Toxic and Nontoxic MOLDS Found in Food Materials

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TOXIC and NONTOXIC MOLDS
FOUND in FOOD MATERIALS[1]

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In this report the terms *toxic* and *toxin* refer to substances produced by fungi that have a detrimental effect on the metabolism of animals and man. The term food materials is quite broad, but here it is restricted to food of plant origin, such as seeds, seed products like flour, meals, mash, bran, and in a few cases forage, hay, straw, and fodder. Mold is used in the collective sense and synonymous with fungus, generally referring to saprophytic or weakly pathogenic fungi rather than aggressive plant pathogens.

This report is a summation of many papers and several excellent reviews of literature that deserve special mention: 1) George Semeniuk, chapter on "Microflora" in Storage of Cereal Grains and Their Products, 1954 *(15)*; 2) C. M. Christensen, "Deterioration of Stored Grains by Fungi" in Botanical Review, 1957 *(5)*; and 3) Forgacs and Carll, "Mycotoxicoses" in Advances in Veterinary Science, 1962 *(7)*. For anyone interested primarily in the subject of aflatoxin, the bibliography of Skau, Robertson, and Mayne *(16)* encompasses the literature from 1960 through 1963.

Fungi (Molds) Associated with Food Materials of Plant Origin

Many species of fungi and bacteria make up the microflora of seed and seed products. Semeniuk *(15)* reported nearly 70 species of bacteria, 7 species of Actinomycetes, more than 40 species of yeasts and yeastlike fungi, and more than 50 species of fungi have been found externally on cereal grains and their products. About 25 species of parasitic fungi and bacteria were reported to be carried internally by cereal grain seeds alone. Colony counts ranged from a few hundred on some seed to as high as 41,600,000 colonies per gram of moldy grain *(6, 15)*. The fungi associated with cereals were divided by Christensen *(5)* into: 1) the field fungi — major genera consist of *Fusarium, Rhizoctonia, Alternaria, Helminthosporium, Diplodia, Cladosporium, and Rhizopus;* and 2) the storage fungi — principally *Aspergillus* and *Penicillum.*

Thus, a great number of genera and species of fungi and bacteria are associated with seed and food materials. Jackson *(9)* recently listed 132 species of fungi that have been reported to be associated with peanut pods and kernels. Because of their great reproductive capacity in favorable environments, these molds occur in huge numbers.

Origin, Nature, and Determination of Fungi

Fungi found in and on food materials are identical to those found in the soil and air, and on living and dead plants and animals. Some are pathogens of living organisms, whereas others are primarily saprophytes (living on soil or organic matter). Field fungi invade developing plants and seeds in the field prior to harvest, especially under moderately warm and moist weather conditions. In order to grow in seeds, fungi require moist environments with a moisture content in equilibrium with relative humidities from 90 to 100 per cent. Most seed crops are harvested at moisture contents and relative humidities below the level favorable for further invasion of seed by these fungi. Therefore, field fungi gradually diminish in quantity as seed moisture declines in curing and depending upon temperature and moisture of the environment around the seed.

Storage fungi consist mainly of 10 to 18 species of *Aspergillus* and *Penicillium* that are saprophytic on a wide variety of plant and animal residues as well as seed and seed products. Seed invasion occurs primarily following harvest when moisture contents are in equilibrium with relative humidities from 75 to 90 per cent and frequently accompanied by temperatures of 25 to 35° C. The determination of fungi present is made by isolation, cultivation on laboratory media, and subsequent identification using a microscope. Field fungi can be cultured on most common laboratory media, such as potato-dextrose agar and Czapek's Dox medium. However, the storage fungi, especially the important *Aspergillus glaucus* group of five to eight species, cannot be cultured satisfactorily on these media. For good growth, sporulation, and development of perithecia, they require culture media containing high concentrations of sucrose or salt (NaCl), such as Thom and Raper's Czapek solution agar with 20 per cent sucrose *(17)*, or Christensen's malt agar with 8 to 20 per cent salt *(4)*. *A. flavus,* the *A. glaucus* group, other Aspergilli, and many *Penicillium spp.* grow well on these media of high osmotic tension.

