

Chapter 13

ADVANCES IN MYCOTOXIN ELIMINATION AND RESISTANCE

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INTRODUCTION

The presence of toxic fungal metabolites (mycotoxins) in food has been a concern of man for many years. However, it was not until 1960 and the onset of the "turkey X disease" syndrome in England that intensive study of mycotoxins began. In that case, over 100,000 turkey poult died after consuming feed that contained Brazilian peanut meal contaminated with a toxic metabolite(s) of *Aspergillus flavus* Link, which came to be known as aflatoxin (Lancaster *et al.*, 1961; Sargeant *et al.*, 1961). The name aflatoxin refers to four naturally occurring metabolites designated B₁, B₂, G₁, and G₂. Aflatoxins B₁ and B₂ are metabolites of *A. flavus*, whereas all four toxins are produced by the closely related fungus, *A. parasiticus* Speare. Aflatoxin B₁ is considered to be the most important of the group since it is the most toxic. It has been demonstrated to be a potent animal carcinogen, and has been classified by the International Agency for Research on Cancer (1987) as a probable human carcinogen. *Aspergillus flavus* and *A. parasiticus* are distributed worldwide in soil and air, and under certain environmental or storage conditions they can invade and produce aflatoxin in several agricultural commodities, including peanuts.

Shortly after the discovery of aflatoxin in Brazilian peanut meal, the U.S. peanut industry began working closely with scientists to develop an integrated system to manage the aflatoxin problem. With the exception of managing preharvest contamination with proper irrigation, this technology has focused more on postharvest techniques such as segregation of aflatoxin-contaminated lots and removal of low quality, high risk peanuts by means of size separations, density segregation, and electronic color sorting alone or in conjunction with blanching (Davidson *et al.*, 1981; Cole *et al.*, 1991). Because of the success of this integrated system, aflatoxin is generally not considered to be a human health problem in the U.S. This is substantiated by a report by The Council for Agricultural Science and Technology (CAST, 1989) which stated that there was no epidemiological evidence to suggest that aflatoxin posed any threat to human health at exposure levels found in the U.S. The integrated management system is continually evolving as new technologies emerge, and it has been very effective in managing the aflatoxin problem, although at enormous expense. The U.S. peanut industry recently established a goal to completely eliminate aflatoxin in U.S. peanuts by the year 2000, and the goal is being supported by enhanced government and private sector funding.

Although many other fungal species isolated from peanuts are toxigenic in culture, the only other mycotoxin that has been reported as a natural contaminant of peanuts is cyclopiazonic acid (Lansden and Davidson, 1983; Urano *et al.*, 1992). Cyclopiazonic acid is a metabolite of *A. flavus* as well as other species of *Aspergillus* and several species of *Penicillium* (Dorner *et al.*, 1985). Natural contamination of peanuts with cyclopiazonic acid has been associated with high concentrations of aflatoxin caused by extensive *A. flavus* invasion and growth that occurred during crop years 1980 (Lansden and Davidson, 1983) and 1990 (Urano *et al.*, 1992). Because cyclopiazonic acid is a common metabolite of *A. flavus*, it would be expected to occur in peanuts that have been subjected to conditions favorable for aflatoxin contamination. However, cyclopiazonic acid is not as toxic as aflatoxin, and steps taken to manage or eliminate aflatoxin in peanuts should be effective for cyclopiazonic acid also. The potential for other mycotoxins in peanuts has been reviewed (Diener *et al.*, 1982) and, because no natural occurrence of other mycotoxins in peanuts has been found, the remainder of this chapter will be devoted to aflatoxin contamination.

A considerable research effort has been conducted to address aflatoxin contamination of peanuts since the review chapter written by Diener *et al.* (1982). Some of this research was discussed at an international workshop (ICRISAT, 1989), and recently a review and extensive literature database were published (Mehan *et al.*, 1991). The objective of this chapter is to present the latest technological advances that may ultimately achieve the goal of aflatoxin elimination from peanuts and peanut products.

PREHARVEST ADVANCES IN AFLATOXIN ELIMINATION AND RESISTANCE

Factors Involved in Preharvest Aflatoxin Contamination

Although many aspects of the preharvest aflatoxin contamination process remain a mystery, considerable insight into the mechanism of contamination has been acquired over the past decade. Preharvest contamination of peanuts requires extensive heat and drought stress during the last 3-6 weeks of the growing season (Sanders *et al.*, 1985; Cole *et al.*, 1989).

Aflatoxin contamination is common when peanuts are deprived of water for 30-50 days during the pod maturation period and soil temperatures are high (average 29-31 C). If the stress period occurs very late in the growing season, there may not be sufficient time for contamination to occur. If the stress period occurs too early, the major impact is on quality and yield.

