NATIONAL CORN HANDBOOK

PEST MANAGEMENT

NCH-52

CR-2105

Aflatoxins and Other Mycotoxins

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Mycotoxins, toxic metabolic by-products of fungi, have received increased attention during the past decade In recent years, aflatoxins B_1, B_2, G_1, and G_2 (a group of closely-related mycotoxins produced by the fungus *Aspergillus flavus* Link ex Fries.) have been given considerable attention in corn. Although aflatoxins have been reported in corn produced in the Midwest, they have been more commonly found in corn produced in the southeast. Aflatoxins were particularly troublesome in 1977, 1980, and 1983 southeastern crops.

In 1960, aflatoxins literally exploded onto the scene when over 100,000 turkeys died after consuming contaminated peanut meal Hepatomas in trout hatcheries, later traced to contaminated cotton seed meal, was almost simultaneously found to be due to aflatoxins in the western United States as was turkey X disease in England. Through the work of several scientists in many disciplines, it was discovered that aflatoxins could be produced by two fungi, *Aspergillus flavus and Aspergillus parasiticus* For many years, it was thought that aflatoxins were produced only in storage However, surveys done in South Carolina in the early 1970's clearly demonstrated that aflatoxins could also be produced prior to harvest Evidence of preharvest contamination of corn with aflatoxins caused additional concerns with regard to potential control measures

THE FUNGUS

Aspergillus flavus and the closely-related species *A parasiticus* are widely distributed in nature. Temperatures ranging from 80° F to 100° F and a relative humidity of 85% (18% moisture in the grain) are optimum for *A. falvus* growth and aflatoxin production. Growth of the fungus is poor at temperatures below 55° F, but slow growth will occur and low amounts of aflatoxins may be produced under favorable moisture conditions at the lower range of temperatures Moisture levels in corn below 12 to 13% inhibit growth of the fungus at any temperature

A. flavus has been reported to occur in most agricultural soils of the south The fungus occurs on many types of organic material in various stages of decomposition including forages, cereal grains, food, and feed products All isolates of the fungus do not produce aflatoxins, thus, the mere presence of *A. flavus* does not mean that aflatoxins will be present in the substrate The fungus has not been associated with causing a yield reduction in corn However, it has been associated with causing a reduction in quality

As is typical with most plant diseases, the amount of corn contaminated with aflatoxins varies from year to year depending upon the environmental conditions under which the grain was produced As an example, based upon surveys done in North Carolina, the percent of the corn samples that contained 100 parts per billion (PPB) or more aflatoxins was as follows: 1976—8%, 1977—12%, 1978—1%; 1979—4%; 1980—18% Other states in the southeast have experienced similar trends In Alabama (6), during a five month period in late 1977, 2,489 corn samples were analyzed for aflatoxin concentration Of these 2,489 samples, 1,556 (62 5%) exceeded the FDA action-level of 20 PPB aflatoxins Of the samples exceeding 20 PPB, 924 (59 4%) exceeded 100 PPB

Losses due to aflatoxin contamination occur at all levels in the production, marketing, and utilization process Contaminated corn and feeds cause economic losses to growers, elevator operators, feed manufacturers, and livestock feeders not only in fewer products and poorer quality products to sell, but also in increased production and operating costs Nichols (13) estimated that the total cost of

aflatoxins in the southeast in 1977 and 1980 were $197,567,000 and $237,834,000, respectively. The majority of these losses were borne by the corn and swine producers

Not only does the extent of the problem vary from year to year, but it also varies a great deal within a state during any particular year For example, producers in the eastern and southern areas of North Carolina more often experience a problem with aflatoxins than do producers in other areas of the state Researchers in Alabama have also reported differences in aflatoxin levels among geographical areas of the state Different levels of aflatoxins between areas appear to be related to differences in plant stress (or insect infestation) in those areas Plant stress in corn, particularly during the time of pollination, is associated with high levels of aflatoxin

