Fungi in U.S. Export Wheat and Corn

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ABSTRACT

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From January 1977 to December 1978, 1,653 samples of wheat and 1,940 samples of corn were obtained from 79 export elevators as ships were being loaded. Percentages of kernels containing viable storage fungi after surface-disinfection were about ten times higher in corn than in wheat. *Aspergillus glaucus* was by far the most common storage mold; it was found in nearly every corn sample and in 80% of wheat samples. *A. glaucus* was present in an average of 21% of 194,000 corn kernels tested, and in 2.6% of wheat kernels. The high incidence of *A. glaucus* in corn compared to wheat is indicative of the relatively higher moisture contents at which corn is stored and marketed. *Aspergillus flavus* was found in only 1% of wheat samples, but in 70% of corn samples. Kernel invasion by *A. flavus* averaged less than 2% in corn and was negligible in wheat. *Penicillium* spp. were found in 7% of corn kernels and 0.1% of wheat kernels. Percent of kernels with *Aspergillus* spp. other than *A. glaucus* and *A. flavus* averaged slightly less than 1% in corn and about 0.1% in wheat. Samples of both corn and wheat from West Coast ports and wheat from the Texas Gulf ports had lower counts of storage fungi than those from other regions. Other regions were similar except that corn samples from southeastern ports had more infection by *A. flavus* and more aflatoxin. Percentages of corn kernels with storage fungi were highest in the summer. Also, samples with higher percentages of damaged kernels tended to be higher in invasion by storage fungi. About 10% of ground corn samples displayed bright greenish-yellow fluorescence (BGYF) under a long-wave ultraviolet lamp. One third of the BGYF positives contained detectable aflatoxin; 9% contained 20 ppb or more.

Additional key word: grain.

Grain importers sometimes complain that the quality of grain they purchase is not of the quality indicated on the grade certificate. Excessive broken grain, dust, and foreign material may be cited, or the problem may be insect infestation, moldiness, or poor storability. There are, however, no comprehensive studies that identify the extent of insect or fungal contamination in U.S. export grain, or in grain exported from other countries.

Early studies of 150 shiploads of U.S. corn showed that extensive moldiness and heating developed in transit because much of the corn had 18–19% moisture content (1,10). The adoption of 15.5% as a maximum moisture level for export corn greatly reduced spoilage in transit. Sauer and Christensen (8) found 375 samples from 35 shiploads of grade No. 2 corn to be relatively uniform in germination, fat acidity, and fungal invasion. The most abundant fungus, *Aspergillus glaucus*, was found in 14% of the kernels. Moisture contents were all below 15.5%, but only 5% of the samples had less than 14% moisture.

More recent studies of export grain quality have not dealt with contamination by fungi or insects. Hill, et al (5) studied changes in the amount of broken corn during shipment, and Hesseltine et al (4) studied the microflora of a shipload of soybeans. The principal storage fungi found were species of the *A. glaucus* group and *Penicillium*.

There is a great range in the keeping quality of No. 2 corn, and the greater the degree of previous invasion by storage molds, the greater the spoilage risk (3).

The study reported here is a comprehensive evaluation of fungi present in wheat and corn from all export locations in the U.S. We determined the percentages of fungal invasion in about 3,600 samples over 2 yr, and related high or low fungal populations to geographic, seasonal, and grade factors. Insect incidence in the same samples is reported elsewhere (12).

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MATERIALS AND METHODS

Source of samples. Grain samples were obtained from the Federal Grain Inspection Service, U.S. Dept. of Agriculture (FGIS) from January 1977 to December 1978. FGIS inspectors were asked to send us a 1-kg sample from every 50th sublot of corn and every 100th sublot of wheat shipped from each export elevator in the U.S. and along the St. Lawrence Seaway in Quebec. Automatic samplers are used to collect samples for official grade determination from each sublot during shiploading. Sublots consist of up to 60,000 bushels. Beginning in late 1977 we increased the sampling frequency by requesting one random sublot sample from each shipload of wheat and corn. Samples were received from 79 export elevators. About 1,000–1,200 g of the sample taken for grading was placed in polyethylene containers and mailed to our laboratory. All samples were numbered to identify the elevator location, loading date, and ship name. Other information such as grade data and shiplot size were obtained when possible. Samples were usually processed at our laboratory the day they arrived.