Stilbene phytoalexins produced by peanut seeds in response to fungal invasion may contribute to the natural resistance of peanuts to *A. flavus* and, thus, aflatoxin contamination (Wotton and Strange, 1985, 1987; Dorner *et al.*, 1989, 1991). Evidence for the role of phytoalexins in peanut resistance include (a) peanut seeds at high water activity (a_w) produce stilbene phytoalexins in response to invasion by fungi, including *A. flavus*; (b) stilbenes produced by peanuts inhibit growth and aflatoxin production by *A.*

flavus (Wotton and Strange, 1985); (c) peanuts grown with adequate moisture are often invaded with *A. flavus* without being contaminated with aflatoxin (Hill *et al.*, 1983), indicating that fungal proliferation with concomitant toxin production after invasion did not occur; and (d) fungal proliferation and aflatoxin contamination apparently can occur only when the a_w of seeds is reduced to 0.95 and the stilbenes are no longer produced (Dorner *et al.*, 1989).

Figure 1 illustrates the possible interrelationship of several factors involved in the natural resistance and contamination processes of peanuts. Under adequate moisture conditions, fungal invasion stimulates the peanut seed to produce phytoalexins, which in turn inhibit fungal growth and, thus, aflatoxin production. However, when peanuts are subjected to drought conditions, the soil temperature increases as the foliage canopy recedes, and seed a_w decreases as the plant becomes stressed. Seed a_w eventually reaches a point at which stilbene biosynthesis no longer can occur. Data suggest that this point is probably between 0.98 and 0.95 (Dorner *et al.*, 1989), which is an a_w that still supports *A. flavus* growth and aflatoxin production. If *A. flavus* is present and viable in a peanut seed when phytoalexin production can no longer occur and the a_w in the seed is still sufficient for growth and aflatoxin production, the peanut seed likely will become contaminated. As drought progresses, seed a_w continues to decrease to a point not supportive of further fungal growth and aflatoxin production. Therefore, for a peanut to become contaminated, it must be in the proper developmental stage and the fungus must be both present and viable in the peanut seed at just the right time. This may help explain why so few peanut seeds actually become contaminated in the absence of damage.

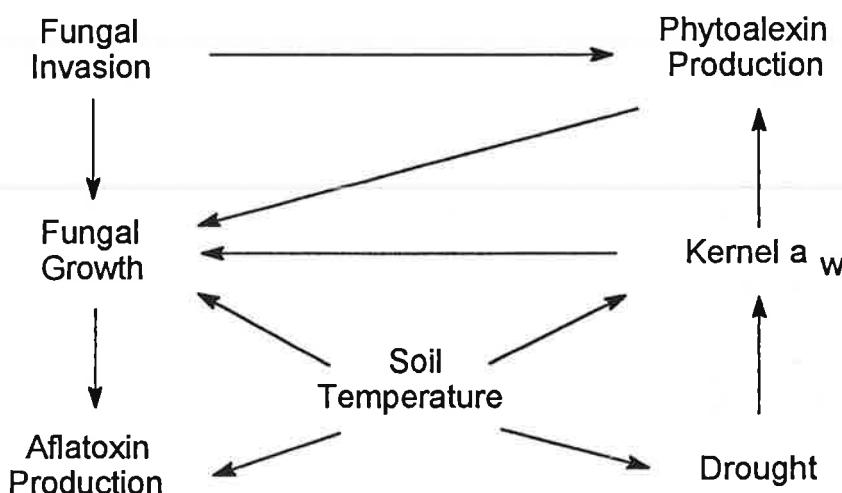


Fig. 1. Scheme showing the interrelationship of several factors involved in preharvest aflatoxin contamination of peanuts. Arrows indicate an influence of one factor upon another.

Table 1 shows that as drought stress progressed, the capacity of all peanut maturity stages to produce phytoalexins ceased by 31 days of stress. By 38 days of stress, aflatoxin was detected in immature peanuts and increased as the period of stress increased. Therefore, the association of reduced seed a_w , loss of phytoalexin-producing capacity, and appearance of aflatoxin contamination provide evidence as to how contamination may occur and also may offer clues on how best to combat the problem.

Irrigation

Because the conditions responsible for preharvest aflatoxin contamination are characterized by high soil temperature and drought during the latter part of the growing season, prevention of aflatoxin contamination can be achieved by proper irrigation (Dorner *et al.*, 1989). Unfortunately, irrigation is not available to all peanut producers and, therefore, is a technology that cannot be utilized fully. In addition, some producers irrigate from limited water sources, such as farm ponds. During years of extensive drought and high ambient temperatures, this limited water supply may be exhausted before the drought conditions have been alleviated. It is also difficult to irrigate peanuts properly under extremely hot and dry conditions.

