Chapter 13

AFLATOXINS AND OTHER MYCOTOXINS IN PEANUTS

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Fungi and their toxic metabolites (mycotoxins) in grain, seed, and feeds have been a problem for man and domesticated animals for hundreds of years. Diseases (mycotoxicoses), caused by the ingestion of foods made toxic by fungi, have resulted in mortality in man and animals since the days of ergotism in the Middle Ages in France to the epidemics of alimentary toxic aleukia (ATA) in the 1930's and 1940's in Russia. A literature review on mycotoxins and mycotoxicoses has been recently published (Wyllie and Morehouse, 1977, 1978 a,b).

The surge of present day interest in mycotoxins developed following the death of 100,000 turkey poults on 500 farms in England in 1960. Investigation revealed a toxic fungal metabolite (aflatoxin) of *Aspergillus flavus* in the Brazilian peanut meal fraction of the feed (Lancaster et al., 1961; Sargeant et al., 1961). *A. flavus* is distributed world-wide in soil and air, and is a seed-inhabiting storage-fungus of many important food crops of man. Aflatoxins have been found not only in peanuts in all major peanut-producing countries, but also in cottonseed, corn, soybean meal, fish meal, and other grains, seed, and feeds. Research on aflatoxin has been extensively reviewed by Goldblatt (1969b) and more recently by other investigators (Jones, 1977; Edds, 1979; CAST, 1979).

Aflatoxin B_1 is the most potent, naturally occurring carcinogenic substance known and has induced cancer and caused mortality in most domesticated and experimental animals. Also, there is direct evidence from India wherein aflatoxin was incriminated as a probable cause of human infantile liver cirrhosis. The most impressive, aflatoxin-related episode is the acute poisoning in India in 1974 involving some 400 people and resulting in 106 deaths (CAST, 1979).

OCCURRENCE OF ASPERGILLUS FLAVUS AND A. PARASITICUS

Fungi (molds) are constantly associated with the peanut pod (geocarp) during its development in the soil, and after digging and during curing and subsequent storage. This review summarizes the status of knowledge on the role of *Aspergillus flavus* Link and *A. parasiticus* Speare in invading peanut pods and seed in the soil before harvest, after digging, and during curing and subsequent storage (shelled or in-shell). After invasion of the seed, *A. flavus* and *A. parasiticus* may under favorable conditions form aflatoxins. This may occur before harvest or after digging during curing in the field or during storage.

In this chapter, a number of synonymous terms are in common usage throughout the world of peanuts: e.g., peanut and groundnut, kernel and

seed, testa and seedcoat, pod and fruit and geocarp, stem and haulm, digging and lifting, and curing and drying. Many of these synonyms are used interchangeably to preserve the terminology employed by the respective authors cited. Similarly, *Macrophomina phaseolina* (Tassi) Goid, *Sclerolium bataticola* Taub and *Macrophomina phaseoli* (Maubl.) Ashby are names for the same fungus, as are *Diplodia gossypina* Cooke, *Botryodiplodia theobromae* Pat. and *D. theobromae* (Pat.) Nowell, and *Rhizopus nigricans* Ehr. and *R. stolonifer* (Ehr. ex Fr.) Lind. *Aspergillus flavus* is used in this chapter in a collective sense for both *A. flavus* and *A. parasiticus*, since many investigators did not differentiate or establish the specific identity of their experimental isolates.

FIELD FUNGI

Fungi associated with peanut pods and seed are broadly classed into 2 groups, field fungi and storage fungi (Christensen, 1957). Peanuts differ uniquely from cereal grains and other legumes in that the peanut flower blooms aboveground on the plant, but the fruit develops under the soil surface. Therefore, the peanut has been selected for its survival in the soil environment, whereas grains and soybeans have been selected for their survival in an aerial environment. Peanut harvesting is a 2-stage process consisting of digging and drying prior to picking, which is the equivalent of combining or threshing of cereal grains and soybeans. At harvest, the damage due to digging and exposure to variable atmospheric conditions of the soilborne peanut pod with seed high in moisture can be equated with that of mature grains and soybeans exposed constantly to the hazards of an aerial environment. Fungi present in the soil and air (including *A. flavus*) may invade the peanut seed before digging, and/or during curing and storage in an ecological succession of microorganisms determined by humidity, temperature, time, light, air movement, and physical and biological damage to the pod.

Austwick and Ayerst (1963) outlined the sequence of those events:

> "During harvesting, drying and storage, the conditions affecting the growth of fungi on groundnuts change so that different groups of fungi become dominant at each stage. In the ground the mature groundnut fruit consists of a senescent shell, which is frequently invaded by a number of saprophytic fungi, and the kernels, which are probably resistant to attack at this time except by specialized plant parasites. After removal from the soil within the shell, kernels begin to dry and may be mechanically damaged, these factors reducing their resistance to infection and rendering them susceptible to attack by a number of saprophytic and weakly parasitic fungi. With further drying, moisture becomes one of the main factors limiting the growth of fungi and the kernels are then attacked only by relatively xerophytic storage fungi."

Most species of field fungi that invade peanut pods in the soil are facultative parasites. The pod appears to be generally resistant to penetration by saprophytic fungi until it matures, unless it has been damaged by cultivation, nematodes, insects, pathogenic fungi, or physiologically affected by environmental extremes (Diener, 1973). Field fungi, especially *A. flavus*, rapidly invade damaged, physiologically unsound, or overmature peanut pods and seed in the soil. After digging, sound unblemished peanut pods may be invaded, if environmental conditions during curing are unfavorable for rapid drying of the pea-

nut pod and seed in windrow or stack. During curing, field fungi may invade pods damaged mechanically by digging even though the environment is generally favorable for rapid drying.

Mycoflora of Peanut Soils

Fungi dominating the mycoflora of peanut field soils and of the rhizosphere and geocarposphere of peanut plants vary throughout the world with soil type and environment as do the fungi predominating during curing. Data from Nigeria, Israel, and Georgia are in general agreement on the relatively high frequency and prevalence of species of *Aspergillus, Penicillium,* and *Fusarium,* whereas *Macrophomina phaseoli* and *Rhizoctonia* spp. were subdominants (Diener, 1973).

In Nigeria, the high numbers of fungal propagules present in the geocarposphere (fruit soils) at 15 and 17 weeks reflected the high incidence of *Penicillium funiculosum* Thom and *A. flavus,* respectively (McDonald, 1969a). The percentage of *A. flavus* in the mycoflora of peanut field and fruit soils was highest during weeks 14-16 in dilution plate tests, whereas in fruit soils, *A. flavus* reached the highest levels, in terms of both numbers of propagules and in percentages of total fungi, during weeks 16-18. *A. flavus* was not observed on Warcup plates of field soil, but was present on plates of fruit soil sampled at weeks 14-16.

In studies on the occurrence of *A. flavus* in peanut field soils of 12 different regions of Israel, Joffe and Borut (1966) noted that the incidence of *A. flavus* was second only to that of *P. funiculosum.* Later, Joffe (1968a) demonstrated with soil inoculations in containers that the frequency of *A. flavus* increased greatly in the geocarposphere during the growing period, while it was dominant early in the season and rapidly decreased in rhizosphere and soil. These results were confirmed in a field study with a number of *A. flavus* concentrating near the geocarps (Joffe, 1969a). He also reported that *A. flavus* was more prevalent in medium and heavy soils than in rhizosphere or geocarposphere soils. *A. niger* Van Tieghem also tended to concentrate near geocarps and was antagonistic to *A. flavus* (Joffee, 1969b), a possible reason for the relatively low occurrence of *A. flavus* in groundnuts in Israel. The rates of germination of *A. flavus* conidia and *A. flavus* populations were virtually identical in geocarposphere soils and nongeocarposphere soils in Virginia. However, *A. flavus* conidia germinated readily in 16 hours in soil adjacent to pods superficially injured and inoculated with infested soil (Griffin, 1972).

In Georgia, Jackson (1968) found that species of *Aspergillus* and *Penicillium* comprised 50% of the total fungal propagules of the mycoflora of geocarposphere soils with *A. flavus* comprising about 10% of the *Aspergillus* isolates.

These data indicate that universally *A. flavus* is associated or becomes concentrated in the geocarposphere, although it is omnipresent in peanut soils but at very low levels compared to *Fusarium* spp. and several other fungi.

Peanut Pod and Seed Mycoflora before Maturity and Digging

In Nigeria, *A. flavus* was isolated weekly from 4-14% of the peanut shells starting 90 days after planting and continuing to maturity (McDonald, 1970a). About 1% of the seed were infected by *A. flavus* during this same period.

No reduction in the levels of total fungi was reported in peanut pods sampled from plots of 16 tillage-organic matter-crop combinations observed in Georgia (Hanlin, 1970). Shells and seed plated out 100 days after planting showed 4.5 and 14.1% contamination, respectively, by *A. flavus.* In Alabama, *A. flavus* was isolated infrequently from peanut shells and seed lifted and picked over a 2-month period from 70 days after planting through curing (Lyle, 1966). Barnes (1971) in Oklahoma rarely isolated *A. flavus* from developing pods and seed. In Virginia Bunch peanuts, Porter and Garren (1968) found that *A. flavus* was isolated more often (4.9%) from seed than shells (1.1%) sampled in August (90 days after planting.) These data support those from Georgia that suggest that *A. flavus* tends to become established in the seed rather than in the shell.

Penicillium, Trichoderma, Chaetomium, and *Fusarium* were the dominant genera of the endogeocarpic microflora of peanuts in Virginia (Porter and Garren, 1968). Isolation density of *A. flavus,* a subdominant, was relatively low (3%) and remained constant in the shell, but increased on the seed as the season progressed. Under gnotobiotic conditions, Wells et al., (1972) observed that *Trichoderma viride* Pers. ex Fr. colonized immature pods, mature pericarps, and testae; the presence of *T. viride* reduced colonization of immature and mature pericarps by *A. flavus. P. funiculosum* not only nullified this antagonistic effect, but also appeared to stimulate colonization of mature peanut pericarps and testae by *A. flavus.*

Data on the mycoflora of immature peanut pods obtained before digging in Nigeria, Georgia, Alabama, Oklahoma, and Virginia agreed in that *Fusarium* spp., *Penicillium* spp., and *Aspergillus* spp. were usually dominants or subdominants (Diener, 1973). *M. phaseoli (Sclerotium bataticola)* was a dominant in Nigeria and a subdominant in Georgia. Data from Nigeria and Georgia also agreed in that *A. flavus* invaded 4 to 14% of the shells, but it was isolated infrequently in Alabama and Oklahoma. Seed invasion by *A. flavus* amounted to 4.9% in Virginia and 14.1% in Georgia.

