Grain Fungal Diseases & Mycotoxin Reference

Preface

This manuscript is a compilation of information that has been available to college professors, researchers, and grain trade specialists for some time. We will try to present this information in a way that will be helpful to non-specialists in an easy to digest manner. The nature of this information tends towards the technical and sometimes keeps needed information out of the reach of those that could use it most. We will try to keep this in mind while presenting the needed information. Also, while not being technically correct, we have kept reference citations to a minimum, and have inserted numbers, i.e.: [*23*], to keep the interruptions to the flow ideas as few as possible.

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Technical Services Division

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Chapter 1
Fungi and Fungal Diseases of Plants

Definition and Overview. Fungi are probably one of the most numerous plant families on earth. By definition they are plants that contain no chlorophyll (can grow in conditions of little or no natural light) and range from single cells to a body of branched hyphae (tubular filaments) that often produce fruiting bodies that form molds, mushrooms, smuts and yeasts. Instead of producing their own food, fungi absorb nutrients from either a living or dead host material. Symptoms and disease development come about from the growth of the fungi through the host-parasite interaction. These fungi sometimes produce metabolites (by-products of growth) that are toxic to animals and humans. Reproduction in fungi occurs through the production of spores. These spores can then reproduce without coming into contact with a different plant (asexual reproduction).

The small size of the spores aid in their dispersal. They can become airborne and move by the action of winds and travel from field to field. They also can become attached to insects and birds which then transport them from plant to plant. Transport can also occur by use of contaminated trucks and equipment.
Fungal infection from spores can occur at any of the various stages of crop production. It can begin in the fields, in or on the crop itself. It can infect healthy products during transportation and storage by coming into contact with contaminated equipment or grain products. The spores can lay dormant (inactive) in the soil or accumulate on equipment or in storage facilities for months or sometimes years until the proper conditions for growth occur and infect generation after generation.

There are many varied environmental conditions that need to be in place before the spores will germinate or begin to grow. Generally relative humidity over 70% and temperatures over 30°C (86°F) for extended periods (several days to a week) are generally needed. Stress to the plants such as periods of drought, flooding, or insect infestation are also common factors in the fungus growth cycle. High moisture content of the crops (20% or higher in corn), as at the optimal times of growth and harvest, give the spores the necessary elements to start the growth process.

Any one of these conditions by itself will not promote the fungal growth. It is only when the right conditions as a group occur, for each particular fungus, do the growth cycles begin. If any one optimal condition is removed, the growth cycle of a particular fungus will stop. But this may then promote the growth of a different or related fungus. Once the growth cycle begins the crop damage has already started and can not be reversed. The drying of corn lowers the moisture content to a point where growth of molds is prevented or at least stops further damage.

Fungi and mold are normally thought of as mushrooms and the grey fuzzy stuff that grows on food left in the refrigerator too long. Molds and fungi do not necessarily have to be

visible to the naked eye to be growing and damaging crops and foods. Many of the spores associated with fungi are microscopic and in their first stages of growth are not visible without the aid of a microscope. Only in the later stages or when large masses of fungi are present, do the fungi become visible to the unaided eye.

The presence of visual mold in grain or feed products does not necessarily mean that mycotoxins are present in the sample. Mold growth indicates that there are some fungi present. Many of the fungi grow under similar conditions and more than one kind of fungi can exist in or on the grain or feed product at the same time. When more than one fungi is present at the same time on the same host, they sometimes increase the toxic effects of each other by attacking different bodily functions when ingested by a human or animal. They can also cancel each other out by growing and attacking each other during the growth process. The lack of visual mold does not mean that there are no mycotoxins present. Many times during harvest or handling the visual aspects of the mold can be brushed off, but the toxic by-products can remain ***in*** the kernel.

Non-Mycotoxic Fungi

Fungi are a major cause of spoilage in stored grain. The Food and Agriculture Association estimates that 25% of the worlds food crops are affected by mycotoxins (the by-products of fungal growth) during growth and storage. The damage of fungi is second only to that caused by insects in stored grain products. Many of the fungi cause damage to the crops themselves with little or no toxic effects on humans and animals [*15*].

Common Smut or Bunt. Common smut is caused by two fungi; *Tilletia tritici and Tilletia laevis.* Because it requires cool moist soil conditions, the disease is less of a problem on spring planted wheat than winter wheat. Common smut reduces wheat yields and grain quality. Wheat contaminated with bunt spores has a pungent, fishy odor and a darkened appearance. Wheat that has an unmistakable odor of smut will be designated "light smutty" on official inspection certificates. In addition, bunt spores released during combining are combustible, and have caused explosions and fires during harvesting with mechanical harvesters. [23]

Plants may be moderately stunted but are not easily distinguished until the heads emerge. Bunted heads are slender and maintain their green color longer than healthy heads. The glumes may spread apart exposing the smut balls they contain. Smut balls are approximately the shape of normal kernels and are dull gray-brown. The small balls often rupture at harvest, releasing black, powdery spores. Wheat containing smut balls will be designated as "light smutty" (6-30 smut balls) or "smutty" (31 or more smut balls) on official inspection certificates.

Dwarf Bunt or TCK Smut. The *Tilletia* species of fungi cause smut or bunt diseases in wheat, rye, and barley. *Tilletia controversa*, commonly known as Dwarf Bunt or TCK Smut, causes dwarfing or stunting in the growth of the plant itself along with reduced yields. The major source of transmittal is by the spores laying dormant in the soil until the next crop year. These spores can lay dormant for up to ten years retaining their infectious properties [*23*].

Karnal Bunt. Karnal Bunt is thought to have originated in India (therefore the name *Tilletia indica*) but has since been discovered in many places worldwide. It is thought to be in Afghanistan, Lebanon, Mexico and now the United States. Karnal Bunt has spores that are primarily wind-borne, but infection can occur through planting in contaminated soil, use of infected seed, and coming into contact with contaminated equipment and machinery. The spores attack the developing kernels within the seed head with little or no outward sign of infection. The infected kernels are shrunken at the germ end and are covered with sori (small hairlike filaments) that will discolor flour made from infected kernels. These kernels will also impart a fishy odor and taste to flour making their commercial use impractical [*10, 23*].

Black Tip / Black Point. Black Tip fungus is another non-mycotoxic fungus that attacks wheat and barley. The name Black Tip or Black Point can generally refer to any of a number molds that form dark brown to black sooty mold. The principal species of infection are *Alternia, Fusarium, and Helminthosporium*, with the species *Helminthosporium* generally being the causal agent in infections associated with wheat [*23*]. The mold generally occurs when seeds are sown in infected soils, but can also occur when the seeds themselves are infected. The most common forms of *Helminthosporium* are generally associated with dry, warm soils. Moisture in the form of rain or extended periods of high humidity (over 90% relative humidity) then start the disease on the maturing kernels. If the mold begins early in kernel growth sterility and germ death occur, often accompanied with a shriveled germ resulting in decreased yields. If the mold attacks in the later stages of kernel development the seed is discolored and often carries an odor causing market discounts.
These molds are also associated with seedling blight and root rot which will effect the overall health of the plant leading to increased susceptibility of other plant diseases and molds.

Blue-Eye Rot. Blue-eye mold occurs in stored corn with high moisture content. Blue-eye damage is caused by species of *Penicillium* and is characterized by a blue-green discoloration in the germ area. The discoloration results when *Penicillium* fungi invade the germ area through the tip of the kernel. Some corn varieties have a purple colored plumule which can be mistaken for the presence of *Penicillium* fungi in the germ area.

Corn Smut. Smut is caused by *Ustilage maydis* and is always present in field corn. No harmful effects have been noted from feeding silage made from smutty corn to livestock.

Chapter 2
What Are Mycotoxins?

Background. In the previous chapter the principal damage caused by the fungi was the mold. There were little or no toxic affects from by-products of the mold. The molds were also limited as to the hosts they attacked. Mycotoxins on the other hand are metabolites (by-products) of the growth of the molds. They have very real toxic side effects to other plants, animals, and humans. They are also generally less selective of the hosts they attack and can cross plant species.

The species *Fusarium* can and will attack both corn and wheat with different effects in each plant. In wheat, they cause scab damage to the kernels and produce deoxynivalenol (DON). In corn, they create Gibberella Ear Rot and produce DON, zearalenone (ZEN) and T-2 toxins. Both DON and ZEN have toxic effects on animals and humans, with differences depending on the species.

Mycotoxin contamination of crops has been a world wide problem for thousands of years. Only in the last thirty or forty years has technology allowed researchers to isolate the fungal mycotoxins and study the effects on feed crops, livestock, and their affects on humans. The study of mycotoxins is a narrow part of the research into naturally occurring toxins and their effects on plants, animals, and humans. Much of the information in print becomes quickly dated as the field expands and grows. With recent improvements in testing methods more research is now being done, and in greater detail, with lower costs than was previously possible. Many of the newest research papers are now posted on the Internet allowing new information to reach more people faster and more efficiently than was possible before. Information that was published only 20 to 25 years ago is now considered dated and questionable. But with the limited nature of the end usefulness of the information all information must be considered.

The problems associated with mycotoxin contamination of grains are world wide and are uncontained by national borders. A look at a map of North America shows that the important grain producing areas stretch from north central Canada to the southern reaches of the United States. These areas in turn export their products to countries in Asia, the Pacific islands, South and Central America, Europe, the Middle East, India and Africa [*15*].

Scientists estimate that there are 300 to 400 mycotoxins presently identified with more being isolated as new techniques and processes evolve. The most frequently found mycotoxins are aflatoxin, deoxynivalenol (DON), zearalenone (ZEN), fumonisin, and T-2 as far as grain crops are concerned. In surveys conducted by the North Carolina Cooperative Extension service in 1990 some amount of aflatoxin, DON, or fumonisin was found in over 70% of the samples tested [*20*].

Mycotoxic molds generally attack the kernels of grain robbing the nutrients and lower the fat, protein, and vitamin content of the grain.

The mold also often changes the color of the kernels, the consistency (texture), and often imparts an odor that causes feed refusal in animals. These effects lead to economic losses due to impaired health in animals and humans, reduced productivity (reduced production of eggs, milk, and weight gain), and in severe cases death to animals and humans. When the grain is processed into final products like flour or feed, the visible mold may be removed, but the majority of toxins are not and can still cause poisoning.

Economic effects. The economic effects attributed to mycotoxin infection are widely felt in all sectors of the production and consumption of grain products. Grain producers are affected by limited yields, restricted end markets, and price discounts. Grain handlers are affected by restricted storage options, cost of testing grain lots, and loss of end markets. Grain processors incur higher costs to due to higher product losses, monitoring costs, and restricted end markets. Consumers end up paying higher end product prices due to increased monitoring at all levels of handling, and in extreme cases health problems due to consumption of contaminated products. Societies as a whole end up paying higher costs due to increased regulations, needed research, lower export costs, and higher import costs.

While these costs are found at every level of the grain production system, it is almost impossible to put a dollar figure on the losses. Estimates of losses to small portions of the grain and related industries are the best that can be accomplished. The North Carolina Cooperative Extension Service published an estimate for 1992 that the losses to the animal production industry in North Carolina were $20 million for poultry, $10 million for swine, $5 million for dairy, $1 million for beef and sheep, and $1 million for horses. In 1990 a vomitoxin (DON) outbreak in New York and other Northeastern states, the Northcentral U.S., and Eastern Canada had widespread economic affect. The New York Corn Growers estimated, conservatively, that $12 million dollars was lost by the corn farmers due to lost markets, decreased crop value, and costs associated with testing for the 1990-91 crop year [*15,20*].

Health Hazards. The health hazards associated with mycotoxin contamination in humans are rarely seen in North America and Europe. This is generally attributed to a higher level of general health than is seen in underdeveloped countries and better control of food and feed storage.

The levels of intake of affected products that are necessary to bring about poisoning in healthy individuals are actually quite high. In the rare cases that humans have been reported to be poisoned by mycotoxins, the populations were consuming limited quantities of other non-tainted foods and lived in areas of economic and environmental stress. The greatest threat of health hazards to humans comes from long term exposures of tainted food products, either from spoilage or from consuming milk or meat from animals that have been fed contaminated feed [*6, 12*].

One possible avenue of concern to humans is the suspected link between aflatoxin and cancer. While the International Agency for Research on Cancer (IARC) has listed aflatoxin B_1 as having a definite link to cancer in animals, it is listed as having a *probable* link to cancer in humans. The studies that have been done on the cancer link to humans have been

done in Africa and Asia and show an association between aflatoxin and cancer but no definitive cause and effect relationship has yet been documented [*21*].

Mycotoxicoses (poisoning due to mycotoxins) have several common symptoms that are shared from species to species and toxin to toxin. These symptoms include:

1. Drugs and antibiotics are not effective in treatment.
2. The symptoms can be traced (associated) to food or feedstuffs.
3. Testing of food/feedstuffs reveals fungal activity. [*12*]
4. The symptoms are not transmissible to control subjects.
5. The degree of toxicity in subjects is influenced by age, sex, and the nutritional status of the host.
6. Outbreak of symptoms is seasonal.