Determination of fungi. Corn kernels were shaken for 1 min in 5.25% NaClO (Clorox), rinsed in sterile water, and 100 were placed on malt agar containing 4% NaCl and 200 ppm Tergitol NPX (Union Carbide Corp.). Wheat kernels were rinsed 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 the aid of a dissecting microscope. A total of 359,300 kernels were plated and examined during the 2-yr study.

Aflatoxin tests. Although the samples were not large enough for reliable estimation of aflatoxin 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 (11). The presence or absence of bright greenish yellow fluorescence (BGYF) was noted. Samples with visible BGYF, and a few without, were then more finely ground for aflatoxin analysis by using thin-layer chromatography techniques (9).

RESULTS

Incidence of fungi. The fungi found in grain can be classified into two groups, field fungi and storage fungi. Field fungi, such as *Alternaria* and *Fusarium* spp., grow in the developing kernels when the moisture content is high (20–40%), but usually not in stored grain. These fungi gradually die during storage, so that old grain generally has low counts of viable field fungi. Low numbers of field fungi can also reflect arid growing conditions or high drying temperatures (corn).

Alternaria was the most common field fungus in wheat, followed by *Fusarium*. Both were present in most samples, and often in the same kernel. *Cladosporium, Epicoccum*, and *Helminthosporium* occurred less frequently. *Fusarium moniliforme* was the most common field fungus in corn samples. Others were *Cephalosporium, Cladosporium, Trichoderma, Nigrospora, Alternaria, Helminthosporium, Mucor, Rhizopus, Chaetomium, Diplodia*, and *Epicoccum*. Field fungi were found in essentially 100% of both corn and wheat samples. The average percent of kernels invaded is shown in Tables 1 and 2.

Storage fungi in both wheat and corn consisted primarily of *A. glaucus* (group species). *A. glaucus* can grow in the marginal moisture content range of 14–16%, and is probably the most prevalent grain storage fungus worldwide. It is less aggressive and destructive than some other *Aspergillus* species, and is not known to be a mycotoxin producer. However, it causes mustiness, loss of germinability, and can create a favorable environment for more destructive fungi (3).

A. flavus was found frequently in corn samples, but usually in only a low percentage of kernels. It can invade corn in the field, particularly in the southeastern U.S., so our data may reflect both preharvest and postharvest invasion. *A. flavus* requires grain moisture in excess of 17% for growth (7). Other species of *Aspergillus*, such as *A. candidus, A. restrictus, A. versicolor, A. ochraceus*, and *A. niger*, were found occasionally, but never in high percentages of kernels. Invasion by *Aspergillus* species other than *A. flavus* and *A. glaucus* averaged 0.8% in corn and 0.1% in wheat.

Average percentages of kernels infected with storage fungi was ten times higher in corn than in wheat (Tables 1 and 2). One or more *A. glaucus*-infected kernels per 100 were detected in essentially all corn samples, and *A. flavus* and *Penicillium* were found in 72 and 98% of the samples, respectively. Some *Penicillium* species invade corn before harvest (2). Nearly 80% of the wheat samples had detectable *A. glaucus*, but only 1% had *A. flavus*. *Penicillium* was also infrequent in wheat compared to corn.

Percentages of *A. glaucus* in corn were higher in 1977 than in 1978, (Table 1) possibly because of slightly higher average moisture contents in 1977 samples. Another factor may have been the time between sampling at the port elevator and arrival of the sample at our laboratory. Long delays occurred most frequently during the early part of the survey. For example, the 42 samples taken in January, 1977 took an average of 40 days to reach our laboratory. Their average *A. glaucus* count was 39%, compared to 29% for the entire year (Table 1). Data for 244 corn samples with moisture contents of 15.0% or higher showed average *A. glaucus* counts of 16% in the 159 that arrived at our laboratory within 20 days. Those taking longer than 20 days averaged 32% *A. glaucus*, indicating the fungus grew in the sample bags. Delays had no effect on samples with lower moisture contents. Average *A. glaucus* counts were no doubt somewhat higher when we tested the samples than they were at the time of ship loading. To that extent, the data reflected the poor storability of the corn rather than the amount of fungal invasion at the time of loading.