Peanut Pod and Seed Mycoflora at Maturity and Digging

Data on peanut pod and seed mycoflora at maturity and digging from Nigeria, Israel, Georgia, and Oklahoma agreed in the most part that *Fusarium* spp., *Penicillium* spp., and *A. niger* were dominant in most experiments (Diener, 1973). *M. phaseoli* was a dominant in Nigeria and Gambia and a subdominant in Georgia and Oklahoma. *Rhizoctonia solani* Kuhn was a dominant in Georgia and Texas and a subdominant in Nigeria and Oklahoma. In general, only a low percentage of seed had been invaded by fungi before lifting.

Although *Rhizoctonia* was dominant in Texas relative to pod damage, *A. flavus* was a prominent invader of seed of undamaged pods, and in pods damaged by *Rhizoctonia* and *Rhizoctonia* plus insect larvae (Ashworth and Langley, 1964). Data from Oklahoma and Nigeria (McDonald, 1969b) revealed little or no *A. flavus* on seed from undamaged pods at digging. However, *A. flavus* occurred frequently in isolations from seed and pods from 10 to 12 fields of different soil types in Israel (Joffee and Borut, 1966). In Gambia (Gilman, 1969),

A. flavus made up 14% of the fungi contaminating seed of mature intact pods at lifting. In Georgia, *A. flavus* was among the more abundant pod surface fungi before and after lifting, but it represented less than 1% of total seed fungi at lifting and after 2 days of drying in the windrow (Jackson, 1965a).

Invasion of peanut pods by *A. flavus* or other fungi before digging following physical or biological damage may result in empty or rotten pods that are lost at digging. At harvest time in Texas, pod examination revealed that only 1 to 4% of the seed from undamaged pods yielded *A. flavus* or any other fungus (Ashworth et al., 1965). On the other hand, 58 to 74% of the seed from damaged pods yielded *A. flavus*. Similarly in the Sudan, *A. niger* and *A. flavus* were isolated from all samples of mechanically damaged shells obtained from three production areas with different soils and irrigation practices (Abdalla, 1974). *A. flavus* occurred in 2.6 to 31.4% of the seed and *A. niger* in 18.4 to 47.4% of the seed from the 53 samples.

The effects of crop sequence and soil types on the mycoflora of peanut seed were investigated in 81 fields over a 3-year period in Israel (Joffe and Kisker, 1970). The mycoflora of peanut seed was richest in fields previously planted with peanuts. In most cropped fields, *A. niger* made up 50 to 60% of the total mycoflora with *P. funiculosum* and *P. rubrum* Stoll being considerably more prevalent on previously fallowed fields. The prevalence of *A. flavus* was generally low and was not influenced by most crop sequences. However, high *A. flavus* infestation of shells and seed was noted in plots previously planted with peanuts in India (Subrahmanyam and Rao, 1974).

Peanut Pod and Seed Mycoflora during Curing in Windrow and Stack

At normal maturity and lifting time, peanut pods are commonly infested with fungi, although very few seed are invaded. While peanuts are curing in the windrow, there is generally an increase in both pod fungus populations and kernel invasion by fungi. In an earlier review Diener (1973) indicated that data for peanut pod and seed mycoflora during windrow and stack curing were fairly comparable throughout the world. Species of *Fusarium, Macrophomina, Diplodia, Rhizopus, Aspergillus,* and *Penicillium* were common in Gambia, Georgia, Nigeria, Oklahoma, Puerto Rico, and Virginia. *M. phaseoli* was dominant in Nigeria, Gambia, Georgia, and Puerto Rico. *D. gossypina (B. theobromae)* was a dominant in Nigeria and in Puerto Rico. *Penicillium* spp. were dominant or subdominant in Georgia, Virginia, Oklahoma, and Puerto Rico, whereas *Rhizopus* spp. were common in Nigeria, Georgia, and Virginia. Seed were invaded by *Aspergillus* spp. and *Penicillium* spp. in Gambia, Georgia, Oklahoma, Virginia, and Puerto Rico.

In Gambia, at lifting only 47% of visibly undamaged peanut pods were invaded by fungi as compared to 97% after 3 days of drying (Gilman, 1969). *A. flavus* made up 14% of the fungi isolated from seed of apparently intact pods. *A. flavus* was found to invade pods more frequently after digging. In Nigeria, there was an increase in seed contamination by *A. flavus* and other fungi with time after liftings; the rate of increase was higher on seed from broken pods than on seed from undamaged pods (McDonald and Harkness, 1964). In Georgia, less than 1% of the shells of stacked peanuts picked 40 days after lifting had been invaded by *A. flavus,* whereas 6% of the seed were contaminated (Hanlin, 1970). Shell invasions by *Aspergillus* spp. decreased from 13-24% at digging to 1-3% after 40 days in stacks, whereas *Fusarium* spp. increased from 1-5% to 60-78%. In Oklahoma (Barnes and Young, 1971), *A. flavus* was rarely isolated in either 1965 or 1966 crop peanuts, although aflatoxin was found in all 1965 samples and in a few 1966 samples.

In Nigeria, at normal maturity and lifting time, shells were commonly infested by fungi, although very few seed were invaded (McDonald, 1970b). Following windrow curing, there was an increase in seed invasion. Seed from pods picked shortly after lifting and sun-dried on mats showed little increase in fungal infection during drying. Pods left in the ground after reaching maturity showed a gradual increase in fungus invasion of the seed. *Macrophomina phaseoli* and *Fusarium* spp. were the dominant fungi in shells and in seed of overmature and windrow-dried pods. *Aspergillus* spp. were not abundant but were frequently present in shells and seed, with *A. flavus* being the most common.

The mycoflora of hydrated, mature, cured peanut fruit from Virginia (VA) was compared with that from Puerto Rico (PR) (Garren and Porter, 1970). *A. flavus* was isolated only at temperatures of 21 and 27 C in shells of VA and PR fruit. Seed with intact testae from VA discolored and unblemished shells were contaminated by *A. flavus* at 50 and 25%, respectively, at 27 C. Only *Diplodia gossypina* was more aggressive than *A. flavus* at 27 C showing 65 and 70% contamination of seed from discolored and unblemished PR shells, respectively. *A. flavus* was rare in PR samples and was not found in VA seed before hydration.

Inverted and Random Windrow Drying

Effects of drying on the mycoflora in peanut pods in random windrows in comparison to windrows where the plants are inverted to expose the pods to sunlight have been studied by several investigators (Diener, 1973). In Georgia, the total number of pod surface fungi was about twice as high in pods from random windrows as compared to pods from inverted windrows. Numbers of fungi isolated from seed of these pods showed little difference relative to pod position. Data from North Carolina confirmed that field-drying was accelerated and conditions were less favorable for fungal growth in inverted windrows than in random windrows.

Researchers in Virginia (Porter and Garren, 1970) reported that the number of shells infested by fungi was reduced 20.5% by drying in inverted windrows in comparison to shells of freshly-dug and random windrow-dried pods. The proportion of seed infested with fungi was reduced by 31.5% in the random windrow and by 60.4% in the inverted windrow. The isolation frequency of *A. flavus* from seed from random windrowed fruit was 3.9% as compared to 2.6% from seed from inverted windrows. In freshly-dug pods, species of *Chaetomium, Penicillium, Trichoderma, Rhizoctonia,* and *Fusarium* were dominant in pericarp, while in windrowed pods, species of *Chaetomium, Rhizoctonia, Fusarium, Sclerotium,* and *Alternaria* were dominant. Seed of freshly dug pods were dominated by *Penicillium* and *Aspergillus,* whereas seed of windrowed pods were dominated primarily by *Penicillium.* Peanut pods and seed from inverted windrows in Tex-

as were also less severely damaged by fungi, whereas pods in contact with the soil surface in random windrows were heavily invaded by fungi and the seed of such pods were frequently invaded (Pettit and Taber, 1970). Thus, inverted windrow drying reduces the potential for mycofloral development by promoting rapid curing to low seed moisture levels, at which fungal growth terminates. However, detrimental effects from rapid curing by this method may result in increased splits and lowered seed germination and field emergence. Thus, utilization of this drying method may vary with the variety of peanut, length of time in the windrow, environmental patterns of the growing area, and other factors.

The role of field (soil) fungi in deterioration of peanuts is not well established except where pathogenesis is involved. Approximately 10-12 of 50 genera of fungi appear to dominate the soils, pods, and seed in peanut-producing areas of the world. The mycoflora of the peanut pod is determined by the mycoflora of the soil, which in turn is influenced by the makeup and structure of the soil, organic matter, moisture, temperature, and other environmental, physical, and biological factors. The mycoflora of the seed is determined by the effect of these factors and certain inherent characteristics of each fungus species making up the pod mycoflora. The activity of many of these fungi is noticeably reduced under conditions of low moisture (drought); and osmophilic fungi, such as *A. flavus* and *A. parasiticus*, increase rapidly to dominate the mycoflora of the soil, pod, and eventually the seed of even undamaged pods. A few field (soil) fungi persist in storage and may invade the seed under conditions favorable for their growth. However, many species do not persist and are important only in seed destruction when physical and/or biological damage to the pod provide entry to the seed in the soil, at harvest, or during curing.

STORAGE FUNGI

The Species

After digging, the field fungi tend to die out during the curing period and species of saprophytic and weakly parasitic fungi proliferate at lower seed moistures and become dominant. This second group of fungi, the storage fungi, dominates the peanut mycoflora during curing, picking and storage, and consists principally of species of *Aspergillus, Penicillium, Rhizopus, Sclerotium,* and *Fusarium*. Although 153 species of fungi have been isolated from stored peanut seed in Israel and 146 species in the USA, data point to a relatively small number of genera being involved in peanut deterioration (Joffe, 1969c; Hanlin, 1973). *Aspergillus* spp. (*A. niger, A. flavus, A. glaucus* group), *Penicillium* spp. (*P. funiculosum, P. citrinum* Thom, *P. rubrum*), *M. phaseolina, R. solani, Fusarium* spp. and *Rhizopus* spp. appear to be most important. *B. theobromae* also appears to be significant in Nigeria. Jackson (1965c) reported that 4 of these species (*A. niger, A. flavus, S. bataticola,* and *Rhizopus* spp.) could rapidly penetrate the pod and invade the seed.

In a 4-year survey of the 6 major peanut-producing states, Hanlin (1973) found no *A. parasiticus* among the fungi isolated from peanut shells and seed from Oklahoma and Texas, but only *A. flavus*. No *A. flavus* occurred among fungi isolated in North Carolina, but only *A. parasiticus* and that from shells

rather than seed. It would appear that in the more arid peanut producing areas of the world, including Israel, *A. parasiticus* is not as prevalent as *A. flavus* (Joffe, 1969d; Taber and Schroeder, 1967).