Infection by *A flavus* and subsequent production of aflatoxin in corn before harvest have been well documented Extensive aflatoxin accumulation in the field is more likely in the southern United States than in the corn belt states, but aflatoxin has been found in preharvest corn in Iowa, Illinois, Indiana, and Missouri. Jones suggested that high temperatures and high relative humidity favor infection in the field and may account for the greater incidence of aflatoxin in southern regions (9)

Taubenhaus first reported the occurrence of *A flavus* on Texas field corn in 1920 (15) He concluded that insect injury to the maturing ear was necessary for infection, however, he never attempted artificial inoculation with the fungus Since that time, insects have been implicated in transporting inoculum to the developing ear, moving inoculum from silks into the kernel region, and providing wounds for establishment of the fungus in damaged kernels The role of insects in the epidemiology of *A flavus* on corn was recently reviewed by Widstrom (17).

Observations in North Carolina in 1976, 1977, and 1978 revealed a high incidence of *A flavus* infection in ears and kernels free of obvious insect damage Subsequently, a study was undertaken to examine the influence of temperature, humidity, and time of inoculation on the ability of *A flavus* to colonize silk tissue and to invade and produce aflatoxin in undamaged kernels The data obtained in this study showed that *A flavus* could colonize silk tissue, invade the corn kernel, and produce aflatoxin in corn grown in the Phytotron (Southeastern Plant Environment Laboratory), greenhouse, and field Since the plants produced in the Phytotron were free of ear-inhabiting insects, insect feeding does not appear to be necessary for establishment of the fungus or aflatoxin production Natural outbreaks of aflatoxins in corn, however, are often associated with higher than normal incidences of ear invading insects Factors influencing infection of corn by *A flavus* has been recently reviewed by Payne (14)

Jones et. al , in 1981 reported on a study conducted to determine the influence of several cultural practices, including planting date and harvest date, on the development of aflatoxin in short-season, mid-season, and full-season cultivars at three locations in North Carolina (10) Records were made at harvest of the number of ears with visible infection by *A flavus*, with damage by European corn borer, with damage by corn earworm, and with sporulation of *A. flavus* associated with insect damage In addition, airborne inoculum, leaf xylem water potential, and weekly determinations of the mycoflora of developing kernels were monitored in irrigated and nonirrigated plots at one location during the 1978 and 1979 growing seasons

The results of these studies showed that corn planted in April contained about one-third of the aflatoxins found in corn planted in May (averaged across the varieties, location, and years) Although the results were influenced by location and year, there was a significant association of high aflatoxin levels with delayed harvest. The short-season and mid-season hybrids used in this study contained less aflatoxins than the full-season hybrid Although these data agree nicely with the trends noted in the surveys conducted in North Carolina, scientists in other states in the southeast have reported opposite trends The reason for this variation has not been adequately explained, but it may be due to the amount of stress that the plants are under at time of pollination

In the study reported on by Jones et al , in 1981, irrigation did greatly reduce the number of kernels infected and the levels of aflatoxins regardless of hybrid The level of aflatoxin contamination at time of harvest in this study was correlated with the number of spores of *A. flavus* in the atmosphere, particularly at the time the full-season hybrids were pollinating The degree of drought stress, particularly at time of pollination, was also correlated with aflatoxin levels (the greater the stress, the higher the aflatoxin contamination) Although drought stress is very important in the aflatoxin problem, it is not the only stress factor that can have an influence. For example, nitrogen stress can also influence the level of aflatoxin Insect damage and other stress factors that alter normal kernel morphology have also been reported as being important contributors to the aflatoxin problem However, in the study reported by Jones et al , in 1981, and in a more recent study, a poor correlation with the insect damage and aflatoxin concentration was found

Therefore, in summary, it would appear that stress on the corn plant at time of pollination is conducive to high aflatoxin levels at time of harvest Although insects may not be involved in the primary infection process, they certainly can be involved in spreading the fungus within infected ears When the pericarp of a kernel is broken, its contents are exposed to invasion by many microorganisms As the moisture content drops rapidly to levels where *A flavus* can compete successfully with other microorganisms, it becomes an excellent competitor

