6 Molds and Mycotoxins in Stored Products

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The ecosystem within stored grain structures is limited in microbial species because of human efforts to maintain grain quality (Wicklow 1995; Sinha 1992). Of the more than 70,000 fungi species that have been described (Blackwell 2011), relatively few are found on grain. At harvest, grain contains populations of field microbes. Once the grain is placed into a storage facility, a succession of new microbial species begins to grow. Without intervention, microbial respiration will increase temperature and moisture, providing optimum growth conditions for even more diverse fungal species.

The type of grain, moisture, and temperature will influence the specific fungi that are associated with grain. The fungi on the maturing seed in the field typically require free water (liquid) for spore germination and high moisture to grow and flourish. Some of these fungi are pathogenic to the plant, while other fungi exist serendipitously. When grain is dried after harvest, most of the fungi on or in the grain before harvest will not be able to grow under the low-moisture conditions within the storage environment. Over time these fungi will slowly die off. In an ideal grain ecosystem, spoilage can be prevented if grain mass moisture is maintained at levels below that at which microbes grow. In practice, temperature and air movement continually change within the grain mass. This causes moisture to migrate, resulting in areas with moisture conditions that allow storage fungi to grow and slowly impact grain quality (Wicklow 1995; Sinha 1992; Dehoff, et al. 1984; Ayerst 1986).

The amount of water available in an environment is often measured in values of water activity (a_w) (Reid 1980). Water activity equals the vapor pressure of pure water divided by the vapor pressure of a solution at a given temperature. The equation for calculating a_w is essentially the same as for relative humidity. The two values are equal when the air and solution are at equilibrium. Unlike aquatic environments where water activity is based on the concentration of solute, in dry environments water in a gas phase is in equilibrium with the surrounding matrix, e.g., soil, lumber, or dry grain (Yanagita 1990).

Describing the water available in these environments is more complex. In most cases it is composed primarily of hygroscopic water that is strongly adsorbed to insoluble particles. The water activity in the dry environment fluctuates in equilibrium with the relative humidity of the air surrounding this matrix (grain mass)(Yanagita 1990). A moisture value often used in grain storage is the equilibrium moisture content (EMC). This value is the water activity (a_w) when the moisture in the grain is at equilibrium with the moisture (humidity) in the air spaces between the grain. Of course the temperature as well as the proportion of starch and oil in the grain greatly impact EMC values. EMC tables are available for each grain type (Tables 1a and 1b). These tables provide information about how humidity (water activity) in the air between the seeds will affect the seed moisture content at different temperatures. They also provide information on how the humidity (water activity) will change when grain temperature increases or decreases in grain stored at a particular moisture content. When using these tables, it is important to remember that the moisture limit for microbial growth is around 65 a_w. Although most field fungi and bacteria require conditions above 95 a_w, some storage fungi can grow at 70 a_w. As the humidity (water activity) approaches 90%, storage fungi grow faster. It is important to pay attention to EMC values of stored grain during summer storage.

Impact of Disease on Grain Storability

Grain quality prior to storage is a major criterion for determining whether the grain will maintain quality during long-term storage. Any breakdown in seed coat integrity allows easy entry for storage fungi. Preharvest grain disease will reduce storability. The greater the number of kernels affected, the greater the probability of spoilage during storage. Such grain must be maintained under the driest conditions and carefully monitored during the warm seasons.

Mycotoxins – There are a number of diseases that affect corn and small grains (Bockus et al. 2010; White 1999). All of them can cause seed coat damage and affect storability, but this chapter will discuss only five diseases, four in which the fungal pathogens produce mycotoxins. Mycotoxins are chemicals produced by fungi that are toxic to animals and humans. They are grouped into classes based on chemical structure. The mycotoxins of most concern in grain are produced by species of *Aspergillus*, *Fusarium*, and *Penicillium* (Council for Agricultural Science and Technology 2003). Field surveys that determine incidence and severity of disease before harvest allow the producer to make proper decisions about selling, testing for mycotoxins, and storing the grain.

Fusarium ear rot – This is an important disease of corn. The disease can be found virtually every-

Table 1a. *Equilibrium moisture content of corn kernels.*

Air Temp. (˚F)	Relative Humidity (%)													
	30	35	40	45	50	55	60	65	70	75	80	85	90	95
30	11.4	12.1	12.7	13.3	14.0	14.6	15.2	16.0	16.7	17.6	18.6	19.8	21.4	24.0
40	10.6	11.3	12	12.5	13.1	13.8	14.5	15.2	16	16.9	17.9	19.1	20.8	23.4
50	9.9	10.6	11.2	11.9	12.5	13.1	13.9	14.6	15.4	16.3	17.3	18.6	20.2	22.9
60	9.3	9.9	10.6	11.2	11.9	12.6	13.3	14.0	14.9	15.7	16.8	18.1	19.7	22.4
70	8.7	9.4	10.0	10.7	11.4	12.0	12.7	13.5	14.3	15.2	16.3	17.6	19.3	22.0
80	8.2	8.9	9.6	10.2	10.9	11.6	12.3	13.1	13.9	14.8	15.9	17.1	18.9	21.6
90	7.7	8.4	9.1	9.8	10.4	11.1	11.9	12.6	13.5	14.4	15.5	16.8	18.5	21.3
100	7.3	8.0	8.7	9.4	10.0	10.7	11.5	12.2	13.1	14.0	15.1	16.5	18.2	21.0

