Chapter 16

Deterioration of Peanut Quality Caused By Fungi

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I. *Introduction*

Fungi (molds) are constantly associated with the peanut pod (geocarp) during its development in the soil, and after digging during curing and subsequent storage. This review will attempt to summarize the status of knowledge on the role of fungi in loss of peanut (groundnut) quality before harvest, after digging, during curing and subsequent storage (shelled or in-shell). Invasion and damage of the peanut pod and plant by fungi in the field have been discussed in the chapter on peanut diseases. However, field fungi associated with the pod, peg (gynophore), and the soil on the surface of the pod, may under favorable conditions continue to proliferate after digging and become a factor in quality deterioration of the kernel during curing and storage. There may be an area of overlap, but an effort was made to exclude consideration of pathogenic fungi associated with pod rots, concealed damage and peg rots, which have been summarized in the literature (60) and a previous chapter. This review will be confined to the saprophytic and weakly pathogenic soil and peanut pod microflorae in the field and during harvest and storage.

In this chapter there are a number of synonymous terms that are in common usage throughout the world for peanuts, various parts of the plant, and peanut harvesting; e.g., peanut and groundnut, kernel and seed, pod and fruit, pod and geocarp,

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stem and haulm, digging and lifting, and curing and drying. Both are used interchangeably in this review usually in the context of citing authors in their own terminology. Similarly, references to *Sclerotium bataticola* and *Macrophomina phaseoli* are citing the same fungus. However, whether *Botryodiplodia theobromae* and *Diplodia theobromae* are the same fungus in any or all cases is not clear.

Fungi associated with peanut pods and kernels may be broadly classed into two groups, field fungi and storage fungi, as they have been categorized by Christensen (14). However, peanuts differ uniquely from cereal grains and soybeans studied by Christensen in that the peanut fruit develops under the soil surface rather than in an aerial environment. Therefore, the peanut has been routinely selected for its survival in the soil environment, whereas grains and soybeans have been selected for survival in aerial environments. Peanut harvesting is a two-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 kernels high in moisture must be equated with that of maturing grains and soybeans exposed constantly to the hazards of an aerial environment. Fungi present in the soil and air apparently invade the peanut kernel during curing and storage in an ecological succession determined by humidity, temperature, time, light, air movement, and physical and biological damage to the pod.

> Austwick and Ayerst (3) wrote that "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, the 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".

II. Field Fungi

Most species of field fungi that invade peanut pods in the soil under certain conditions are facultative parasites. The pod appears to be generally resistant to penetration by saprophytic fungi until it matures near digging time, unless it has been damaged by cultivation, nematodes, insects, pathogenic fungi, or physiologically affected by environmental extremes (1, 4, 86, 87, 88, 91, 112, 113). Field fungi rapidly invade damaged, physiologically unsound, or overmature peanut pods and kernels in the soil. After digging sound unblemished peanut pods may be invaded, if environmental conditions during curing are unfavorable for rapid drying of the peanut pod and kernel in the windrow or stack. Field fungi particularly invade pods damaged mechanically by digging during field curing even though the environment is generally favorable for rapid drying.

A. *The Species*

The species of field fungi that dominate the mycoflora of the soil of peanut fields, and the rhizosphere and geocarposphere of developing peanuts in the soil, at maturity, and during curing vary throughout the world with soil type and environment.

Jackson (49) reviewed the literature and listed 132 species and 17 genera of fungi on peanut pods and kernels. Joffe and Borut (68) isolated 95 species in 42 genera from soils of Israeli peanut fields. In other studies of the mycoflora of peanut rhizosphere and geocarposphere on light, medium and heavy soils of Israel, Joffe (66) identified 44 genera and 157 species. He (67) later identified 109 species from freshly-dug kernels. McDonald (82) identified 51 genera and at least 87 species from Nigerian peanut soils. A summary of all these fungi is not presented in this paper. The more important genera and species wil be noted from frequency and/or quantitative data that is cited in the research of each investigator.