Factors That Determine Mold Invasion

Moisture is the most critical factor, whether it is the water content of the material or the relative humidity around the food stuff, since in a restricted, unaerated, or a stable environment they come to equilibrium (i.e.-hygroscopic equilibrium). Most fungus species remain dormant and/or slowly die out under conditions of low moisture. Under these conditions they perish faster at high temperatures than at low. The degree of invasion by the fungus prior to storage of food materials will affect the moisture relationship, since fungi directly produce water as a product of respiration as do most other living things. Heavily-infested seeds, dried to safe moisture levels, have been reported to continue being deteriorated by fungi.

Storage fungi and field fungi generally grow well at temperatures of 25 to 30° C. However, many species of storage fungi grow well up to 35 to 38° C. *A. flavus* grows well on certain substrates up to 45° C. (113° F). Growth rates of most mesophilic fungi are greatly reduced at temperatures below 20° C., although certain *Penicillium*

species and a few Aspergilli will grow slowly at 2 to 5° C. under conditions of high moisture.

Time is an important factor as it relates to storage of food materials with suboptimal environments that permit only limited mold development. During extended periods, fluctuations of moisture and temperature result in changing environmental conditions that may favor mold growth for short periods of time, during which deterioration is progressive and accumulative in its effect.

Contamination of food materials with foreign matter of high moisture content, or of a hygroscopic nature can be a factor in promoting mold development. Insect and/or animal infestation may introduce moisture and produce substrates favorable for mold development.

Molds are typically aerobic in nature and growth rates decrease with lowering of oxygen tensions. High levels of CO_2 concentration (above 14 per cent) inhibit growth of many molds.

Deteriorative Effects of Fungi

Seeds lose germinability, germs become discolored, and musty odors develop in stored grain as a result of the presence of molds. Christensen (5), Milner and Geddes (11), and others have documented these facts.

Food nutritive value is destroyed by molds. Zeleny (19) has reviewed the literature on chemical, physical, and nutritive changes during storage. Nagel and Semeniuk (13), Milner and Geddes (10), and Ward and Diener (18) have demonstrated the biochemical changes in corn, soybeans, and peanuts caused by fungi. These and other investigations have shown that losses of organic matter, carbohydrates, and oil are accompanied by increases in free fatty acids and development of off-odor, changes in flavor, and changes in color of oil. Thus, quality goes down as rancidity goes up. Edibility or palatability of food or feed may be reduced.

Changes in respiration, moisture content, and heating of damp grain as caused by fungi have been reviewed by Milner and Geddes (11).

Economic losses in terms of value of food for animal and human consumption, caused by deterioration as result of mold, have been estimated at 1-2 per cent of the annual crop. Seed losses, poor stands, and land replanting are all economic losses caused by fungus deterioration of seed germinability.

Production of Toxins by Fungi

Mycotoxicoses are poisonings after entrance into the host's body of toxic substances of fungal origin, Forgacs and Carll (7). Most micro- and macro-organisms secrete or excrete a toxin or toxins in normal metabolic processes that are toxic or have detrimental effects on one or more other organisms. However, this discussion is limited to fungus-toxins affecting animals and man.

One of the earliest known fungus toxins is that found in "ergot" sclerotia on ryegrass produced by Claviceps purpurea. Rye was the main food cereal in the Middle Ages. Ergot contains alkaloids that affect the central nervous system causing convulsions and gangrene. Rye bread eaten by peasants in France in severe ergot years resulted in gangrenous infections and death to many people. Ergot, caused by other species of Claviceps, occurs on barley, wheat, dallis, brome, and other grasses. Ergot also causes death and abortions in livestock.

Barley and corn infected with head smut or scab contains toxic substances formed by the pathogenic fungus, Gibberella zeae (Fusarium spp.) during invasion of the living plant (12). Scabby grain is prevalent in warmer parts of humid Eastern and Central States. Swine losses are greatest following severe scab years because of feeding infested grain, especially barley.

Stachybotryotoxicosis is the result of ingestion of feeds (hay, straw, fodder) upon which strains of Stachybotrys atra have grown and formed a toxin. Horses were severely affected in Russia and Siberia in 1931. Cattle, domesticated and experimental animals (mice, guinea pigs, rabbits and dogs) as well as humans are susceptible to this toxin. Man may develop a rash.