Mycotoxins have been linked to birth defects in many animals, nervous system problems (tremors, limb weakness, staggering, and seizures), and tumors of the liver, kidneys, urinary tract, digestive tract, and the lungs [*6*].

Aflatoxin primarily attacks the liver, with secondary effects shown in decreased production, and immune system suppression. The trichothecene group of mycotoxins (DON and T-2) cause necrosis and hemorrhage of the digestive tract with decreased blood production in the bone marrow and spleen along with changes to the reproductive system as the secondary sites of attack.

Zearalenone has affects different from other mycotoxins in that it mimics the bodies production of estrogen causing feminization of male animals and interference with conception, ovulation, and fetal development in female animals [*6*].

One of the primary similarities in mycotoxicosis is the health status of the host before infection, which will to a large extent determine the degree to which the host is attacked. In both animals and humans if the host is healthy the final prognosis is generally very good except in cases of acute poisoning where very large doses of toxin are eaten over a very short period of time.

Many variables affect the degree of susceptibility of various hosts. These include:

General health. A healthy individual is more able to fight the toxins than a one that starts out malnourished or diseased.

Age. The very young and very old have weakened immune systems that are less able to fight the effects of toxicoses.

Sex. In general female animals, and to a degree female humans, seem to be more susceptible to the effects of mycotoxicoses.

Environment. Hosts exposed to harsh living conditions of neglect or squalor have an added burden on their systems.

Adequate food storage. If grain is left in open storage to the effects of weather the grain is further weakened allowing for continued fungal growth. If the grain is also not adequately dried, the conditions for fungal growth continue to persist.

Exposure Level. Very high doses of aflatoxin attack the host quicker than lower doses.

Exposure duration. A very short time of exposure (a single dose) allows the host time to fight the infection where a continued exposure tends to have cumulative effects.

Other food sources. If contaminated grain is the only or main source of nourishment, the host has less nutrients available to feed their system.

Lack of Regulatory and Monitoring systems. Areas that lack the means to regulate and monitor the presence of mycotoxins in general, and aflatoxin in particular, leave their inhabitants open to unknown poisonings that can continue over long periods of time unchecked. [21]

Although the mycotoxins have been greatly researched over the course of the last forty years, little research has been done on the interactions of the mycotoxins and their combined effects. Almost all research has been in determining the effects of pure strains developed in the laboratory. Animal feed can contain several different grains and grain in storage can contain several different strains of the various mycotoxins.

Studies done with natural field contaminated DON and ZEN have produced results that have varied from those that have been carried out in the laboratory. This leads researchers to believe that there are unknown strains of toxins in the field or that the toxins interact with each other to produce effects greater than or different from what laboratory tests have predicted would occur [*15*].

Regulatory Control. In 1965, the Food and Drug Administration (FDA) established action limits of 20 parts per billion (ppb) of aflatoxin in all food and feeds to limit the inclusion of this contaminate into the food chain. But since aflatoxin can occur both in raw products and in finished by-products, this has necessitated the testing for aflatoxin and other mycotoxins at many points in the food chain. As technology has progressed in recent years testing has become simplified, faster, and cheaper (using ELISA methods) allowing more testing to be accomplished in all phases of food handling.

The Grain Inspection; Packers and Stockyards Administration (GIPSA) tests all corn that is exported for aflatoxin with an action level of 20 ppb. If a sample tests above 20 ppb, the FDA must be notified. Testing for vomitoxin is done as a service to customers with no FDA action level set. FGIS will institute new testing services as the tests are certified for reliability and repeatability, and as customer demand requires.

Aspergillus Toxins

Aspergillus is an important genus in foods with most species occurring as spoilage or biodeterioration fungi. *Aspergillus* is a large genus containing more than 100 recognized species, several of which are capable of producing mycotoxins.

Nearly 50 species of *Aspergillus* have been listed as producing toxic metabolites. Those of greatest significance in feed and foods include: aflatoxin, ochratoxin A, sterigmatocystin, cyclopiazonic acid, citrinin, patulin, and tremorgenic toxins.

Aspergillus species produce toxins that exhibit a wide range of toxicities, with the most

significant effects being long term. Aflatoxin B_1 is a potent liver carcinogen. Ochratoxin A and citrinin both affect kidney function. Cytopiazonic acid has a wide range of effects and tremorgenic toxins affect the nervous system.

While there is a known link between aflatoxin and cancer in animals, it should also be noted that the *Aspergillus* species of fungi also have many beneficial uses. One of the largest commercial uses of *Aspergillus* fungi is in the production of soft drinks. The extraction of pure citric acid from fruits and vegetables has proved to be to expensive, so the manufacturers have developed a way to use large vats to ferment *Aspergillus niger* to form artificial citric acid [*22*].

The Japanese have developed methods of fermenting rice with strains of *Aspergillus flavus* (aflatoxin) to cause the enzymes in the kernels to breakdown the carbohydrates into simple sugars, to produce a sweetened rice drink. These drinks or Koji, are marketed under many different brand names [*7*].

The family of products that includes miso, soy sauce, and sake also use strains of *Aspergillus oryzae* to ferment grain products into usable food and drink products [*22*].

Penicillium Toxins

Penicillium is a large genus with over 150 species recognized and at least 50 species of common occurance. The discovery of penicillin in 1929 gave impetus to a search for other *Penicillium* metabolites with antibiotic properties. This search led to the recognition of "toxic antibiotics" or mycotoxins.

Nearly 100 *Penicillium* species have been reported as toxin producers. Of these the following nine mycotoxins produced by 17 *Penicillium* species are potentially significant to human health: citreoviridin, citrinin, cyclopiazonic acid, ochratoxin A, patulin, penitrem A, PR toxin, Roquefortine C, and Secalonic acid D.

The toxins produced by *Penicillium* species can be placed in two general groups: those that affect the liver and kidney function, and those that are neurotoxic. The *Penicillium* toxins that affect liver or kidney function are asymptomatic or cause generalized debility in humans or animals while the neurotoxins are characterized by sustained trembling.

Fusarium Toxins

Fusarium species are the most important group of mycotoxigenic molds other than *Aspergillus* and *Penicillium*. Many *Fusarium* species are plant pathogens and most can be found in the soil.

Fusarium species are most often encountered as contaminants of cereal grains, oilseeds, and beans. Corn, wheat, barley and products made from these grains are most commonly contaminated although rye, triticale, millet, and oats can also be contaminated.

F. graminearum is a plant pathogen found worldwide in the soil and is the most widely distributed toxigenic *Fusarium* species. It causes various diseases of cereal grains such as gibberella ear rot in corn and head blight or scab in wheat and barley. The mycotoxins produced by *F. graminearum* include:

deoxynivalenol, zearalenone, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, diacetyldeoxynivalenol, nivalenol, T-2, neosolaniol, and diacetoxyscirpenol.

F. moniliforme is a soilborne plant pathogen that is found in corn growing in all regions of the world. It is the most prevalent mold associated with corn. It has also been found in rice, sorghum, yams, hazelnuts, pecans, and cheeses.

F. moniliforme has long been suspected of being involved in animal and human diseases. Animal diseases associated with *F. moniliforme* include equine leukoencephalomalacia (ELEM) a liquefactive necrosis of the brain of horses, pulmonary edema and hydrothorax in swine, liver cancer in rats, and abnormal bone development in chicks and pigs.

The main human disease associated with *F. moniliforme* is esophageal cancer. Several studies have linked the presence of *F. moniliforme* and fumonisins in corn to high incidences of esophageal cancer in humans in certain regions of the world including an area around Charleston S.C. Mycotoxins that have been associated with *F. moniliforme* include fumonisins, fusaric acid, fusarins, and fusariocins.

Alternaria Toxins

Alternaria species may be significant as potential contaminants of food. *Alternaria* infects the plant in the field and may contaminate wheat, sorghum, and barley. *Alternaria* species also infect various fruits and vegetables and can cause spoilage of these foods in refrigerated storage.

Alternaria toxins include: alternariol, alternariol monomethyl ether, altenuene, tenuazonic acid, and the alertoxins. Little is known about the toxicity of these toxins; however, cultures of *Alternaria* that have been grown on corn or rice and fed to rats, chicks, turkey poults, and ducklings have been shown to be quite toxic.

Claviceps Toxins

The ergot mold, *Claviceps purpurea*, is the cause of the earliest recognized human mycotoxicosis, ergotism. Ergot has been reported in sporadic outbreaks in Europe since 857, with near epidemic outbreaks in the Middle Ages.

Ergot is a disease of cereal grains such as rye and wheat in which the grains are replaced by ergot sclerotia that contain toxic alkaloids. The main ergot alkaloid, ergotamine, has vasoconstrictive properties that can cause swollen limbs, and alternating burning and cold sensations in the fingers, hands, and feet (St. Anthony's Fire).

Chapter 3
Mycotoxins in Grain and Feed

Aflatoxin

Pathogen. The name aflatoxin comes from A(Aspergillus) + FLA(flavus) + toxin. Modern research into aflatoxin had its beginnings in 1961, looking into what caused the deaths of 100,000 young turkey poults in England. The research traced the poison to contaminated Brazilian peanut meal that had been used as feed. When the feed was given to ducks and pheasants, the same outcome was produced.

Research found that there were four different metabolites formed from aflatoxin. When the contaminated grain in question was viewed under a black light, the metabolites glowed either blue (B metabolite) or green-yellow (G metabolites). Of the two distinct color varieties there were isolated two distinct toxins ($B_{1,}$ $B_{2,}$ $G_{1,}$ G_2). The subscripts refer to their separation patterns on TLC plates. Of the four metabolites B_1 was the most predominate and the most toxic [*17*].

Further research in the years since has found other metabolites, with two found in the milk (M_1 and M_2) and urine of lactating mammals. Aflatoxins M_1 and M_2 are produced from their respective B aflatoxins by hydroxylation in lactating animals and are excreted in milk at the rate of approximately 1.5% of the rate of ingested B aflatoxins. Research has also found that aflatoxin is most commonly found in tree nuts, peanuts, and oilseeds including corn and cottonseed.

Ecology. The greatest problems associated with aflatoxin are in corn production and foodstuffs. These problems occur for two reasons. Corn is grown in climatic areas that give the fungi/mold the greatest opportunity for growth and dispersal, and the areas that grow corn consume it as a main part of the diets of both animals and humans [*17*].

Aflatoxin grows best at temperatures of 80° to 90° F, but can survive at temperatures as low 40° F. The mold also needs a high moisture content in the host, either through kernel moisture or in the form of rainfall. Kernel moisture of 20% or greater is optimal, but the fungus can survive and grow in grain with moisture content as low as 15% [*17*]. In corn, insect damage to developing kernels allows entry of aflatoxigenic molds, but invasion can also occur through the silks of developing ears.

Research conducted into the growth of aflatoxin has indicated that the fungus needs some form of associated stress in the plants for the fungus to invade. The stress may be in the form of drought that weakens the plant system, extended periods of high temperature, damage from insects or birds, high crop density, or competition from weeds. All of these conditions weaken the host or provide a means of entry to the spores to establish a foothold in/on the host [*17*].

As was indicated in Chapter One, the spores need a means of transmittal to spread and grow. Insects and birds can carry spores on

their bodies and when moving through a field contaminate many plants. Raindrops hitting infected plants and splashing to another plant have also been shown to be a means of transmittal. Poor field management in cleaning away contaminated residual crop debris leaves the spores in the fields to further continue the growth cycle in later crops.

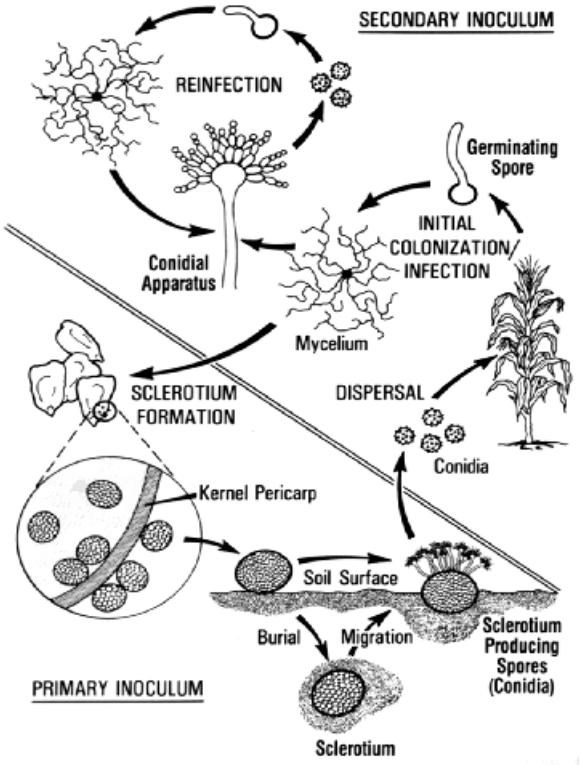


Figure 1. ***Aspergillus flavus*** **Infection of Corn (Wicklow and Donahue, 1984)**

Infection of harvested crops also occurs once the grain reaches storage. If corn is not cleaned and dried to adequate levels (moisture content of less than 15%), the fungus will grow and contaminate healthy clean grain. "Hot spots" containing spores and moisture can occur in storage bins that create a self-sustaining environment of moisture (respiration) and heat (decaying grain) that provide prime growing conditions for the aflatoxin fungi [1].