B. *Mycoflora of Peanut Soils*

In Nigeria, McDonald (82) investigated the mycoflora of peanut soils, of soils adhering to the fruit (pod), and of geocarposphere soil throughout the season using both the dilution plate technique and Warcup soil plate method. He identified 51 genera and at least 87 species of fungi from these soils. Data from the dilution plate method showed the dominant fungi in Nigerian field soils were *Fusarium* spp. (42.5%) and *Aspergillus* spp. (44%) for the 6 weeks prior to lifting. *Fusarium* spp. (36%) were dominant in soil associated with peanut pods with *Penicillium* spp. (19%), *Aspergillus* spp. (16.5%), and *Macrophomina phaseoli* (11%) being strong subdominants. *Myrothecium verrucaria* (6%) and *Botryodiplodia theobromae* (5%) constituted a lesser portion of the fungi from pod soil. Geocarposphere soil was obtained by washing the pods and plating out the washings; *Aspergillus* spp. (34.5%), *Fusarium* spp. (23.3%), and *Penicillium* spp. (20.5%) were dominant in this soil with *Macrophomina* (5.2%) and *Myrothecium* (4.1%) constituting 9.3% of the fungal population. The dominant fungi in all three soil environments were *Fusarium* and *Aspergillus*. The former being predominant in field and pod soils, and the latter in geocarposphere soil. *Penicillium* and *Macrophomina* were important in pod and geocarposphere soils.

In 1964, Joffe and Borut (68) reported that the soil mycoflora was more or less uniform in fields of 12 different regions of Israel. They isolated 95 species representing 42 genera of fungi from these soils. Seventeen species were isolated from at least 10 of the 12 locations. Five species of *Aspergillus*, two species of *Penicillium*, *Fusarium solani*, and *Rhizoctonia* spp. were isolated from soils from all locations. In 1969, Joffe (66) reported a detailed investigation of the mycoflora of the peanut rhizosphere and geocarposphere on light, medium, and heavy soils in Israel. He identified 44 genera and 157 species of fungi, 10 of which had not been previously reported. Of these 157 species, 120 were found on light soil, 116 on medium, and 92 on heavy soils. Of these fungi, 133 occurred in the rhizosphere, 96 in field soil, and 86 in the geocarposphere. *Aspergillus* spp. were most numerous in heavy soil, *Penicillium* spp. in light soil, and *Fusarium* spp. in medium soil.

In Georgia, Jackson (59) determined the mycoflora of geocarposphere soils and found that species of *Aspergillus* and *Penicillium* comprised over 50% of the total fungal propagules isolated from pod surfaces by washing. *Penicillium* spp. were more abundant than *Aspergillus* spp. *Fusarium* spp. were common and varied little in abundance during the maturation of peanut fruit.

Data from Nigeria, Israel, and Georgia were in agreement on the high frequency and prevalence of species of *Aspergillus*, *Penicillium* and *Fusarium*, but *M. phaseoli* and *Rhizoctonia* spp. were subdominants in Nigeria and Israel, respectively.

C. *Peanut Pod and Kernel Mycoflora before Maturity and Digging*

In Nigeria, McDonald (84) found that the dominant fungi in the shells of variety Samaru 38, lifted weekly for 9 weeks before harvest, were *Fusarium* spp. and *M. phaseoli;* other fungi included *Aspergillus* spp., *B. theobromae, Penicillium* spp., and *Rhizopus* spp. Dominant fungi in the kernels before harvest were *Fusarium* spp., *Aspergillus* spp., and *Penicillium* spp. The percentage of seed infected was only a small fraction of the percentage of shells of immature and just-mature pods infected by fungi.

In Alabama, Lyle (80) found that *Rhizoctonia, Fusarium, Rhizopus,* and *Penicillium* were predominant among numerous fungi isolated from peanut shells and kernels from pods lifted and picked over a 2-month period from 70 days after sowing through curing.

