Seed contributes about 15% of farmer income from cotton. Dairies and oil mills compete for cottonseed and largely determine the ultimate price of seed. If prices of competing commodities such as corn are sufficiently high, cattle feedlots also may be important outlets for seed. However, the use of cottonseed and cottonseed meal for feed is limited by the presence of the natural plant compound gossypol, which is toxic to monogastric animals. Growers can influence the value of seed sold to oil mills by selecting cultivars with large seed and seed with high oil content because trading rules permit seed lacking these characteristics to be discounted. Factors that influence lint yield also influence seed yield, but two important characteristics that indicate seed quality and value do not relate to lint value; these are free fatty acid content and aflatoxins.

**Seed Deterioration**

Seed deterioration refers to the breakdown of the cotyledon and embryo tissue within the seed. All seed with moisture content over 12% is assumed to be in the process of deteriorating. In some seasons, all seed from an area may be over 12% when harvested. Free fatty acid content is the most commonly applied measure of seed deterioration, and therefore, high free fatty acid content may be used as a criterion for rejecting seed for both non-oil-mill and oil-mill use. Another indicator of preginning deterioration may be seed from which patches of the linters have been ripped during ginning, also known as bald seed. Bald seed can result from microbial activity, which weakens fiber and linter attachment to the seed coat.

Free fatty acid is expressed as a percentage of the extracted cottonseed oil. These acids are formed when the oil's constituent triglycerides break down. For oil mills, increased free fatty acid content causes reduced oil yield and increased refining costs. Oil mills therefore begin to discount seed prices for seed yielding 2% free fatty acids and increase discounts as fatty acid content increases.

**The Process**

A wide range of fungi and bacteria may participate in the process of "seed deterioration." However, the process also refers to loss of seed reserves and seed viability independent of microbes. Free fatty acids form when mature seed is exposed to high moisture. Seed with moisture above 12% will deteriorate. Warm temperatures speed the process, which may be catalyzed by both plant and microbial enzymes. Deterioration and formation of free fatty acids may occur in the field as the mature crop awaits harvest, in storage modules where the harvested seed cotton is stored before ginning, or in storage after ginning.

**Control**

Procedures to minimize canopy humidity reduce preharvest deterioration. Many recommendations outlined under aflatoxin control and boll rot control also reduce seed deterioration.

Harvesting seed cotton that is dry (below 12% moisture), harvesting as early as possible, and constructing proper modules help to reduce seed deterioration. Moist trash in modules can increase seed cotton moisture and temperature and accelerate deterioration. Unfortunately, the extent of deterioration is largely determined by the environment and, in very wet years, deterioration begins before harvest.

Use of mold inhibitors and preservatives to prevent seed deterioration after harvest has been investigated by researchers but is not routine. To prevent seed deterioration, seed cotton should be stored in covered cars or properly constructed tarped modules and ginned in a timely manner. Wet seed cotton should not be placed in modules and should be ginned as soon as possible. After ginning, seed should be rapidly transported to storage facilities where it can be cooled, dried, and protected from further wetting. Storage facilities should have an adequate aeration system to cool and properly aerate the stored tonnage. Aeration should be timed to remove moisture rather than to introduce it. Temperatures of stored seed should be monitored and minimized, ideally to below 16°C (60°F). High-moisture seed should be fed or processed as rapidly as possible.

**Aflatoxin**

Aflatoxins are a group of chemical compounds produced by certain fungi. These fungal metabolites are toxic and can cause serious disease in many animals, including humans. The primary target of aflatoxins is the liver. Aflatoxin B1, the most common aflatoxin (Fig. 11), is known to induce cancer in certain animals at concentrations as low as 1 ppb (ppb = µg/kg). This makes aflatoxin B1 one of the most potent carcinogens known. Aflatoxins are transmitted from feed to the milk of dairy cows. To prevent exposure of humans, particularly children, to aflatoxins, the sale of milk with aflatoxin concentrations greater than 0.5 ppb is prohibited. To ensure that aflatoxins do not occur in excess of 0.5 ppb in milk, dairy cows may not be fed cottonseed containing more than 20 ppb aflatoxin B1. Dairies found to be producing milk with excess aflatoxins
have been subjected to milk disposal and quarantine. Mature beef cattle and nonlactating dairy cattle may be fed cottonseed containing aflatoxin up to 300 ppb. Since dairies typically pay a premium for cottonseed, in areas where aflatoxin contamination of cottonseed is common, the aflatoxin content of whole cottonseed is the most important factor determining its value.