Various Russian workers reported that extracts of A. flavus, A. fumigatus, A. nidulans, A. niger, A. calyptratus, Mucor albo-alter, and M. hiemalis were toxic to rabbits and other animals. Other workers have reported that various species of Aspergillus, Penicillium, and Rhizopus were toxic to swine, ducks, rabbits, and guinea pigs. Carll and coworkers implicated A. chevalieri, A. clavatus, and A. fumigatus as causes of bovine hyperkeratosis in cattle, horses, rabbits and mice.

Moldy corn toxicosis in swine was reported by Burnside, Sippel et al. (3) in Georgia and Florida. They found corn cultured with A. flavus, P. purpurogenum, and P. rubrum was toxic to swine and P. rubrum also affected the horse and goat. This toxicosis was probably identical to hepatitis X in dogs (1).

Moldy feeds are toxic to poultry — hemorrhagic syndrome. Alternaria sp., Aspergillus clavatus, A. flavus, A. fumigatus, A. glaucus, P. purpurogenum, P. rubrum, P. citrinum, and several other fungi have been shown to induce mycotoxicoses in chickens by Forgacs and Carll (7).

Facial eczema in ruminants (sheep and cattle) was first reported in 1942 to be caused by Sporodesmium bakeri growing on dead perennial ryegrass in New Zealand and Australia. Moldy bermudagrass toxicosis in fields of Southeastern United States also produces facial eczema in cattle. The fungus, Periconia minutissima, (Alabama, Florida) develops on bermudagrass after a killing frost or drouth is followed by warm rain and the dead grass becomes infested with various fungi. Any similar combination of conditions caused by drouth or other environmental factors will produce an outbreak in ruminants foraging such pastures.

Alimentary toxic aleukia (ATA) develops in human beings, who have ingested overwintered moldy grain or its by-products. Russia reported high mortality rates among those afflicted in 1913 in far eastern Siberia; in 1932 in western Siberia; and in 1941-45 in the Ukraine, the area south and west of the Ural Mountains, in central Asia, and in far eastern Siberia. The causal fungus was Fusarium sporotrichioides growing on cereal grains primarily proso millet. The fungus develops after a mild winter with abundant snows followed by frequent alternate freezing and thawing in the spring on millet allowed to overwinter on the ground. Farm animals that eat toxic grain are also affected. Mostly rural people are affected. It has been experimentally produced in cats, guinea pigs, dogs, and monkeys. Uniquely, this fungus grows slowly at −10° C., optimally at 24° C., with an optimal temperature for toxin production between 1.5 to 4° C. The toxin is stable for 30 minutes at 125° C. and 18 hours or longer

at 110° C. Moldy grain has remained toxic for 6 years.

Finally the fungus more or less responsible for this symposium — Aspergillus flavus — and its mycotoxin, aflatoxin, which is also produced by a closely related species, A. parasiticus, and possibly by Penicillium puberulum.

In 1960 (2) an apparently new disease occurred in turkey poults in England causing a loss of more than 100,000 birds at 500 locations. Outbreaks in ducklings, young pheasants, swine, and calves were subsequently reported. Brazilian groundnut (peanut) meal was found to be involved in all cases. Similar disease outbreaks occurred in East Africa and India from local peanuts. Sargeant et al. (14) isolated a toxin-producing strain of Aspergillus flavus from toxic Uganda peanuts. Considerable data have established that toxic groundnut meals and feeds had been present in England since 1951. In the USA, moldy corn toxicosis in 1957 (3) and hepatitis in dogs in 1959 (1) had already been shown to be caused by A. flavus and P. rubrum.

Aspergillus flavus has been found to be prominent in the microflora of cereal grains, oilseeds, other seed crops and seed products (5, 15). It grows profusely and produces aflatoxin on most seed crops and seed products in the laboratory. A fairly adequate method for determining aflatoxin has been developed and aflatoxin has subsequently been found in low quality stocks of most cereals, oilseed crops, and their products. Toxin-producing strains are probably country-wide and world-wide in distribution.

Elimination and Control

The first step in control is the recognition and awareness that the problem and threat exists. Prevention and control of mycotoxins are dependent on control of factors influencing mold growth, such as the following:

Harvest seed crops at maturity and follow curing and drying techniques to rapidly reduce seed moistures to safe levels for storage. Utilize modern drying methods.

Store cleaned and sound seed in dry, aerated, insect-free storage facilities. Foreign matter, whether soil or plant debris, damaged seed, and immature seed may affect aeration and moisture availability unfavorably.