Mycoflora of Stored Seed

The number of ecological studies of storage fungi in peanuts involving quantitative mood counts of populations is limited (Diener, 1973). In Alabama, quantitative data on the mycoflora of seed from farmers stock peanuts stored in 26 farm-size bins were related to initial moisture, seed damage, type of bin, and length of storage. Species of the *A. glaucus* group, *A. flavus* group (*A. tamarii* Kita), and *P. citrinum* Thom were dominant in the mycoflora of the seed of farmers stock peanuts stored 8-56 months.

The number and kind of fungi associated with seed plated after being stored for 1-6 months have been determined (Welty and Cooper, 1968). Initially, *A. repens* deBary (*Eurotium repens* deB.) was isolated from 54% of the seed, *Penicillium* spp. from 80% and *A. flavus* from 7%. After 2-3 months of storage, the percentage of seed with *A. repens* and *Penicillium* dropped to about 10% and then gradully increased to approximately 40% after 6 months. *A. flavus* remained at 7% for the first 3 months and after 4-6 months it was isolated from 18% of the seed. *A. ruber* (Konig, Spieck., and Bremer) Thom and Church (*E. rubrum* Konig, Spieck., and Bremer) and *A. amstelodami* (Mangin) Thom and Church (*E. amstelodami* Mangin) were isolated from a low percentage of the seed (4.4%) throughout the 6 months storage.

In Texas, the rate of invasion in damaged pods was 6 times that in sound pods. *A. glaucus* species, undetected in the first week of storage, rapidly became the dominant fungi of seed in sound pods and a major part of the mycoflora of seed in damaged pods (Schroeder and Boller, 1971). *A. candidus* Link ex Fr. was found after 6 weeks in about 60% of the seed in damaged pods. The penicillia became prevalent in 4 weeks with a 74% recovery after 6 weeks. Neither penicillia or other aspergilli, including *A. flavus*, were recovered from an appreciable number of seed from sound pods.

In Israel, *A. niger* was found to be the most common species of 71 fungi occurring in 114 stored peanut samples of 1963 and 1964 crops (Borut and Joffe, 1966). *A. niger* occurred in 97.4% of the samples, while *A. flavus* was detected in 78.4% of the 1963 samples and in 63.5% of the 1964 samples. However, *A. niger* occurred in large numbers (40% of the total colonies) in 114 samples, whereas *A. flavus* made up only 5.7% of the mycoflora of stored seed. *A. niger* was the dominant species in the mycoflora of 419 samples of stored seed examined over a 5-year period in Israel (Joffe, 1969c). It was even more predominant in stored seed than on fresh ones. *P. funiculosum* and *P. rubrum* were prevalent, but *Rhizopus nigricans, Rhizoctonia* spp., *Sclerotium* spp., *F. solani* (Mart.) Sacc., and *A. flavus* were present in relatively small quantities in comparison to *A. niger*.

In Egypt, peanut seed were adjusted to 8.5, 13.5, 17.5, and 21% moisture levels and stored for 6 months at 5, 15, 28, and 45 C (Moubasher et al., 1980). *A. fumigatus* was the dominant fungus in this experiment followed by *A. flavus, A. niger, A. terreus* Thom, and *P. funiculosum*.

The high incidence of *A. niger* in stored seed that were disinfected with mer-

curic chloride was considered a selective removal of antagonists by Joffe (1968b). However, this could have been due to the mercury tolerance of *A. niger*, whereas the disinfectant is quite toxic to *A. flavus*, *F. solani*, *P. rubrum*, and *P. funiculosum*.

In evaluating the published data on the mycoflora of peanuts that have been stored after curing, the isolation medium determines to a great extent the range of species that are recovered, particularly if any kind of quantitative count of fungal propagules is made (Diener, 1973). Christensen's malt-salt medium used by Diener (1960) and Welty and Cooper (1968) is selective for osmophilic tolerant storage fungi and reduces the rate of growth of field fungi. Joffe and his colleagues in Israel and Jackson and Hanlin in Georgia used media (Czapeks, RBM-2, Sabourauds, malt) in their investigations that were appropriate for the field fungi found in the soil, on pod surfaces, and in seed from freshly-dug immature and mature peanuts (Diener, 1973). However, for isolations from peanuts during curing and storage, these media were probably inadequate for the isolation of the *A. glaucus* group. Christensen (1957) noted:

> *"It is almost axiomatic in microbiological work that any culture medium used to isolate microorganisms from materials, in which they are present, is to a certain extent selective, but this very obvious principle has at times been disregarded in work with fungi related to deterioration of stored grains."*

Christensen (1957) further states:

> *"No one medium or technique is sufficient to disclose all of the organisms that might be present in a given lot of seeds."*

Garren (1964) had made this same point in the isolation of the terrestial mycoflora of peanut pods. Besides the medium, quantitative and qualitative mycofloral data are also confounded by difference, in the natural substrate, preisolation environment, surface sterilant or washing, plating and isolation technique, temperature and length of incubation, and the examination date after plating. Despite all of these variables, there is considerable agreement in the mycofloral data from stored seed as well as those previously summarized from soil, pod, and freshly dug seed.

Environments Favoring Invasion of Peanuts by Storage Fungi

The main factors influencing the growth of storage fungi in peanut pods and seed are moisture (relative humidity), temperature, time, and gaseous composition of the atmosphere. Data on the relation of environment to growth and sporulation of many storage fungi have been summarized by several investigators (Diener, 1973).

Given species of fungi have very similar moisture optima and limits on a great variety of natural substrates when moisture is measured in terms of relative humidity (RH) equilibrium. It should be kept in mind that, as a result of the metabolism of seed substrates of low moisture by some fungi, the moisture content rises enabling a new succession of fungi to develop. Moisture data for peanut seed and meals at 30 C were reported by Austwick and Ayerst (1963) and are re-presented in Table 1.

High mycofloral counts were associated more often with high initial moistures of peanuts going into storage than any other factor (Diener, 1960). Austwick and Ayerst (1963) reported the growth of *A. flavus* and *A. chevalieri* (Mangin) Thom and Church (*E. chevalieri* Mangin) of the *A. glaucus* group at different RH (% moisture) and temperatures. Other studies with *A. niger*, *A. tamarii*, *A. fumigatus* Fres., and *Penicillium martensii* Biourge gave results similar to *A. flavus* in that they will not grow below 80-85% RH. The early literature on the influence of moisture, temperature, and other factors on the growth of *A. flavus*, an important storage fungus in grains, peanuts, and other agricultural commodities, has been reviewed (Diener and Davis, 1969, 1977).

Table 1. The moisture equilibrium of groundnuts.

Relative Humidity at 30 C (%)	Moisture Content Seed (%)	Meal (wet weight)
98	30.5	-
95	20.0	-
90	14.3	23.5
85	11.3	19.0
80	9.3	16.3
75	8.0	14.0
70	7.0	12.3

Changes in frequency of 6 species of fungi isolated from seed stored at controlled humidities 75, 85, and 99% for 6 months at 22-28 C were investigated by Welty and Cooper (1968). At 85% RH and seed moisture content (SMC) of 10.5%, the percentage of seed infested by *A. ruber* ranged from 0 in 1 month to 97-100% after 3-4 months of storage. Prevalence of *Penicillium* spp., *A. repens*, and *A. flavus* decreased from 80%, 57%, and 7% to 10%, 10%, and 1%, respectively, after 2 months. All decreased to negligible levels in 4 months. At 99% RH, the SMC increased slowly to 28% with monthly variations in the dominant fungus. Under the circumstances of competition between these storage fungi, it was concluded that *A. ruber* grew best at 12-15% SMC, *A. repens* at 18.5%, *A. flavus* and *Penicillium* spp. at 20% and *A. amstelodami* at 20-28%. *Fusarium* was detected in the fourth month and became the dominant fungus as SMC increased above 18%.

Most storage fungi found on peanuts, as well as some field fungi that may survive in soil or in the pods, grow well at temperatures of 25-35 C (Semeniuk, 1954). However, a few grow well at temperatures of 35-45 C. Growth rate is reduced at lower temperatures for most fungi, but some continue to grow slowly at 5-15 C. Jackson (1965b) found invasion of seed in intact, surface-sterilized pods by *A. flavus*, *A. niger*, *S. bataticola*, and *R. stolonifer* was favored by temperatures of 26-38 C. At temperatures below optima, fungi will grow and with time can cause extensive deterioration, if other conditions are met.

The combined interaction of temperature and RH determines spore germination rate; the higher the temperature at a given RH the less time is required for germination (Panasenko, 1967). In the storage of various seeds, the water content of the seed itself is not as important as the hygroscopic capacity of the seed and the RH of the microenvironment around the stored seed. The water content of peanut pod and seed will change until a hygroscopic balance (RH

equilibrium) has been established between the seed and its aerial environment.

Storage fungi are highly aerobic organisms and their activity depends on the oxygen in the air. Landers et al. (1967) investigated the effect of various concentrations of carbon dioxide (CO_2), nitrogen (N_2), and oxygen (O_2) on growth and sporulation of *A. flavus* in high moisture peanut seed. Growth was also measured by the increase in free fatty acids (FFA) in fungus-contaminated seed as compared to control peanuts. Striking reductions in fungal growth and sporulation occurred when 0_2 concentrations were reduced from 5% to 1% in combination with 0, 20, or 80% CO_2. Fungus growth and sporulation were reduced with each 20% increase in CO_2 from 40 to 80%. No growth occurred in 100% CO_2. FFA formation closely paralleled growth of *A. flavus*. In similar experiments, Sanders et al. (1968) evaluated the combination of RH and temperature in reducing fungal growth with CO_2. Visible growth and FFA formation by *A. flavus* were inhibited at 86% RH by 20% CO_2 at 17 C and by 60 and 40% CO_2 at 25 C. FFA levels decreased as RH decreased from 99% to 92% to 86%.

In related experiments, Jackson and Press (1967) studied the effect of air, 92-99% nitrogen, and 77-89% CO_2 at 4 and 27 C on the mycoflora of shelled and unshelled peanuts over a 12-month storage period. They found that the composition of pod surface mycoflora did not change. However, a significant reduction in the number of fungus propagules occurred after 27 C storage regardless of the gaseous environment. The mycoflora isolated were principally *A. flavus, A. niger, Chaetomium* spp., *Cladosporium* spp., *Fusarium* spp., *Penicillium* spp., *R. stolonifer,* and *T. viride.* At 4 C the number of pod surface fungi remained relatively unchanged through the 12-month period.

Storage fungi increase in the mycoflora of peanut seed during curing and storage until the SMC drops to 8-10%; a level at which they slowly disappear. Increases in moisture from rewetting in storage or exposure to high humidity for extended periods of time will result in rapid buildup of mycofloral fungi including *A. flavus*. Natural buildup of CO_2 and decreased oxygen levels in closed storage reduce mycofloral development.