In Georgia, Hanlin (43) noted that 93.7% of the peanut pods sampled 60 days after planting already contained fungi. He (45) then sampled peanut fruits from experimental plots of 16 tillage-organic matter-crop combinations for fungi. No treatment reduced the levels of total fungi on the pods. About 85-95% of the pods at two locations yielded fungi 21 days before digging. The predominant fungi were *Penicillium* spp. (53.7%) and *Aspergillus* spp. (18%). In this study, the percentage of kernels invaded by *A. flavus* and *A. niger* was twice that of the same species invading the shells, whereas there was a 30% decrease in seed invaded by *Penicillium* spp. *Fusarium* was not a significant factor in the mycoflora of preharvest samples.

In 1966, Hanlin (44) isolated fungi from developing peanut fruit at biweekly intervals from 45 to 14 days before harvest. Over 90% of the shells were invaded by fungi. The predominant fungi were *Penicillium* spp. (44%) with *Fusarium* spp. (14%) and *Gliocladium* spp. (10%) relatively abundant. Total fungi invading kernels was about 40% of the number isolated from the shells. Over 72% of the kernel invasion was by *Penicillium* spp. plus another 7% by *Gliocladium.* Fungi were not isolated from kernels until about 30 days before harvest or maturity.

Jackson (59) stated that the fungal community from shells of peanuts in Georgia was dominated by species of *Fusarium* and *Penicillium* with subdominant genera of *Thielavia, Chaetomium, Rhizoctonia,* and *Sclerotium.* Immature kernels from pods taken up before harvest had a variable community dominated by *Aspergillus* and *Penicillium.* In Argentine cultivar, *Chaetomium* was a co-dominant, while in Early Runner and Florigiant, *Thielavia* was a co-dominant genus. *Fusarium* was a subdominant genus throughout the study. In 1965-67, fungi of the endogeocarpic microflora of peanut fruits in Virginia were determined by Porter and Garren (103) and divided into dominant and subdominant groups according to isolation density. The density of bacteria decreased, while the density of fungi increased as fruits matured. The dominant genera of fungi were *Penicillium, Trichoderma, Chaetomium,* and *Fusarium,* with the subdominants of *Aspergillus* spp., *Thielavia* spp., and *Rhizopus* spp.

Barnes (5) found that the immature shells of Starr and Argentine cultivars were readily invaded in the field by diverse fungi. Species of *Fusarium* spp., *Penicillium* spp., *A. niger,* and *Trichoderma viride* were dominant in pod and shell in Oklahoma. There was a sharp population increase in *Fusarium* spp. and *T. viride* in developing pods in 1966 experiments accompanied by similar sharp decreases in *Penicillium* spp. and *A. niger* with time. Only rarely were fungi isolated from kernels.

Data on immature peanut pod mycoflora before digging from Alabama, Nigeria, Georgia, Virginia and Oklahoma agreed in that *Fusarium* spp., *Penicillium* spp. and

Aspergillus spp. were usually dominants or subdominants. *M. phaseoli (Sclerotium bataticola)* was a dominant in Nigeria and a subdominant in Georgia. Kernel mycoflora were dominated by *Aspergillus* spp. and *Penicillium* spp., with *Fusarium* spp. also being important in Nigeria, Alabama, and Virginia.

D. *Peanut Pod and Kernel Mycoflora at Maturity and Digging*

McDonald (85) found that the dominant fungi at normal maturity (digging) in shells and kernels in variety Samaru 38 in Nigeria were *Fusarium* spp. and *M. phaseoli,* although very few kernels were invaded. Other fungi isolated from over 5% of the shells or seed were *B. theobromae, Rhizopus* spp., *Rhizoctonia solani,* and *Phoma* spp.

In Israel, Joffe and Lisker (71) investigated the effects of crop sequence and soil types on the mycoflora of freshly-dug, mature kernels from 81 fields over a 3-year period. The general mycoflora was richest in fields previously sown to peanuts. *A. niger* made up 50-60% of the mycoflora. *Penicillium funiculosum* and *P. rubrum* were considerably more abundant in kernels of pods from previously fallowed fields. Total kernel mycoflorae were consistently higher on medium and heavy soils than on light soils. Similar data were reported by Pettit and Taber (99), who found that peanuts harvested from land planted to successive crops of peanuts in Texas were more highly infested with fungi than peanuts grown on land planted to rye, oats, melons, or potatoes the previous year.