Table 1b. *Equilibrium moisture content of wheat.*

Air Temp. (˚F)	Relative Humidity (%)													
	30	35	40	45	50	55	60	65	70	75	80	85	90	95
30	10.9	11.3	11.8	12.2	12.7	13.2	13.7	14.2	14.8	15.4	16.2	17.1	18.4	20.4
40	10.4	10.8	11.3	11.7	12.2	12.7	13.2	13.7	14.3	15.0	15.8	16.7	18.0	22.0
50	9.9	10.4	10.8	11.3	11.8	12.3	12.8	13.3	13.9	14.6	15.4	16.3	17.6	19.6
60	9.5	10.0	10.4	10.9	11.4	11.9	12.4	12.9	13.5	14.2	15.0	16.0	17.2	19.3
70	9.2	9.6	10.1	10.6	11.0	11.5	12.1	12.6	13.2	13.9	14.7	15.7	16.9	19.0
80	8.8	9.3	9.8	10.2	10.7	11.2	11.7	12.3	12.9	13.6	14.4	15.4	16.7	18.7
90	8.5	9.0	9.5	10.0	10.4	10.9	11.5	12.0	12.6	13.3	14.1	15.1	16.4	18.5
100	8.2	8.7	9.2	9.7	10.2	10.7	11.2	11.8	12.4	13.1	13.9	14.9	16.2	18.3

Source: Postharvest Pocket Guide (1995), Purdue University Extension ID 215 where corn is grown. Most often the disease is associated with insect damage on the ear, but the pathogen can infect kernels without insect damage. Infected kernels can be scattered on the ear and appear tannish or salmon-pink. Often, white streaks referred to as "starburst" are visible on the top of the kernel (Figure 1). Fusarium ear rot is most often caused by *Fusarium verticillioides* and *F. subglutinans*. Although it is impossible to visibly distinguish these two pathogens by disease symptoms, *F. verticillioides* produces the group of mycotoxins known as fumonisins. Fumonisins have many adverse effects on animals that consume the contaminated grain. Equine (horses) are most sensitive, but swine are also affected at relatively low levels (Voss et al. 2007). The FDA has set advisory levels for fumonisin in food and feed (Table 2).

Figure 1. *Fusarium ear rot showing starburst.*

Table 2. *FDA advisory limits for fumonisins.*

Human Foods	
Product	Total fumonisins (FB_1+FB_2+FB_3)
Degermed dry milled corn products (e.g., flaking grits, corn grits, corn meal, corn flour with fat content of <2.25%, dry weight basis)	2 parts per million (ppm)
Whole or partially degermed dry milled corn products (e.g., flaking grits, corn meal, corn flour with fat content of ≥2.25%, dry weight basis)	4 ppm
Dry milled corn bran	4 ppm
Cleaned corn intended for masa production	4 ppm
Cleaned corn intended for popcorn	3 ppm

Animal Feeds	
Corn and corn by-products intended for:	Total fumonisins (FB_1+FB_2+FB_3)
Equines and rabbits	5 ppm (no more than 20% of diet)**
Swine and catfish	20 ppm (no more than 50% of diet)**
Breeding ruminants, breeding poultry, and breeding mink*	30 ppm (no more than 50% of diet)**
Ruminants ≥3 months old being raised for slaughter and mink being raised for pelt production	60 ppm (no more than 50% of diet)**
Poultry being raised for slaughter	100 ppm (no more than 50% of diet)**
All other species or classes of livestock and pet animals	10 ppm (no more than 50% of diet)**

* Includes lactating dairy cattle and hens laying eggs for human consumption
** Dry weight basis
Source: U.S. Food and Drug Administration
http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation

Gibberella ear rot – This disease of corn is caused by the fungus, *Gibberella zeae* (*Fusarium graminearum*). This disease occurs in cool, wet areas during the first 21 days after silking begins. Extended periods of rain in the fall before harvest often increases disease severity. Ear rot is most severe in fields where corn follows corn or corn follows wheat. The pathogen survives on residue left from the previous crop, and during wet conditions the pathogen releases spores into the air. The disease is easy to recognize in the field by pulling back the husk to reveal the pinkish rot at the tip of of the ear and silks that adhere tightly to the ear (Figure 2). The pathogen produces two mycotoxins in the infected kernels: deoxynivalenol and zearalenone. These mycotoxins can affect the health of many monogastric animals, with swine being most sensitive. If ear rot is present, assume that mycotoxins also are present. The FDA has set advisory levels for deoxynivalenol in food and feed (Table 3).