When harvesting an irrigated peanut field, it is important to harvest only those areas that have received adequate water. In the Southeastern U.S., many irrigation systems pivot around a central point producing a circular irrigation pattern. In a square or rectangular field, such a system can leave the corners unirrigated. Therefore, it is necessary during harvest to keep

Table 1. Phytoalexin production and aflatoxin contamination detected in 29 C treatment in five maturity groups of peanuts grown under drought stress (from Dorner *et al.*, 1989).

Days of stress	Maturity group				
	Yellow 1	Yellow 2	Orange	Brown	Black
<u>Total phytoalexins</u>					
17	17000	10700	8600	4800	9200
24	0	13700	11800	2900	3200
31	0	0	0	0	0
38	0	0	0	0	0
45	0	0	0	0	0
51	0	0	0	0	0
59	0	0	0	0	0
<u>Total aflatoxins</u>					
	ppb				
17	0	0	0	0	0
24	0	0	0	0	0
31	0	0	0	0	0
38	861	15	0	0	6
45	1190	624	0	12	0
51	2419	118	329	0	0
59	785	1915	33	291	0

peanuts from irrigated and nonirrigated areas separate to avoid mixing aflatoxin-free peanuts with those that are potentially contaminated.

Insect Control

A significant factor in preharvest aflatoxin contamination is insect damage, notably damage caused by the lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller) (Lynch and Wilson, 1991; Bowen and Mack, 1993) and termites, *Odontotermes* spp. (Lynch *et al.*, 1990). The former insect proliferates during hot, dry environmental conditions that are conducive also for preharvest aflatoxin contamination. Under these environmental conditions the insect moves to the peanut pods (Huang *et al.*, 1990). Peanuts damaged by insects under drought conditions become highly contaminated. Control of this insect is difficult since most insecticides require moisture to be effective. Recommended control measures usually involve prophylactic treatment with a residual insecticide, a practice with possible ecological and economic drawbacks. In the future, biological control of the lesser cornstalk borer or development of insect-resistant peanut cultivars may be possible. In any event, practical insect control must be an integral part of any strategy that seeks the elimination of preharvest aflatoxin contamination of peanuts (see Chapter 4).

Expert Systems and Aflatoxin Risk Forecasting

Another preharvest aflatoxin management approach available to the producer is to carefully monitor the growing conditions of the peanut crop (soil temperature and precipitation) and to harvest prior to extensive contamination. As previously alluded to, peanuts become at risk for aflatoxin contamination after approximately 3 weeks of drought stress with a mean geocarposphere temperature of 29 C (Sanders *et al.*, 1985). The longer the crop is under stress after this period, the greater the contamination level. In the near future "aflatoxin early warning systems", which are expert systems that automatically monitor soil temperature and moisture conditions, may provide advanced warning for growers to harvest the crop earlier than normal, but before extensive contamination occurs (Thai *et al.*, 1990).

Biological Control

A high degree of protection from extensive preharvest contamination of peanuts under drought stress conditions can be achieved through biological control (Cole and Cotty, 1990; Dorner *et al.*, 1992). This management technology employs the concept of competitive exclusion by the addition of nontoxicogenic, yet competitive, strains of *A. flavus* and *A. parasiticus* to peanut soil just after planting. Studies initiated in 1987 in which nontoxicogenic strains of *A. parasiticus* were added to peanut soil early in the growing season resulted in consistently lower levels of aflatoxin in peanut seeds from treated soils compared with those from untreated soils (Dorner *et al.*, 1992). Population build-up of toxicogenic strains of *A. flavus* and *A. parasiticus* (particularly during the latter part of the growing season) were much lower in treated soils than in untreated soils, indicating that addition of the nontoxicogenic strains effectively interfered with the buildup of toxicogenic

strains in the soil during the latter part of the growing season. The apparent effect of this was a 10- to 100-fold reduction in aflatoxin contamination. More recent studies employing a combination of nontoxigenic strains of *A. flavus* and *A. parasiticus* have provided the most effective control thus far, with aflatoxin in edible peanuts averaging 4.0 ppb from five treatments compared with 93.8 ppb from five nontreated controls (Dorner *et al.*, 1993). Even though biological control agents provide an attractive alternative to pesticides, the ultimate approval and subsequent implementation of biological control agents by peanut producers must be extensively evaluated relative to human safety issues, since *A. flavus* is reportedly a human pathogen (aspergillosis) in immuno-compromised individuals.

Genetic Resistance

The most desirable solution to preharvest aflatoxin contamination of peanuts would be the discovery and development of resistant cultivars. Studies to demonstrate the existence of resistance to aflatoxin contamination are difficult because the fungi involved are not plant pathogens, but rather saprophytes or facultative parasites (Diener *et al.*, 1982). Contamination of peanuts in the field is extremely variable. Even when optimal conditions for aflatoxin contamination exist, the vast majority of peanuts do not become contaminated. A small percentage of highly contaminated peanut seeds usually accounts for most of the contamination. Therefore, when small samples are taken for analysis, it is not unusual to find several samples that are negative for aflatoxin and a few that are highly contaminated (Cole *et al.*, 1988). This makes it difficult to identify true resistance using classical plant pathological approaches.