PRODUCTION OF AFLATOXIN IN PEANUTS

The Fungus

Research has associated at least 173 species and 51 genera of field and storage fungi with peanuts in Israel and 146 species and 70 genera in the USA (Joffe, 1969c, Hanlin 1973). However, in the *A. flavus* group of species *A. flavus* and *A. parasiticus* produce aflatoxin and *A. oryzae* (Ahlb) Cohn and *A. tamarii* do not (Hesseltine et al., 1970). A summary of data from investigations in England, Holland, India, Israel, South Africa, and the USA indicated that about 60% of 1,400 isolates of the *A. flavus* group were aflatoxin producers (Diener, 1973). Isolates of *A. flavus* and *A. parasiticus* vary widely in the amount of aflatoxin produced on peanuts, and some isolates (strains) produced none (Diener and Davis, 1966; Taber and Schroeder, 1967).

Although some fungi lose their toxin-producing ability, Indulkar and Sen (1972) found that Indian isolates of *A. flavus* and *A. parasiticus* did not lose aflatoxin-producing ability when subcultured on natural substrates for 30 years. In Texas, aflatoxin-producing ability of room-temperature dried conidia of these two fungi remained relatively unchanged for 21 to 34 months in laboratory experiments, although viability of the conidia decreased (Boller and Schroeder, 1974). However, aflatoxin-producing ability and viability of moist conidia were lost rapidly within 21 to 60 days.

Of 1,626 isolates of *A. flavus* tested in Israel, 1.7% produced only aflatoxin B_1 (AFB_1), 95% produced both AFB_1 and AFB_2, 8.4% produced AFB_1, AFB_2, AFG_1, and AFG_2, and 10.4% produced no aflatoxins. Thus, about 9% were probably *A. parasiticus,* which is the primary producer of AFG_1 and AFG_2 (Joffe, 1969a). Doupnik (1969a) screened 244 *A. flavus* isolates from 3 major varieties of peanuts, and 34% produced no aflatoxins. About 18.6% produced only AFB_1, 31% produced AFB_1 and AFB_2, and 50.3% produced both AFB_1 and AFG_1 and in addition some produced AFB_2 and/or AFG_2. Thus, it appears that about 50% of the Georgia isolates were *A. parasiticus*. Summary of these data indicated that about 7 and 10% of the isolates produced less than 1 ppm; 70 and 40% produced from 1 to 100-125 ppm, and 23 and 50% produced more than 100-125 ppm (data for Israel and Georgia, respectively).

Moisture and Relative Humidity

The most important factor in growth and aflatoxin production by *A. flavus* is the moisture in or relative humidity (RH) surrounding the substrate (Austwick and Ayerst, 1963). It is probably arbitrary to separate the interrelationships of temperature and time from that of RH or moisture. Under precisely controlled conditions in the laboratory, the limiting RH for aflatoxin production in heat-killed Early Runner peanuts was 85 ± 1% at 30 C for 21 days (Diener and Davis, 1967). Aflatoxin production in living seed from freshly dug, surface-sterilized Early Runner peanuts was limited at a similar RH (Diener and Davis, 1968). In stored, unsterile living peanuts, low levels of aflatoxin were formed in immature and broken mature (damaged) seed after 84 days at 84% RH and 30 C, but not in sound mature and unshelled peanuts (Diener and Davis, 1970). No aflatoxin occurred at 83% RH in any treatment. This limiting relative humidity is in equilibrium with 10.5-11.0% SMC. In pure culture studies, Austwick and Ayerst (1963) reported that *A. flavus* was limited by 80% RH (9% SMC) and the growth rate on culture media between 80-85% RH (9-11% SMC) was very slow (0.1-1 mm/day).

Research has indicated that certain conditions may result in either preharvest or post-harvest infection and aflatoxin formation by *A. flavus* in peanuts. Fifteen years ago, most experimental data (Diener, 1973) indicated that there was little invasion of seed in intact and undamaged immature and mature pods in the ground by *A. flavus,* and little or no aflatoxin present in seed before lifting at the normal harvest time, although there was some contradictory data (Norton et al., 1956; McDonald et al., 1964; Joffe and Borut, 1966). It appeared that invasion of peanut pods and seed by *A. flavus* and other fungi usually occurred during curing when the variety was dug near maturity (Austwick and Ayerst, 1963; McDonald and Harkness, 1964; Diener et al., 1965). When dehydration (drying) of the peanut lowers the seed moisture content rapidly and steadily downward within 4 or 5 days to safe storage moisture, little opportunity for fungal invasion occurs. No varietal resistance was observed

(McDonald and Harkness, 1963). After lifting, peanuts were most rapidly invaded by *A. flavus* during drying or dehydration in windrow and stack at 14 to 30% SMC (McDonald and Harkness, 1964; McDonald et al., 1964; Dickens and Pattee, 1966); apparently *A. flavus* does not become established rapidly in peanuts at higher or lower moisture contents (Austwick and Ayerst, 1963; McDonald and Harkness, 1964, 1965). This conclusion is also supported by the data of Porter et al. (1972), who found that the isolation frequency of *A. flavus* from partially dried fruit with a SMC of 10% and 30% was 20% and 25%, respectively, following incubation of inoculated fruit in comparison to 12% frequency from seed of fruit with a 50% SMC. Whether this is a moisture-based response or is based on the fact that moisture levels of peanuts 2 days after lifting are usually in this range and this is also coincident with the time required for spore deposition, germination, penetration, and development of the fungus is a matter of conjecture. Where seed moisture was high there was some resistance to invasion by *A. flavus* (McDonald and Harkness, 1967). Data from field studies at Mokwa, Nigeria, showed that in the 1963 crop, which had matured and was lifted well before the rains had ended, toxicity at lifting was rare (McDonald, 1969b). However, at Kano where the rains ceased before harvest, toxicity increased as the moisture content of the seed at lifting decreased.

When peanuts that are being cured are in the general range of 14 to 24% SMC, interruption and retardation of the field drying cycle by showers or overcast humid weather, or a regain of moisture after picking and storage, usually result in the development of *A. flavus* with subsequent toxin formation (Austwick and Ayerst, 1963; Bampton, 1963). In other experiments (McDonald and A'Brook, 1963), artificial drying after 4 to 6 days in the field gave toxin-free seed, but peanuts left for 8 to 12 days or sun-dried for 10 to 16 days contained from 25 to 500 parts per billion (ppb) aflatoxin. Under tropical conditions peanuts that were initially free of toxin at digging contained detectable toxin in 48 hours (Bampton, 1963). Also, seed with testae damaged during shelling exhibited sporulating *A. flavus* in 4 days. In northern Nigeria, no aflatoxin was detected in peanuts left windrowed for 6 days after lifting, whereas those left for longer periods contained toxic seed (McDonald and A'Brook, 1963). The longer the crop was in the field before artificial drying, the higher the aflatoxin content. Some seed contained aflatoxin at harvest and this was associated with pod damage and low SMC (McDonald et al., 1964). Other field studies showed that contamination of seed with aflatoxin occurred at least 5 to 6 days after lifting (McDonald and Harkness, 1964). The extent of aflatoxin contamination in peanuts varies from year to year at specific sites. More *A. flavus* and other fungi were isolated from seed from slowly dried pods (attached to plant) than from rapidly dried pods (McDonald and Harkness, 1965). In Georgia, high levels of aflatoxin as well as high percentages of seed invasion by *A. flavus* occurred experimentally in slowly dried pods as compared to rapidly dried pods (Jackson, 1967).

Soil population levels of *A. flavus* gradually increased in the field during the 1973 growing season in Georgia (Wilson and Flowers, 1974). Less than 0.1% of the peanut seed sampled daily from random windrows contained *A. flavus* when collected from digging to combining (0-7 days). However, the day after combining and drying to 10-12% moisture, *A. flavus* was recovered from 15-25% of the seed. Even though there was low *A. flavus* seed infection at harvest, aflatoxin from 2 to 15 ppb was found in 21 of 37 samples collected during windrow drying from 0-7 days. The aflatoxins may have been produced by early infestations of *A. flavus* that did not persist or were already present at digging. In North Carolina, peanut samples harvested from random and inverted windrows contained little or no aflatoxin after being dried to 15% SMC at 32 C and 50% RH, while the majority of 128 samples dried at 32 C and 85% RH contained from 6 to 960 ppb of AFB_1 (Dickens and Pattee, 1966). Other research has demonstrated that moisture percentage decreased more rapidly in inverted windrows than in random windrows; lower *A. flavus* invasion occurred in seed from inverted windrows (Page, 1964; Pettit and Taber, 1970; Porter and Garren, 1970; Porter and Wright, 1971).

Data demonstrating that invasion by *A. flavus* and aflatoxin formation in peanuts frequently occurs in the field before harvest have been steadily accumulating. In Texas, *A. flavus* was reported to be the dominant fungus in unblemished spanish peanuts, having been isolated from 16% of the seed and shells over a 6-week period before and after harvest (Norton et al., 1956). Fungi grew from 22.2% of the seed plated out with *A. flavus* occurring 79.3% of the time. Generally, seed invasion by *A. flavus* and aflatoxin accumulation before harvest have been associated with drought stress, overmaturity, and physical and biological damage to the pod by fungi, insects, and possibly nematodes.

Drought stress has been the factor most frequently associated with aflatoxin occurrence in peanuts before digging. Drought stress probably increases susceptibility to fungal invasion, since it decreases the moisture content of the pod and seed and greatly lowers the physiological activity of the peanut. In Nigeria, aflatoxin was detected in seed in a late planted crop in a sandy soil in a semi-arid region, although the plants had been in the ground for only the normal length of time (Bampton, 1963). Physiological activity of these peanuts probably had been reduced by a 9-week drought. In South Africa, the pods and seed of peanuts that had not recovered from drought were conspicuously invaded by *A. flavus* (Sellschop, 1965).

A survey of 282 lots of segregation-3 peanuts in North Carolina in 1968 revealed a good correlation between drought stress occurring after peanuts were formed but before they were dug and *A. flavus* infection before digging (Dickens et al., 1973). Data from an irrigation experiment also indicated that incidence of aflatoxic seed, insect damage, and concentrations of aflatoxin in farmers stock peanuts were related to drought conditions before digging. In Texas, it was found that peanuts grown under dryland conditions, where drought stress occurred, accumulated more aflatoxin before digging than peanut grown under irrigation (Pettit et al, 1971a). It appeared that when SMC was above 30% or below 10% *A. flavus* activity was restricted as previously noted. Seed became more susceptible to *A. flavus* invasion when the soil moisture in the pod zone approached levels at which moisture moved from the pod into the soil and SMC dropped below 31% (Dickens and Pattee, 1966).