Health Effects. Aflatoxins are both acutely and chronically toxic in animals and humans. The disease primarily attacks the liver causing necrosis, cirrhosis, and carcinomas. No animal has been found to be totally resistant to the effects of aflatoxin, although susceptibility differs from species to species. Aflatoxin B_1 has been shown through research to be the most potent naturally occurring carcinogen in animals, with a very strong link to human cancer incidence [*21*].

Scientists have conducted studies that have shown a positive correlation between consumption and the level of intake of contaminated food and feed, to liver cancer in Kenya, Mozambique, Uganda, and Swaziland in Africa, and China and Thailand in Asia. Studies conducted in the Southeastern United States where foods that possibly contain aflatoxin are grown also show an increase in liver cancers. While this increased incidence of cancer is statistically apparent, there is no confirmed laboratory link of cancer to humans, only animals [*11*].

Aflatoxicosis refers to poisoning from the ingestion of aflatoxins in contaminated food or feed. It can occur from acute exposure of very high doses of contaminated grain over a short period of time, or from the chronic ingestion of low levels of aflatoxin over longer periods of time [*21*].

Acute aflatoxicosis in humans is rare; however, several outbreaks have been reported. In 1967, twenty-six people in two farming communities in Taiwan became ill with apparent food poisoning. Nineteen were children, three of whom died. Rice from affected households contained about 200 ppb of aflatoxin which was probably responsible for the outbreak.

An outbreak of hepatitis in India in 1974 affected four hundred people, one-hundred of whom died. The outbreak was traced to corn containing up to 15,000 ppb. It was calculated the affected adults may have consumed 2 to 6 mg on a single day, implying that the lethal dose for adult humans is on the order of 10 mg.

Perhaps of greater significance to human health are the immunosuppressive effects of aflatoxins. Immunosuppression can increase susceptibility to infectious diseases, particularly in populations where aflatoxin ingestion is chronic, and can interfere with production of antibodies in response to immunization in animals and perhaps also in children.

As stated before aflatoxicosis primarily attacks the liver but does cause other health effects. Acute symptoms include vomiting, abdominal pain, pulmonary edema, convulsions, coma, and cerebral edema. Many of these conditions can only be treated with medical care which is beyond that available to developing or third world areas, leaving their populations at great risk.

Research has shown that animals that develop aflatoxin poisoning have compounding effects that can effect the human population around them. Cows' reduced milk and meat production limits the variety of diet in third world cultures. The milk products can also have metabolites that are passed on through the milk and milk end products including nonfat dry milk, cheese, and yogurt. In poultry, chickens lay fewer eggs, and can pass on aflatoxin in the yolks of their eggs further adding to the problem. Both the animal and human populations can develop secondary immune system problems that leave them susceptible to further unassociated diseases and viruses [*17*].

Tolerance Levels. The Food and Drug Administration (FDA) has established action levels for aflatoxin present in food or feed. These limits are established by the Agency to provide an adequate margin of safety to protect human and animal health.

Table 1. FDA Action Levels for Aflatoxin

Species	Commodity	Action Level
Humans	Milk	0.5 ppb (M_1)
Humans	Any food except milk	20 ppb
Immature animals (including poultry), dairy animals, or when end use is not known.	Corn and other grains	20 ppb
All Species	Animal feed other than corn or cotton seed meal	20 ppb
Breeding beef cattle, breeding swine, or mature poultry	Corn and other grains	100 ppb
Finishing swine of 100 lbs. or greater	Corn and other grains	200 ppb
Finishing beef cattle	Corn and other grains	300 ppb
Beef cattle, swine, poultry	Cottonseed meal	300 ppb

Detoxification. Many companies and researchers have tried to find a means of detoxifying aflatoxin contaminated grains. Most of the treatments have been found to be either too expensive to use in storage facilities or to have side effects on the end products that cancel the benefits of detoxification.

Dietary supplements to strengthen the general health of affected animals and humans have shown the most promise. The addition of vitamins, proteins, and trace elements, along with inorganic absorbents added to feed, have the most positive effects. The absorbents bind and immobilize the toxins holding them in the intestinal tract, allowing them to be eliminated in urine and fecal matter [*17*].

Occupational Risks. It must be emphasized that there have been no definitive links between aflatoxin and cancer in humans, but studies have shown associative risks in grain handling and processing occupations. Research conducted in Holland and the United States showed elevated risk of respiratory and liver cancer to those exposed to dust associated with grain and flour production. The greatest risk appeared in flour baggers and employees working at dump pits unloading grain into storage facilities. Accordingly care should be taken when handling raw grain products or fine end products to avoid inhalation of fine particulates [*12*].

Ergot

Pathogen. Ergot is another fungi that attacks wheat, rye, barley, and oats. *Claviceps purpurea*, or ergot, is the cause of the earliest recognized human mycotoxicosis, ergotism.

Ecology. *C. purpurea* has three different means of transmittal. Windborne ascospores can attack the immature kernels and grow into sclerotium, purple-black hornlike structures, that replace kernels on the heads of grain. If these sclerotium mature on the stalk, they ripen and grow small club like stromata that in turn grow and release more ascospores. As the sclerotium mature they release a substance called honeydew (sugary dew-like liquid) that encourages insects to feed. This liquid contains small conidiophores that will attack the immature grain heads and start the process over and therefore spread the spores from plant to plant. The sclerotium, what are commonly called ergot, must be exposed to cold (36 - 37° F) for several weeks before they can germinate. The sclerotium can overwinter in the soil or stay dormant in storage until the proper conditions exist to again start the process over again [*9*].

The damage of ergot is two fold. It decreases the yields of infected crops by replacing healthy kernels and robbing the host plant of needed nutrients. The sclerotium contain alkaloids that can have adverse health affects on humans and animals. The grains are also discounted in market value if the sclerotium are present after harvest.

Health Effects. Animals can suffer severe blood vessel constriction that can result in dry gangrene. Internal bleeding, vomiting, constipation, diarrhea, and intestinal inflammation are also common problems in livestock. Swine that are fed ergoty feed may abort any fetuses that they hold.

Humans can suffer gastrointestinal distress and convulsions, abortion of fetuses, or a necrotic gangrenous condition of the extremities if the ergot is ingested in sufficient quantities. Ergotism was known as St. Anthony's fire during the Middle Ages because it was believed that pilgrimages to a shrine of St. Anthony could lead to a cure of the disease. It is likely that as pilgrims traveled to the shrine, they left areas where bread was contaminated with ergot and traveled to areas

where ergot was not a problem. The main ergot alkaloid, ergotamine, has vasoconstrictive properties that cause swollen limbs and alternating burning and cold sensations in fingers hands and feet, hence the term "fire" in St. Anthony's fire. Convulsive ergotism may also have been the reason for the Salem witchcraft trials of 1692. In recent history, outbreaks of ergotism have occurred in Russia in 1926, Ireland in 1929, France in 1953, India in 1958, and Ethiopia in 1973.

The ancient Chinese and Europeans used ergot alkaloid compounds to reduce bleeding after childbirth and to induce abortions when needed. Modern science has found ways to control these alkaloids. Several useful medications have been developed to treat bleeding, muscle spasms, and migraine headaches. Two alkaloid compounds of ergot have shown remarkable effectiveness in treating migraine headaches. Research is continuing to find more and varied medical uses for these ergot alkaloids [*23*].

Trichothecenes

Pathogen. The trichothecenes are a chemically related family of compounds that are produced by fungi such as *Fusarium, Trichoderma, Myrothecium,* and *Stachybotrys.* The trichothecene mycotoxins have been isolated and found in Canada, England, Japan, South Africa, and the United States. The most common mycotoxins in the trichothecene family found in grain are DON and T-2, with ZEN and Fumonisin also commonly found [*12*]. *Fusarium gramminearum*, the parent fungi that produces DON, causes both Gibberella Ear Rot in corn, and head scab in wheat.

Over a ten year period the Mycotoxin Laboratory at North Carolina State University found *Fusarium* species of fungi in almost every lot of corn tested. DON was detected in over 60 percent of poultry and dairy feed tested, and ZEN was found in 15 to 20 percent of feeds tested.

The *Fusarium* species of fungi are capable of producing 70 different mycotoxins, with some species producing as many as 17 strains of mycotoxins simultaneously. Research is finding new strains of previously unknown mycotoxins as testing methods improve and more research is conducted. Fumonisin is a recent discovery that research indicates is very toxic to horses, but little is known of its incidence or range [*20*].

Health Hazards. The trichothecene family of mycotoxins affects each species of animals in different ways. Testing done so far indicates that poultry has the highest resistance to these toxins. Cattle, sheep, and goats have some level of resistance due to their multiple digestive process, while animals of the monogastric digestive process seem to have the least resistance to the toxins. Swine seem to be the most sensitive, partly due to their increased sense of smell which leads to feed refusal [*15*].

The greatest problems associated with these toxins are from prolonged feed intake at low contamination levels. The effects depend on the specific toxin, the duration of exposure, and the type of animal involved. All animal species suffering from chronic toxicoses show very good to excellent signs of improvement when the contaminated feed is removed. Few long term side effects remain with most of this group of toxins if diagnosis is made quickly

before the general health of the affected animals is compromised [*12*].

The processing of grain with toxins in the trichothecene group generally does little to remove the toxin. Milling, baking or boiling has only a slight effect in removing the toxins. Tests conducted on finished products contaminated with these mycotoxins have shown that 50 to 60 percent of the toxins are transmitted to the finished product. In some cases, such as the tempering of grain to reach a desired moisture level, the toxins have actually increased due to the proper environmental conditions needed for toxin production. The toxins can be transmitted to final products such as flour, bread, crackers, and cereal [*12*].

Deoxynivalenol (DON)

Pathogen. *Fusarium graminearum* is the parent fungi of deoxynivalenol (DON) or vomitoxin. Wheat and barley are the most commonly effected grain crops but the same fungus does infect corn. In the field, it shows up as a brown discoloration at the base of barley glumes, a pink to reddish mold on the glumes and kernels of the wheat heads and the tips of the ears of corn. Spores from the mold stage of the fungi can stay dormant on infected residues left on or in the soil. Contamination is most severe in fields where corn follows corn, or where corn follows wheat, especially if the previous crop was infected [*24*].

Ecology. The optimal temperature range for the DON mold is 70 to 85 F with moisture levels preferred to be greater than 20 percent. There are exceptions to be noted. The mold can survive temperatures as low as 0 F for short periods of time. This particular fungi has two distinct growth cycles, with the mold growing during the warm temperatures of daytime, while the toxins are produced during the cooler temperatures of the night [*2*].

Health Effects. The symptoms associated with DON poisoning are many and varied which sometimes leads to its misdiagnosis as a problem. At low levels of toxicoses the symptoms may include behavioral and skin irritations, feed refusal, lack of appetite, and vomiting. In later stages, symptoms may include hemorrhage and necrosis of the digestive tract, neural problems, suppression of the immune system, lack of blood production in the bone marrow and spleen, and possible reproductive problems including birth defects and abortion [*15, 20*].

Table 2. FDA Advisory Levels for DON

Class of Animal	Portion of Diet	Maximum DON Level
Humans	Finished wheat products (flour, bran, &germ)	1 ppm
Beef and feedlot cattle older than 4 months	Grain and grain by-products not to exceed 50% of diet	10 ppm
Chickens	Grain and grain by-products not to exceed 50% of diet	10 ppm
Swine	Grain and grain by-products not to exceed 20% of diet	5 ppm
All other animals	Grain and grain by-products not to exceed 40% of diet	5 ppm

As stated earlier, DON stays in end feed products even after processing. In 1995, 16,000 tons of dog food was produced using wheat by-products (most probably dust). After it reached the consumer level it was found to contain DON in excess of 30 ppm. The products were recalled costing the company in question a loss of approximately $20 million [*16*].

Tolerance Levels. Canada has set action levels for DON in grain and finished products, while the United States has set only advisory levels. Canada's tolerance levels are set at less than 2 ppm in wheat for human consumption, and less than 1 ppm for wheat destined for use in infant food products.

The United States has set their advisory levels at less than 2 ppm for wheat destined for human consumption, and less than 5 ppm for most animal feed products [*12*].

Zearalenone (ZEN)

Pathogen. Zearalenone is very similar to deoxynivalenol (DON) in most aspects with a few exceptions.

Ecology. The growing conditions of ZEN are very comparable to DON, with the optimal temperature range of 65° to 85° F. A drop in temperature during growth also stimulates the production of toxins [*2*].

The moisture content required by ZEN is also similar to DON at 20 percent or greater. But if the moisture content during growth drops below 15 percent the production of toxins is halted. This is one of the reasons that corn for storage must be dried to moisture levels less than 15 percent [*24*].