To develop peanut cultivars with resistance to aflatoxin contamination, a reliable screening technique must be developed to identify resistance and it must be determined if genetic variation for resistance exists within the large collections of *Arachis hypogaea* L. germplasm.

Screening Techniques. Early efforts to identify resistance to aflatoxin contamination in peanuts involved laboratory screening techniques based on fungal colonization of rehydrated, mature, sound peanut seeds. Mixon and Rogers (1973) developed the dried seed laboratory inoculation method to screen peanut genotypes for resistance to *A. flavus* sporulation. Several other researchers have used Mixon and Rogers' method, or modifications of it, to screen peanut germplasm for resistance to *A. flavus* and aflatoxin contamination (LaPrade *et al.*, 1973; Bartz *et al.*, 1978; Mehan *et al.*, 1981, 1983, 1986b, 1987, 1988; Zambettakis *et al.*, 1981; Tsai and Yeh, 1985). Although this screening technique has been used to identify genotypes that possess resistance to *in vitro* seed colonization, serious limitations of the technique have been poor correlations between *in vitro* seed colonization and field colonization and between *in vitro* seed colonization and field aflatoxin contamination.

Kisyombe *et al.* (1985) evaluated 14 genotypes for resistance to *in vitro* seed colonization and for resistance to field colonization. They observed a poor correlation between ranking for resistance to *in vitro* colonization and

resistance under field conditions. Several genotypes which appeared to have moderate levels of resistance to *in vitro* seed colonization were highly susceptible under field conditions. Genotype PI 337409 showed high resistance to *in vitro* colonization, as it has in numerous other studies (Mixon and Rogers, 1973; Mehan *et al.*, 1981, 1983; Zambettakis *et al.*, 1981), but was susceptible in field tests for seed infection. One genotype was consistently rated as susceptible to *in vitro* colonization but was rated as highly resistant under field conditions.

Davidson *et al.* (1983) conducted field studies to evaluate Sunbelt Runner and Florunner. Sunbelt Runner had been reported to be 50% more resistant than Florunner to seed colonization by *A. flavus* in the *in vitro* laboratory screening method (Mixon, 1981). Microflora and aflatoxin data from this study (Davidson *et al.*, 1983) showed that Sunbelt Runner (reported to be resistant to *in vitro* seed colonization) and Florunner (reported to have only moderate resistance) differed little in levels of *A. flavus* and subsequent aflatoxin contamination under field conditions highly conducive to aflatoxin contamination.

Blankenship *et al.* (1985) used computer-controlled environmental control plots to examine four peanut genotypes previously selected as resistant to *in vitro* colonization. This study found all genotypes were highly contaminated with aflatoxin when subjected to preharvest drought and temperature conditions that were highly conducive to *A. flavus* invasion and aflatoxin contamination. It was concluded that *A. flavus* invasion and subsequent preharvest aflatoxin contamination of peanuts cannot be simulated easily under laboratory conditions.

Mixon (1986) observed a good match in the rankings of resistance to *in vitro* seed colonization and aflatoxin contamination under field conditions conducive to moderate levels of aflatoxin contamination. Over 3 years of testing he observed virtually no aflatoxin in six genotypes with resistance to *in vitro* seed colonization. Two check cultivars and a line with high susceptibility to *in vitro* seed colonization had moderate levels (6 to 66 ppb) of aflatoxin contamination in the field. Mixon proposed that resistance to *in vitro* seed colonization may be a measure of short-term resistance to field colonization but may be ineffective when peanuts are grown under extended drought stress.

Mehan *et al.* (1986b) and Waliyar *et al.* (1994) observed a significant reduction in the level of seed infection by *A. flavus* under field conditions in genotypes reported as resistant to *in vitro* colonization in comparison to genotypes reported as susceptible. Resistant genotypes also appeared to have reduced aflatoxin contamination under field conditions conducive to very low levels of contamination. Mehan *et al.* (1987) evaluated 11 peanut genotypes, six resistant and five susceptible to *in vitro* seed colonization, for resistance to field colonization and for resistance to aflatoxin contamination. Five of the lines with *in vitro* resistance also had significant resistance to preharvest colonization, and this resistance was stable across seven environments. These five lines also had reduced aflatoxin contamination under field conditions conducive to moderate levels of aflatoxin contamination.

Waliyar *et al.* (1994) also observed reduced aflatoxin contamination in genotypes with resistance to *in vitro* seed colonization. Mehan *et al.* (1988) conducted field studies with lines resistant and susceptible to *in vitro* seed colonization. Thirty-three percent (two of six) of the lines with resistance to *in vitro* seed colonization were susceptible to field seed colonization and 10% (one of 10) of the lines with susceptibility to *in vitro* seed colonization was resistant to field colonization. The only highly susceptible line tested also had reduced levels of field colonization. As the authors stated, these results emphasize that it should not be assumed that all genotypes resistant to *in vitro* seed colonization also will be resistant to colonization under field conditions.

