 1980. *Aspergillus glaucus* was found in 84% of the corn, 70% of the oat, and 37% of the wheat samples. Corn, oat, and wheat samples with *A. glaucus* had averages of 26, 20, and 14% of the kernels invaded, respectively. Fungal contamination varied little in corn from four crop years. Wheat from the most recent (1979) crop year was low in storage fungi and high in field fungi compared with earlier crop years. Storage fungi averaged higher in samples with higher as-received moisture contents, even though most moisture contents were too low to permit mold growth. *A. glaucus* was also higher in northern compared with southern states in the central plains. Other storage fungi found included *A. restrictus* and other species of *Aspergillus* and *Penicillium*. *A. flavus* was found in an average of 1.2% of all corn kernels and in less than 0.1% of the oats and wheat. Samples heavily invaded by storage fungi were more likely to also contain insects than were lightly invaded or uninvaded samples. This report provides the first extensive documentation of the incidence and extent of fungal invasion in farm-stored grain in the United States.

The amount of grain stored on U.S. farms in recent years has been increasing both in actual volume and as a percentage of total grain stocks. Reasons for this include a desire on the part of grain producers to improve their marketing flexibility, government programs that have encouraged construction of on-farm grain storages, and loan programs for long-term storage of grain in a "farmer-owned reserve." Grain stored on farms as of 1 January ranged from 6 to 8 billion bushels in 1979–1983, representing about 67% of the total U.S. grain stocks (1). Those figures represent an increase of more than 50% in volume compared with farm storage a decade earlier.

Freshly harvested grain is relatively free of storage fungi, and grain arriving at terminal elevators is usually infected to varying degrees (3). Quantitative data on the extent of invasion by these fungi in farm or other primary storage facilities is lacking.

There have been no comprehensive or systematic studies of farm-stored grain to determine the incidence of damaging fungi; however, recent limited surveys in several states have indicated that many farm storages are insect-infested, mold-invaded, or both (2; R. Mills and J. Pedersen, *unpublished*; A. Foudin, *personal communication*).

In Canada, a questionnaire survey of 2,919 elevator managers during the 1970–1971 crop year recorded 11,618 known cases of insect and mite infestation, 12,596 moldy grain bulks, and 11,289 cases of hot spots, all in farm storages (10). Although no percentages were given, the report clearly indicated widespread insect and mold problems in farm-stored grain.

In a recent survey of export corn and wheat samples, *Aspergillus glaucus* was found in nearly all corn samples, with an average of 21% of the kernels invaded (7). *A. glaucus* was present in 80% of the wheat samples but in an average of only 2.6% of the kernels.

In this study, we sought to determine the extent of invasion by storage fungi in grain stored on the farm and to see if fungal populations could be related to geographic factors, time in storage, moisture content, or insect infestation. Data on insect incidence and management practices relating to the same grain samples are published elsewhere (11,12).

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MATERIALS AND METHODS

Grain samples. Samples were obtained by Agricultural Stabilization and Conservation Service (ASCS) personnel from bins of wheat, oats, and corn stored under the USDA's farmer-owned grain reserve program. Bins were selected for sampling with a random-sample procedure used by ASCS for periodic quality checks of farm-stored reserve grain. Bins were selected for sampling at random so that the amount of grain sampled in any state or area was proportional to the amount of farmer-owned reserve grain stored in that area. County ASCS employees were directed to use a probe to obtain a representative sample from each bin. About 2 kg of grain was drawn from each bin and divided into two equal parts. One part was sent by mail to the U.S. Grain Marketing Research Laboratory; the other part was submitted to licensed inspection agencies for grading. Wheat and oats were sampled during June and July 1980 and corn was sampled during September and October. A total of 8,139 samples (4,171 wheat, 2,918 corn, and 1,050 oats) from 628 counties in 27 states were received. Crop years represented were 1976–1979. Moisture content and test weight were determined for each sample on a GAC-II grain tester (Dickey-John Corp., Auburn, IL). Subsamples for fungal testing were kept in paper envelopes at room temperature for several weeks, then those not yet tested were moved to a room at 5 C. Samples were randomly selected from states that submitted large numbers of samples, whereas all were tested from states that submitted only a few samples.

Determination of fungi. Corn kernels were shaken for 1 min in 5.25% NaClO (Clorox), rinsed in sterile water, and 100 were placed on Difco malt agar containing 4% NaCl and 200 ppm of Tergitol NP-10 (Sigma Chemical Co., St. Louis, MO). Wheat and oat kernels were rinsed for 10–15 sec in 100% ethanol, shaken for 1 min in 2% NaClO, rinsed in sterile water, and 100 were placed on malt-salt-Tergitol agar containing 8% NaCl. After incubation at 25 C for 5–7 days, fungi growing from the kernels were identified and counted with a dissecting microscope. A total of 255,700 kernels were plated.