Health Effects. The greatest difference between ZEN and DON is the way the toxin acts in animals. ZEN mimics the hormone estrogen in the way it effects animal tissue. Swine are the most sensitive to its effects with levels of 1 ppm causing feed refusal. Continued consumption of contaminated grain will cause estrogenism (health problems related to the reproductive system). These effects include swelling of the reproductive organs including the genital and mammary glands, interruption of the reproductive cycles, birth defects, and atrophy of the ovaries and testes. In male animals, feminization occurs with enlargement of the mammary glands and loss of sex drive [*20, 2*].

Poultry show little or no effects from ZEN consumption. Cattle also show very little effect except in cases of prolonged consumption of high levels (greater than 15 ppm) of ZEN. The effects produced are reduced milk production, swollen reproductive organs, and in some cases infertility. The ZEN is passed through the system with very little absorption shown in milk, urine, or body tissues [*2*].

Tolerance Levels. The FDA has issued no advisory levels for zearalenone recommending only that the levels of concern for DON be observed (See above). Levels of as little as 0.1 ppm to 5 ppm have been shown to cause reproductive problems in swine so great care should be used when feeding wheat that is possibly contaminated to pigs [*24*].

Fumonisin

Pathogen. *Fumonisin moniliforme* is the parent fungi species of fumonisin. This fungus causes Fusarium Ear Rot in corn which is the most common disease of corn in the United States Midwest region. Testing of corn fields has shown that over 90 percent of fields are affected by this fungi in one of its various strains [*24*].

The mold appears on the corn ears as a cottony white to light grey filaments between the corn kernels. As the mold progresses the kernels will turn grey to light brown.

The fumonisin toxin can grow in the kernels even with no apparent outward signs of mold. Testing of the grain is the only positive means of verifying whether fumonisin is present or not [*24*].

Ecology. Growing conditions vary widely. The temperature and moisture ranges are so wide spread as to include most of the Northern and Southern Hemispheres. The one common factor associated with fumonisin is that higher incidence of infections seem to occur after periods of drought which stress the plants immune system [*24*].

Fumonisin causes the corn kernels to become brittle and crack more frequently than is normal. The more the grain is handled the more cracking and breaking occurs, giving the fungi more host material to grow on. For this reason corn screenings should be very suspect when used as feed, especially in horses. Testing has shown that screenings contain a higher level of fumonisin toxin (and mycotoxins in general) than the whole grain product [*24*].

Health Effects. Fumonisin is one of the mycotoxins that has only recently been discovered and has been little studied. The related health effects have shown few effects in humans and most animals other than swine and horses. While in depth research is lacking, fumonisin has shown a high degree of toxicity in preliminary studies conducted in horses. Swine have shown little or no effects with the only preliminary symptoms to be possible respiratory problems and possible links to the liver and kidneys. [*20*].

Toxin levels of as low as 5 ppm have shown direct links in horses with symptoms which include: disorientation, walking/agitation, derangement, colic, head pressing, blindness, and death. The toxin seems to attack the liver and kidneys, which is similar to other mycotoxins except in the severity. Fumonisin has also been linked to equine leukoencephalomalicia, also known as "Blind Staggers" (a complete breakdown of the neural system in the brain) which has a high mortality rate [*20*].

Tolerance Levels. Currently there are no action or advisory levels in place by the FDA because so little is known about the effects. Industry levels have recommended levels to be no higher than 5 ppm in horses, 10 ppm for swine, and 50 ppm for cattle [*24*].

Nivalenol (NIV)

Pathogen. Nivalenol is produced by the *Fusarium nivale* fungi and has also only recently been isolated. Little is known of its growth cycle or habitat range. Studies have shown it to be much rarer in occurrence and has only been found in a few samples of barley, wheat, wheat flour, and rice [*12*].

Health Effects. Though little actual test data has been produced, the results so far cause scientists to be extremely cautious. Preliminary testing shows that NIV is thought to be 10 times more potent than DON. If the advisory level for Don is used as a guide for toxicity, NIV would have an advisory level of only 0.2 ppm [*12*].

Ochratoxin

Pathogen. Ochratoxins were initially associated with *Aspergillus ochraceus* but are produced primarily by *Penicillium verrucosum*.

Ecology. *A. ochraceus* is widely distributed in dried foods such as peanuts, pecans, beans, dried fruit, and dried fish. Ochratoxin contamination of foods is of greatest concern in Scandinavia and the Baltic states where it is produced by *Penicillium verrucosum*. It is found in barley and wheat crops infected in the field or in storage crops used for both bread making and animal feeds.

Health Effects. Ochratoxin is the most important toxin produced by a *Penicillium* species. It can cause listlessness, huddling, diarrhea, tremors, and other neural abnormalities in poultry and has been associated with kidney disease in swine in Scandinavia and northern Europe.

Because ochratoxin A is fat soluble and not readily excreted, it accumulates in the fat of affected animals and from there is ingested by humans eating pork. A second source is bread made from infected barley or wheat.

T-2

Pathogen. *Fusarium tricinctum* and some strains of *F. roseum* produce T-2 . T-2 has been found in corn in the field, silage, and prepared feeds made with corn.

Health Effects. During WWII, a very severe human disease occurred in the former Soviet Union. Alimentary toxic aleukia (ATA) is believed to have been caused by T-2 and HT-2 in grain left to overwinter in the field. When this grain was consumed, severe mycotoxicosis occurred. ATA results in a burning sensation in the mouth, tongue, esophagus, and stomach. Eventually the blood making capacity of the bone marrow is destroyed and anemia develops. In the final stages hemorrhaging of the nose, gums, stomach, and intestines develops and the mortality rate is high. In poultry, T-2 may produce lesions at the edges of the beaks, abnormal feathering, reduced egg production, eggs with thin shells, reduced body weight gain, and mortality.

Cyclopiazonic Acid (CPA)

Pathogen. CPA is produced by *A. flavus* and several *Penicillium* species. It has been found in corn and peanuts in Georgia. The principle *Penicillium* species producing CPA, *P. commune*, is a cause of cheese spoilage around the world.

Health Effects. CPA is a highly toxic compound that causes fatty degeneration and hepatic cell necrosis in the liver and kidneys of domestic animals. Chickens are particularly susceptible. When the compound is injected into experimental animals, central nervous

dysfunction occurs and high doses can result in death. CPA and aflatoxin may act synergistically when consumed together by animals.

Citrinin

Pathogen. Citrinin is primarily a metabolite of *Penicillium citrinum* but is also produced by *P. expansum* and *P. verrucosum.* These three species are the most commonly occurring penicillia, and so citrinin is probably the most widely produced *Penicillium* toxin.

Ecology. Citrinin has been isolated from almost every kind of food surveyed for fungi. The most common sources are cereals such as rice, wheat, and corn, milled grains, and flour.

Health Effects. Citrinin is a kidney toxin which has been associated with mycotoxicoses in swine, horses, dogs, and poultry. No toxic effects to humans have been noted when citrinin is ingested in the absence of other toxins, however, there is a possibility of synergism if ingested with other toxins.

Chapter 4
Sampling

The first step in mycotoxin analysis is obtaining a representative portion. Great care should be taken when sampling, since sampling error is often the greatest source of variance in the analytical procedure.

The sampling and testing of grain crops infected with mycotoxins presents several problems. Unlike protein or moisture content in corn or wheat, where every kernel tested has some level of content (a uniform distribution), mycotoxin content does not occur in every kernel. In the extreme it may only occur in or on a few ears or heads in an entire field (an nonuniform distribution). The greater the extent of contamination the more likely that the distribution will be uniform and test results accurate.

Protein			
12	13	12	14
13	13	14	12
15	11	12	12
13	14	11	9
13	12	12	13

Protein Avg. 13%

Aflatoxin			
0	0	0	0
0	0	0	0
0	0	0	0
0	200	0	0
0	0	0	0

Aflatoxin Avg. 10 ppb

Sample Size. There is a smaller sampling error associated with processed commodities, such as flour, than is associated with whole seeds. This is because of the smaller particle size which increases the sample population (number of possibly infected particles), and the greater degree of mixing associated with the production process.

There is a higher incidence of error in whole grains because the number of infected particles may be as little as 0.1 percent of the total population of the sample. When the fact that a single kernel of corn was tested and found to contain aflatoxin at a level of 400,000 ppb, it is apparent why the accuracy of the representative sample is so critical [*6*].

Each pound of corn contains approximately 1,530 kernels. Ten pounds of corn contains about 15,300 kernels of corn. With the greater number of kernels in a larger sample comes a higher probability of correct test results for that sample.

GIPSA has determined that the optimum aflatoxin sample size for corn is a minimum of 10 pounds. This sample is then ground and a subsample of 500 grams obtained. This subsample is then mixed and a 50 gram sample obtained to be tested.

GIPSA has not established an optimum aflatoxin sample size for wheat, barley, sorghum and other grains. Currently the ten pound sample size is used by default for all grains when testing for aflatoxin.

GIPSA established reduced sample sizes for domestic shipments of corn by truck and rail. The smaller sample sizes were adopted in response to industry concerns that the ten pound

sample size would significantly increase the cost of inspection. The GIPSA minimum sample sizes are listed in Table 3.

Table 3. GIPSA Minimum Sample Sizes

Carrier	Minimum Sample
Trucks	Two pounds (908 grams)
Railcars	Three pounds (1362 grams)
Sublots/Barges	Ten pounds (4540 grams)
Submitted Samples	Ten pounds recommended

It should be noted that using the reduced sample sizes significantly increases the sampling variability. Table 4 illustrates how reducing the sample size affects sample variability.

Table 4. Truck containing 20 ppb aflatoxin contaminated corn (Romer Labs 1995).

Sample Size	Kernels	Variability (ppb)
10 lbs.	30,000	11.6 - 28.4
5.0 lbs.	15,000	8.1 - 31.9
2.5 lbs.	7,500	3.2 - 38.8
1.0 lbs.	3,000	1 - 46.9

However, a 1998 GIPSA study of DON contaminated barley have shown that increasing the sample size does not appear to significantly decrease the variability of DON results in barley. This does not mean that sample size is unimportant for DON analysis of barley. For some sufficiently small sample, size would become a significant factor.

The sample size required by GIPSA for barley and wheat is a minimum of 200 grams and preferably larger. This sample is then ground and a 50 gram subsample obtained. The effect of sample sizes smaller than 100 grams is unknown, but larger sample sizes do not appear to appreciably improve the precision of DON test results in barley. Grains with larger kernel size such as corn would require larger sample sizes to maintain the same level of uniformity.

Sampling Variability. The next variable that enters the picture is sampling variability. To ensure that the sample that is tested is accurate, proper sampling techniques must be used to obtain a representative sample. A “boot” sample from the exposed layer of grain in a hopper car or truck, or a “bucket” sample as a truck or railcar is unloaded does not give a representative sample of the lot as a whole.

In fact, nearly 90% of the error associated with aflatoxin testing can be attributed to how the original sample was obtained. This is due to only one to three percent of the kernels in a contaminated lot containing mycotoxin, and these contaminated kernels are not generally distributed evenly in the lot [20]. A study conducted by Michigan State University found that the variability of DON measurements in trucks of newly harvested soft red winter wheat was significantly higher if less than four

probes were taken from the lot [8].

Representative Sample. Obtaining a representative sample from a lot of grain is an important and essential part of mycotoxin analysis. If the sample is not representative the analysis result will not represent the true quality of the lot. In order for a sample to be considered representative, it must be:

1. Obtained with equipment/procedures designed to obtain sample from all areas of the lot;
2. Of appropriate size;
3. Adequately identified;
4. Handled in such a way as to maintain representativeness.

Sampling Methods. Of all the sampling devices available, the mechanical sampler obtains the most representative sample from lots of grain. They are powered either pneumatically, electrically, or hydraulically.

The diverter-type (D/T) mechanical system is used for commodities with large particle size such as whole grain. Even though D/T's vary in design, all operate on the same principle.

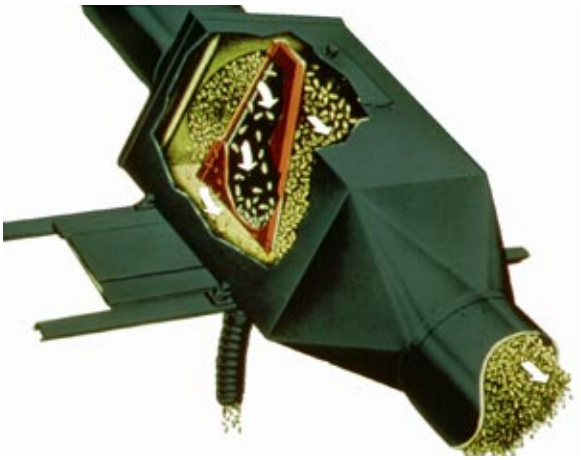


Figure 2. Diverter-Type sampler installed in spout.