Incidence of storage fungi was lower in wheat and corn samples from the Pacific region compared to the other three regions (Tables 1 and 2). Within the regions there were differences among port areas (Tables 3 and 4). Corn from the Toledo-Maumee area had the highest *A. glaucus* counts, whereas the Norfolk area was highest in *A. flavus*. Some corn exported from the Norfolk and Mobile areas is grown in the southeast where preharvest invasion by *A. flavus* is

TABLE 1. Average percentages of surface-disinfected U.S. export corn kernels from which various fungi grew

Region	Year	Samples (no.)	Kernels (%) with *Aspergillus* *glaucus*	*Aspergillus* *flavus*	*Penicillium*	Field fungi
Pacific	1977	11	20	0.8(45)[a]	4.9	23
	1978	126	15	1.0(53)	6.9	24
Great Lakes	1977	165	30	2.0(76)	7.0	12
	1978	337	20	1.4(69)	7.4	16
Atlantic	1977	232	28	2.2(79)	5.7	13
	1978	291	16	2.0(75)	7.0	27
Gulf	1977	233	30	2.4(82)	6.6	15
	1978	545	17	1.4(69)	7.8	29
All regions	1977	641	29	2.2(79)	6.3	13
	1978	1,299	17	1.5(69)	7.4	25

[a] Numbers in parentheses are percentages of samples with 1% or more of the kernels containing *A. flavus*.

TABLE 2. Average percentage of surface-disinfected wheat kernels from which various fungi grew

Region	Year	Samples (no.)	Kernels (%) with *Aspergillus* *glaucus*	*Aspergillus* *flavus*	*Penicillium*	Field fungi
Pacific	1977–1978	464	1.5(63)[a]	0.02(1)	0.12(9)	33
Great Lakes	1977–1978	537	3.5(90)	0.01(1)	0.07(7)	57
Atlantic	1977–1978	77	4.0(95)	0.05(5)	0.19(11)	44
Gulf	1977–1978	575	2.4(80)	0.01(1)	0.08(6)	31
All regions	1977	291	2.3(76)	0.02(2)	0.09(7)	39
	1978	1,362	2.6(80)	0.02(1)	0.10(7)	41

[a] Numbers in parentheses are percent of samples in which 1% or more of the kernels contained the fungus.

TABLE 3. Fungi and aflatoxin found in U.S. export corn samples from ten port areas, 1977–1978

Port area	Samples (no.)	Kernels (%) with *Aspergillus* *glaucus*	*Aspergillus* *flavus*	Samples (no.) with aflatoxin ≥3 ppb	≥20 ppb
California	59	17	0.6	0	0
Seattle-Portland	78	15	1.3	1	1
Duluth-Superior	82	16	1.6	1	0
Chicago-Milwaukee	141	20	1.4	0	0
Toledo	276	27	1.6	1	0
Philadelphia-Baltimore	337	23	1.7	1	0
Norfolk	183	17	2.8	33	10
Mobile	78	18	2.1	12	5
New Orleans	575	22	1.7	17	1
Houston	119	14	1.5	1	0

TABLE 4. Fungi found in U.S. export wheat samples from ten port areas, 1977–1978

Port area	Samples (no.)	Kernels (%) with *Aspergillus* *glaucus*	*Aspergillus* *flavus*	Field fungi
California	48	0.2	0.02	35
Seattle-Portland	416	1.6	0.01	33
Duluth-Superior	323	2.4	0.01	62
Chicago-Milwaukee	31	6.4	0.06	29
Toledo	65	6.7	0.02	57
Quebec	118	3.9	0.01	52
Philadelphia-Baltimore-Norfolk	77	4.0	0.05	44
Mobile	8	4.0	0.0	47
New Orleans	191	3.7	0.03	41
Houston	376	1.8	0.01	25

common. Wheat from the Toledo area was highest in *A. glaucus*, while the Texas Gulf (Houston) area and the West Coast were lowest. The Texas and West Coast areas account for about 75% of the U.S. wheat exports. The low counts at Houston probably reflect the low moisture content of wheat from the southern Great Plains. New Orleans wheat comes from areas further north and east, where moisture contents tend to be higher.

An attempt was made to correlate grade factors with populations of *A. glaucus*, the principal storage mold. Only the corn data were used, since invasion percentages were consistently low in wheat. The grade factor "damaged kernels" includes mold damage, so it might be expected to correlate somewhat with *A. glaucus* counts.