Figure 2. *Gibberella ear rot.*

Head blight of wheat (head scab) – Like the corn disease Gibberella ear rot, this infection occurs during flowering when the weather is wet and humid. The clearest symptoms of the disease is the early bleaching of heads when healthy plants are still green. At harvest, diseased kernels are often shriveled, lightweight, and pinkish. Both deoxynivalenol and zearalenone will be found in the shriveled kernels, but mycotoxins are often found in kernels that appear healthy. The FDA has set advisory levels for deoxynivalenol in food and feed (Table 3).

Aspergillus ear rot – Caused by *Aspergillus flavus*, this disease commonly occurs during hot, dry years in fields under drought stress. Ear-invading insects also contribute to disease development. To identify the disease, peel back the husk and look for an olive-green fungus on the ears. The fungal spores, which are the olive-green material, will appear powdery and may disperse like dust when the husk is pulled back. Symptoms are mostly observed at the tip, but when the disease is severe, kernels all the way to the base of the ear can be infected (Figure 3). The mycotoxin, aflatoxin, will be found in grain with this disease. Aflatoxin is a potent liver toxin and carcinogen. The presence of aflatoxin will affect livestock health if the grain is consumed. Feeding aflatoxin-contaminated grain to dairy cattle is a concern because the mycotoxin will pass into the animals' milk. There are strict legal limits on the amount of aflatoxin in grain and milk products (Table 4).

***Figure 3**. Aspergillus ear rot.*

Table 3. *FDA advisory limits for deoxynivalenol.*

Deoxynivalenol/Vomitoxin	FDA Advisory Level
Humans (finished product)	1 ppm
Cattle and chickens (all grains, distillers grain)	10 ppm (not to exceed 50% of diet)
Swine (all grains and grain products)	5 ppm (not to exceed 20% of diet)

Source: U.S. Food and Drug Administration
http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation

Table 4. *Action levels established by the FDA for the use of aflatoxin-contaminated corn.*

Action Level (parts per billion)	Commodity
20 ppb	Corn for animal feed and feed ingredients intended for dairy animals
20 ppb	Corn for human consumption
100 ppb	Corn grain intended for breeding cattle, breeding swine, and mature poultry
200 ppb	Corn grain intended for finishing swine of 100 pounds or greater
300 ppb	Corn grain intended for finishing beef cattle

Source: U.S. Food and Drug Administration
http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation

Diplodia ear rot – The fungus, *Stenocarpella maydis,* causes this disease. Affected ears have grayish or grayish-brown fungus on and between the kernels and tan or brown kernels. With severe disease, the entire ear will be affected and will be very lightweight. Another diagnostic sign of the pathogen is the presence of small black specks, called pycnidia, which will be scattered on the husks, cobs, and sides of kernels (Figure 4). No major mycotoxins are associated with Diplodia ear rot.

Figure 4. *Diplodia ear rot.*

Storage Fungi

The distinction between field and storage fungi is based not on taxonomic classification, but rather on life habits. Storage fungi have adapted to grow well under dry conditions. Debris, such as dirt, chaff, and green tissues (pods and beans) are a reservoir for storage fungi and moisture. Although a few of the fungi from the field can grow under storage conditions, most will invade the grain after harvest. The fungi enter the grain through breaks in the seed coat caused by mechanical damage, insects, or preharvest diseases. Grain stored for only a few weeks at any combination of moisture content and temperature that permits even moderate invasion by storage fungi will be at high risk if kept in continued storage. Research has shown that grain moisture level will greatly influence the fungal species that can attack and grow on the grain (Sauer et al. 1992). Under the driest conditions, species such as *Aspergillus restrictus* and *A. glaucus* can grow. As the grain moisture rises to 16 to 18% in corn and 15 to 17% in soybeans, *Penicillium* species and even the aflatoxin-producer *A. flavus* can grow. All of these fungi can cause germ damage, mustiness, caking, and attract fungal feeding insects. Determination of what fungal species are on the stored grain requires laboratory examination and culturing, which may take several days to determine. Once storage fungi become established in the grain, they continue to develop at moisture and temperature levels below those required for the initial invasion of sound grain. Preventing them from infecting the grain is essential.

Blue eye in corn – A common type of spoilage in corn, known as “blue-eye,” often appears when the grain is not properly dried before storage. A blue-green line will appear on the surface of the germs, under the seed coat (Figure 5). The visible color is actually the spores of either an *Aspergillus* species or a *Penicillium* species, which has invaded the germ tissues. The spores are produced in that restricted area because the fungi are growing strictly in the germ tissues. A laboratory identification of the fungal species causing the blue-eye can provide information about why the damage occurred. In corn stored at about 20% moisture or more and temperatures of 41° to 50°F (5°to 10°C), these spore masses are often *Penicillium. Penicillium* blue-eye sometimes develops when whole ears (with cob) are stored. Some species of *Penicillium* produce the mycotoxin ochratoxin, a potent kidney toxin. Poultry are very sensitive to ochratoxin. In corn stored at moisture contents of about 14.5 to 15.5% and temperatures of 50° to 59°F (10° to 15°C) or higher, the spore masses are those of *Aspergillus restrictus* or *A. glaucus*.

Figure 5. *Blue-eye of corn.*

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