Installed at the end of a conveyor belt or within a spout, they draw their sample by periodically moving a pelican (named for its resemblance to the beak of a pelican) through the entire grain stream.

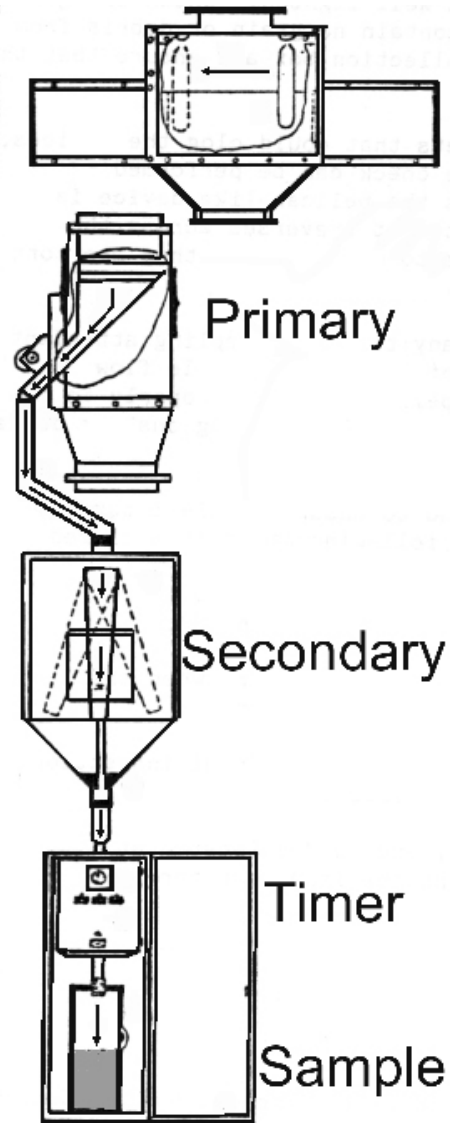


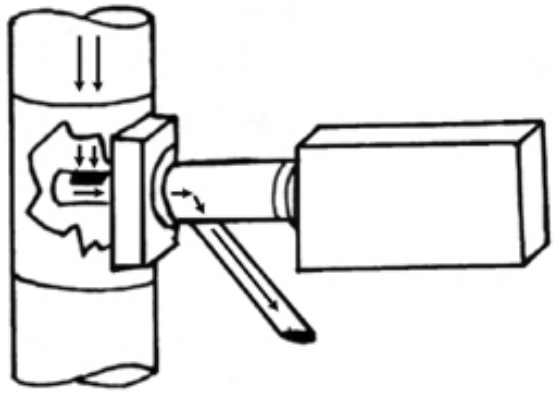
Figure 3. Diverter-type mechanical sampling system used for whole grain.

The frequency of these "cuts" is regulated by timer controls. After the grain enters the primary sampler, it flows through a tube into a secondary sampler. The secondary sampler

reduces the size of the sample. From the secondary sampler, the sample flows to a collection box or sample bucket.

Point-type (P/T) mechanical sampling systems are commonly used for powdered commodities. These commodies are more homogeneous than whole grain and have less particle segregation. They do not use a pelican to completely cut across the stream of commodity through a spout. Instead they use a tube with a hole or slot and an auger delivery system.

Figure 4. Point-type mechanical sampling system used for powdered commodities.



A large percentage of grain, as it travels from the farm to the final consumer, is sampled with a probe sometimes referred to as a trier. The probe is the only sampling method approved by GIPSA for stationary lots. If probe sampling is performed correctly, the samples drawn are considered representative.

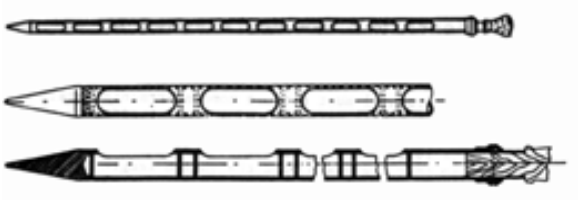


Figure 5. Grain probe or trier

Probes are constructed of brass or aluminum and come in various sizes with standard legnths of 5, 6, 8, 10, and 12 feet. The type of carrier dictates which probe length is used. Probes consist of two tubes, one inside the other.

GIPSA approved grain probes are 13/8 inches in diameter (outer tube). The inner tube is divided into compartments. The outer tube has slots which match the compartment openings of the inner tube. When the tubes are aligned, grain may enter into or be emptied from the compartments of the probe.

The lengths of double-tube compartmented probes approved by GIPSA for sampling lots of bulk grain can be found in Table 5.

Table 5. GIPSA approved probe sizes for sampling stationary lots of grain.

Carrier	Length (feet)
Flat-bed truck/trailer	5 or 6
Hopper bottom trailer	6, 8, or 10
Box car	6
Hopper car	10 or 12
Barge	12

Sampling patterns. GIPSA has established a sampling pattern for each type of carrier. Each lot should be probed in as many additional locations as necessary to assure that the sample is the required size and representative of the lot. Additional probes should be drawn in a balanced manner. For example, one compartment of hopper car should not be probed

twice unless the other compartments are also probed twice, regardless of the amount of grain in any one compartment or the amount of additional sample needed.

The following diagrams indicate the standard sampling patterns. Insert the probe at the points marked, with the tip of the probe angled ten degrees in the direction of the arrow. When two arrows are shown, the tip of the probe may be pointed in either of the indicated directions at the samplers discretion.

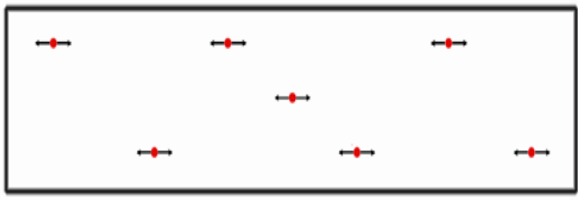


Figure 6. Seven probe pattern for flat-bottom trucks or trailers containing grain more than four feet deep.

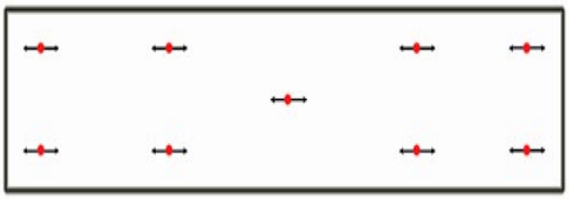


Figure 7. Nine probe pattern for flat-bottom trucks or trailers containing grain less than four feet deep.

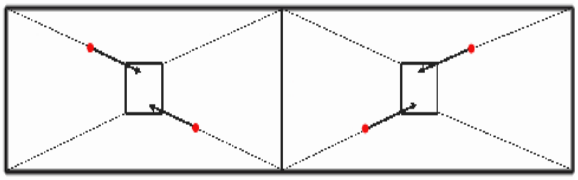


Figure 8. Hopper bottom trailers or containers.

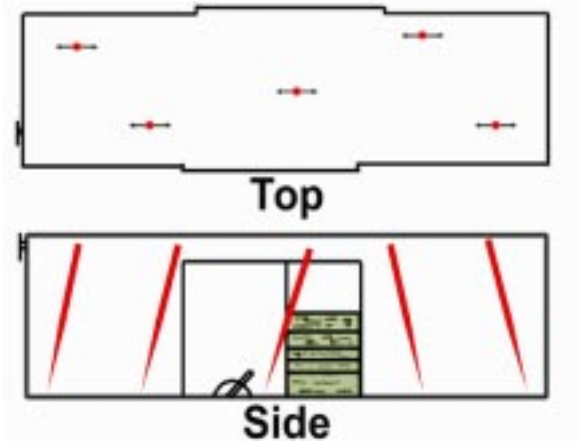


Figure 9. Boxcar

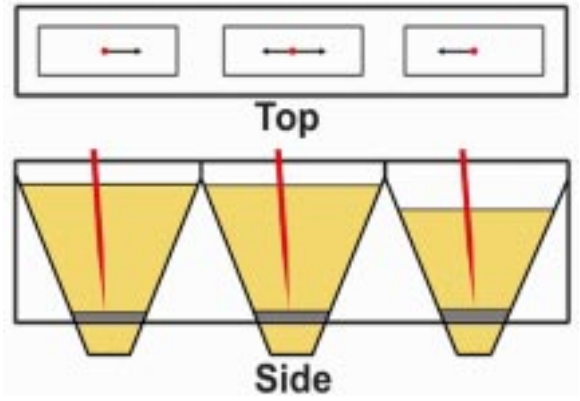


Figure 10. Hopper car

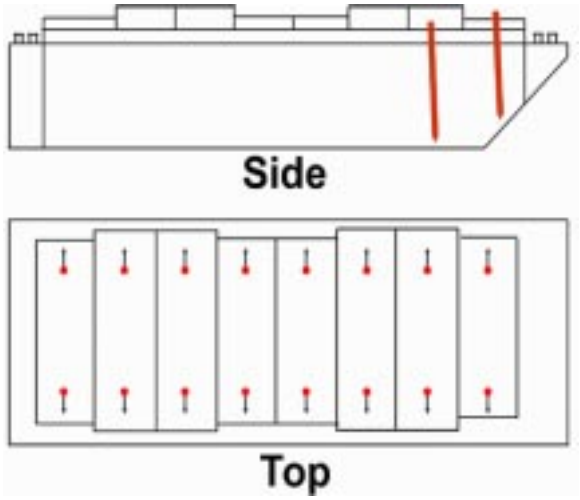


Figure 11. Roll-top barge

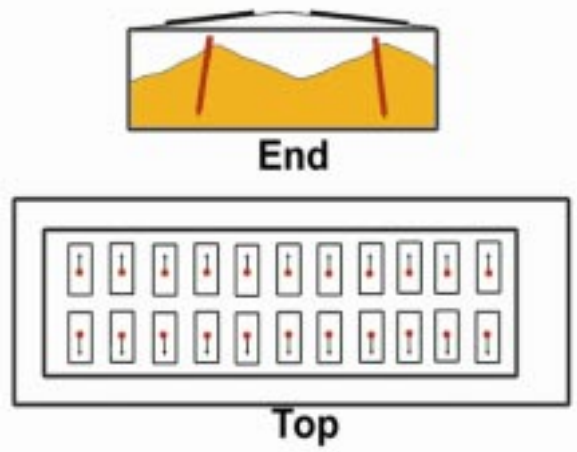


Figure 12. Flat-top barge

Sacked Grain. When grain is sampled from sacks, a double-tubed, compartmented grain probe (4 feet minimum length) should be used. Stand the sacks up on end and insert the probe into the top corner of the sack. Move the probe diagaonally through the sack until the probe touches the bottom of the opposite corner.

Chapter 5
Analytical Procedures

Laboratory Safety. In the previous discussion of the toxicity of the mycotoxins it should be apparent that the toxins attack the systems of animals and humans in many different and harmful ways. Caution should be the guide whenever dealing with crops directly from the field and in the laboratory.

Mycotoxins can cause three types of illness:

Infection. Invasion of living tissue by microorganisms.

Allergies. Hypersensitivity of tissues to bacteria and fungal agents.

Toxicoses. Chronic or acute disease from exposure to mycotoxins [*19*].

All samples that are being prepared for analysis should be treated as being toxic until proven otherwise. Personal protective equipment should be used whenever handling samples in the lab or when dealing with hazardous chemicals. This should include the use of non-permeable gloves, safety glasses, and dust masks whenever possible [*19*].

Dust creation should be kept to minimum whenever possible. Laboratories should never be dry swept but instead vacuumed with vacuums equipped with high efficiency particulate filters. Grinding of samples should occur either in negative pressure rooms or in enclosed areas with exhaust hoods that will remove as much of the fine particulates as possible [*19*].

Grinding. Once the sample is obtained it is once again "enlarged" by grinding to a fine particle size. The grinding opens up infected kernels and distributes the particles throughout the sample giving an increased chance of detecting contaminated particles. To increase the probability of finding contaminated grain, more samples could be obtained to increase the sample size yet further. By doubling the sample size the sampling variance is reduced by 50 percent. But the larger the sample the more cumbersome the process becomes. If a ship lot of corn is comprised of 20 sublots, a single sample of 200 pounds could be ground and analyzed, but it is statistically better to analyze 20 samples of 10 pounds each to better use resources and obtain more accurate results [*6*].

Various types of grinders exist to handle different products and the varying degrees of humidity they are subjected to in storage. Some grinders allow the screen size to be changed, others provide removable disc heads and various hammer speeds. Grinders such as the Romer mill, Bunn Model G3, Viking Hammermill, Falling Number Mill, and UDY grinder are a few examples.

Care should be taken to always use a clean grinding system. More care must be taken after grinding a "hot" sample than after a "clean" one. Some labs run a "clean" sample between lots for the purpose of cleaning the mill. Other labs run a few grams of the next sample and discard it to "purge" the mill.

These practices are no guarantee that cross contamination will be eliminated. Only if the grinder can be completely opened up and cleaned can cross contamination between successive samples be avoided. The use of a vacuum can make this procedure effortless.