Ashworth and Langley (1) investigated the cause of pod damage averaging 56% in 10 peanut fields in Texas from 1960-1962. They found that *R. solani* caused about 83% of the pre-harvest pod damage in Texas-grown Spanish peanuts with *S. rolfsii* invading another 10%.

In Georgia, Hanlin (44) isolated fungi from freshly-dug peanut pods of the cultivar Argentine at maturity and found that shells were invaded by *Penicillium* spp. (67%), *R. solani* (44%), *Fusarium* spp. (28%), *S. bataticola* (16%) and *Phoma* spp. (8%). Kernels contained only 20% of the total colonies isolated from shells. The dominant fungus in the kernels was *P. funiculosum,* which made up nearly 84% of the isolates.

In Virginia and Spanish pods, Garren (35) found that *T. viride* and *Penicillium* spp. were dominant in quiescent climax endocarpic community of sound mature peanut fruits. *Fusarium* spp. and *A. flavus* persisted throughout the season at a low level in sound pods.

Barnes (5) reported that *Fusarium* spp., *T. viride, A. niger,* and *Penicillium* spp. were dominant fungi in pods and shells at digging time in Oklahoma. Populations of *Fusarium* and *T. viride* increased with time, while populations of *Penicillium* and *A. niger* declined. Barnes and Young (6) found that the dominant fungus with "Starr" cultivar at digging in Oklahoma in 1965 and 1966 was *Fusarium* spp.; *Penicillium* spp., *Alternaria tenuis, T. viride, Rhizopus* and *Mucor* were subdominants. *S. bataticola* and *R. solani* were present at low levels. There was little difference in mycoflora between hand picked and machine-combined peanuts at digging.

Data on peanut pod and kernel mycoflora at maturity and digging from Nigeria, Israel, Georgia, and Oklahoma agreed for the most part that *Fusarium* spp., *Penicillium* spp., and *A. niger* were dominants in most experiments. *M. phaseoli* was a dominant in Nigeria and a subdominant in Georgia and Oklahoma. *R. solani* was a dominant in Georgia and Texas and a subdominant in Nigeria and Oklahoma. Only a low percent of kernels were invaded by fungi at lifting.

E. *Peanut Pod and Kernel Mycoflora during and after Curing in Windrow and Stack*

At Mokwa, Nigeria, in 1964, McDonald (83) found that fungal infection of kernels in pods at digging did not reach 2%. Dominant fungi were *M. phaseoli, Fusarium* spp., and *B. theobromae.* In a further trial in 1965 (83), peanuts were harvested at 2-day intervals. With the long season variety Samaru 38, the percentage of kernels infected in windrowed pods was 25% after 2 days of drying and increased to 85-95% in the next 4-11 days. Dominant fungi in both shells and kernels were *Fusarium* spp., and *M. phaseoli.* Total fungus-infected kernels from comparable liftings of overmature pods from the soil rose from 1% to 61% after six liftings over 12 days. Thus, the longer the pods were left in the ground after maturity the greater was the invasion of kernels by fungi. He found that the percentages of kernels infected with *Fusarium* increased rapidly from 1-2% at normal digging to about 14-16% in 2 days and rose steadily from 10-12 days. However, the percentage of *M. phaseoli* invasion of undamaged pods increased steadily about 3% every 2 days for 10 days and then jumped from 12% on day 10 to over 30% kernels infected at day 13. McDonald (83) found in 1965 and 1966 that although fungi were more abundant in shells of windrowed pods than in shells of freshly-lifted overmature pods with the same planting date, the composition of the shell mycoflorae was very similar. *Fusarium* spp. and *M. phaseoli* were the dominant fungi in shells of windrowed and of overmature pods in Nigeria. They were similarly dominant in the kernels from overmature and windrowed-dried pods. *Rhizopus* spp. was the subdominant fungus in these experiments. These results are similar to those of Jackson (59).