Detection

Detection of aflatoxins in corn lots is necessary for regulatory agencies, producers, and the grain buyers for obvious reasons The detection of aflatoxins is not exact and there are opportunities for error in all of the steps involved Perhaps the greatest chance for error is in the sampling process, either in the field or from truckload lots The data obtained in this area indicate that at least a 10 lb. sample should be obtained from the area to be sampled, and the sample should be as representative of the total lot as possible

Once the main sample has been obtained, a sub-sample must be obtained This is probably the second greatest source of error. The final analysis for aflatoxin is done on a 50 to 100 gr sample, which again must be representative of the larger sample. The sub-sampling error can be reduced if the total sample is ground before the sub-sample is obtained. However, in many laboratories neither time nor equipment is available to grind the entire 10 lb. sample Thus, a sub-sample of the intact kernels is taken before grinding

Although there is a chance for error in the analytical process, this is the most accurate step in the detection procedure There are several ways of detecting aflatoxin once the sub-sample has been obtained Detection methods range from procedures as simple as visual observation of the toxin-producing fungi to complicated chemical analyses of the toxins themselves

Ultraviolet light. This is the so-called black light method and is used by several buying stations An ultraviolet light of 365 nm is normally used However, it is not a reliable method of detecting aflatoxin since the compound that produces the bright, greenish-yellow fluorescence is kojic acid and not aflatoxin It may be used as a presumptive screening method, but not as an analytical method since fluorescence may occur without aflatoxin being present

Minicolumn method. Velasco devised a minicolumn method employing florisil for rapid screening of aflatoxin B_1 (16) This procedure has been modified and is used by several buying stations to determine whether or not to purchase a lot of corn. Elevators frequently use this method to follow up on black light positive samples, particularly during years when aflatoxin problems are common The method can detect B_1 as low as 5 PPB in cottonseed products, but cannot be used analytically because it lacks resolution, and more importantly, because it does not definitely identify B_1 Normally, a sample is called positive for B_1 if an aflatoxin-like fluorescing material is found absorbed to the florisil layer of the column Generally, an unknown sample is compared to one or more known aflatoxin positive samples (usually at 20 and 100 PPB)

Fluorometric-iodine method. Davis and Diener developed a method for detecting aflatoxins in which iodine is used to convert aflatoxin B_1 into a more intensely fluorescent derivative which is then quantitated using a comparatively simple photofluorometer and filter combination. The instrument is adjusted to read directly in micrograms per kilogram (PPB) of aflatoxin. This method also has the advantage of using less solvents, which makes it much safer for the operator

Thin layer chromatography. This method is approved by the Association of Official Analytical Chemists and is referred to commonly as the CB method In this method, the aflatoxins are extracted from corn using solvents concentrated and spotted on chromatograms The presence of spots on thin layer chromatograms with RF values similar to or identical with those of aflatoxins B_1, B_2, G_1, or G_2 is a tentative identification. To confirm the presence of aflatoxins, the suspect spot is reacted with trifluoroacetic acid or glacial acetic acid, and developing the reaction products in a new solvent system and comparing with known standards This method is used by several laboratories, but is not used by buying stations.

High performance liquid chromatography. This is a relatively new method of detecting aflatoxins and is very reliable. Again, it is used by several research laboratories, but not by buying stations A recently developed HPLC procedure is more rapid, more sensitive, and more precise than the TLC procedure at high toxin levels

Mass Spectrometry. There is no more definitive confirmation of the aflatoxins than mass spectroscopy because this method is a direct molecular characterization of the molecule However, this method is used by only a few research laboratories

Preventive Measures

Growers and feeders can utilize several practices to minimize aflatoxin contamination. Some of these are as follows

Use recommended production practices. Everything should be done to maximize yields It appears that aflatoxins are not as much of a problem in corn where the yields are high as they are when yields are low Therefore, only recommended practices should be followed.