Aflatoxins also cause losses when cottonseed is processed for oil production. During processing, hulls (seed coats) and kernels are separated. Most of the aflatoxins are within the kernels, and when kernels are physically disrupted and the oil is extracted, the aflatoxins are concentrated in the solids, the meal. Hulls are also a common feed, and hulls from highly contaminated seed also may contain unacceptable aflatoxin levels. Cottonseed meal is a high-protein feed ingredient and an important source of income for mills. Meal with high aflatoxin levels must be used as fertilizer, which greatly reduces its value. It is important for oil mills to know the aflatoxin content of their seed upon receipt, so that seed with varying levels of aflatoxin may be stored separately and production of acceptable quality meal can be optimized. Culls from planting seed (immature, damaged, and lightweight seed) also may be marketed to oil mills, feedlots, and dairies and can contain higher aflatoxin concentrations than presorted seed.

All regions of the United States may experience some aflatoxin contamination of cottonseed in some years (Fig. 12). The occurrence of unacceptable aflatoxin levels in cottonseed is infrequent in most of the Cotton Belt, but in some regions, aflatoxins are a perennial concern. These regions include the production areas of Arizona, southern Texas (including the Lower Rio Grande Valley and the Coastal Bend area), and the Imperial Valley of California.

Symptoms

A distinct symptom of many seed lots containing unacceptable aflatoxin contamination is a distinct bright green yellow fluorescence (BGFY) under ultraviolet light on the linters of a small subset of the seed (Plate 1). Although aflatoxin also fluoresces, the compound causing BGFY is a high molecular weight substance that forms when kojic acid (another fungal metabolite) reacts with cotton peroxidase. Presence of BGFY does not indicate that the seed lot is contaminated with unacceptable aflatoxin levels, and absence of BGFY does not mean that the crop has acceptable aflatoxin levels. However, seed exhibiting BGFY usually contains much greater concentrations of aflatoxins than nonfluorescent seed. In general, gins sorting seed for marketing to dairies need not analyze seed piles with greater than 0.5% BGFY seed since these piles generally have an unacceptable aflatoxin content. BGFY also may be found on the lint of bolls infected with Aspergillus flavus, even after ginning. This is sometimes called "cat-eye." Fiber with cat-eye generally has acceptable spinning quality, and the fluorescence is readily removed from the resulting yarn and fabric during scouring and bleaching, which are normal preparations for dyeing and finishing.

Aflatoxin content is routinely determined with laboratory analysis. Contamination is highly variable; it is not unusual for individual seeds containing over 500,000 ppb aflatoxin B1 to be produced on the same plant as seeds containing no toxin. If just one seed in 25,000 is above 500,000 ppb, the seed is unacceptable for dairy use. Thus, in order for analyses to be reliable, relatively large samples, 10-20 kg (22-44 lb), must be carefully taken from multiple portions of a seed pile. Some states mandate specific sampling schemes for tests to be used in commerce. Commercial laboratories may perform either chromatographic or immunological analysis. Relatively simple immunological test kits are available for rapid screening outside of a laboratory. However, these tests still require careful sampling, dehulling of seed, grinding of kernels, and extraction of the toxin with a solvent.

![Fig. 12. Distribution of Aspergillus flavus on the cottonseed crop in the United States, 1991 and 1992. Bars represent the percentage of the crop with different numbers of A. flavus propagules per gram. The numbers result from five 2-kg samples for each participating oil mill. (Courtesy P. J. Cotty)](image-url)
Causal Organism