In the Gambia, Gilman (38) studied the mycoflora of visibly undamaged peanut pods at lifting and 3 days after harvest to determine the extent of fungal contamination. At lifting only 47% of the pods had been invaded by fungi as compared to 97% after 3 days of drying. *M. phaseoli* was the dominant fungus in 61% of the isolates at lifting and 72% of the isolates 3 days after harvest. *A. flavus* made up 14% and *Fusarium* spp. 11% of the fungi contaminating kernels of apparently intact pods. *A. flavus* was more frequently found to invade after digging. Overmature pods were invaded rapidly in the soil by a number of fungi.

In Georgia, the data of Jackson (50) indicated that *Fusarium* spp. were the dominant fungi in the soil (89%), at lifting (83%), and throughout windrow-drying for 9 days (avg.-58%). *Rhizopus* spp. (13%) and *Penicillium* spp. (7%) were also common. In other studies, Jackson (59) found that the windrow community was dominated by *Fusarium, Rhizopus,* and *Sclerotium. Rhizoctonia, Trichoderma, Nigrospora,* and *Curvularia* were subdominant genera.

Hanlin (44) isolated fungi from Argentine pods in Georgia that had been field-dried in the stack for 11 days after digging at maturity (119 days). The dominant fungi in the shells were *Penicillium* (63%), *Fusarium* (48%), and *Rhizoctonia* (15%). *Sclerotium, Rhizopus* and *Aspergillus* had each invaded over 5% of the shells. *Penicillium* dominated the kernel isolations being present in 83% of the seed invaded by fungi. The number of fungal isolations from kernels was less than 25% of the total for shells. In other experiments, Hanlin (45) found that the percentage of fruits invaded by fungi increased with time from 20 days prior to digging to after 40 days in the stack. However, with *Aspergillus* spp., shell invasion percentage decreased notably from 13-23% to 1-3%, whereas with *Fusarium* it increased from 1-5% to to 60-78% (data are for 2 locations). There was also a decrease in *Penicillium* isolation from 51-55% to 28-44%. Higher percentages of kernels were invaded by

Aspergillus spp. than were shells. Fungal populations were lower after 40 days in the stack with *Aspergillus* spp. decreasing from 29-51% to 8-13%. *Penicillium* spp. decreased slightly from 37-48% of kernels invaded to 21-25%. Again, there were notable increases of *Fusarium* in kernels in the stack with time as fungal populations increased from 1-4% to 17-20%.

Windrowed "Starr" cultivar peanuts were dried in the field in Oklahoma (6) until kernel moisture dropped to about 25%. The dominant fungi isolated from these peanuts were *Fusarium* spp., *Alternaria tenuis,* and *T. viride.* Subdominant were mucoraceous spp., *S. bataticola, R. solani,* and *Aspergillus niger.* Other studies in Oklahoma by Barnes (5) have identified species of *Fusarium* and *Penicillium, A. niger,* and *T. viride* as dominants in 1966. Only rarely were fungi isolated from kernels.

Garren, *et al.* (37) stressed the quiescent or static nature of peanut endocarpic mycofloral communities and the need for careful handling and storing to prevent the proliferation of these fungi in mature peanuts after lifting. Several of these fungi are toxicogenic and constitute a real mycotoxin potential. The genera posing the greatest threat appear to be *Penicillium, Aspergillus, Alternaria,* and *Fusarium.* Garren and Porter (36) further compared the quiescent mycofloral communities in hydrated, mature, carefully-cured peanut fruit grown as far north as they are grown commercially (Virginia), with those of fruit grown in the tropics (Puerto Rico). Fungi classified as dominants because they had isolation frequencies of 5% or greater were *A. flavus, Alternaria tenuis, Chaetomium globosum, Cylindrocladium* spp., *Diplodia gossypina, Fusarium* spp., *Penicillium* spp., *Macrophomina phaseoli, Rhizoctonia solani,* and *Rhizopus stolonifera.* These ten species or genera of fungi were characteristic of one or more of eight endocarpic communities. In determining the extent the comunities were dominated by toxicogenic or pathogenic fungi, *A. flavus* was found to be rare in PR samples and was not found in VA samples before hydration. *A. flavus* appeared to be dominant in kernels from unblemished and discolored VA pods. It appeared to be suppressed by *Fusarium* spp. and *Rhizopus stolonifera* in discolored VA shell communities.