Plant early. Research has demonstrated that corn pollinated during periods of adequate moisture and moderate temperatures has less risk of high aflatoxin contamination Such conditions are more likely to occur when the corn is planted early Early planted corn is also generally exposed to lower insect populations

Reduce stress on the corn. Corn exposed to stress, particularly drought stress has a greater risk of contamination with aflatoxins than non-stressed corn Thus, producers should consider irrigation or other means of reducing drought stress, particularly during the period of pollination

Harvest early. *Aspergillus flavus* is not a good competitor until the moisture content of the grain is at 20% or below. Thus, if the grain can be harvested above this level and dried quickly, there is less chance of aflatoxin contamination

Avoid damage during harvest. *Aspergillus flavus* can spread from infected kernels to other kernels, particularly damaged kernels, under the right environmental conditions The possibility of this happening can be greatly reduced if the combine is properly adjusted to avoid kernel damage.

Dry and store corn properly. *A. flavus* cannot grow in corn with a moisture content less than 12 to 13%. Therefore, if the corn is dried below this level, no additional growth of the fungus or production of aflatoxin will occur if proper storage practices are followed

Keep storage and feeding facilities clean. The fungus can survive in residues left in storage and feeding facilities and can rapidly produce aflatoxins under such conditions Corn and feed residues should be discarded as soon as possible and storage and feeding facilities should be decontaminated. Materials are available for decontamination.

Utilization of Contaminated Corn

The current FDA action level on aflatoxins in corn is 20 parts per billion (PPB). This means that corn that contains more than 20 PPB aflatoxins may be seized if offered for sale in interstate commerce Also, corn that contains more than 20 PPB aflatoxins should not be fed to lactating animals, used in starter rations, or under any circumstances be milled into corn meal or other human food Decisions to feed aflatoxin-contaminated corn should be based on (I) contamination level, (2) age and species of the livestock to be fed, (3) willingness to risk toxic effects on livestock, and (4) balancing the value of contaminated feed and risk of livestock poisoning against the cost of non-contaminated feedstuffs

The questions of a safe contamination level in animal feeds is complex. Safety to one person may not mean the same thing to another since some measure aflatoxin effects in terms of mortality while others measure effects in terms of feed conversions or weight gains The most conservative approach is to realize that we do not know what levels of aflatoxins are completely safe However, the greater the concentration the greater the risk involved If aflatoxins cannot be totally avoided, accept or use as little contaminated corn as possible The following table is reproduced from the publication, "Reducing the Effects of Aflatoxin on Livestock" (Frank T. Jones, editor) simply as a guideline It is not to be accepted as recommendations

"An aflatoxin level of zero is recommended However, the following guidelines are offered to those producers who have decided to risk feeding aflatoxin-contaminated feeds "

Species	Aflatoxin level (PPB) less than
Swine	
Birth to 75 lb	20
75 lb to market	20
Brood sows (gestating or lactating)	20
Boars	20
Poultry	
Turkeys	20
Turkey breeders	20
Broilers	20
Broiler breeders	20
Layers (commercial)*	20
Beef Cattle	
Brood cows and bulls	20
Growing-finishing cattle over 400 lb **	100
Creep feeds	20
Stress feeder cattle	20
Dairy Cattle	
Lactating cows*	20
Calves	20
Pregnant non-lactating cows	20
Open heifers	20
Horses	20

* Special attention must be paid to these animals since their products are promptly used as human food

** Feed aflatoxin-free rations for at least 3 wk prior to slaughter

Detoxification procedures. Several detoxification procedures are presently being studied. While these procedures are promising, they are not to be recommended at this time. Roasting may reduce aflatoxins, but may char corn kernels and affect feeding value Detoxification procedures involving aqueous and anhydrous ammonia are being developed. Since the toxicity of breakdown products of aflatoxins has not been determined, *the use of detoxification procedures has not been approved by the Food and Drug Administration* to date; however, the agency is evaluating new data which may influence a change in detoxification regulations Aqueous ammonia procedures, which should be carried out under controlled conditions, are corrosive and expensive Procedures involving anhydrous ammonia are less costly, but may be hazardous because of toxic fumes and the danger of explosions In addition, detoxified corn must be thoroughly aerated in order to prevent feed refusal or reduced feed intake due to residual ammonia Farmers are advised to obtain more details from their local Extension office before initiating this procedure.