The *in vitro* screening technique also may not identify material with reduced aflatoxin contamination under postharvest conditions conducive to aflatoxin contamination. Wilson *et al.* (1977) examined aflatoxin contamination that developed under high humidity conditions in shelled and nonshelled peanuts of two genotypes with resistance to *in vitro* colonization and a susceptible check genotype. All genotypes had appreciable levels of aflatoxin after 9-10 days of storage in relative humidities of 87-95% at 23-26 C.

Other screening techniques also have been examined. Kushalappa *et al.* (1979) examined pod resistance after inoculation and incubation of intact pods. They concluded that the resistance of pods to *A. parasiticus*, as measured by this technique, was not a true form of plant resistance since genotypes that were relatively resistant 1 year were susceptible the next and *vice versa*. They proposed that the pod resistance observed may have been due to antagonistic microflora that were in the shell before inoculation. Mixon (1980) observed more consistent results using a similar technique. However, based on a significant genotype by environment interaction, he concluded that the *in vitro* seed colonization technique was equal to or more effective than the pod inoculation technique for identifying genotypes resistant to *A. parasiticus*. Mehan *et al.* (1986a) examined a screening technique based on aflatoxin production in seed which had been inoculated and incubated under laboratory conditions. They observed genetic differences in levels of aflatoxin production that were consistent in seed lots grown in three environments.

Holbrook *et al.* (1994a) developed a large-scale field screening technique to directly measure field resistance to preharvest aflatoxin contamination. This technique uses subsurface irrigation in a desert environment to allow an extended period of drought stress in the pod zone while keeping the plant alive. In initial field tests conducted in the desert environment without subsurface irrigation, peanut plants died and their seeds rapidly dehydrated in the soil before contamination could occur. The concept of using subsurface irrigation was first used by Sanders *et al.* (1993) to show that high levels of aflatoxin occurred when peanuts in the pod zone were artificially stressed with heat and drought while keeping plants nonstressed by providing root zone irrigation. By utilizing this concept in the desert field screening tests, the mean aflatoxin contamination was increased and the variation among samples was reduced, thus providing a more valid screening technique.

Drought tolerance may serve as an indirect selection tool for resistance to preharvest aflatoxin contamination; however, conflicting results have been reported in the literature. Kisyombe *et al.* (1985) examined the colonization of seed by *A. parasiticus* in drought stress and nondrought stress plots. They examined 14 genotypes including three which had been reported to have some drought tolerance. Although the drought-tolerant lines were found susceptible to *A. parasiticus*, infection of two of these genotypes was not enhanced by drought stress. Mehan *et al.* (1987) and Mehan (1989) also observed that several drought-tolerant genotypes were susceptible to colonization and subsequent contamination by aflatoxin. However, Mehan (1989) did observe relatively low levels of seed infection in one drought-tolerant genotype and concluded that more research is needed to determine if drought tolerance can reduce stress on pod and seed to a level which would reduce aflatoxin contamination.

More promising results on the correlation of drought tolerance with reduced levels of aflatoxin contamination have been observed by Cole *et al.* (1993) and Holbrook *et al.* (1994b). Although similar levels of aflatoxin contamination have been observed in field studies involving Southern Runner and Florunner (Wilson *et al.*, 1991; Anderson *et al.*, 1995), differences in drought tolerance and aflatoxin resistance between these two cultivars were observed over three different crop years (1991-93) in studies using environmental control plots at the National Peanut Research Laboratory (NPRL) (Cole *et al.*, 1993). For example, the average aflatoxin concentration for 140 Southern Runner plants in the 1992 study was 8.0 ppb, while the average for the same number of Florunner plants was 334 ppb. Furthermore, 1.4% of the Southern Runner plants contained aflatoxin concentrations of >100 ppb while 9.3% of Florunner plants were contaminated at this level. In these studies that considered only undamaged seeds on an individual plant basis, the enhanced resistance of Southern Runner was at least in part associated with its drought tolerance as measured by its ability to maintain high seed moisture under extended drought conditions. The same observations were made in the 1991 and 1993 year comparison.