Feed and food processors should determine toxin presence by chemical procedures before products are manufactured and processed.

Research may eventually yield methods for detoxifying mycotoxin-contaminated feeds and food materials. Improved techniques for growing, harvesting, drying, and storing food materials to prevent mold development are being further investigated. Basic research on the influence of environment on mold growth in food products and effect of chemicals on mold development are being studied.

Summary

Despite thousands of plant diseases and species of fungi associated with food materials, relatively few have proved to be toxic to man and animals. Many species of fungi contribute to the welfare of mankind in destroying organic waste, formation of mycorhizae, alcohol and organic acid production by fermentation, direct utilization as food (mushrooms), food production such as cheese and bread making, livestock feed as fermentation byproducts, antibiotics, medicinal compounds such as vitamins, and chemical synthesis of enzymes, glycerol, and fats. These benefits are in opposition to harmful activities of fungi such as

plant diseases, decay of timber, tropical deterioration of textiles, food spoilage, allergens, and toxin-production.

Storage fungi are important in stored food materials because they grow at low moistures, fairly high temperatures, and are universally present in air and soil. Fungi adversely affect seed viability, storage quality, nutritive value, edibility, and occasionally produce toxins in seed and food products.

Although toxin production is characteristic of most micro-organisms, only a few toxins affect animals. The present concern over mycotoxins has been emphasized because aflatoxin is a carcinogen at very low levels in the diet of experimental animals.

Control probably will be accomplished by utilizing knowledge of moisture, temperature, and aeration to prevent development of fungi during harvest, curing, storage, and processing of food materials. Fungicidal chemicals may afford some promise for mold and toxin control.

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Histological Studies
of Root and Shoot from
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Histological Studies of Root and Shoot from

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T RIFLURALIN (α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) is known to cause marked effects on morphology of roots (1,3,4,5).

The presence of multinucleate cells and the interference in cell division in roots following trifluralin treatment have been reported in several species (1,5). Laboratory studies at Auburn (3) showed that in some species trifluralin also caused swelling of shoots. The study reported here was designed to examine the nature of swelling caused by trifluralin both in roots and shoots of corn (*Zea mays* var. Dixie 18).

The corn used in this study was germinated and grown in the dark for 3.5 days in 5 p.p.m. trifluralin solution and in water at 82 F. Treated corn showed an abnormal swelling of root tips and first internodes (Fig. 1) Three 5 mm. segments of root beginning at the tip and three 5 mm. segments of shoot from the base of first internode were sampled and killed in FAA. The tissue was dehydrated, infiltrated, and finally embedded in wax. Transverse and longitudinal microtome sections 10 μ in thickness were cut. Sections were stained by the tannic acid-ferric chloride method of Foster (2). Radii of the sections were measured from a camera lucida sketch. The number of cells were counted in these radii and the average cell diameter was calculated.

Swelling in roots was maximum at about 3 mm. from the tip. In this region the radius of roots in treated seedlings was about 3 times larger than those of the check (Fig. 2). Increase in size of the cortex was larger than in the vascular tissue. The number of cells was slightly higher in trifluralin-treated seedlings, but the diameter of the cortex cells was more than double and they were also longer. Similar effects of trifluralin treatment on radius, number, and diameter of cells were noticed in next two segments (5 to 10 mm. and 10 to 15 mm.) of the roots. Length of the cortex cells, however, did not increase because of treatment in these segments.

In shoots the characteristic swelling from the trifluralin treatment was more obvious in 5 to 10 mm. segments of

first internode (Fig. 3). As in roots the larger part of the swelling was the result of increase in cortex cell size.

The epidermis in both roots and shoots of treated seedlings was either disorganized or not present (Fig. 2b and 3b). This could be attributed to the fact that the increase in epidermal cell size could not keep pace with the rate of increase in size of the cortex. Cortex cells near the edge were also very irregular in shape.

Multinucleate cells were noticed both in roots and the first internode sections of treated seedlings (Fig. 4). Nuclei, in some cases four to five, were generally aggregated and some of them were different in size. The frequency of multinucleate cells was greater in the apical 5 mm. section of roots to the 5 to 10 mm. segment of the first internode.