Alcohol. Aflatoxin contaminated corn could be utilized to produce alcohol for the production of gasohol. In fact, some has been utilized in this process However, the residue from such a process remains contaminated with aflatoxins and should not be fed to livestock unless it is decontaminated. Apparently, the aflatoxins do not interfere with the fermentation process in producing alcohol.

OTHER MYCOTOXINS

In the southeastern United States, the word aflatoxin has tended to become synonymous with the word mycotoxin with laymen and many scientists

alike This tendency has led to surprise when farmers have been introduced to mycotoxins other than aflatoxin through contaminated lots of corn or other grain On the other hand, farmers and scientists outside the southeastern United States have tended to feel that aflatoxin contamination is not a major problem in the midwestern and northern corn belts Mycotoxins produced by species of Fusaria are considered to be more prevalent in these areas. Interpretation of recent data indicates that there is, as we would expect, considerable overlap among the geographic areas involved and that the mycotoxin "problem" in the United States is due to the growth and production of secondary metabolites by many different species of fungi when suitable conditions exist By its very nature, mycotoxicology, a relatively recent multidisciplinary field, is complex

There are many other genera and species of fungi which have been isolated from both grain and other commodities When these fungi have been tested for their ability to produce toxins in culture in the laboratory, many of the isolates of nearly all the species examined have been toxigenic That is, cultures of these fungi or extracts of these cultures have been poisonous to test animals in a biological assay Along these same lines, it has been estimated that there are between 200 and 300 described mycotoxins produced by various fungi It is easy to see then the potential for mycotoxin problems in grain, however, only a few of these mold metabolites have been definitely proven to cause discrete, characteristic, identifiable, easily diagnosed mycotoxicoses The "slobber syndrome" caused by the mycotoxin slaframine, which may be found in second cutting red clover infested with the fungus *Rhizoctonia leguminicola* (Gough and Elliot), is an example of a characteristic and easily diagnosed mycotoxicosis Slaframine is also an example of a mycotoxin that is produced both in storage and in the field

The concepts of field and storage fungi have been very useful in assisting laymen and scientists grasp that production, handling, and storage of grain are biologically dynamic in terms of insect and fungal spoilage of corn For fungi, the moisture content and temperature of the grain are critical factors governing the length of time a given bin of corn or feed can be safely stored without molding Both factors are important to the physiology of the fungus and to giving competitive advantages to different groups of fungi Mycotoxin contamination of grain can arise in the field before harvest or after harvest during handling, storage, feed-making, etc

There are at least four other important groups of mycotoxins that may occur in corn 1) the zearalenones and related compounds, 2) the trichothecene toxins, 3) ochratoxins and the *Penicillium viridicatum* (PV) toxins, and 4) other toxins produced by *Aspergillus* and *Penicillium* spp

Fusarium toxins. In the United States, two major *Fusarium* mycotoxin groups, zearalenones and trichothecenes, are possibly equal to aflatoxins in importance to agriculture. However, a formal assessment of economic losses due to contamination of corn with *Fusarium* toxins has not been nearly as well documented as the losses due to aflatoxin contamination The fungal genus *Fusarium* is comprised of soil-inhabiting species and includes some important plant pathogens There were serious infections of midwestern corn in recent years with *Fusarium graminearum* which caused stalk and ear rots. When ears are infected, "gib" corn is the result Most outbreaks of "gib" corn seem to occur in years when wet conditions prevail during the 21 days after pollination and when cool, wet conditions occur at harvest