Data for peanut pod and kernel mycoflora during curing and drying in windrow and stack were fairly comparable throughout the world. *Fusarium* spp. was a dominant or subdominant in shells and kernels in Nigeria, Gambia, Georgia, Oklahoma, Virginia, and Puerto Rico. *M. phaseoli* was a dominant in Nigeria, Gambia, Georgia, and Puerto Rico. *B. theobromae* was a dominant in Nigeria, *Diplodia gossypina* was a dominant in Puerto Rico. *Penicillium* spp. were dominant or subdominant in Georgia, Virginia, Oklahoma and Puerto Rico, and *Rhizopus* spp. were common in Nigeria, Georgia, and Virginia. Kernels were invaded by *Aspergillus* spp. and *Penicillium* spp. in Gambia, Georgia, Oklahoma, Virginia, and Puerto Rico.

F. *Inverted Windrow Drying, a Recent Development*

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. Jackson (50) in Georgia described experiments in 1964 wherein plants were placed in the windrow by hand with the pods down against the ground or inverted into the air. The total number of pod surface fungi was about twice for the down position as compared to the up or inverted position. Numbers of fungi isolated from kernels of these pods showed little difference relative to pod position. Dickens and Pattee (16) found that field-drying was acceler-

ated considerably in the inverted windrow and was less favorable for fungal growth than conditions in random windrows.

Porter and Garren (104) in Virginia (1966-69) found that the number of shells infested by fungi was reduced by 20.5% by drying in inverted windrows in comparison to shells of freshly-dug and random windrow-dried pods. The proportion of kernels infested with fungi was reduced by 31.5% by drying in the random windrow and by 60.4% by drying in the inverted windrow from the number of kernels infested from freshly-dug pods. Over the 4 years, 73% of the shells were infested as compared to only 36% of the kernels. *Chaetomium* spp. (21.4%), *Penicillium* spp. (17.5%), and *Trichoderma* spp. (14.1%) were the dominant fungi in shells of freshly-dug fruit. *Rhizoctonia* and *Fusarium* infested 6-6.2% of the shells. The dominant mycoflora of the shells of random windrowed peanuts consisted of *Chaetomium* (20.5%), *Fusarium* (14.4%), *Rhizoctonia* (10.7%), *Alternaria* (9.5%), and *Sclerotium* (8%). Results were similar for inverted windrows except the level of invasion by these five genera was about 20% lower. Kernels of freshly-dug fruit were dominated by *Penicillium* spp. (27.8%) as were windrowed fruit. *Aspergillus* spp. were subdominants in kernels of windrowed pods. *Rhizoctonia* was a subdominant in the windrow, but was only infrequently isolated from freshly-dug kernels.

Pettit and Taber (100) in Texas (1967-69) also found that peanuts from the inverted windrows were less severely damaged by fungi, whereas peanut pods in contact with the soil surface in random windrows were heavily invaded by fungi.

Inverted windrow drying appears to reduce mycofloral development by promoting rapid curing to low kernel 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 (9). Thus, utilization of this new 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 fungi in deterioration of peanut quality is not well established except where pathogenesis is involved. Not more than 10-12 of at least 46 genera of fungi appear to be dominant in soils, pods, and kernels in peanut-producing areas of the world. Likewise, only a limited number of species of each genus appears to be involved. Several of these fungi are involved in diseases of the peanut pod and plant. Some field fungi persist in storage and may invade the kernel under favorable conditions for their growth. However, many species do not persist and are important only in kernel destruction when physical and/or biological damage to the pod provide entry to the kernel in the soil, at harvest, or during curing. Research on the effect of field fungi on seed viability and germinability both pre- and post-storage, processing and storage quality, nutritive value, and edibility of peanuts appears to be sparse at this time.