Only members of Aspergillus flavus are known to produce aflatoxins. Over 90% of aflatoxin contamination of cottonseed in the United States is caused by A. flavus LinkFr. A. flavus is commonly associated with lint and rotted bolls worldwide. In regions where A. parasiticus Speare is a common soil inhabitant (i.e., the southeastern United States), this species also may infect and contaminate cottonseed. A. nomius is a third aflatoxin-producing species. On Czapek agar, most aflatoxin-producing fungi form conidiomata that appear yellow-green to dark green due to profuse production of globose to subgbose conidia on conidial heads after 5–10 days at 30°C (86°F). Most A. flavus isolates produce light green colonies with smooth to slightly roughened conidia (3–6 μm) and only B aflatoxins; A. parasiticus produces generally darker-green colonies, conidia (3.5–6.5 μm) with distinctly roughened walls, and both B and G aflatoxins. A. nomius, which occurs relatively infrequently, produces colonies similar to those of A. flavus, but it is distinguishable from A. flavus by production of both B and G aflatoxins and elongate sclerotia. Most, but not all, aflatoxin-producing isolates produce sclerotia, but even isolates within the species A. flavus vary widely in sclerotal size and shape and in the quantity of sclerotia they produce.

Two distinct strains of A. flavus infect and contaminate cottonseed in the United States. Typical or L strain A. flavus isolates form colonies as described above. However, S strain isolates produce relatively few conidia, profuse quantities of small (<400 μm) sclerotia, and larger quantities of aflatoxins than L strain isolates (Plate 2). S strain isolates vary widely in the quantity of conidia that they produce. Colonies of S strain isolates growing from cottonseed may initially appear white and fluffy with few or no mature conidiophores, which may cause the S strain to be overlooked. In 5–7 days, however, regions of the white colony mature, producing tiny black sclerotia interspersed with conidial heads, which can be observed under a dissecting microscope. In some western desert valleys in the United States, S strain isolates compose over 50% of the A. flavus community.

A. flavus is a highly variable fungus. Both the L and S strains are composed of many isolated genetic populations, or vegetative compatibility groups (VCGs), based on the ability of hyphae of similar isolates of a fungus to fuse. These groups vary greatly in aflatoxin-producing ability, and some groups are composed of isolates that produce no aflatoxins. This variability contributes to the differences observed in aflatoxin content of A. flavus-contaminated seed. Seeds are often colonized by more than one VCG, and the quantity of aflatoxin produced by a strain during seed infection is greatly influenced by coinfecting strains. Thus, identification of the specific etiologic agents of a contamination episode may be complex.

Disease Cycle and Epidemiology

Unlike corn and peanut, in which aflatoxin contamination is reduced or eliminated by irrigation, cotton undergoes the most consistent and severe contamination in regions with irrigated cotton production. The fungi that produce aflatoxins occur in all agricultural soils where cotton is produced, but they occur in greater numbers in warmer areas. Hot, dry conditions favor these fungi and permit them to outcompete other microbes in both the soil and crop. A. flavus is an aggressive saprophyte capable of utilizing a wide range of organic substrates. It also is an opportunistic pathogen of predisposed plants, insects, and mammals. A. flavus may survive between seasons in the soil as free conidia and sclerotia, on colonized organic matter, and in association with insects. Wind, rain, and insects readily disperse the fungus to the crop.

Aflatoxin contamination of cottonseed occurs in two phases. The crop is first contaminated when A. flavus infects the developing bolls through wounds or cracks. The best-documented infection port is pink bollworm damage. However, any wound in the developing boll may be sufficient to permit A. flavus infection. Suture cracks andnectaries also have been suggested as possible entry points. Bolls infected by A. flavus through wounds before boll opening may produce seed with fluorescent staining (BGYF) on the lint and linters. Seed from these bolls, usually with linter BGYF, may contain very high aflatoxin concentrations, in excess of 500,000 ppb. Bolls heavily colonized by A. flavus, and many other fungi, before boll maturity tend to produce one or more "tight locks," which are inefficiently picked by spindle pickers. This low efficiency is partially responsible for the lower aflatoxin content of spindle-picked cotton compared with stripper or ginned cotton.