The mycotoxins produced by *Fusarium* spp (trichothecenes and zearalenones) in corn are second only to the aflatoxins in attracting the attention of scientists and farmers. The most familiar trichothecenes include T-2, deoxynivalenol (DON), and diacetoxyscirpenol (DAS), monoacetoxyscirpenol (MAS), nivalenol, and fusarenone-X. The trichothecenes as a group are strong irritants and have been associated in naturally occurring outbreaks with vomiting, feed refusal, and possibly gastric ulcers when consumed Zearalenone and zearalenol, on the other hand, are *Fusarium* metabolites possessing estrogenic activity which when consumed by animals have been associated with reproductive problems such as abortions, false heat, recycling, reabsorption and mummies, and vulval-uterine prolapse

There are other biologically active metabolites produced by *Fusarium* which are less well known. Moreover, new active metabolites (toxins) of *Fusarium* are being discovered and characterized. For example, *Fusarium moniliforme* infection of corn has been strongly linked to a condition in horses called equine leucoencephalomalacia A portion of the symptoms of this disease can be reproduced by administration of moniliformin, a mycotoxin named after *F moniliforme,* but the complete etiology remains unknown

Ochratoxins and PV Toxins. Ochratoxin, a contaminating feed grain in Denmark, is a serious problem to the swine industry there In fact, in the Balkan countries there is a disease in humans associated with ochratoxin A in the grain consumed by the population In the United States, however, outbreaks of ochratoxin A as a contaminant of corn are not well documented One factor limiting research on this mycotoxin is that rapid methods of analysis are not yet available The PV toxins, xanthomegnin, and viomellein, are produced by *Penicillium viridicatum* A lower temperature storage situation seems to favor growth of *P viridicatum* and production of the PV toxins The PV toxins and ochratoxin A are nephrotoxins, that is, the kidney is the target organ for these toxins Production problems in swine caused by the PV toxins plus small concentrations of ochratoxin A have been particularly well described in Indiana.

Other toxins produced by *Aspergillus and Penicillium.* There are several other groups of mycotoxins which may become more important as mycotoxin research continues These are mainly some of the toxins produced by *Aspergillus* and *Penicillium.* Some are produced by only one or a few fungi and some may be produced by several fungi in both genera.

Citrinin is a mycotoxin which has been found as a natural contaminant of corn associated with mycotoxicoses in swine, horses, and poultry. It is a kidney toxin which can sometimes be found in rather high amounts in corn Frequency of occurrence and economic impact on agriculture are not well known even though there seems to be several documented cases in the literature

The tremorgens are toxins, produced mainly by *Aspergillus* and *Penicillium* species, possessing activities which give strong central nervous system effects or tremors in test animals. Although tremors are often reported with possible mycotoxicoses in farm animals, there is simply not enough information available to assess their importance to agriculture

Other alkaloids similar to those produced by *Claviceps* sp , the ergot fungus, are produced by *A flavus.* Whether the indole alkaloids or other compounds produced by *A. flavus* contribute to toxicity to animals is presently unknown.

The *A. flavus* toxin cyclopiazonic acid (CPA) has been found in corn and peanuts in Georgia leading some researchers to suspect that aflatoxins and CPA are acting in concert when consumed by animals It has been demonstrated that two or more mycotoxins often act synergistically when consumed together in animal rations

SUMMARY

Solutions to mycotoxin problems are certainly no more elusive than solutions to other problems in plant and animal production. True solutions can be based only on application of research data For example, active programs at several locations around the country are conducting broadly-based research programs in detoxification/decontamination of mycotoxin-contaminated grain, improved grain storage technology appropriate for different geographies and efforts to prevent mycotoxin contamination before harvest through breeding and cultural practices. Even application of present knowledge has led to important ways of alleviating the adverse impact of mycotoxins on producers

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