It has been suggested that in overmature peanut seed there is a drop in moisture content to a more susceptible state associated with the physiologic change from active growth to one of a low metabolic state (McDonald an

Harkness, 1964). In 1963, Nigerian peanuts left in the ground 4 weeks after maturity contained alfatoxin (McDonald and Harkness, 1967). Data from Alabama in the same year show that a much higher percentage of *A. flavus* invasion occurred in overmature seed and pods than in immature and mature seed and pods from the same plants at harvest (Diener et al., 1965). Overmature kernels also contained a high incidence of other fungi. In Nigeria, aflatoxin was associated with overmature pods and seed and with pods from dead plants with seed of low moisture content (McDonald et al., 1964). In 1963 and 1964, peanut crops in northern Nigeria harvested at or earlier than normal were free from aflatoxin, whereas late harvesting usually resulted in toxin in some peanuts. Peanuts stored for 1 year were more rapidly invaded by *A. flavus* than freshly dug immature and mature pods and seed in the laboratory (McDonald and Harkness, 1964). In Israel, the high mycofloral counts from fresh and stored peanut seed were attributed to (a) the advanced maturity of the crop at harvest, (b) peanut after peanut rotation, (c) a 3-day time lag from field to laboratory, and (d) a long incubation period (3 weeks vs. 1 week) on a rich nutrient medium (Joffee and Borut, 1966; Joffee and Lisker, 1969). Likewise, the research of Parker and Melnick (1971) reinforces the importance of digging peanuts at matruity, since delayed harvest resulted in a high incidence of mold contamination of the crop associated with a 30 to 40 times increase in aflatoxin content.

Drought stress, overmaturity, SMC, and the level of physiological activity in the peanut are interrelated and moisture-related, although other factors may also be involved. The high level of pod invasion by *A. flavus* in the soil has been associated both with overmaturity and with low SMC due to a lack of rain. Decreased vigor of the plant and reduced physiological activity in seed coincide with the drop in SMC and overmaturity resulting in increased susceptibility of pod and seed to *A. flavus* invasion and aflatoxin formation (McDonald and Harkness, 1964, 1967). Pods collected from dead plants of "Samaru 61" at Kano, Nigeria, contained aflatoxin-contaminated seed 5 to 14% SMC, whereas pods from living plants at harvest had no toxic seed and 24 to 32% SMC (McDonald, 1969b). It was concluded that delayed lifting could have undesirable results. Apparently decreased physiological activity associated with maturity or from low moisture in the soil environment favors invasion of seed and pods by *A. flavus* and the production of aflatoxin in the seed of such peanuts.

Thus, *A. flavus* propagules in the soil may invade an intact developing peanut pod and seed under conditions of SMC and maturity in the field favorable for the fungus. If pod and seed are not infected in the field, invasion by *A. flavus* may occur after digging during curing in the windrow or stack or afer picking during storage, if the drying rate is slow and the SMC and/or relative humidity around the pod is high enough. Little opportunity for fungal invasion of pod and seed occurs after digging when drying (dehydration) proceeds rapidly lowering the SMC steadily downward within 4 or 5 days to safe the storage moisture levels. Once below 30% SMC, interruptions that slow the field drying rate may result in *A. flavus* infection and subsequent toxin formation. Thus, *A. flavus* invasion and subsequent aflatoxin elaboration may occur in the field before harvest or after digging during curing and storage.

Temperature and Time

Aspergillus flavus has been classified (Semeniuk, 1954) as a mesophilic fungus having cardinal growth temperature as follows: minimum 6-8 C, optimum 36-38 C, maximum 44-46 C. The minimum and maximum temperatures for growth are affected by moisture, oxygen concentration, availability of nutrients, and other factors. *A. flavus* has a higher maximum temperature for growth on natural substrates than on synthetic media (Christensen, 1957).

The optimal temperature and time for aflatoxin production by *A. flavus* on sterilized peanuts in culture flasks were 25 C and 7-9 days (Diener and Davis, 1966). At 30 C the optimum was reached in 5-7 days, while at 20 C the maximum amount of aflatoxin was produced in 11-13 days. With *A. parasiticus*, maximum AFB_1 was produced at 35 C in 11-13 days and maximum AFG_1 at 25-30 C in 11-15 days. Large amounts (10+ ppm) of AFB_1 and AFG_1 were produced at 25-30 C during incubation periods of 7-15 days.

In more critical studies using 1-1.5 kg samples of heat-treated peanuts, Diener and Davis (1967) found that the lower limiting temperature for aflatoxin production by *A. flavus* to be 13 ± 1 C for a 21-day incubation at 98 ± 1% RH. The upper limiting temperature was 41.5 ± 1.5 C. With surface-sterilized, living unshelled peanuts, which were inoculated and incubated at high moistures shortly after digging, the lower limiting temperature for aflatoxin production was 17.5 ± 2.5 C for Early Runner and below 15 C for Florigiant peanuts for a 21-day incubation period (Diener and Davis, 1968). The upper limiting temperature was slightly above 40 C, since small amounts of aflatoxin were formed at that temperature. Burrell et al. (1964) noted that a constant temperature of 45 C inhibited growth of *A. flavus* in peanuts and an exposure of 2-4 hours at 50 C checked growth for about 24 hours. Dickens and Pattee (1966) found that in 10 days aflatoxin developed rapidly in peanut samples of 15-30% moisture held at 32 C, but developed in few samples held at 21 C. In these studies, the minimum time for aflatoxin production was 21 days after inoculation.

Research on the relationship of time to aflatoxin formation in peanuts after digging has given varying results. Under tropical conditions in Africa, peanuts that were free of toxin at digging contained detectable toxin in 48 hours (Bampton, 1963). McDonald and A'Brook (1963) found that artificial drying after 4-6 days in the field resulted in toxin-free seed, but that samples left for 8-12 days or sun-dried for 10-16 days resulted in moderate aflatoxin contamination (25-500 ppb). In other studies, McDonald and Harkness (1964) found that contamination of seed with *A. flavus* and aflatoxin did not occur until at least 5-6 days after lifting. They also reported more *A. flavus* and other fungi in seed from slowly dried pods (attached to plant) than from rapidly dried pods (McDonald and Harkness, 1965). Jackson (1967) also reported high levels of aflatoxin in slowly dried pods as compared to rapidly dried pods.

In controlled environment studies with cured peanuts, Diener and Davis (1970) found that the upper limiting temperature for growth and aflatoxin production by *A. flavus* was 40.5 C ± 0.5 C at 99% RH in 21 days for sound and broken mature seed, immature seed, and seed of unshelled peanuts. The

lower limiting temperature was 13 ± 1C for sound and broken mature seed incubated up to 84 days at 99% RH. Some aflatoxin developed in immature seed at 15 C in 21 days, although none was found at 14 C in 42 and 84 days. A negligible amount of aflatoxin developed in seed from intact pods at 20 C in 21 days. In 42 days a large amount of aflatoxin developed at 18 C, but none occurred at 16 C. These data parallel results with freshly dug Early Runner peanuts (Diener and Davis, 1968) in that some aflatoxin was found at 20 C, but none at 15 C in 21 days. Thus, living peanut seed stored in intact pods were much less susceptible to invasion and aflatoxin formation by *A. flavus*.

Aeration

In an investigation of the influence of carbon dioxide (CO_2), oxygen (O_2), and nitrogen (N_2 on growth, sporulation, and aflatoxin formation by *A. flavus* for 2 weeks at 30 C and 99% RH, Landers et al. (1967) observed that no visible change in fungus growth and sporulation occurred when CO_2 concentration was increased from 0.03% (air) to 20%, although aflatoxin formation was reduced 75%. *A. flavus* growth, sporulation, and aflatoxin formation were reduced with successive 20% increases in CO_2 from 20 to 100%. No growth of *A. flavus* or aflatoxin production occurred in peanuts in 100% CO_2. The reduced growth rates of *A. flavus* at CO_2 concentrations ranging from 20-80% was similar to that reported for *A. flavus* by Golding (1945) and for other fungi by Brown (1922). However, CO_2 has also been reported to be one of the essential factors for initiating germination of *Aspergillus conidia* (Tsay, 1965). With the same experimental design as Landers et al. (1967), Sanders et al. (1968) found that aflatoxin production was inhibited for at least 2 weeks by atmospheres composed of 20% CO_2 at 17 C and 86-92% RH, but not at higher RH (99%) and higher temperatures (25 C). Likewise, aflatoxin production was inhibited at 25 C by 60% CO_2 in 86 and 92% RH and by 40% CO_2 in 86% RH, but not at higher RH (92%) and higher temperatures (30 C). Data of Barnes et al. (1970) showed that 100% CO_2 inhibited the growth of *A. flavus, Fusarium* spp., and *Rhizopus* spp. for at least 16 days on high moisture pods at 21-24 C and for 30 days at 2-4 C.

No striking decrease in growth or sporulation by *A. flavus* was noted until O_2 was decreased from 5 to 1%, whether in combination with 0, 20, or 80% CO_2 (Landers et al., 1967). In general, reducing the P_2 concentration decreased aflatoxin, although the most sizable decreases occurred when O_2 was reduced from 5 to 1% with 0, 20, or 80% CO_2. No measurable aflatoxin was formed in peanuts stored for 6 weeks at 15 C under 40% CO_2 and 5% O_2. This inhibitory effect of low O_2 on *A. flavus* growth was reported by Miller and Golding (1949) to be inporportion to oxygen solubility in the medium or mycelium. However, the growth of fungi in oxygen concentrations of less than 1% has been reported (Brown 1922). Landers et al. (1967) found that growth and aflatoxin produciton occurred at 1% O_2:99 N_2 and 1% O_2:79% N_2:20% CO_2, but was completely inhibited by 1% O_2:19% N_2:80% CO_2. Pattee et al. (1966) reported that aflatoxin production was limited to 10-80 ppb by O_2 levels below 1%. In similar experiments, Wilson and Jay (1976) remoistened shelled peanuts to 16.7%, stored them for 4 weeks at 27 C in air and 3 modified atmospheres containing varying levels of CO, CO_2, O_2 and N_2. Peanuts incubated in air and in an atmosphere containing 11% O_2 accumulated high levels of aflatoxin (<1,857 ppb) in 1 and 2 weeks, respectively. Treatments containing 0.3 and 0.6% O_2 accumulated a maximum of 12 and 21 ppb, respectively. However, none of the treatments eliminated infection by *A. flavus*. Thus, the conclusion of Stotsky and Goos (1965) that soil microorganisms are generally tolerant to condition of high carbon dioxide and low oxygen applies to *A. flavus*, which is a soil organism (field fungus) as well as a storage fungus.