Holbrook *et al.* (1994b) evaluated resistance to preharvest aflatoxin contamination in a set of genotypes that had been documented as having varying levels of drought tolerance (Rucker *et al.*, 1995) and determined the correlation of drought tolerance characteristics with aflatoxin contamination. The 20 genotypes were tested for 2 years under drought stress conditions at Yuma, AZ and Tifton, GA. Drought tolerance was very effective in reducing aflatoxin contamination in Tifton; however, it was not effective in reducing aflatoxin contamination in Yuma. They proposed that the ability of drought tolerance to serve as a mechanism to reduce aflatoxin may have been overwhelmed by the low relative humidity at the Yuma location. At the Tifton location, significant positive correlations were observed between aflatoxin contamination and leaf temperature and between aflatoxin contamination and visual stress ratings. A significant negative correlation was observed also between aflatoxin contamination and yield under drought stress conditions. Leaf temperature, visual stress ratings, and yield are all less variable and

cheaper to measure than aflatoxin contamination. These characteristics may be useful as indirect selection tools for reduced aflatoxin contamination.

Genetic Variation. Very little is known about the amount of genetic variation in peanut that affords resistance to aflatoxin contamination. However, there are large, genetically diverse collections of *A. hypogaea* germplasm. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has a collection of over 13,000 accessions and the U.S. has a collection of over 7000 accessions. These germplasm collections have served as valuable sources of diversity for resistance to many pathogens of peanut. However, it is not feasible to evaluate either of these collections in their entirety for resistance to preharvest aflatoxin contamination using available screening techniques. Therefore, a core collection (a sample from a germplasm collection that represents the genetic variability of the entire collection) has been selected for the U.S. germplasm collection (Holbrook *et al.*, 1993), and research is underway to examine this collection for resistance to preharvest aflatoxin contamination (Holbrook, 1993).

Recombinant DNA Technology. Programs recently have been initiated that involve recombinant DNA technology and plant breeding for resistance to preharvest aflatoxin contamination. A successful transformation system for peanuts has been developed and transgenic peanut germplasm has been regenerated (Ozias-Akins *et al.*, 1993). Studies are now directed toward producing transgenic peanut germplasm with enhanced resistance to *A. flavus*. These may provide a more effective genetic resistance strategy for prevention of preharvest aflatoxin contamination of peanuts under drought stress conditions (see Chapter 3).

POSTHARVEST ADVANCES IN AFLATOXIN ELIMINATION

Segregation of Aflatoxin-Contaminated Lots

After harvesting and windrow drying, peanuts are brought to a "buying point" to be marketed. The USDA Peanut Marketing Agreement (Peanut Administrative Committee, 1994) requires that all farmers' stock loads be inspected by the Federal State Inspection Service for the presence of visible *A. flavus* in a sample drawn from the load. When *A. flavus* is found on any one seed, the entire load (*ca.* 4000 kg) is designated Segregation 3 and cannot be utilized for edible purposes because the assumption is made that there is a high probability that aflatoxin is present in the load. When *A. flavus* is not detected in the sample, the load is designated Segregation 1 and can be moved into the normal marketing channels for edible purposes provided subsequent chemical testing (official thin-layer chromatography method conducted by USDA- and Peanut Administrative Committee-approved labs) shows the peanuts meet regulatory guidelines. In fact, the load may contain aflatoxin even though visible *A. flavus* was not found in the sample. This is because of the small sample size and assessing aflatoxin contamination by examining the sample for visible *A. flavus* is an indirect method. Sampling errors are quite large because the sample size is relatively small (*ca.* 500 g out

of a load weighing *ca.* 4000 kg) (Dickens and Whitaker, 1986). Since aflatoxin-contaminated loads sometimes pass as Segregation 1 loads, a subsequent commingling of aflatoxin-free and aflatoxin-contaminated loads in warehouses creates a major problem for peanut processors, who must meet regulatory aflatoxin guidelines based on a direct chemical analysis for aflatoxin.

Direct chemical analysis for aflatoxin in farmers stock peanuts at the buying point could improve the identification and proper segregation of loads based on aflatoxin risk. A direct chemical test is better than an indirect visual test because with a chemical test a much larger sample of peanuts (2.5 kg) could be evaluated for aflatoxin, thus reducing the sampling error. Studies during the 1990 and 1991 harvest seasons supported the feasibility and methodology of a chemical test for aflatoxin at peanut buying points (Blankenship and Dorner, 1991a,b). The Aflatoxin Assay Project of 1990 installed testing laboratories at five buying points in the U.S. (Blankenship and Dorner, 1991a). All loads that were marketed at these buying points were analyzed for aflatoxin in addition to the visual inspection. Immunoassays were used to determine if the chemical procedure was feasible within the time frame currently required for official grading. The aflatoxin data generated from the laboratories indicated the need for such a test because at one location the average aflatoxin concentration of all loads determined to be Segregation I by the visible *A. flavus* method was 168 ppb and at another location the average was 135 ppb. Therefore, it was found that many of the loads determined to be Segregation 1 contained little or no aflatoxin, but some of the Segregation 1 loads were highly contaminated and were being commingled with the noncontaminated loads in warehouses.