Attaching a plastic bag to the outlet spout of the grinder will capture all of the sample without releasing dust into the air. The Romer mill is capable of automatically subdividing large ten pound samples into a representative 500 gram subsample. When using other grinders a riffle divider must be used to reduce ten pound samples to 500 grams. Once the 500 gram sample is obtained, blend the sample by lifting or rolling the ends of the bag to the opposite side and repeating at least ten times. Allow the dust to settle before opening the bag. The 50 gram portion can now be removed and weighed.

Blacklighting. Screening of corn for possible aflatoxin contamination using a "black light" was popular in the 1970's. In spite of the widespread use of black lighting, research has shown that the technique detects materials that are not mycotoxins. In addition, mycotoxins such as DON do not fluoresce; therefore blacklighting is inappropriate. **The black light should no longer be used for any type of mycotoxin screening** [20].

Minicolumn. The minicolumn is a small column containing silica gel and florisil to which sample extracts are applied for detection of aflatoxin. Minicolumns were very popular until quick-test kits became available over the last few years. Although capable of giving good results under the proper conditions, the minicolumn is no longer a GIPSA approved official method for mycotoxin analysis..

TLC/HPLC. Since 1990 the officially approved method of testing by the Association of Official Analytical Chemists (AOAC) for mycotoxins, and aflatoxins in particular, has been thin-layer chromatography. In recent years High Pressure Liquid Chromatography (HPLC) has replaced TLC. TLC is no longer a GIPSA-approved official method for mycotoxin analysis.

Quick Tests. Newer processes have been developed for "quick" tests that will give results in a shorter period of time with less use of hazardous or toxic chemicals and procedures. Four tests that will be covered in more detail that use these methods are the Neogen Veratox, Romer AccuTox, Vicam Aflatest, and Romer Fluoroquant.

The Neogen Veratox and Romer AccuTox methods use direct competitive Enzyme Linked Immunosorbent Assay (ELISA) technology. Vicam's Aflatest procedure and Romer Fluoroquant process use fluorescence technology.

All of these tests are quicker than HPLC, with accuracy that is acceptable in the grain processing and feed industries. HPLC is used as a reference method to gauge the accuracy of the other tests and in laboratory use where greater accuracy is needed.

NEOGEN (Aflatoxin and DON)

Neogen's "Veratox" (DON) and "Veratox-AST" (Aflatoxin) use ELISA technology. Antibodies specific for a mycotoxin are adhered to the bottom of a microwell.

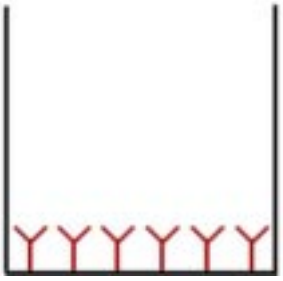


Figure 13. Neogen Microwell.

A solution of mycotoxin, chemically conjugated to an enzyme, is provided with the kit. A sample to be tested for mycotoxin is ground and extracted. The extract is then filtered and mixed with a fixed amount of the mycotoxin-enzyme conjugate solution in a mixing well.

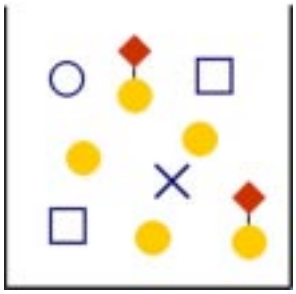


Figure 14. Mixing well containing extract and conjugate.

A portion of the mixture is then transferred to a microwell. The mycotoxin from the extracted sample and mycotoxin-enzyme conjugate then compete for binding to the antibodies in the microwell.

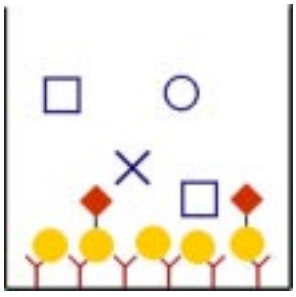


Figure 15. Free toxin and conjugate compete for binding sites in microwell containing antibodies.

The assay procedure measures how much of the conjugate actually binds to the antibodies by first thoroughly washing the microwell then adding a colorless substrate.

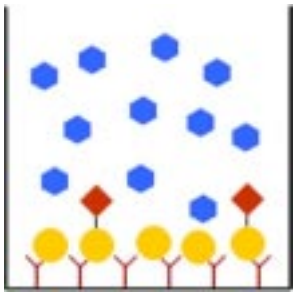


Figure 16. After washing substrate is added.

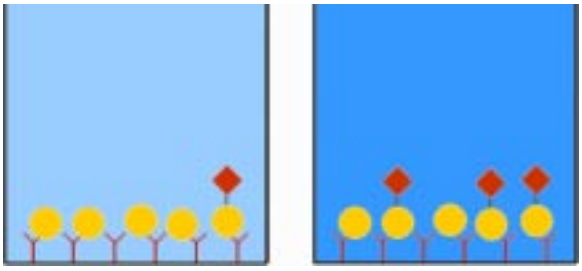


Figure 17. The conjugated enyme and substrate react to form a blue color.

The enzyme present in the microwell converts the substrate to a blue colored product; the more mycotoxin-enzyme conjugate in the microwell, the more intense the blue color. Because samples with high mycotoxin will result in less binding of the mycotoxin- enzyme conjugate, positive samples will be lighter blue. Quantitative measurements are obtained by measuring the intensity of the color with an optical density reader.

ROMER (Aflatoxin)

Romer Lab's FluoroQuant test for aflatoxin uses fluorescence technology. A sample to be tested is ground and extracted with methanol/

water (80/20). The extract is filtered and a portion of the filtrate is placed in the top of a UniSep 2001 column. An equal portion of diluent is added and the solutions are thoroughly mixed by shaking. The column is placed into a cuvette and a syringe barrel attached. The extract is slowly pushed through the column.

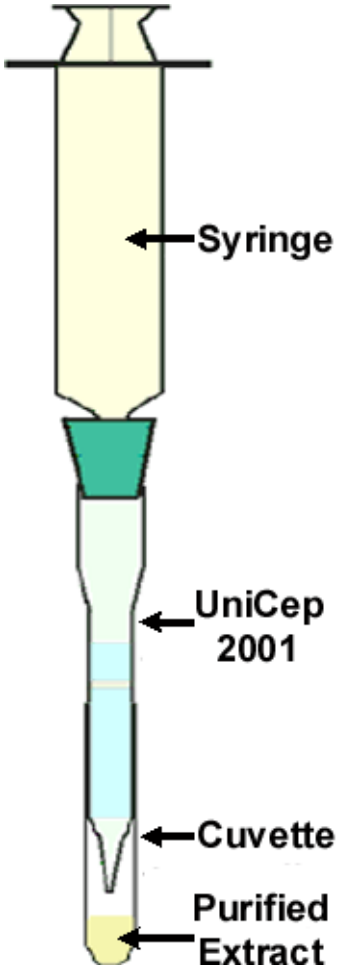


Figure 18. The filtrate and diluent are placed into a UniSep column, mixed, and forced through the column

A portion of the purified extract is transferred to a clean pipet and a developer reagent is added to enhance the fluoresence of the aflatoxins. The solution is mixed by vortexing and the sample is read in a fluorometer.

ROMER (Deoxynivalenol)

Romer Lab's AccuTox test for deoxynivalenol is an ELISA method. Antibodies specific for a mycotoxin are adhered to the bottom of a tube.

Figure 19. Romer Antibody Tube.

A sample to be tested for DON is ground, extracted with distilled water, and filtered. A solution of DON, chemically conjugated to an enzyme, is provided with the kit. The filtered extract is mixed with an equal amount of the mycotoxin-enzyme conjugate in an antibody tube.

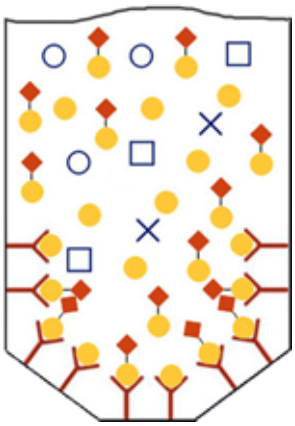


Figure 20. Free toxin and conjugate compete for binding sites in microwell containing antibodies.

After the toxin and conjugate have had time to attach to the antibodies, the remaining solution is discarded and the tube rinsed with distilled water.

After tapping the tube on a paper towel to remove the water, substrate solution is added to the tube. The conjugated enzyme present converts the substrate to a blue colored product; the more conjugate in the tube, the more intense the color.

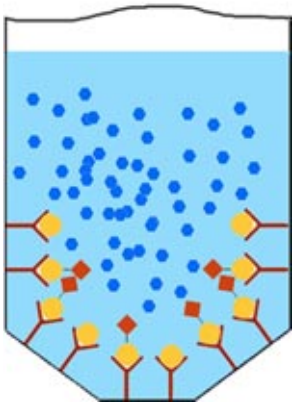


Figure 21. After washing. substrate is added which reacts with the conjugated enzyme to form a blue color.

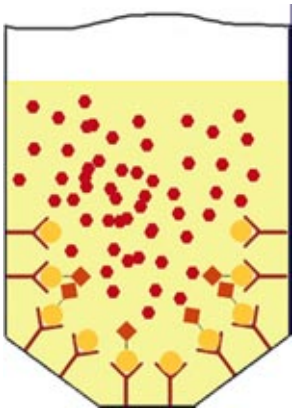


Figure 22. The solution turns yellow when the stop solution is added.

After allowing the color change to develop for five minutes, “stop solution” is added. This stops the reaction and changes the color of the solution to yellow. Quantitative measurements are obtained by measuring the intensity of the color with a Hach spectrophotometer. Because samples with high DON levels will result in less binding of the conjugate, positive samples will be lighter color.

VICAM (Aflatoxin)

Vicam’s columns use immuno-affinity chromatography technology. The columns contain antibodies specific for the mycotoxins chemically fused to beads.

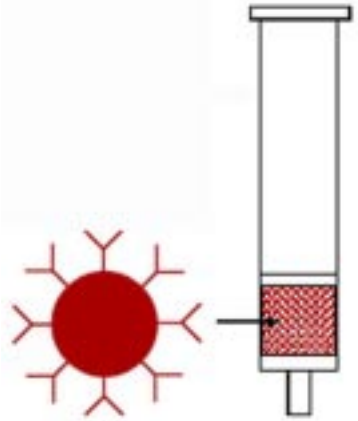


Figure 23. The aflatest column contains antibodies fused to glass beads.

A sample is ground and extracted with a methanol/water solution. The extract is then diluted and run through the affinity column.

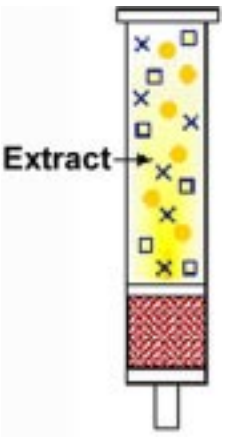


Figure 24. The filtered and diluted extract is run through the affinity column.

The mycotoxin sticks by binding to the antibody beads. Other materials in the extract do not stick and are washed off the column.

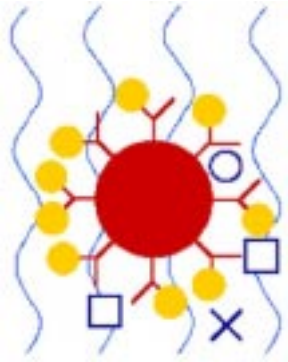


Figure 25. Impurities do not stick to the antibodies and are washed off.

The mycotoxin is then removed from the column using methanol.

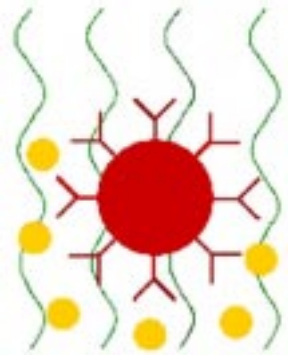


Figure 26. The toxin is removed with a methanol wash.

A developer containing bromine is added to enhance the fluorescence by making a derivative of the mycotoxin. The level of mycotoxin is measured with a fluorometer.