Pod and Seed Damage

Another factor that influences the invasion of peanuts by *A. flavus* with subsequent aflatoxin production is physical and biological damage to the peanut shell and seed. The thesis that seed of unblemished, intact immature and mature pods are rarely invaded by *A. flavus* and other fungi before digging under most growing conditions is widely accepted (Diener, 1973). In Texas, less than 1% of the seed of such pods had visible necroses and only a few such seed from undamaged pods yielded fungi in agar culture. In Nigeria, seed of broken pods showed extensive fungal contamination in comparison with that of seed from undamaged pods with preharvest development of aflatoxin occurring only in seed of broken pods. In Texas, aflatoxins were found in much higher concentrations in seed of pods damaged mechanically and by growth cracks than in seed of pods showing insect and rot injury. Growth cracks were also notable sources of *A. flavus* invasion in South Africa. *A. flavus* invasion and aflatoxin formation were closely associated with pod damage caused by termites (*Odontotermes latericius, O. badius)* in the ground in South Africa (Sellschop et al., 1965). Lesser cornstalk borer (*Elasmopalpus lignosellus)* in Texas, mites in South Africa (Aucamp, 1969), and termites in Nigeria have been suggested as *A. flavus* vectors, but field data showing aflatoxin contamination are not available nor are they available to clearly implicate nematodes (Widstrom, 1979). Pod openings caused by *Rhizoctonia solani* and *Sclerotium rolfsii* Sacc. provided entrance for *A. flavus* and subsequent aflatoxin development.

Experimentally, it was demonstrated that the percentage of seed colonized by *A. flavus* increased with shell damage and incubation time (Wright et al., 1976). The time required for aflatoxin to exceed 25 ppb in seed of sound pods and microscopically damaged pods (damage detected by staining) was 96 and 24 hours more, respectively, than in seed from visibly damaged pods. Other workers have reported that the levels of *A. flavus* infestation and aflatoxin in the seed of damaged pods always far exceeded that of seed from sound pods (McDonald and Harkness, 1963, 1964, 1976; McDonald et al., 1964; Schroeder and Ashworth, 1965; Dickens and Khalsa, 1967; McDonald, 1969b; Schroeder and Boller, 1971; Porter et al., 1972; Subrahmanyam and Rao, 1974).

Bampton (1963) concluded that damage to the peanut shell is a major factor in *A. flavus* invasion and aflatoxin development in the seed during drying. He observed that shelling (decortication) of peanuts at harvest resulted in testa damage and usually increased fungal invasion and aflatoxin contamination, unless the seed were dried rapidly to safe moisture levels after shelling. The in-

tact shell serves as a barrier to fungus invasion and some protection is also afforded by an intact testa. In Georgia, it was found that the mycoflora of mature peanut seed from unblemished intact pods developed in or beneath the dead cells of the testa (Jackson, 1964). Nigerian scientists have reported that seed from damaged pods were more frequently contaminated with *A. flavus* and aflatoxin than seed from undamaged pods during sun- and artificial-drying (McDonald and A'Brook, 1963; McDonald and Harkness, 1964; McDonald et al., 1964). Under gnotobiotic conditions, it was demonstrated that *A. flavus* readily penetrated intact shells, but was limited in its invasion of the kernel by the testa (Lindsey, 1970). Colonization of the embryos by *A. flavus* was limited.

Damage to shell or testa affords increased opportunities for rapid and direct invasion of the seed, which increases the possibility of aflatoxin formation. Pattee and Dickens (1965) found that "analysis of the damage segment in peanuts may be used as a sensitive indicator for detecting the presence or absence of aflatoxin in farmers stock peanuts." Seed damage also increases nutrient availability. Thus, the extent of fungus growth and toxin formation at minimal temperatures and RH ultimately will be determined by time.

Crop Sequence

In Texas, peanuts harvested from land planted to peanuts the previous year were more highly infested with fungi and contained more aflatoxin than peanuts grown on land planted to rye, oats, melons, and potatoes the previous year (Pettit and Taber, 1968). However, continuous cropping of peanuts did not select for high aflatoxin producing isolates (Pettit et al., 1973). Corn or peanut residues left undisturbed on the soil resulted in a buildup of *A. flavus* populations. Soils from fields with a winter cover crop planted in the crop residues or in rotation with grasses or sorghum contained fewer *A. flavus* propagules. Also, they reported that new land with soil previously free of *A. flavus* became contaminated during the latter part of the second year peanuts were grown.

Microbial Interaction

Aspergillus flavus is frequently found associated with numerous other microorganisms in stored grains and seeds. Thus, the possibility arises that microbial competition between fungi for the substrate under favorable environmental conditions will restrict or reduce the amount of aflatoxin formed (Diener, 1973). *A. flavus* or competing fungi might absorb or degrade aflatoxin following its formation in the substrate. Research suggests that microbial competition or microbial breakdown may be responsible for smaller amounts of aflatoxin in the seed of parasite-damaged pods than in seed from broken pods. It was demonstrated that several fungi could break down aflatoxin in peanuts and in an aflatoxin-containing liquid medium; *A. niger* and *Rhizoctonia solani* appeared to limit the development of *A. flavus* and aflatoxin production in the substrate. In Israel, it was noted that a large number of colonies of *A. niger* occurred in the geocarposphere with moderate penetration into seed, while there was very little penetration of seed by *A. flavus*, *Fusarium solani*, *Penicillium fu-*

niculosum and *P. rubrum*. The rapid penetration into seed by *A. niger* may have prevented the development of other fungi. The relationship between the dominant fungi *A. flavus*, *A. niger*, *P. funiculosum*, *P. rubrum*, and *F. solani* was studied in 234 samples and 5,850 culture plates made with fresh and stored peanut seed from 2 groundnut crop years in Israel. The number of colonies developing in individual plates showed antagonism between *A. flavus* and *A. niger* and slightly less but still marked antagonism between each of these species and *P. funiculosum*, *P. rubrum*, and *F. solani*. In Georgia it was noted that *A. flavus* invaded pods infested by *Sclerotium bataticola*, but that the reverse did not occur. *A. flavus* had a pronounced despressing effect on the rate and extent of seed infection by *S. bataticola*. On malt-extract agar, growth of *S. bataticola* was strongly inhibited by *A. flavus*, but lysis was not observed.

Under gnotobiotic (aseptic) conditions *A. flavus* penetrated and colonized a high percentage (9 to 77%) of shell tissues of living, attached, immature and mature pods (Lindsey, 1970). Under natural conditions, *A. flavus* was found in only a small percentage of shells (Garren, 1966; Jackson, 1968) and less *A. flavus* was isolated from the shell portion of freshly dug pods than from the seed contained in those shells (Porter and Garren, 1968). Either the normal endogeocarpic mycoflora of the shells was antagonistic to *A. flavus*, which limits its colonization of shell tissue, or the faster growing components of the mycoflora mask *A. flavus* when shells were plated on agar media. Using surface-disinfected attached pods, Jackson (1965b) obtained similar results in that *A. flavus* as well as *A. niger*, *S. bataticola*, and *R. stolonifer* readily penetrated and colonized a large percentage of pods, especially at high temperatures (26 to 38 C). In the gnotobiotic study (Lindsey, 1970) there was no evidence of natural resistance to seed invasion in living attached pods as suggested by Austwick and Ayerst (1963). Thus, the presence of normal endogeocarpic mycoflora appears to provide a barrier to the invasion of peanut seed by *A. flavus*.

CONTROL OF *ASPERGILLUS FLAVUS* AND AFLATOXIN IN PEANUTS

Preventing aflatoxin in peanuts is of vital concern to the peanut industry. Industry-wide efforts have greatly reduced the incidence of aflatoxin contaminated peanuts by the use of production, harvesting, handling, and storage practices that restrict the growth of the aflatoxin-producing aspergilli. Major production practices of prime importance are: (1) limiting fungus growth in peanut field soils and peanut pods and seed, (2) irrigation, and (3) harvesting, curing, handling, drying, and storing procedures that minimize fungal growth. In addition, inspection and diversion programs have been maintained to ensure that peanut products moving in market channels are free of aflatoxin. Contaminated peanuts and/or peanut products can be processed to remove or detoxify aflatoxin in contaminated seed or peanut meal or must be destroyed.

Reducing *Aspergillus flavus* Activity in the Field

Production practices that limit *A. flavus* development include irrigation (previously discussed), crop rotation, fertilization, and insect and nematode control. To restrict *A. flavus* growth in the soil, those crop residues (e.g., pea-

nuts, corn, beans), which are commonly metabolized by *A. flavus* in the top soil, are reduced to a minimum. Some research indicates that crop rotations have little influence on the activity of *A. flavus*, but data show that levels of this fungus were relatively low in the soil (Barnes and Young, 1971; Griffin and Garren, 1976). In hot and arid environments, *A. flavus* levels in the soil can become quite high and crop rotations appear to have an influence on fungal activity (Hsi, 1967; Pettit and Taber, 1968). Planting peanuts following peanuts tends to maintain or increase the incidence of peanut plant pathogens and *A. flavus* (Kulkarni et al., 1967; Pettit and Taber, 1968; Diener and Davis, 1977). Planting of grasses, small grains, potatoes, and melons in rotation with peanuts helps reduce the incidence of some soil-borne peanut plant pathogens (Pettit and Taber, 1968; Griffin and Garren, 1974). Planting a winter cover crop of oats or rye followed by peanuts every third year maintains soil fertility and reduces the incidence of peanut plant pathogens in Texas (Pettit et al., 1975). Fertilization of rotational crops and the addition of organic matter and/or humus to peanut soils have been helpful in improving soil fertility. Also, the addition of crop residues and humus improves the water-holding capacity of the soil and provides nutrients for soil microorganisms that can compete with *A. flavus* (Pettit and Taber, 1968, 1970; Wells et al., 1972). However, any year peanuts are planted in subtropical regions, the chances for *A. flavus* buildup are fairly high.

Practices that lower the incidence of soil insects, mites, and nematodes will certainly contribute to improved yield and quality of peanuts, but their value in reducing aflatoxin contamination has not been demonstrated except in the case of large termites in South Africa (Sellschop et al., 1965). Research on the relationship of lesser cornstalk borer (*Elasmopalpus lignosellus* Zeller), southern corn rootworm (*Diabotrica undecimpunctata howardi* Barber), small termites (*Microtermes* spp.), mites (*Caloglyphus* spp., *Tyrophagus* spp.), and several species of nematodes as vectors of *A. flavus* and aflatoxin contamination has been reviewed by Widstrom (1979). Although insect, mite, and nematode damage was involved, increased invasion by *A. flavus* and increased aflatoxin contamination were not established.

Planting Peanut Varieties with Greater Resistance to *A. flavus*

Several researchers have identified certain peanut varieties that were less susceptible to *A. flavus* penetration and aflatoxin elaboration. Reports from India indicated that accessions of the peanut varieties Koboka (US 26) and Asiriya Mwitunde carried resistance to aflatoxin contamination by *A. flavus* (Kulkarni et al., 1967; Mixon and Rogers, 1973). However, these same varieties were found susceptible to aflatoxin contamination in Georgia, although the variety Koboka was more tolerant than the Starr variety (Doupnik, 1969b). Several investigators have reported that an Indian variety (Nagarajan and Bhat, 1973), a native Iranian peanut (Tavasolian, 1977), runner type varieties (Florunner), several breeding accessions, and valencia peanuts have low levels of resistance (Mixon and Rogers, 1973; Zambettakis, 1977; Bartz et al., 1978; Hsi, 1978). However, when grown under nonadapted environmental conditions or when heavily stressed, these varieties became contaminated with aflatoxin (Wilson et al., 1977; Bartz et al., 1978).