Aflatoxin tests. Although samples were not large enough for maximum reliability of aflatoxin determination on a per-sample basis, the large number of samples warranted an attempt to screen for aflatoxin. Corn samples of about 1 L were coarsely ground in a burr mill under a high-intensity longwave ultraviolet lamp. The presence or absence of bright greenish yellow fluorescence (BGYF) was noted (9). Samples with visible BGYF, and a few without, were then ground further for aflatoxin analysis by thin-layer chromatography (8).

RESULTS

Incidence of fungi. Table 1 summarizes the incidence of fungi in all of the wheat, oat, and corn samples tested. *A. glaucus* was found in 84% of the corn samples and in 37% of the wheat samples. All *Aspergillus* species designations in this report are "group species" as classified by Raper and Fennell (6). In wheat samples with detectable *A. glaucus*, an average of 15% of the kernels were invaded; percentages were higher in oats and corn. *A. flavus* was found in 35% of the corn samples but in almost none of the wheat and oats. Other species of *Aspergillus*, including *A. candidus, A. restrictus, A. versicolor, A. ochraceus, A. niger, A. terreus, A. fumigatus,* and *A. clavatus*, along with *Penicillium* spp., were found in occasional samples.

Data on the various field fungi have been combined and labeled simply as "field fungi." *Alternaria alternata* (Fr.) Keissl. was the most common in wheat and oats, followed by *Fusarium* spp. Both were present in most samples and both could often be seen growing from the same kernel. Others such as *Cladosporium, Epicoccum,* and *Helminthosporium* occurred less frequently. *Fusarium moniliforme* Sheldon was the most common species in corn samples; other genera identified included *Cephalosporium, Penicillium, Cladosporium, Trichoderma, Nigrospora, Alternaria, Helminthosporium, Mucor, Rhizopus, Chaetomium, Diplodia, Trichothecium,* and *Syncephalastrum*.

Effect of storage time. Field fungi were most abundant in newer crop grain as was expected. The trend was quite clear in wheat and oats, whereas corn from all crop years had low numbers of field fungi. *A. restrictus* was detected in 11% of the wheat samples and the percentage of infected kernels increased from a mean of 0.2% in 1-yr-old grain to 1.1% in 4-yr-old grain. The 4% salt content of the agar medium used for corn samples was not high enough to promote growth of *A. restrictus*; the few infections seen were included in the *A. glaucus* counts. *A. glaucus* did not vary according to crop year, except 1979 wheat had about half as much as that from the three previous crop years.

Effect of moisture content. For the wheat and corn data, there was a positive relationship between moisture content and percentage of kernels with storage fungi. This was true even though moisture contents as received were too low to permit fungal growth. Wheat samples with less than 11% moisture averaged 3% kernel invasion by *A. glaucus*, whereas those above 13% moisture had 14% *A. glaucus*. *A. restrictus* averaged 0.0% in the 545 samples with less than 11% moisture and 4.9% in the samples with higher than 13% moisture. Corn samples with less than 11% moisture averaged 14% kernel invasion by storage fungi (all *Aspergillus* and *Penicillium* plus the Mucorales) and those with higher than 13% had 44% invasion. The correlation coefficient between moisture content and percentage of *A. glaucus* plus *A. restrictus* was 0.27 for wheat and 0.32 for corn. Similarly low but highly significant correlations were obtained when analyzing data from individual states from which large numbers of samples were tested.

Geographic differences. There was a clear trend toward higher storage mold levels in grain from northern states compared with