GIPSA Approved Mycotoxin Methods

April 1999

Kind	Manufacturer	Model	GIPSA Certificate	Distributor	Location
Aflatoxin (quantitative)	Neogen	Veratox-AST	94-101A	Neogen	Lansing, MI
Aflatoxin (quantitative)	Vicam	Aflatest	91-103B	Vicam	Waterstown, MA
Aflatoxin (quantitative)	Romer	FluoroQuant	98-101	Romer	Union, MO
DON (quantitative)	Neogen	Veratox	95-102	Neogen	Lansing, MI
DON (quantitative)	Romer	FluoroQuant	95-101	Romer	Union, MO
DON (quantitative)	Romer	AccuTox	99-101	Romer	Union, MO
Aflatoxin (qualitative)	Editek	EZ-Screen	Per Memo	Diagnostix	Mississauga, Ontario
Aflatoxin (qualitative)	International Diagnostic	Afla-20-Cup	Per Memo	Romer	Union, MO
Aflatoxin (qualitative)	Neogen	Agriscreen	Per Memo	Neogen	Lansing, MI
DON (qualitative)	Neogen	Agriscreen	95-102	Neogen	Lansing, MI

NEOGEN 620 Lesher Place, Lansing, MI 48912, (800)-234-5333

VICAM, L.P. 313 Pleasant Street, Watertown, MA 02172, (800)-338-4381

Romer Labs 1301 Stylemaster Drive, Union, MO 63084, (314) 583-8600

International Diagnostic Systems Corp. P.O. Box 799, St. Joseph, MI 49085, (616) 428-8400

EDITEK 1238 Anthony Road, Burlington, NC 27215, (800) 334-1116

Diagnostix 5730 Coopers Avenue, Unit #27, Mississauga, Ontario, Canada L4Z2E9, (905) 890-6023

Glossary

(Derived from underlined words in text)

Aflatoxicosis A poisoning that results from ingestion of aflatoxins in contaminated food or feed.

Ascospores A sexual spore formed in an ascus.

Ascus Saclike structure in which ascospores are borne.

Blight General term for sudden, severe, and extensive spotting, discoloration, wilting, or destruction of leaves, flowers, stems, or the entire plant.

Carcinogen A substance or agent producing or inciting cancer.

Carcinoma A new growth or malignant tumor enclosing cells in connective tissue.

Cirrhosis A chronic disease of the liver characterized by progressive destruction and regeneration of liver cells, ultimately resulting in liver failure and death.

Coleoptiles Ephemeral, nonpigmented tissue sheathing the first true leaf of a grass (maize) seedling.

Clums Stem of a grass plant.

Cultivars Cultivated variety.

Embryo Seed “germ”; the rudimentary plant within a seed.

Florets Small flower enclosed in a spikelet.

Fungicide Chemical or physical agent that kills or inhibits the growth of fungi.

Fungus (fungi) Organism having no chlorophyll, reproduces by sexual or asexual spores and not by fission, and, generally, a mycelium with well-marked nuclei.

Germination Begin growth as of a seed, spore, sclerotium, or other reproductive body.

Glumes Empty bract at the base of a spikelet.

Host	Living plant attacked by (or harboring) a living parasite and from which the invader is obtaining part or all of its nourishment.
Hybrids	Offspring of two individuals of different genetic character.
Hyphae	A tubular, threadlike filament of fungal mycelium.
Inoculum	Spores or other diseased material that may cause infection.
Lesions	Well-marked but localized diseased area; a wound.
Metabolite	A product of the chemical changes in living cells by which energy is provided for vital activities and processes and new material is assimilated.
Mycelium	Mass of hyphae constituting the body (thallus) of a fungus.
Mycotoxicoses	Literally, fungus poisonings; current usage limited to poisoning of people and animals by various food and feed products contaminated (and some times rendered carcinogenic) by toxin-producing fungi.
Necrosis	Death of plant or animal cells, usually resulting in tissue turning dark; commonly a symptom of fungus, nematode, virus, or bacterial infection.
Pathogen	Organism or agent that causes disease in another organism.
Pericarp	Outer layer of a seed or fruit.
Perithecia	A small fruiting body in certain fungi, containing ascospores.
Sclerotia	Hard, frequently rounded, and usually darkly pigmented resting body of a fungus composed of a mass of special hyphae cells. The structure may remain dormant for long periods. Sclerotia germinate upon return of favorable conditions to produce stroma, fruiting bodies and mycelium.
Sori	Compact fruiting structure of rust and smut fungi.
Spikelet	Spike appendage comprised of glumes and florets; unit of inflorescences in grasses.
Spore	One-to-many-celled reproductive body in fungi and lower plants that can develop into a new plant.

Subcrown internode	Short, culm-like connection between the crown and seed roots of wheat.
Teliospores	Thick-walled resting spore of rust and smut fungi that germinates to form a basidium.
Toxin	A poisonous substance, having a protein structure, that is secreted by certain organisms and is capable of causing toxicosis when introduced into the body tissue. Toxins are also capable of inducing an antitoxin.
Trichothecene	A group of chemically related compounds produced by fungi such as *Fusarium.*

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   Plant Disease Volume 82 No.6 June 1998

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11. "**Known Carcinogen: Aflatoxin**" [Cas. No. 1402-68-2] (nd): 2 pag. Online. Internet. 20, Feb. 1997.

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17. **Saad, Nabil.,** "Aflatoxins: Occurrence and Health Risks" (nd): 7 pag. Online. Internet. 20, Feb. 1997.

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20. "**Understanding and Coping With Effects of Mycotoxins in Livestock Feed and Forage"** North Carolina Extension Service (nd)

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Internet Resources

U.S. Government Sites

Grain Inspection, Packers and Stockyards Administration
"http://www.usda.gov/gipsa"

Food Safety and Inspection Service
"http://www.fsis.usda.gov/index.htm"

Animal and Plant Health Inspection Service
"http://www.aphis.usda.gov"

The Cooperative State Research, Education, and Extension Service of USDA
"http://www.reeusda.gov"

Agricultural Research Service
"http://www.ars.usda.gov"

The National Agricultural Library
"http://www.nal.usda.gov"

GrainGenes: A Database for Small Grains and Sugarcane
"http://wheat.pw.usda.gov"

FDA
“http://www.fda.gov”

State Sites

Auburn University Grain Crops Homepage
"http://www.acesag.auburn.edu/department/grain/Frame.htm"
Mycotoxins And Mycotoxicoses, Auburn University, Auburn, Alabama
"http://www.acesag.auburn.edu/department/grain/ANR767.htm"

University of Arizona Plant Pathology Home
"http://ag.arizona.edu/PLP/plphome.html"

Cornell University Animal Science Department
“http://www.ansci.cornell.edu:80/Nabil2/ansci625.html”
Wheat Diseases and Pests
“http://greengenes.cit.cornell.edu/wpest.html”

Iowa State University Index of Available Articles About Plant DiseasesI
“http://www.ipm.iastate.edu/ipm/icm/indices/plantpathindex.html’
Iowa State University-Identifying Ear rot Diseases
“http://www.ipm.iastate.edu/ipm/icm1996/10-7-1996/earrotid.html”

Kansas State University Extension Plant Pathology
"http://www.ksu.edu/plantpath/extension"
KSU Plant Pathology Database
“http://www.lib.ksu.edu/resource/ppat/ppat0004.htm”

University of Maryland "United States National Dairy Database"
"http://www.inform.umd.edu/EdRes/Topic/AgrEnv/ndd"

Michigan State University Extension Home Page
"http://www.msue.msu.edu"
MSU Field Crop Newsletter Info
"http://www.msue.msu.edu/ipm/fieldCAT.htm"

Missouri Agriculture Experiment Stations
"http://aes.missouri.edu”
Aflatoxin in Corn
"http://aes.missouri.edu/delta/croppest/aflacorn.htm"

NC State University: CALS Home Page
"http://www.cals.ncsu.edu/"
World Wide Web Agriculture Library
“http://imp www.ncsu.edu.cernag/cern.htm”

North Dakota State University Extension Service
"http://www.ext.nodak.edu"
FUSARIUM HEAD BLIGHT at NDSU
"http://www.cc.ndsu.nodak.edu/instruct/stack/FHB/FHB.html"

Ohio State University Extension
"http://www.ag.ohio-state.edu"
Ohio State University Ohioline: Your Link to Information, News, and Education
"http://www.ag.ohio-state.edu/~ohioline"

Ohio State University Ohioline: Agronomic Crop Disease Factsheet Index
"http://www.ag.ohio-state.edu/~ohioline/ac-fact"

Oregon State University Cereals Extension Page
"http://www.css.orst.edu/cereals"
Wheat Diseases - Oregon State University Cereals Extension
"http://www.css.orst.edu/cereals/Wheat/Diseases"

Purdue University Agricultural Communications
"http://www.agcom.purdue.edu/AgCom"
Purdue Extension Publications
"http://www.agcom.purdue.edu/AgCom/Pubs/menu.htm"
Purdue Ag Answers Home Page
"http://www.aes.purdue.edu/AgAnswrs/AgAnswers.html"
Purdue University Plant Pathology Publications & Newsleters
”http://www.btny.purdue.edu/Pubs/btnynews.html”
Crop Diseases in Corn, Soybeans, and Wheat
“http://www.btny.purdue.edu:80/extension/pathology/cropdiseases/crops.html”

Texas A&M University Agriculture Program "AgNews"
"http://agnews.tamu.edu"

Commercial Sites

Neogen
"http://www.neogen.com"

VICAM
"http://www.vicam.com"

Romer Laboratories Inc.
"http://www.romerlabs.com"

Other Sites

Equine Veterinary Network
"http://iaep.com"
Index of /pages/nutrition/toxicosis
"http://iaep.com/pages/nutrition/toxicosis"

Welcome to the Small Grains Website
"http://www.smallgrains.org"

Samuel Roberts Noble Foundation
"http://www.noble.org/Navigate/Navi_bar.htm"

McGill University: "Toxigenic Molds and Mycotoxins"
"http://dietetics.mcgill.ca/staff/chan/420/lecture7/sld001.htm"

UK Ministry of Agriculture,Forestry, & Fisheries
"http://www.maff.gov.uk/maffhome.htm"

Ontario Ministry of Agriculture, Food & Rural Affairs (OMAFRA)
"http://www.gov.on.ca/OMAFRA/english/index.html"

The Plant Pathology Internet Guidebook
“http://www.lfgb.uni.hannover.de/extern/ppigb/ppigb.htm”

Fungal Disease Fact Sheet

Disease Name: Aspergillus Ear Rot

Grain Affected: *Primarily* Corn/Corn products, Peanuts/Peanut products; *Secondarily* Pecans, Walnuts, Almonds, Cottonseed meal, Sorghum, Barley and Oats.

Mycotoxin: Aflatoxin

Pathogen: Aspergillus flavus, A. parasiticus, A. nomius, A. niger, A. glaucus.

Synptom: Yellow-Green mold found on ears of corn in the field or on kernels in storage.

Conditions: Preharvest moisture >18%, Temperature 12 -40 C (54 -104 F), Humidity >85%, Severe drought stress, nitrogen deficiency, and significand insect damage. Stored grain should be dried and stabilized to <14% moisture.

Inoculumn Dispersal: Waterborne via rain, splashing water, airborne and also transmitted through insect and bird damage.

Inoculumn Survival: Overwinters on or near soil surface on decaying host plant debris.

Effect on Crop: Decreased yeilds, grain quality

Management: Minimize crop stress from drought and insect damage, harvest early, and reduce storage moisture levels to <15%.

FDA Action Level: 20 parts per billion (ppb) for grain and feed products, and 0.5 ppb for milk. Recommended limits in feed are: 20ppb for dairy cattle; 100ppb for breeding cattle, breeding swine, and mature poultry; and 300 ppb for finishing cattle and swine.

Livestock Affected: No animal species is resistant to the acute toxic effects of aflatoxins according to FDA literature.

Livestock Symptoms: Liver damage, decreased reproductive performance, reduced milk or egg production, embryonic death, birth defects, tumors,suppressed immune system functions. Aflatoxin is a very potent carcinogen.

Human Symptoms: Historical outbreaks of aflatoxicosis where contaminated corn was he major dietary food 397 persons were affected and 108 persons died. The patients experienced high fever, rapid progressive jaundice, edema of the limbs, pain, vomiting, and swollen livers. Histopathological examination of humans showed extensive bile duct proliferation and peripirtal fibrosis of the liver and gastrointestinal hemorrhages. A 10-year follow up of the outbreak found the survivors fully recovered

Photo 1. *Aspergillus sp.* growing on corn kernel (R. Friedrich)

Photo 2. *A. flavus* on insect damaged ear of corn (J.L.Richard)

Photo 3. Ear of corn infected with *A. flavus*. (G. Munkvold)

Fungal Disease Fact Sheet

Disease Name: Black Tip / Black Point

Grain Affected: Wheat, Barley

Mycotoxin: None

Pathogen: Cochliobolus sativus,
Helminthosporium sativum (asexual stage)

Synptom: Brown lesion on coleoptiles, subcrown internodes, roots, and culms of seedlings. Black-brown leaf lesions on maturing plants.

Conditions: *Seedlings:* warm, dry seedbeds. *Maturing heads:* humidity >90%, Rainfall during seed maturation, seed moisture >20%

Inoculumn Dispersal: Almost exclusively by planting infected seeds. Occasionally soilborne.

Inoculumn Survival: Primarily through storage of infected seed grain. Can overwinter in soil.

Effect on Crop: Discolored grain is discounted in value due to undesirable color and odor. Possible decrease in yield, test weight, and germinability. Causes seedling blight, root rot, and spot blotch. Barley may be unacceptable for malting.

Management: Plant non-diseased seed. Deep soil tillage.