The 1991 Aflatoxin Assay Project was designed to gather data at 37 buying points throughout the U.S., and essentially all loads graded at those locations were visibly examined and chemically analyzed for aflatoxin (Blankenship and Dorner, 1991b). Results again supported the rationale for implementing chemical testing in conjunction with larger sample sizes to reduce sampling error at the buying point as an effective management tool (Whitaker *et al.*, 1994a,b). With chemical testing, farmers stock peanuts could be more accurately segregated for proper storage. Aflatoxin-free loads could be segregated from loads with low levels of aflatoxin destined for subsequent processing and clean-up and from loads that are highly contaminated.

Storage

The postharvest phase that contributes most to the aflatoxin problem in peanuts is storage. Aflatoxin management during storage can be achieved by good warehousing practices (Smith, 1989). The primary goal for aflatoxin prevention in storage is to prevent rehydration of the peanuts due to condensation or leaks in the warehouse. A properly ventilated warehouse with a good roof, double sidewalls, and a floor is required to prevent rewetting of the peanuts. Uniform loading of the warehouse allows excessive heat and moisture to escape and reduces favorable areas for insect infestation, which can cause heat build-up and moisture accumulation with resultant

mold growth and aflatoxin contamination.

Painting warehouse roofs with white paint greatly reduces the solar heat load when compared to conventional galvanized metal; thus, the overspace temperature and condensation potential is decreased (Smith, 1994). The double roofing concept of installing a new roof over a defective, existing roof with an air space between the two roofs, has proven effective in controlling warehouse condensation (see Chapter 12). Removal of high moisture foreign material plus fine material (sand, soil, etc.) that concentrates in the center third of the peanut mass greatly reduces the potential for aflatoxin production by providing better air flow through the peanut mass and quicker removal of excess moisture (Smith *et al.*, 1985).

That aflatoxin contamination can and does occur during warehousing of peanuts is generally accepted. The extent to which it occurs is difficult to assess, particularly since the first official chemical analysis conducted on peanuts occurs after storage and, therefore, represents both preharvest and postharvest contamination.

Processing

During peanut processing, aflatoxin is managed through the removal of contaminated seeds by size separation, density segregation, and electronic color sorting. Aflatoxin contamination in a contaminated lot of peanuts is not uniformly distributed, and is associated primarily with certain high risk components (Cole *et al.*, 1988; Dowell *et al.*, 1990). These high risk components include loose-shelled seeds (seeds shelled during combining), small immature seeds, and damaged seeds. In addition, aflatoxin contamination in a contaminated lot is more likely to be associated with lighter, less dense seeds (Davidson *et al.*, 1981; Cole, 1989; Kirksey *et al.*, 1989). Therefore, removal of these high risk components during processing constitutes effective aflatoxin management.

High capacity belt screens are very efficient in removing loose-shelled seeds and small pods prior to storage or shelling. This can reduce aflatoxin concentrations in years when loose-shelled seeds are highly contaminated (Cole *et al.*, 1988). In a study conducted in 1987, belt screening of 17 trailer loads reduced aflatoxin levels by an average of 35% (Dowell *et al.*, 1990).

Rucker *et al.* (1994) developed a sorting method using pod density to partition peanut seeds into maturity related segregations. Along with improvements in maturity distributions, they found that the higher test weight fractions (higher pod density) had less aflatoxin than the low test weight fractions. They tested many density sorting devices, including air columns, pod cleaners, and gravity tables. All of these devices were capable of sorting pods into maturity groups, but the gravity table was the most precise.

After peanuts are shelled, they are sized over vibratory screens. Because aflatoxin is more likely to be found in small, immature peanuts than in large, mature ones, the sizing process tends to concentrate aflatoxin in size categories that are not used for edible purposes (oil stock). This reduces the aflatoxin concentration of the edible peanuts. In shelling plants, peanuts also

are passed over gravity tables, which separate peanuts on the basis of density. Separation of the lighter, less dense seeds further lowers the aflatoxin content of the heavier seeds.

The most effective procedure for reducing aflatoxin during processing is the use of high speed, electronic color sorting equipment to remove discolored seeds. Peanuts that have been invaded by fungi are often discolored, and electronic color sorters can remove a large percentage of these seeds. Many seeds that have been damaged by aflatoxigenic fungi and typically contain a very high concentration of aflatoxin are removed by this process, making it an effective postharvest aflatoxin management tool. Currently, shelling plants in the U.S. are utilizing all of these processes to remove low quality, aflatoxin-contaminated seeds from contaminated lots.

Peanuts that still are not acceptable for edible purposes following shelling are diverted to blanching plants for further clean-up. The peanut blanching process removes the peanut skin, or testa. The use of gravity tables and electronic color sorting in conjunction with blanching is very efficient in removing aflatoxin contaminated seeds. Table 2 shows the effectiveness of aflatoxin removal of the various processing stages from a 40,000 kg Segregation 1 lot of contaminated peanuts (Cole *et al.*, 1991). These data demonstrate that the most effective aflatoxin management strategy available in the processing phase is electronic color sorting. The process was effective both during the shelling process or later as part of the blanching process. Color sorting yielded a 70% reduction in aflatoxin contamination in the shelling plant and a reduction of 91% after blanching.