Increasing percentages of damaged kernels were associated with higher *A. glaucus* counts (Fig. 1). However, neither factor would accurately predict the other, because storage fungi are only one of several causes of damaged kernels, and mold invasion has to be severe before the kernels would grade visibly damaged. As indicated in Fig. 1, 65% of the samples had less than 3% damaged kernels, which is the limit for grade No. 1 corn. Most of the samples were actually grade 2 or 3 because of broken corn and foreign material (BCFM).

One would expect moisture content to correlate with storage mold counts in corn, because it is the critical factor in determining whether or not fungi will grow. We found somewhat the opposite relationship. Samples in the moisture content ranges 13.0–13.9, 14.0–14.4, 14.5–14.9, and ≥15.0% had corresponding average *A. glaucus* counts of 28, 24, 18, and 21%. The four moisture content ranges represented 18, 29, 29, and 24%, respectively, of the 1,023 samples with grade data available.

Lowest average moistures and highest average *A. glaucus* counts occurred in about August and September, just before harvest of the new crop. New crop corn averages highest in moisture and lowest in storage fungi (Fig. 2). Low storage temperatures in winter months probably account for the continued low *A. glaucus* counts until summer weather provided favorable growing conditions. Mold counts increased during the summer, even though average moisture content was declining.

Aflatoxin. Our samples were not large enough to provide reliable quantitative data on aflatoxin in individual lots, but the large number of samples tested should provide a reasonable sample base for making generalizations.

When 2,322 corn samples were ground under a long-wave UV, approximately 10% had visible bright greenish yellow fluorescence (BGYF). In many cases the BGYF was limited to a single kernel or piece of a kernel. The Atlantic region and specifically the Norfolk and Mobile port areas accounted for most of the aflatoxin positives (Tables 3 and 5). Of the 17 samples with more than 20 parts per billion (ppb), 15 came from the Norfolk and Mobile areas. Our data confirm other published and unpublished information indicating that the southeastern U.S. is the principal aflatoxin problem area (6).

Slightly over one-third of the BGYF positive samples contained detectable levels of aflatoxin, but only 9% had 20 ppb or more. That means about 1% of the samples had aflatoxin levels above the 20 ppb guideline level established by the U.S. Food and Drug Administration. Limited testing of BGYF negative samples revealed no aflatoxin and it is unlikely that many aflatoxin positives could have been found in BGYF negatives.

Insect-fungus interactions. One or more live insects were found in 22% of the corn samples and 18% of the wheat samples used in this study (12). There was no relationship between the presence of storage fungi and the presence of insects in the samples. We related insects to the presence of fungi indirectly, as follows: As a general rule, samples high in field fungi represent recently harvested grain and those low in field fungi represent old grain (3). Such a generalization is weak in samples such as ours, because we know nothing of their history, and must assume that they are mixtures from different sources. Nevertheless, if we look at corn during the period October-December of both years (the time new crop and old crop corn would most likely be in the market at the same time) the percent of insect infested samples is higher in samples low in field fungi (Table 6). Using 10 and 35% field fungi as cutoff points, insect infestation was more than twice as high in the "older" corn. Using 16 and 30% as cutoffs, many more samples were included, and there was still a large difference in insect infestation.

We found a similar relationship of high insect incidence in Great Lakes wheat samples that were low in field fungi. However, there was no such relationship in wheat from other regions. Wheat

TABLE 5. Bright greenish yellow (BGY) fluorescence and aflatoxin in 1977–1978 U.S. export corn samples

Region	Samples: tested (no.)	Samples: with BGY (%)	BGY positives (%): with aflatoxin	BGY positives (%): with ≥20 ppb
Pacific	137	5	12	12
Great Lakes	572	5	8	0
Atlantic	615	13	52	15
Gulf	998	10	34	7
All regions	2,322	10	36	9