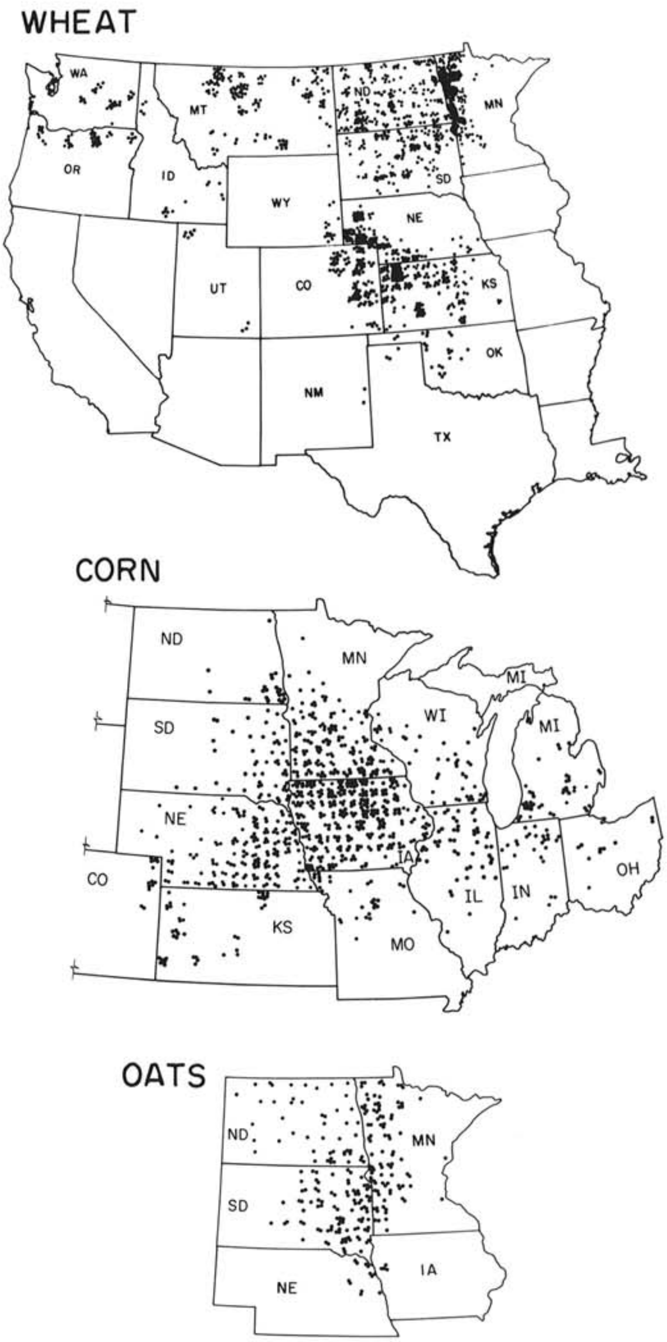

Fig. 1. Origin locations of farm-stored wheat, corn, and oat samples tested for fungi. Twenty eight samples were from states not shown.

TABLE 1. Incidence of principal fungi found in farm-stored grain samples

Grain	No. of samples	Samples (%) with: *Aspergillus glaucus*	Samples (%) with: *Aspergillus flavus*	Samples (%) with: Field fungi	Average (%) kernels with: *Aspergillus glaucus*	Average (%) kernels with: *Aspergillus flavus*	Average (%) kernels with: Field fungi
Wheat	1,230	37	···	89	6(15)[a]	···	25(29)
Oats	257	70	···	80	15(21)	···	15(18)
Corn	1,070	84	35	69	22(26)	1.2(3.5)	4(6)

[a]Numbers in parentheses are average kernel infection percentages in samples that had one or more kernels invaded by the fungus.

grain from southern states within the central plains region. Each dot in Fig. 1 represents the origin of a sample tested for fungi. Three percent of the oat samples, 1.7% of the corn, and 0.2% of the wheat plated were from states outside the areas shown in Fig. 1. The wheat samples can rather easily be divided into geographic areas, but such division would be more arbitrary for corn and oats. Dividing the wheat data into north central (Minnesota, North Dakota, South Dakota, and eastern Montana), south central (Kansas, Nebraska, Wyoming, Colorado, Oklahoma, and Texas), and northwest (Washington, Oregon, and Idaho panhandle) regions, showed *A. glaucus* kernel invasion to average 2.1% in the northwest, 3.0% in the south central, and 7.8% in the north central regions. The 36 Montana samples that appear to fit in with the Dakotas (Fig. 1) averaged 6.3% invasion by *A. glaucus*, whereas the 98 samples from central and western Montana averaged 1.5% *A. glaucus* and were similar to those from Washington and Oregon. Average invasion by storage fungi in samples from principal states surveyed is summarized in Table 2. As indicated by the median and 90th percentile values, many wheat samples were free or nearly free of storage fungi.

Fungal invasion was higher in corn samples from northern and eastern states than from southern and western states in the corn belt (Table 2). As indicated in Table 2, there were significant differences among states with respect to the tendency for populations to yield relatively high or relatively low values. Several nonparametric analyses of ranks or medians produced similar statements of difference among states. The 256 samples from Kansas and Nebraska averaged 18% *A. glaucus*, 0.9% *A. flavus*, and 22% for all storage fungi combined. The same three fungus categories averaged 26%, 1.5%, and 32% in the 313 samples from Wisconsin, Minnesota, and South Dakota, respectively.