The second phase of contamination occurs when mature seed is exposed to both conducive temperatures, usually above 30°C (86°F), and either high relative humidity, above 85%, or wetting at or after boll opening. This phase is characterized by increases in aflatoxin content of seeds infected during the first phase, as well as infection of new seed. In areas where aflatoxin contamination is a perennial problem, crop surfaces become coated with A. flavus, and seed cotton becomes associated with the fungus shortly after boll opening. If seed moisture exceeds 15%, seed infection may proceed. Seed infected during this phase often does not exhibit BGYF on either the linters or linter. Aflatoxin increases associated with this phase may occur both before and after harvest, including in modules, in seed piles, and in the hands of the end user. Thus, while the crop is held in the field awaiting harvest or in modules awaiting ginning, the toxin content may gradually increase. Rank cotton growth, dense canopies, dried, and late irrigations increase the severity of the second phase.

A. flavus colonizes tissues most readily and is most competitive against other microbes at temperatures above 30°C (86°F) and continues to grow up to 42°C (108°F). Severe contamination is associated with periods of high night temperature, with a minimum greater than 24°C (75°F). High night temperatures are associated with periods of increased humidity in the western desert valleys of the United States, and so these contamination episodes reflect the importance of the second phase of contamination. The percentage of the seed exhibiting BGYF along with the quantity of toxin in both the BGYF and the non-BGYF seed may be used to indicate the relative importance of the two phases to a particular contamination episode. Because both mature and developing bolls frequently occur simultaneously, both contamination phases may occur at the same time.

Control

Since aflatoxins can form during boll development, management must begin with the producer. However, because A. flavus remains with the cottonseed until use, all handlers of the crop must participate in management. In some regions, A. flavus inocula are always associated with the mature crop, and when conditions that favor contamination are severe, contamination over 20 ppb may be unavoidable. However, even under severe conditions, careful management can minimize the extent of contamination.

Fertilizer and irrigation applications should be regulated to prevent rank growth. Short plants with open canopies reduce the second phase of contamination. Other practices to reduce humidity in the canopy also may help, including irrigating only alternate furrows and skip-row planting.

Insect damage to developing bolls should be prevented, particularly during the first half of the crop.

Irrigation should be terminated early. Late irrigations expose early-formed bolls to high humidity and increase the second phase of contamination. In areas with supplemental irrigation, the soil profile should be saturated before initial boll opening.

The crop should be harvested as early and as dry as possible. This prevents exposure of the crop to rain and dew.
and reduces overwintering of insects that may damage developing bolls the following season.

Proper modules should be formed with dry seed cotton, and modules should be covered.

In areas with frequent contamination, the first spindle-picked cotton should be segregated and ginned separately to maximize the quantity of seed with acceptable contamination levels. Seed from ground-gleaned cotton should also be kept separate, since this seed generally has much higher contamination levels. When possible, keep ginned seed from separate fields distinct to avoid mixing highly contaminated seed with clean seed.

Seed should be shipped and processed in a timely manner. Keep seed dry during transportation and in on-site dairy storage to prevent postharvest increases in aflatoxin.

Nonaflatoxin-producing A. flavus strains competitively exclude aflatoxin-producing strains and reduce aflatoxin during both phases of contamination. These nonaflatoxin-producing strains may provide a new aflatoxin management tool for growers.

**Detoxification**

Once aflatoxin contamination has occurred, several options remain. Cottonseed with aflatoxin in excess of 20 ppb cannot be used as dairy feed, but if the aflatoxin concentration is less than 300 ppb, it may be fed to mature beef cattle. Cottonseed with unacceptable aflatoxin levels may not legally be mixed with clean seed with the intent of reducing the aflatoxin content to acceptable levels. All cottonseed may be sold to an oil mill that will remove the oil and sell the meal according to its aflatoxin content. In some states, an additional alternative is detoxification.

Aflatoxin-contaminated commodities are detoxified by ammoniation (Fig. 13) in several regions of the world, and importation and use of meal detoxified by ammoniation is allowed by many countries. In the United States, ammoniation is routinely used to detoxify aflatoxin-contaminated cottonseed in both Arizona and southern California. However, the ammoniation process is not approved by the U.S. Food and Drug Administration, and cottonseed products detoxified by ammoniation may not be shipped across state lines. After ammoniation, seed should be stored carefully and utilized rapidly. Ammoniation increases seed moisture (and weight), and the seed may remain vulnerable to subsequent contamination by A. flavus spores.