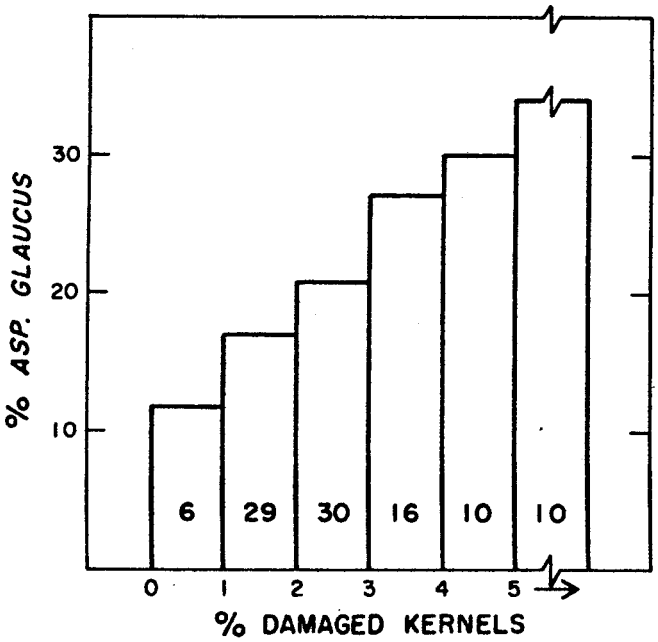

Fig. 1. Average percent invasion by *Aspergillus glaucus* in 1,023 U.S. export corn samples with different amounts of kernel damage. Percentages of samples in each category are indicated on the bars.

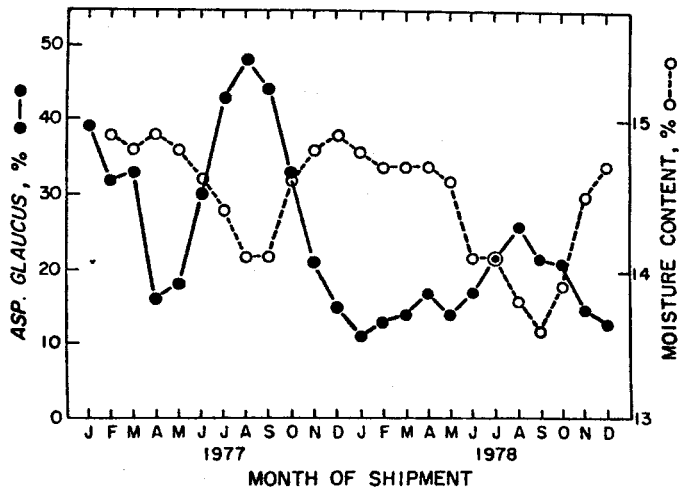

Fig. 2. Monthly variations in average moisture content and average percent invasion by *Aspergillus glaucus* in samples of U.S. export corn.

TABLE 6. Insect infestation in U.S. export corn samples with varying degrees of invasion by field fungi. Samples are from October–December of 1977 and 1978

	Field fungi (%)	Samples (no.)	Insect infested (%)
Probable older corn	≤10	66	38
	≤16	157	35
Probable newer corn	≥30	98	20
	≥35	54	17

shipped from the Great Lakes is primarily spring wheat, whereas Gulf wheat, for example, is more variable in class and origin. Estimating the age of grain based on its mycoflora is most reliable when the grain is from a known region and harvested at the same time of year.

DISCUSSION

A. glaucus was found in 21% of the corn kernels and 2.6% of the wheat. Even though the percentage of invasion was low in wheat, it was present in most (79%) of the samples. This would suggest that most bins or lots of wheat have a small amount of mold development, possibly in local areas where moisture migration or leaks occur. More likely, our samples were mixtures of grain lots, some of which may have had high mold populations, and others that were free or nearly free of storage fungi.

A large part of the U.S. wheat crop is harvested at moisture contents too low to support fungal growth. Proper storage and management will usually keep such grain relatively mold-free. Corn, however, is harvested, stored, and marketed at moisture contents favorable for molds. Grade No. 2 corn is the market standard, and its 15.5% allowable moisture content is widely used as a basis for adjusting the price of corn with higher moisture content. This has probably contributed to complacency about 15.5% moisture content, even though it is too high for long-term storage of corn. To the extent that mold growth is considered undesirable in corn, current moisture content guidelines for storage and marketing should be reduced. Mold invasion that takes place in a few weeks at 15.5% moisture may only be reached after several months at 14.5%. The establishment of a 14.0 or 14.5% moisture standard for corn would greatly reduce the average level of mold contamination found in commercial or export corn. It would also reduce mold problems that occur during delays in shipping or unloading. Lower moisture may, however, cause problems with increased brittleness, especially if corn is not dried carefully.

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