FDA Action Level: None

Livestock Affected: None

Livestock Symptoms: None

Human Symptoms: None

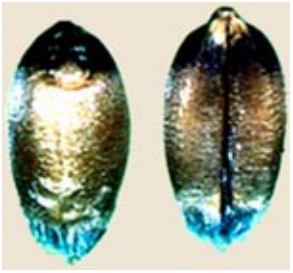

Photo 4. Wheat kernels infected with black tip fungus (FGIS)

Photo 5. Black tip damage (FGIS W-1.0)

Photo 6. Barley kernels severely infected with black tip (E. Banttari)

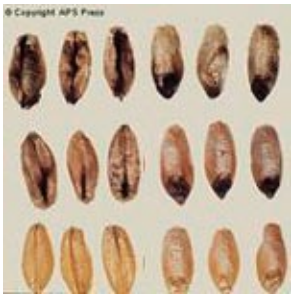

Photo 7. Wheat kernels severely infected with black tip (APS)

Fungal Disease Fact Sheet

Disease Name: Blue-Eye Mold

Grain Affected: Corn

Mycotoxin: None

Pathogen: Penicillium oxalicum

Synptom: Powery green or blue-green mold on and/or between kernels usually at the ear tips. Discoloration of germ indicates kernel death.

Conditions: Primarily a storage mold. Enhanced by prolonged wet-holding periods, especially on cob stored corn in cribs. Moisture/Temp. >14%, 25 C (75 F) for A. glaucus and >18%, 5 C(40 F) for P. oxalicum. Humidity >70%. Can occur in field if introduced through insect/bird damage.

Inoculumn Dispersal: Soil and airborne, insects, birds, equiptment and storage facilities.

Inoculumn Survival: Overwinters on/near soil surface in host residues. Equiptment and storage facilities.

Effect on Crop: Decreased feed and market value.

Management: Early harvest, areation and drying to reduce moisture <15%

FDA Action Level: None

Livestock Affected: None

Livestock Symptoms: None

Human Symptoms: None

Photo 8. Blue-eye mold damage (FGIS C-1.0)

Photo 9. Purple plumule; not damage (FGIS C-1.1)

Photo 10. Penicillium ear rot (APS)

Photo 11. Conidia and conidiphores of *Penicillium sp*. (M. Brown & H. Brotzman)

Fungal Disease Fact Sheet

Disease Name: **Ergot**

Grain Affected: Wheat, Rye, Triticale, Barley, Oats, Cultivated & Wild Oats

Mycotoxin: Ergot Alkaloids - Ergotamine, Ergotpeptide pyrroloidine and Lysergic Acid Alkaloids.

Pathogen: Claviceps purpurea, Claviceps paspalli and Claviceps fusiformis.

Synptom: Purple-black, hornlike sclerotia (ergot bodies) that replace one or more seeds in the head, up to 10 times larger that normal seeds. Infected florets exude a sugary slime in sticky yellow droplets.

Conditions: Ergot is favored by wet, cool weather that accompanies and pro longs flowering periods. Germinates during spring and early summer.

Inoculumn Dispersal: Airborne, splashing rain and insects.

Inoculumn Survival: Sclerotia remain viable for approximately 1 year in soil and longer for grain in storage.

Effect on Crop: Decreased yields, discounted market value.

Management: Deep soil tillage, crop rotation clean seeds, use of modern grain cleaning equipment, clean cultivation, and eliminate potential ergot sources by mowing headlands and roadways, before grasses mature.

FDA Action Level: None

Livestock Affected: Cattle and Sheep

Livestock Symptoms: Blood vessel constriction of extremities followed by dry gangrene. Digestive tract inflammations, internal bleeding, vomiting, constipation or diarrhea. Abortion may occur in swine.

Human Symptoms: Historical accounts indicate gangrene, convulsions and gastro-intestinal disorders were common with approximately 50% resulting in death. Modern cultivation techniques along with education have virtually elimi nated this threat.

Photo 12. Ergot sclerotia (FGIS OF-12.0)

Photo 13. Wheat head with ergots (F.J. Zilinsky)

Photo 14. Barley head with honeydew and ergots (APS)

Photo 15. Ergot sclerotia (APS)

Fungal Disease Fact Sheet

Disease Name: **Fusarium Ear Rot**

Grain Affected: Corn

Mycotoxin: Fumonism

Pathogen: Fusarium moniliforme

Synptom: Salmon-pink to reddish-brown spore mass starting at the ear tip or on groups of kernels scattered thoughout the ear, progressing to a powdery or cottony-pink mold. Has also been found in seemingly healthy corn.

Conditions: Infection tends to follow injury by insects or birds. Disease development favors dry, warm weather. High kernel moisture at time of harvest. Affects as much as 90% of midwest corn crops.

Inoculumn Dispersal: Soil and airborne, insects, birds, corn borers and earworms. Contaminated storage facilties and equipment.

Inoculumn Survival: Overwinters on/near soil surface in host plant debris. Contaminated storage facilties and equipment.

Effect on Crop: Decreased feed and market value. Reduced yield, test weights, and baking qualities.

Management: Crop rotation, deep soil tillage, resistant hybrids, reduce nitrogen levels in fields, and use of clean equipment and storage facilities.

FDA Action Level: None; Advisory levels are 5ppm for horses, 10ppm for swine, and 50ppm for cattle.

Livestock Affected: Horses, donkeys, mules, swine and cattle.

Livestock Symptoms: Equine leukoencelhalomalacia (blind staggers). Loss of appetite.

Human Symptoms: On going research indicates potential for adverse health problems (cancer).

Photo 16. Corn infected by *Fusarium sp*. (G. Munkvold)

Photo 17. Fusarium ear rot (APS)

Fungal Disease Fact Sheet

Disease Name: **Gibberella Ear Rot**

Grain Affected: Corn

Mycotoxin: Deoxynivalenol (Vomitoxin, DON), Zearalenone, T-2

Pathogen: Fusarium graminarum, Fusarium roseum (sexual stage), Gibberella zeae

Synptom: Pink to reddish mold beginning at the ear tip. Occasional blue-black specks (perithecia) found on husk and ear shank.

Conditions: Enhanced by cool wet periods within 3 weeks after silking. Moisture >20%, Temperature DON 21 -29 C (70 -85 F), Temperature ZEA, T-2 <15 C (59 F), Humidity High

Inoculumn Dispersal: Waterborne via rain, splashing water, airborne and also transmitted through insect and bird damage.

Inoculumn Survival: Overwinters on / near soil surface in host residues such as grasses, corn, and wheat stubble.

Effect on Crop: Decreased yields, grain quality and lower test weights.

Management: Crop rotation, deep soil tillage to bury crop residues. Post harvest drying to <18% moisture for whole ear storage, <15% for shelled corn. Early harvest and resistant hybrids.

FDA Action Level: No Action Level; FDA has issued DON advisory levels
1ppm finished wheat products,
5ppm grain/ grain by-products for swine (<20% of diet),
10ppm grain/ grain by products for cattle/poultry (<50% of diet).
5ppm grain/ grain by-products all other animals (<40% of diet),

Livestock Affected: Predominately swine with concentrations as low as 1ppm.

Livestock Symptoms: DON-Vomiting, decreased weight gain, diarrhea, lethargy, blanched skin color, dermal irritation, hypothermia, intestinal hemorrhage, and ultimately feed refusal.

ZEA-Infertility, abortion and other breeding problems.

Human Symptoms: None shown to date. On going research.

Photo 18. Gibberella ear rot (G. Munkvold)

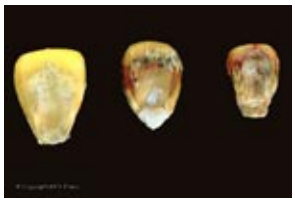

Photo 19. Kernels infected with *Gibberella zeae* (APS)

Photo 20. Gibberella ear rot (APS)

Fungal Disease Fact Sheet

Disease Name: **Karnal Bunt**

Grain Affected: Wheat, Triticales and Rye

Mycotoxin: None

Pathogen: Tilletia indica

Synptom: Dark brown teliospores affect only a few seeds per head and usually at their embryo end. Larger sori may extend along the crease and occasionally envelope the whole kernel. Spores may impart a fishy odor to the grain.

Conditions: Wet conditions required for teliospores germination. Furrow irrigation or rainfall, followed by 3-4 days of cool, humid weather are required for sporidia penetration.

Inoculumn Dispersal: Primarily airborne during harvest when the pericarp of bunted kernels is broken. Also through use of contaminated equipment.

Inoculumn Survival: Spores overwinter in soil then germinate at or near surface in response to favorable conditions. Spores can survive in the soil up to 5 years.

Effect on Crop: Reduced crop yield and quality. Flour made from bunted kernels are discolored with an unpleasant odor and taste.

Management: Chemical seed treatments can inhibit the germination of seedborne teliospores. Some fungicides applied at heading protect against infection.

FDA Action Level: None

Livestock Affected: None

Livestock Symptoms: None

Human Symptoms: None, though wheat containing >3% bunted kernels is considered unfit for human consumption.

Photo 21. Wheat infected with karnal bunt (F.J. Zilinski)

Photo 22. Seed infected with karnal bunt (APS)

Photo 23. Teliospore of *T. indica* (APS)

Photo 24. Infection begins at the embreyo and progresses along the crease. (APHIS)

Fungal Disease Fact Sheet

Disease Name: **Scab (Head Blight)**

Grain Affected: Wheat, Barley

Mycotoxin: Deoxynivalenol (Vomitoxin, DON)

Pathogen: Fusarium graminarum, Gibberella zeae, Zearalenone

Synptom: Wheat-Spikes appear bleached with brown / purplish discoloration of stem. Pink to salmon-orange spore mass appears on glumes and kernels
Barley-The first indication of infection is a small water-soaked, somewhat brownish spot at the base of middle of the glume or on the rachis. Water soaking and discoleration then spread in all directions.

Conditions: Moist periods, Moisture >20%, Temperature 21 -30 C (70 -86 F), Humidity high. Also infection can occur at temperatures as low as 15 C (59 F) if humidity remains high for up to 72 hours.

Inoculumn Dispersal: Airborne. During rainy seasons spores can be splashed onto other heads of cereal crops or windblown. Soil borne spores overwinter from previous host crops. Also insect and bird damage.

Inoculumn Survival: Overwinters on / near soil surface in host residues such as grasses, wheat and barley stubble.

Effect on Crop: Decreased yields, grain quality and lower test weights. Adversely affects flavor and baking qualities.

Management: Crop rotation, deep soil tillage to bury crop residues. Post harvest drying to <15% moisture, <13% for scabby grain going into storage.

FDA Action Level: None: DON advisory levels are 1ppm finished wheat products, 5ppm grain/ grain by-products for swine (<20% of diet), 5ppm grain/ grain by-products all other animals (<40% of diet), 10ppm grain/ grain by products for cattle and poultry (<50% of diet).

Livestock Affected: Predominately swine with concentrations as low as 1ppm. DON can also cause problems in horses, breeding and lactating animals, but only at high concentrations. Cattle and poultry are more tolerant of vomitoxin and zearalenone.

Livestock Symptoms: DON Vomiting, decreased weight gain, diarrhea, lethargy, blanched skin color, dermal irritation, hypothermia, intestinal hemorrhage, and feed refusal.
ZEA-Enlargement or swelling of vulva. Severe reproductive and infertility problems. Decreased milk yield in cattle.

Human Symptoms: Induced muscle spasms and vomiting

Photo 25. Plants infected with scab (APS)

Photo 26. Spikes infected with scab (APS)

Photo 27. Wheat spiklet with orange spore mass (C.R. Luzzard)

Photo 28. Scab damage (FGIS W-2.0)

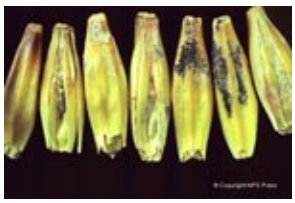

Photo 29. Barley kernels infected with G. Zeae (APS)

Fungal Disease Fact Sheet

Disease Name: **TCK Smut (Dwarf Bunt)**

Grain Affected: Wheat, Rye, Barley, Wild & Cultivated Grasses

Mycotoxin: None

Pathogen: Tilletia controversa

Synptom: Plants infected are ¼ to ½ the normal size. The glumes are conspicuously spread apart exposing plump bunt (smut) balls.

Conditions: Temp. 3 - 8 C (38 - 46 F). Limited to areas where winter is subject to prolonged snow cover.

Inoculumn Dispersal: Primarily soilborne, then germinates under snow or at soil surface.

Inoculumn Survival: Can persist in soil up to 10 yrs.

Effect on Crop: Reduced yield and grain quality. Imparts pungent, fishy odor and darkened appearance. Bunt spores released during threshing are combustible, occasionally resulting in fires sparked by harvesters.

Management: Resistant wheat varities, apply fungicides to soil surface after seeding.

FDA Action Level: None

Livestock Affected: None

Livestock Symptoms: None

Human Symptoms: None

Photo 30. head infected with dwarf bunt (APS)

Photo 31. Teliospores of *T. controversa* (APS)

Photo 32. Teliospore cloud during harves of dwarf bunt infected wheat (APS)

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