**Other Toxins**

Fusarium species commonly infect cottonseed, and Fusarium mycotoxins, such as the trichothecone T-2 toxin, have been detected in cottonseed. Aflatoxins, however, are the only mycotoxins currently regulated in cottonseed. Cottonseed contaminated with other mycotoxins has not, to date, been clearly implicated in incidences of livestock toxicity. However, the potential exists, and cottonseed producers have suffered litigation over alleged toxicity to dairy cows from Fusarium-infected cottonseed. Seed contamination by Fusarium toxins is most likely to occur when harvest is severely delayed, causing the crop to be exposed to prolonged weathering or even overwintering in the field. Seed from such crops should be identified at market.

Animal nutritionists are aware that most cottonseed carries a level of natural toxicity resulting from high concentrations of gossypol. This may lead to cottonseed being scrutinized on occasion. Because cottonseed may be blended with other feed ingredients and may deteriorate after shipping, gins and seed brokers may wish to retain samples of cottonseed sold to dairies to guard against litigation concerning alleged feed toxicity or poor feed utilization. If litigation should arise, such samples may be used to establish cottonseed quality at the time of shipping. Cottonseed samples should consist of multiple sub-samples taken according to a published protocol, such as the one established for aflatoxin sampling by the state of Arizona in its Commercial Feed Law (see Park and Pohland, 1989). Only dry seed (with moisture less than 12%) should be sampled, and samples should be stored dry in a sealed, watertight container. They should be held for a minimum of 1 year.

**Planting Seed**

The most profitable cottonseed market is for planting seed. Growers and gins receive premiums for producing planting seed for seed companies. However, to qualify, seed must meet specific criteria. If it fails to qualify, it must be sold on the open market. Free fatty acid content is a prominent criterion among seed companies for evaluating seed quality. A free fatty acid content below 0.5% is preferred, and a content above 1% is generally unacceptable. This general measure of seed quality provides some assurance that the seed retains food reserves, will store well, and will produce vigorous seedlings. Seed companies also section seed (make "cut tests") to examine internal morphology. Germinable seeds are white to yellowish green. Other important criteria include maturity (based on seed coat color and seed and embryo size) and seed coat integrity. Immature seeds in excess of 20% and mechanically damaged seeds in excess of 15% generally lead to the seed being rejected.

After acid delinting, seed is sorted by cleaning and gravity grading. Other methods of examining seed quality include using a tetrazolium vital stain or examining the conductivity of water in which seeds are soaking to measure the amount of leaching. Germination tests may be conducted on moist germination paper, sand, or soil at alternating temperature ranges of...
20–30 or 20–25°C (68–86 or 68–77°F). A cool germination test, indicating physiological quality, is frequently conducted at 18°C (64°F). Size and appearance of seedlings also are considered, as indicators of vigor.

Phytosanitary certifications for *Glomerella gossypii* are required by some countries. Thus, seed from fields exhibiting anthracnose symptoms may be rejected by state phytosanitary agencies or seed companies producing any seed for export. Fields with bacterial blight may similarly be excluded because of international restrictions on movement of seed contaminated with *Xanthomonas campestris pv. malvacearum*. Other potentially seed-transmitted pathogens include *Ascocysta gossypii* and *Fusarium* spp. Fortunately, with current production, storage, delinting, and conditioning practices in the United States, evidence of economically important seedborne pathogens is virtually undetectable in commercial seed lots. Seed also may be rejected by seed companies based on weed seed content.

Optimizing planting seed quality begins with reducing exposure of the mature crop to humidity before harvest. Many of the recommendations listed for control of aflatoxin are useful. Planting seed should be harvested dry (moisture less than 11%) and as early as possible. To reduce the percentage of immature seed, the crop may be harvested twice. For the first pick, the spindle picker should be set to pick only fully fluffed bolls. This pick may be completed before chemical boll openers have acted on less-mature bolls. Proper module construction and timely ginning can further optimize planting seed quality. Module temperature should be monitored, and if temperatures do not stabilize, preferably below 32°C (90°F), within one week, the seed cotton should be ginned immediately. Seed should not be stored for planting unless the moisture content is below 11%. Storage should follow the recommendations in the section on control of free fatty acid content.

Selected References


(Prepared by P. J. Cotty)