Variability among samples. In the farm survey, there were many samples with no storage fungi and some that were more than 75% invaded. Our recent survey of export corn (7) showed much less sample-to-sample variability, reflecting the blending of grain from different sources that occurs throughout the marketing system. Among the farm-stored wheat samples, 61% had no detectable invasion by *A. glaucus* or *A. restrictus*, 16% of the samples had 1–2% kernel invasion, and 4% had 50% or more invasion. Corresponding percentages for corn were 16% with no invasion, 15% with 1–2% invasion, and 17% with 50% or more.

Only 8% of the corn samples contained 5% or more *A. flavus*-infected kernels. *Fusarium* and/or *Cephalosporium* are usually present in more than 20% of freshly harvested corn kernels (5), but in this study, 20% invasion or higher was found in fewer than 5% of the samples.

Insect-fungus relationships. Samples high in storage-mold invasion were also higher than average in insect infestation. Increasing *A. glaucus* counts were associated with increasing percentages of samples with live insects (Tables 3 and 4). In wheat, flat grain beetles, saw-toothed grain beetles, lesser grain borers, and flour beetles all occurred in higher percentages of samples as *A. glaucus* increased. Of those four insect groups, only flat grain beetles showed a similar relationship in corn. The foreign grain beetle and hairy fungus beetle increased with increasing storage fungi, as might be expected, because these insects feed on fungi and are commonly associated with moldy or high-moisture grain (4). The fungus feeders and other insects were also abundant in samples that were relatively high in other storage fungi (Table 4). Of all corn samples plated, 27% contained one or more insects that are generally associated with damp or moldy grain. Storage fungi were

TABLE 2. Storage fungi and average moisture in farm-stored wheat and corn samples from principal states surveyed

State[y]	Samples (no.)	Kernels (%) with storage fungi[z] Mean	Median	90th percentile	Average moisture (%)
Wheat					
Nebraska (a)	120	1.8	0	5	11.1
Kansas (a)	179	2.8	0	4	10.8
Montana (a)	134	4.0	0	9	11.3
Colorado (ab)	98	5.8	0	20	10.6
South Dakota (b)	138	6.8	0	26	11.4
North Dakota (b)	201	8.8	0	26	11.6
Minnesota (c)	228	11.5	2	43	11.9
Corn					
Nebraska (a)	219	22	10	65	11.6
Iowa (ab)	288	24	11	71	12.4
South Dakota (abc)	54	28	20	75	11.6
Illinois-Indiana (bc)	80	31	19	92	12.5
Wisconsin (bc)	45	32	19	82	11.8
Minnesota (c)	214	32	22	85	11.9

[y] States not followed by the same letter were significantly different ($P=0.05$) in counts of storage fungi according to the Kruskal-Wallis test.
[z] Species of *Aspergillus* and *Penicillium* plus the Mucorales.

TABLE 3. Moisture content and insect incidence in farm-stored wheat samples with varying percentages of kernels invaded by *Aspergillus glaucus*

Kernels with *A. glaucus* (%)	No. of samples	Average moisture (%)	With live insects (%)	Samples with selected insects (%)[a] FR	STGB	FB	LGB
0	770	11.1	20	9	6	2	3
1–4	243	11.3	28	13	9	2	5
5–20	113	11.7	32	22	11	5	6
21–100	97	12.1	41	23	12	5	9

[a] FR = flat and rusty grain beetle, *Cryptolestes* spp; STGB = saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.); FB = flour beetle, *Tribolium* spp; and LGB = lesser grain borer, *Rhyzopertha dominica* (F.)

TABLE 4. Moisture content and insect incidence in corn samples with different populations of storage fungi

Fungus	Kernels invaded (%)	No. of samples	Average moisture (%)	With live insects[a] (%)	Samples with selected insects (%)[b] FR	STGB	FB	WV	FoG	HFB
Aspergillus glaucus	0–3	363	11.6	70	41	6	13	8	9	2
	4–10	160	11.8	76	54	10	22	9	12	2
	11–20	138	12.1	85	66	5	22	14	19	2
	21–40	162	12.2	88	70	11	27	8	23	3
	41–70	151	12.3	93	83	11	23	10	30	4
	71–100	78	13.1	96	86	6	19	20	45	8
A. flavus	⩾5	84	12.2	92	82	13	27	24	25	7
Other *Aspergillus* spp.	⩾3	70	12.7	91	86	11	27	16	29	7
Penicillium spp.	⩾7	75	13.1	84	75	11	23	12	33	7
Phycomycetes	⩾3	95	12.1	87	80	10	30	20	32	7
All samples		1,052	12.0	73	60	8	20	10	19	3