Resistance has been related to specific features of the peanut seedcoat and pod that slow penetration by the aspergilli and other fungi. In Nigeria, research revealed that peanut seedcoats containing higher levels of tannin tended to inhibit the growth of *A. flavus*, but once the fungus became established the inhibitory effect was overcome (Carter, 1970). Research in Georgia has verified that seedcoat tannins slow *A. parasiticus* invasion (Sanders and Mixon, 1978). Also, specific amino acids have been associated with resistance in seedcoats (Amaya et al., 1977).

Peanut cultivars with seedcoat resistance attributed to specific morphological factors have been identified. Some of these factors are: compact arrangement of seedcoat cells, slower water uptake rates of seeds following drying, a smaller hila with minimum of exposure of parenchyma cells, and more uniform deposition of wax on the seedcoat (LaPrade et al., 1973; Taber et al., 1973; Ketring, 1976; Dieckert and Dieckert, 1977).

Peanut pods with resistance to soil-borne fungi have surface cells that are more compact, have a higher concentration of lignin and phenolic compounds within these cells, have a dense layer of lignified sclerenchyma cells within the central region of the pod, and have sufficient strength to resist cracking under normal handling procedures (Pettit et al., 1976, 1977; Zambettakis, 1977). The most disease resistant cultivar tested in Texas has been PI 365553, an introduction from Uruguay (Pettit et al., 1977). Resistant cultivars have been crossed with production type peanuts for varietal development (Mixon, 1977).

Although peanut varieties resistant to *A. flavus* invasion and aflatoxin formation potentially afford the most effective and economical control, the degree of success to date has not been encouraging. In the case of *A. flavus* and *A. parasiticus*, scientists are not dealing with an aggressive plant pathogen, but with a saprophyte that may become a weak pathogen under conditions of stress, which increases the susceptibility of peanut pod and seed to fungus invasion followed by aflatoxin formation. Several morphological and biochemical factors in pod and seed have been associated with resistance, but it takes years to optimize and combine genes from several lines into a variety with commercial potential. Then, it still must be tested in the field under stress conditions in several peanut-producing areas. Breeding for disease resistance in plants is a sound, long-term approach to disease control. In this instance, the problem is complicated by the fact that the fungus is more of a saprophyte than a pathogen.

Optimizing Harvest, Curing, and Storage Conditions

One of the most promising of several procedures, developed to reduce aflatoxin contamination during peanut harvest, is to invert the peanut pods in the windrow with a digger-shaker-inverter. Peanut pods are exposed to direct sunlight and air currents and tend to dry more rapidly after showers. After inverting, pods are unable to take up moisture from the soil. Consequently, the severity of the aflatoxin problem is significantly reduced by the use of inverted windrows (Dickens and Khalsa, 1967; Porter and Wright, 1969; Pettit et al., 1971b; Diener, 1973).

Since 1967, scientists have attempted to control or reduce *A. flavus* activity in peanut pods with fungicides applied to the peanut foliage, soil, and wind-

rows (Diener, 1973; Madaam and Chohan, 1978). To date, no known fungicide, combination of fungicides, or acidic type chemical treatments have been found to be of practical value for controlling *A. flavus* and aflatoxin formation in the field.

The longer peanuts remain in the windrow the greater the chances of fungus invasion and aflatoxin formation (McDonald, 1969b). If mold damaged areas are detected in the field, they should be harvested and marketed separately in order to avoid contamination of noninfested peanuts (Dickens, 1975, 1977a). If adverse drying weather is forecast or peanuts are curing too slowly, they should be combined (removed from the vine) and dried rapidly with heated forced-air dryers (Dickens and Pattee, 1966).

Inspection and Diversion of Aflatoxin Contaminated Lots

To ensure that peanuts utilized for food or feed contain less than 20 ppb aflatoxin, a USDA Marketing Agreement is administered by the Peanut Administrative Committee (PAC) (PAC, 1975; Dickens, 1977a). One PAC requirement is that farmers stock peanuts be graded by the Federal-State Inspection Service upon delivery to the buying points. Peanuts are obtained from several points within each load the producer markets and a sub-sample taken for grading. During grading, loose shelled kernels (LSK) and seed shelled from intact pods are examined externally and internally; and lots with visible mold growth are placed in segregation-3 and diverted from direct use in foods or feeds (Dickens and Welty, 1969). Lots with no mold growth, but with more than 2% damaged seed or more than 1% concealed damage are placed in segregation-2. All other peanuts (U. S. No. 1) are placed in segregation-1. Although the visual subjective grading procedure used to designate segregation-3 peanuts has been challenged, experimental evidence indicates that the inspection procedure pinpoints peanut lots with aflatoxin contamination (Dickens and Satterwhite, 1971; Dickens, 1975, 1977b).

The most promising alternative to visual inspection is a rapid chemical assay that requires chemical extraction and visual detection of the toxin by its fluorescence (Holaday and Barnes, 1973). Such a method detects low aflatoxin levels but requires special equipment, chemicals, and trained personnel; the sampling problems become difficult in attempting to obtain duplicate representative samples (Dickens, 1975, 1977a).

In contaminated lots of peanuts, LSK contain a higher concentration of aflatoxin than unshelled seed (Dickens and Satterwhite, 1971). Thus, the PAC requires shellers to remove all LSK from farmers stock peanuts prior to shelling. Such LSK can be used for wildlife feed or rodent bait, if they do not contain more than 25 ppb aflatoxin.

Peanuts placed in segregation-3 are generally crushed for oil or used for seed (Tiemstra, 1977). Following crushing, the oil is processed using an alkali method that renders the oil free of aflatoxin. Peanut meal frequently contains aflatoxin and must be diverted from the food and feed trade or the toxin destroyed before being used in feeds.

A second phase of the PAC program requires that all segregation-1 peanuts sold for human consumption be sampled by the Federal-State Inspection Service for aflatoxin analysis before entering market channels (PAC, 1975). The sampling procedure is carried out after shelling, photoelectric color sorting, and hand sorting and immediately before the peanuts are bagged. Under the current program, the PAC indemnifies peanut shellers for most of the losses related to aflatoxin contamination in lots of segregation-1 edible peanuts that meet grade requirements. The buyer is guaranteed that the purchased peanuts contain less than 25 ppb aflatoxin. However, the final responsibility for marketing wholesome peanut products lies with the manufacturer. The Food and Drug Administration has the responsibility for inspecting the marketed products. If these are found to contain more than 20 ppb aflatoxin, they are removed from the market at the manufacturer's expense.

The PAC program has allowed the peanut industry the option of purchasing peanut lots that have met certain established standards. The current program is workable primarily because of a surplus of peanuts and because the peanut producer absorbs the economic loss when his peanuts are designated segregation-3. The primary need is to prevent aflatoxin contamination and secondly to improve the inspection, diversion, and separation procedures so as to divert only those seed that are contaminated.

Sorting Techniques for Removal of Aflatoxin Contaminated Seed

Segregation-1 peanuts certified to contain aflatoxin can be processed to remove the aflatoxin (PAC, 1975). One of the most reliable procedures used by shelling plants for removing contaminated seed (with existing equipment) is blanching followed by photoelectric color sorting and hand picking. Properly operated photoelectric sorting equipment can remove badly discolored peanut seed without blanching, although the effectiveness of removing aflatoxin contaminated seed is highly variable, since they may not appear moldy, discolored or shriveled (Dickens and Whitaker, 1975; Whitaker et al., 1976; Tiemstra, 1977). Tiemstra (1977) has reported that processing a lot of peanuts containing 25 ppb aflatoxin will result in a reduction to 16 ppb aflatoxin as a result of destruction due to roasting, while photoelectric sorting following roasting will further reduce the levels to 8 ppb. If the raw peanuts had contained more than 25 ppb aflatoxin, the finished product would have been unacceptable (Whitaker et al., 1974). Careful hand picking for abnormal seed was more selective and accurate compared to the use of photoelectric color sorting (Dickens and Satterwhite, 1971). With a combination of photoelectric color sorting and subsequent hand picking, an average of 72% of the aflatoxin has been removed. However, before the added expense of photoelectric color sorting is contracted, the peanut lots considered for clean-up should first be tested for aflatoxin levels and the expected clean level calculated.

The potential exists to improve existing electronic sorting equipment for sorting fresh shelled peanuts. If polarized laser beams are directed towards a peanut surface and the reflected signal analyzed, the presence of peanut surfaces that are roughened causes a phase shift in the return signal (Pettit and Chan, 1980). Such a detector could identify shriveled seed that lack color changes detectable by photoelectric cells. Also, since the dielectric properties of mold-damaged peanut kernels differ from that of sound mature kernels, it is possible to detect hidden damage without splitting the seed by measuring the dielectric properties of individual peanut seed (Pettit and Geiger, 1980).

Processing for Aflatoxin Removal or Inactivation

Removal or inactivation of aflatoxin in peanut and peanut products is the last step in the efficient utilization of a potential food source; otherwise, their main use is for organic fertilizers (Goldblatt, 1970). Several economically feasible processes for aflatoxin removal and inactivation have been developed (Stoloff and Trager, 1965; Paulsen et al., 1976).

One technique involves roasting of peanuts, which has reduced aflatoxin levels in contaminated peanuts by 45 to 83% depending on the initial aflatoxin levels and roasting conditions (Cucullu et al., 1966; Lee et al., 1969; Waltking, 1971). It was noted that a 3-month storage period had no significant influence on the aflatoxin levels of peanut products; however, as fatty acid oxidation occurred later in the year, aflatoxin levels decreased in storage (Waltking, 1971; Baur, 1975). In heating crude peanut oil containing 260 ppb aflatoxin at 150 C for 20 minutes, the aflatoxin level was reduced 61%. When heating the oil at 250 C (above the smoke point), the aflatoxin level was reduced to 10 ppb aflatoxin (Goldblatt and Dollear, 1977a). These conventional processes, using heat and moisture levels, reduced aflatoxin levels, but not always to levels below the FDA administrative guideline of 15 ppb.