Table 2. Effectiveness of postharvest aflatoxin management techniques at the processing level.^a

Processing level	Aflatoxin μg/kg	Reduction %	Cumulative reduction %
Farmers stock (initial concentration)	217	—	—
Technology:			
Shelling plant:			
Belt separator	140	35	35
Seed size separation ^b	100	29	54
Color sorting ^b	30	70	86
Gravity table ^b	25	16	88
Blanching plant:			
Blanching/color sorting ^b	2.2	91	99.0
Re-color sorting ^b	1.6	27	99.3

^aResults from the processing of a 40,000-kg Segregation 1 lot of contaminated peanuts.

^bData based on medium category peanuts only (seeds ride 18/64" screen).

The last possibility for utilizing contaminated peanuts involves detoxification, including inactivation, destruction, or irreversible binding of aflatoxin. Treatment with chemicals, such as ammonia, formaldehyde, or sodium hypochlorite can reduce the aflatoxin concentration or completely eliminate it. However, such an approach is not feasible for peanuts that are intended for human consumption.

The addition of sequestering agents to feeds and foods has been proposed as a protective detoxification strategy against mycotoxin contamination (Phillips *et al.*, 1987; Kubena *et al.*, 1990, 1991, 1993). This relatively new strategy involves the addition of nonnutritive, adsorptive materials to contaminated food or feed to bind and immobilize aflatoxin in the gastrointestinal tract of animals. Dietary hydrated sodium calcium aluminosilicate (HSCAS) reduces the bioavailability and toxicity of aflatoxin in chickens (Kubena *et al.*, 1990, 1993), turkeys (Kubena *et al.*, 1991), lambs (Harvey *et al.*, 1991), mink (Bonna *et al.*, 1991), and swine (Colvin *et al.*, 1989; Harvey *et al.*, 1989). Sodium bentonite, another proposed sequestering agent, likewise diminished the effects of aflatoxin in growing swine when used as a dietary supplement (Lindemann *et al.*, 1990).

Volclay NF-BC, a bentonite clay, was used in a rat study to investigate its efficacy to protect against the effects of a diet consisting of aflatoxin-contaminated peanut butter (Voss *et al.*, 1993). Addition of 1.0% Volclay to the diet provided protection against hepatotoxicity and reduced the extent and severity of liver lesions in rats. The authors concluded that Volclay could provide protection against aflatoxin in peanut butter.

Because the Volclay study (Voss *et al.*, 1993) and a previous study with swine (Lindemann *et al.*, 1990) suggested a stoichiometric relationship between the sequestering agent and aflatoxin levels in feed, effective protection may be attained with the addition of very low, organoleptically acceptable levels of the sequestering agent in commercial peanut butter.

SUMMARY

The conditions that predispose the peanut plant to preharvest aflatoxin contamination are characterized by severe and prolonged heat and drought stress during pod development. Also, for some as yet unknown reason, immature peanuts are considerably more susceptible to preharvest contamination than are mature peanuts. The resistance is probably based on a rather complex interaction between the fungus, the environment, and the plant's own natural resistance (totally or partially based on its capacity to produce stilbene phytoalexins).

Irrigation can prevent contamination in peanuts if it is applied properly and adequately. However, irrigation is not the complete solution since it is not uniformly available. Another significant factor that must be addressed before the problem can be totally resolved is that of insect control during drought conditions. Peanut seeds damaged by insects under these conditions contribute considerably to overall contamination, and resistance strategies that do not take this into account probably will not be entirely successful.

New technologies that should be available to the peanut producer in the near future are expert systems called "aflatoxin early warning systems", biological control, and cultivars with enhanced resistance. Perhaps the most exciting new technology is the potential use of recombinant DNA technology that may produce absolute or near absolute field-resistant varieties.

The most advanced technologies related to aflatoxin management exist in the area of postharvest elimination. Contaminated lots can be decontaminated by size separation, density segregation, and electronic color sorting. The latter technology has been the most effective decontamination technology used in both the shelling and blanching processes.

Numerous advances have been made in the last decade toward effective management of aflatoxin contamination of peanuts. However, the long-term goal of total elimination of the aflatoxin problem in the peanut industry has not yet been achieved, and may not, in fact, be achievable. Nevertheless, the goal remains and much effort is being expended to accomplish the goal. In the short-term, it will be necessary to utilize every technology available in an integrated approach to reduce aflatoxin to the lowest possible levels. Such an approach must begin in the field and continue throughout all phases of production and processing.

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