ACKNOWLEDGMENTS

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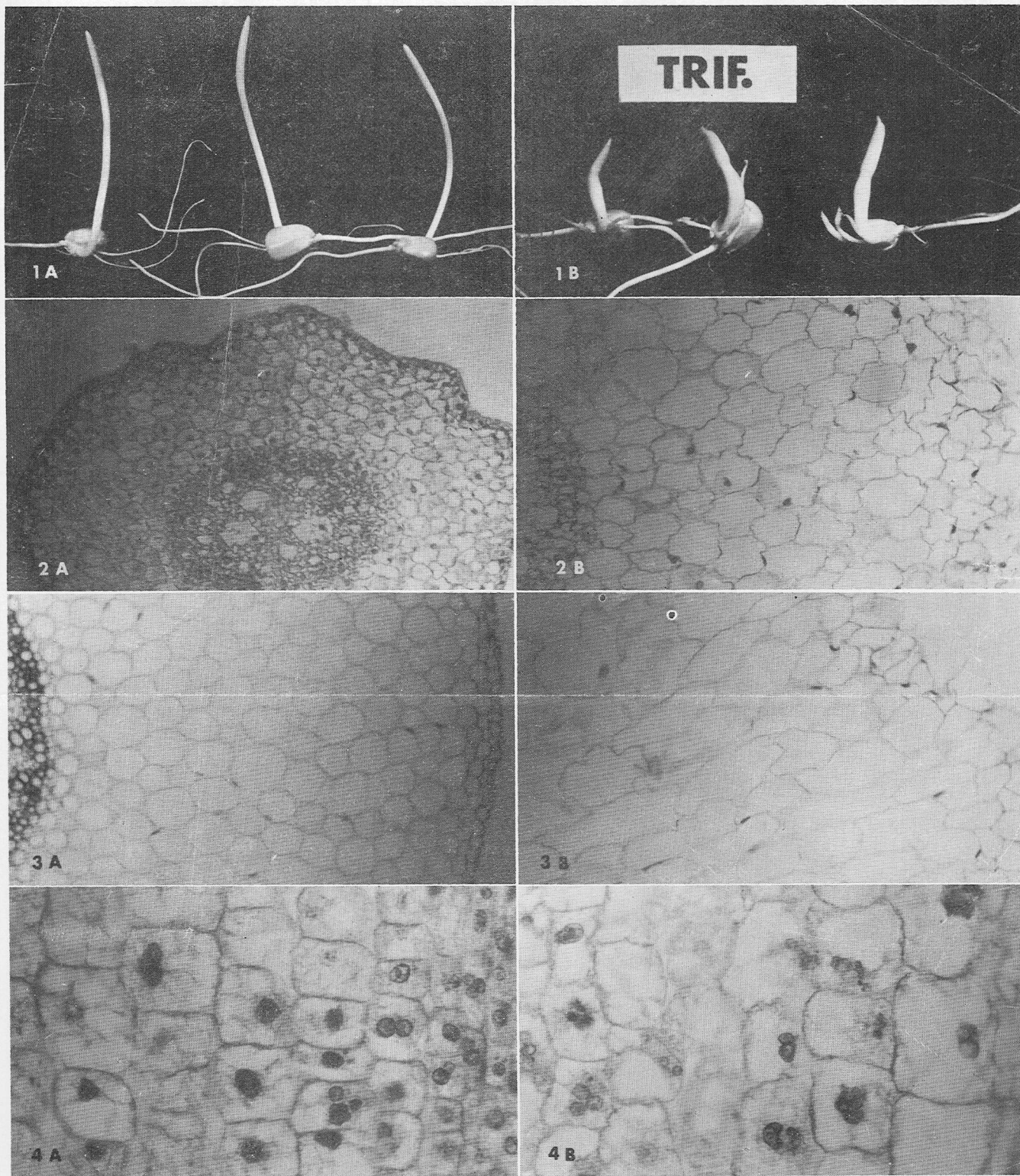
FIGURE 1. Corn seedlings 3.5 days old (A) grown in water, and (B) grown in 5 p.p.m. trifluralin. FIGURE 2. Cross section of root 3 mm. from tip, magnification 120X (A) check and (B) treated. FIGURE 3. Cross section of first internode from 5 to 10 mm. section, magnification 120X (A) check, and (B) treated. FIGURE 4. Longitudinal sections showing multinucleate cells in trifluralin-treated seedlings, magnification 610X (A) root and (B) first internode.