Aflatoxin has been removed successfully from peanut meal by solvent extraction. In Japan, a 96% aqueous solution of dimethyl ether (methoxymethane) was used to extract peanut oil and aflatoxin from peanut meal (Aibara and Nobumitsu, 1977). In this process, the dimethyl ether can be continuously recycled and the resulting meal is free of the solvent. When peanuts are crushed for oil, the major portion of the aflatoxin is concentrated in the peanut cake (Goldblatt and Dollear, 1977b). The small amount of aflatoxin in the unrefined (crude) oil is removed when it is treated with 0.15% excess of 16_0 Baume sodium hydroxide and washed with water. The aflatoxin originally in the crude oil ends up in the soapstock as an alkali salt (Goldblatt and Dollear, 1977a). Treatment of the soapstock at low pH levels with acid converts the salt of aflatoxin B_1 to B_{2a} and G_1 to G_{2a}, 2 aflatoxins of low toxicity, permitting the soapstock to be used in animal feeds (Pons et al., 1972).

A procedure for reducing the aflatoxin content of unrefined peanut oil was developed in India (Sreenivasamurthy et al., 1965) by treating the oil with a 10% aqueous solution of sodium chloride (table salt) at 90 C for 30 minutes. The aflatoxin content was reduced by 80 to 85% (Shanta and Sreenivasamurthy, 1975). If the initial levels of aflatoxin in the unrefined oil are quite high, an 80 to 85% reduction is certainly of value; however, unless the oil is subjected to additional treatments, aflatoxin levels may still exceed 20 ppb.

Aflatoxin can be extracted with hexane-methanol, hexane-ethanol-water, aqueous isopropyl alcohol, hexane-acetone-water, and aqueous ethanol with minor changes in product quality. However, these solvents are expensive and difficult to remove from the final product in comparison to other possible porcedures that destroy the aflatoxins (Goldblatt and Dollear, 1977a.

Inactivation of aflatoxins in oilseed meals has been relatively successful with sodium hydroxide, methylamine, hydrogen peroxide, and sulfur dioxide (Goldblatt and Dollear, 1977b). Commercially their use is limited due to solvent cost, deleterious residues, or reduction in nutrient value of the product (Goldblatt and Dollear, 1977a). Hydrogen peroxide has been used and patented in India to inactivate aflatoxins in peanut proteins used for human consumption (Parpia and Sreenivasamurthy, 1971).

The 3 most promising treatments for the inactivation of aflatoxins in oilseeds are formaldehyde plus calcium hydroxide, sodium hypochlorite, and ammonia. A formaldehyde solution of 0.5% plus a 2.0% calcium hydroxide added to peanut meal containing approximately 600 ppb aflatoxin reduced the aflatoxin level to less than 5 ppb aflatoxin in 1 hour (Codifer et al., 1976). To speed the reaction process, the moisture content of the meal was increased to 25% and the treatment carried out in a steam-jacketed reactor with impeller agitation. Aflatoxins G_1 and G_2 were found to be more susceptible to inactivation than aflatoxins B_1 and B_2.

Sodium hypochlorite has been used for aflatoxin inactivation in conjunction with the aqueous extraction of peanut meal to obtain protein concentrates and isolates (Stoloff and Trager, 1965; Natarajan et al., 1975). Treatment of ground raw peanuts initially contaminated with 725 ppb aflatoxin B_1 and 148 ppb aflatoxin B_2 with 0.4% sodium hypochlorite at pH 8 produced protein isolates with only trace amounts of aflatoxins B_1 and B_2.

Ammonia is the most effective and economically feasible reagent for inactivation of aflatoxin in oilseeds (Gardner et al., 1971; Mann et al., 1971). The effectiveness of the treatment is influenced by the ammonia level, moisture in the seed, temperature and time of the reaction. Peanut meal containing 709 ppb aflatoxin B_1 was moistened to 9.6% and 14.6% and treated at 94 C for 60 minutes with 1.4 kg/cm^2 anhydrous ammonia, which reduced aflatoxin levels 96.4% and 97.6%, respectively (Masri et al., 1969). Ammoniation of peanut and cottonseed meal for livestock feeds has been widely reported (Mann et al., 1970, 1971; Gardner et al., 1971).

More than 1,000 microorganisms have been screened for their ability to destroy aflatoxin (Ciegler et al., 1966). One bacterium (*Flavobacterium aurantiacum* ?) was found to be capable of inactivating aflatoxin.

OTHER MYCOTOXINS IN PEANUTS

Although *Aspergilluus flavus* is best known for its ability to produce the aflatoxins, this fungus is capable of producing other toxins (Cole, 1976). Perhaps the most economically important toxin produced by *A. flavus* other than the aflatoxins is cyclopiazonic acid. Species of fungi known to produce cyclopiazonic acid have been isolated for peanuts and several other important agricultural commodities. Cyclopiazonic acid was initially discovered being produced by strains of *P. cyclopium* Westling during routine screening for toxigenic fungi from domestic cereal products in South Africa (Holzapfel, 1968). It was subsequently found being produced by isolates of *A. versicolor* (Vuill.) Tiraboschi (Ohmomo et al., 1973), *A. oryzae* (Orth, 1977), *P. camemberti* Thom (Still et al., 1978; LeBars, 1979) and *A. flavus* (Kirksey and Cole, 1973; Luk et al., 1977; Gallagher et al., 1978).

The last 3 fungus species are important in potential feed and food contamination. The report that 28 of 54 aflatoxigenic and non-aflatoxigenic strains of *A. flavus* produced cyclopiazonic acid increases the toxic potential of *A. flavus* (Gallagher et al., 1978). This discovery indicates that cyclopiazonic acid con-

tamination, especially in commodities frequently invaded by *A. flavus*, may be more common than currently recognized.

An unusual gross clinical sign of cyclopiazonic acid toxicity in poultry is the posture of animals following death (Cole, unpublished). Especially characteristic was the rigid extension of the legs similar to that described by Goldblatt (1969a) for "turkey X disease." Since many *A. flavus* isolates can produce cyclopiazonic acid, it is plausible that cyclopiazonic acid may have been involved in the "turkey X disease" syndrome.

In 1964, Wilson and Wilson reported that several strains of *Aspergillus flavus* produced a tremorgenic toxin when cultured on oats, millet, rice, potatoes, and corn. The purified toxin, when dosed orally or intraperitoneally into mice, rats and guinea pigs, caused clinical signs, including tremors that persisted for as long as 3 days. High doses of the toxin initially caused tremors followed by convulsions and death. The toxin was named aflatrem. Chemically aflatrem is closely related to the tremorgenic toxins found in sclerotia of *Claviceps paspali* Stevens and Hall (Cole et al., 1977). A naturally occurring neurological disorder, Dallisgrass poisoning, also called "paspalum staggers," occurs when cattle graze *Paspalum dialatatum* infected with *Claviceps paspali*. The tremorgens contained in the sclerotia are thought to be involved in the disease (Cole et al., 1977). It has been recently found that the aflatrem in *A. flavus* cultures is contained only in the sclerotia (Wicklow and Cole, unpublished). Thus, aflatrem would only be a problem when *A. flavus* produces the sclerotial stage.

Several years after *Indigofera endecaphylla* (creeping indigo) was introduced into Hawaii and Latin America as a forage and cover crop, it was observed that this legume produced severe toxic signs in herds of dairy cattle. It was shown that the toxic principle of creeping indigo was β-nitropropionic acid (Morris et al., 1954). Bush et al. (1951) isolated and identified β-nitropropionic acid from toxic culture extracts of several strains of *A. flavus*. In toxicity studies, the LD_{50} of crude β-nitropropionic acid to 25 g mice dosed orally was approximately 250 mg/kg. Clinical signs were apnea, convulsions, congestion in subcutaneous vessels and lungs, and mottled liver.

Kojic acid, a relatively common antibiotic metabilite of *Aspergillus* spp. and in particular *A. flavus*, was first reported to be produced by *A. oryzae* (Saito, 1907; Parrish et al., 1966; Cole, 1976). Kojic acid has been evaluated for its potential as an antibiotic, an insecticide (Beard et al., 1971) and a mycotoxin (Morton et al., 1945). These results, in conjunction with other studies, have been negative (Wilson, 1971). The major significance of kojic acid lies in its use as an indicator for potential aflatoxin contamination in cotton and especially corn (Shotwell, 1972).

Aflatoxin-producing isolates of *A. flavus* produced aspertoxin (Rodricks et al., 1968; Waiss et al., 1968), O-methylsterigmatocystin (Burkhardt and Forgacs, 1968) and dihydro-O-methylsterigmatocystin (Cole et al., 1970). All are chemically-related to the mycotoxin sterigmatocystin. The former showed little or no acute toxicity to animals, while aspertoxin was acutely toxic to chick embryos. Chronic effects have not been investigated in any of these metabolites.

Other species of fungi found invading peanuts are *Aspergillus niger, A. terreus, Penicillium funiculosum, Macrophomina phaseolina, Fusarium* spp., *Rhizopus* spp., and various bacteria. Data on the toxigenicity of most of these fungi and bacteria are not available. However, *A. niger* isolated from peanuts produced nigragillin (Cole, unpublished). Ghosal et al. (1979) recently reported that *A. niger* isolated from mango produced 6 toxic naptho-v-pyrones. *A. terreus* is known to produce the toxins citrinin (Saito et al., 1971), patulin (Wilson, 1976), and terretonin (Springer et al., 1979).

Members of the genus *Fusarium* are best known for their ability to produce the highly toxic trichothecene mycotoxins. Trichothecenes have been isolated from corn, wheat, and other small grains; however, they have not been found to occur naturally on peanuts (Pathre and Mirocha, 1976).

SUMMARY

Of the mycotoxins that have played important roles in human and animal health in the last century, aflatoxin appears to be the most significant threat to modern agriculture. The causal fungi are *A. parasiticus* and *A. flavus*, which are omnipresent in soils and air throughout the world. Peanuts, corn, and cottonseed and their products are constantly monitored for aflatoxin contamination. When environmental conditions are favorable for growth of the fungus, *A. flavus* may rapidly invade and contaminate peanuts with aflatoxin in the field both before digging and after harvest during curing in the windrow. Drought stress has been the factor most frequently correlated with aflatoxin contamination in peanuts before harvest, as exemplified by the 1980 crop in the United States. Control of aflatoxin in peanuts in the field can probably be accomplished most successfully with well-managed irrigation practices that prevent drought stress. The use of rotations, fungicides, and soil insecticides has not been demonstrated to control or greatly reduce pod invasion by *A. flavus* and/or aflatoxin contamination in the field or windrow. Breeding for resistance to *A. flavus* invasion and/or aflatoxin formation is a sound but long-term approach to control, with no early success in sight.

Inversion of peanut pods in the windrow reduces the possibility of aflatoxin contamination during curing. However, peanuts contaminated before harvest must be diverted from the human food chain by inspection or eliminated by sorting and other techniques during processing. Aflatoxin removal or inactivation by chemical treatments is feasible, but the product may only be suitable for animal feeds and not for human consumption. Aflatoxin appears to be the only mycotoxin problem in peanuts of any consequence at this time.

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