[a] Not including Indianmeal moth, *Plodia interpunctella* (Hubner).
[b] FR = flat and rusty grain beetle, *Cryptolestes* spp.; STGB = saw-toothed grain beetle, *Oryzaephilus surinamenis* (L.); FB = flour beetle, *Tribolium* spp.; WV = weevils, *Sitophilus* spp.; FoG = foreign grain beetle, *Ahasverus advena* (Waltl.); and HFB = hairy fungus beetle, *Typhaea stercorea* (L.).

found in an average of 22% of the kernels in samples without "high-moisture insects" compared with 40% in samples with the insects. Among the corn samples with high-moisture insects, those with higher than 12.5% moisture averaged 54% invasion by storage fungi.

Aflatoxin in corn. BGYF was detected in 5.5% of 1,613 samples tested. Chemical analysis showed 43% of the BGYF positive samples contained detectable aflatoxin B_1, and those with 20 ppb or more represented 0.4% of the samples screened for BGYF. It is possible that a few of the BGYF negatives contained low levels of aflatoxin. Our survey of export corn (7) showed about twice as much BGYF and aflatoxin compared with farm samples, probably a reflection of more corn from the southern U.S. in the export samples. An attempt was made to distinguish relative amounts of BGYF in the samples, dividing them into four categories: N = negative, P− = barely visible, P = visible but not abundant, and P+ = visible and abundant. In each category, the percentage of samples containing more than 5 ppb of aflatoxin was as follows: N = 0%, P− = 13%, P = 24%, and P+ = 54%.

DISCUSSION

The storage fungi observed in this study consisted primarily of members of the *A. glaucus* group. *A. glaucus* grows in the marginal moisture content range of 14–16% and is probably the most prevalent of the storage fungi in all grains anywhere in the world. It is not as aggressive or destructive as some other *Aspergillus* species and is not a known mycotoxin producer. However, it does result in mustiness and loss of germinability, produces grade-reducing "blue eye" mold damage, and can help create a favorable environment for more destructive fungi (3).

A. flavus is usually considered a storage fungus but it can invade corn in the field, so our data may reflect preharvest or postharvest invasion or both (3).

Viable field fungi were found in most samples of all grains but the average percentage of kernels was relatively low. Low numbers of field fungi can reflect arid growing conditions, high drying temperatures, or long storage. Samples in our 1977–1978 survey of export grain had 21 and 41% field fungi in corn and wheat (7) but the same two grains in this study averaged 4 and 25%, respectively. The average age of the export grain was probably less than for the farm samples.

Storage fungi were found in about one-third of the wheat samples, and in those samples, the invasion level was often quite high. In both wheat and corn, there was a tendency for moldy samples to be infested with insects as well. The much higher incidence of storage molds in corn compared with wheat is undoubtedly a reflection of higher average moisture contents in corn. These findings indicate that good management practices, including storing grain at low moisture contents, prevent both insect and mold problems.

Fungi will not grow in grain at 11–12% moisture, yet samples in that range contained storage fungi, and to a greater degree than in drier samples. We must assume that grain in many of the bins had moisture contents high enough for mold growth, then dried during storage. The data indicate that all bins may have dried somewhat during storage, but on the average, grain that was initially high in moisture was still relatively high compared with grain with initially lower moisture content.

The southern plains area had relatively low storage mold invasion in wheat, probably because of low average moisture contents at harvest. This is in spite of the fact that average temperatures are warmer and therefore more favorable for fungal growth in the southern states. Higher mold incidence and higher moisture contents in the cooler northern states indicate that improved management practices should be encouraged in those areas. Aeration of sufficient capacity could be used to gradually reduce moisture contents and maintain temperature uniformity.

Many growers probably put corn into long-term storage with average moisture contents of 15–15.5%, which are acceptable in commercial channels and within the standards for no. 2 corn. Such corn will remain moldfree for many months only if the grain temperature is kept low or if the moisture level is gradually reduced. During warm weather, mold will grow in grain at 14–15% moisture, and if temperature differences exist in the grain mass, areas of high moisture can develop through moisture migration and spoilage can develop rapidly.

There is no evidence from this study to suggest that a significant aflatoxin problem exists in corn stored in the Midwest. Both the incidence and toxin level were low in our samples. This agrees with other information showing that aflatoxin is much less of a problem in the main corn belt states than in the southeastern states (13).

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