III. Storage Fungi

A. *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 the lower kernel moistures and soon become dominant. This second group of fungi that is associated with peanuts during curing, picking, and storage consists principally of species of *Aspergillus* and *Penicillium*, but *Rhizopus*, *Sclerotium bataticola*, and *Fusarium* are frequently found. Joffe and co-workers (10, 62-71) in Israel have carried out extensive research on peanut

mycoflora in recent years particularly with stored peanuts. These workers have isolated and identified numerous fungi from kernels taken aseptically from whole unblemished pods that had been surface-sterilized in 0.1% mercuric chloride, rinsed in sterile water, and plated on Czapek's medium at pH 4.0. In a summary of research (1963-1968), Joffe (67) listed 173 species of fungi, of which 153 species were isolated from stored kernels. Borut and Joffe (10) found 71 species in the 1963 and 1964 peanut crops. In the 1965 and 1966 crops, Joffe (63) identified 83 fungi with 35 species being deep-seated enough to survive kernel disinfection. Forty-six species had not been previously recorded in peanuts. Most papers reviewed by Jackson (49) were investigations primarily determining the predominant species of fungi associated with stored peanuts. He listed 132 species of fungi that had been isolated from peanut pods and kernels. Diener (18) and Jackson (47) reported 23 and 16 species, respectively. Although at least 150 species of fungi have been isolated from stored peanut pods and kernels, the data point to a relative small number of genera being involved in storage deterioration. *Aspergillus* spp. *(A. niger* group, *A. flavus* group, *A. glaucus* group), *Penicillium* spp., *(P. funiculosum, P. rubrum), Macrophomina phaseoli (S. bataticola, R. solani, Fusarium* spp. and *Rhizopus* spp. appear to be the most important. *B. theobromae* also appears to be significant in Nigeria.

Table 1 shows the species of fungi that occurred most frequently in peanuts during storage. Jackson (51) reported that four of these species *(A. niger, A. flavus, S. bataticola,* and *Rhizopus* spp.) could rapidly penetrate the pod and invade the kernel. There is some agreement in this summary even though the media used by Jackson and Joffe and their co-workers were selective for mesophilic and hydrophilic fungi, whereas that used by Diener (18) and Welty and Cooper (121) (Christensen's malt-salt) was selective for xerophilic and some mesophilic fungi. Comparisons of results are difficult because of the differences in the raw material, pre-isolation environment, plating and disinfection technique, media, and the temperature and length of incubation.

Table 1. Frequency of peanut mycoflora in stored peanuts

Fungus species or group	References[a]							
Aspergillus niger group		2,	3,	4,	5,	6,	7,	
Aspergillus flavus group	1,	2,	3,	4,		6,	7,	8
Aspergillus glaucus group	1,			4,				8
Penicillium rubrum				4,	5,	6,	7,	
Penicillium citrinum	1,	2,						
Penicillium funiculosum	1,	2,		4,	5,	6,	7,	
Sclerotium bataticola		2,	3,			6,	7,	
Rhizoctonia solani		2,		4,	5,	6,	7,	
Rhizopus spp.	1,	2,	3,	4,	5,	6,	7,	8
Fusarium spp.		2,		4,	5,	6,	7,	8

[a]1 — 18; 2 — 47; 3 — 49; 4 — 10; 5 — 63; 6 — 70; 7 — 67; 8 — 121.

B. *Mycoflora of Stored Kernels*

The number of ecological studies of storage fungi in peanuts involving quantitative mold counts of populations is limited. In 1960, Diener (18) quantitatively determined the density and relative density of the mycoflora of kernels from unshelled farmers stock peanuts in 26 farm-size bins. Fungal populations were compared with initial moisture, seed damage, type of bin, and length of storage. He found that species of the *A. glaucus* group, *A. flavus* group *(A. tamarii),* and *Penicillium citrinum* were dominant in the mycoflora of the kernels of farmers stock peanuts stored 8-56 months. These fungi were present in amounts of 4059-7266 propagules per g of peanut kernel and made up 37.1%, 20.7%, and 30.6%, respectively, of the total propagule load in the 26 bins sampled. Other fungi with relative densities exceeding 1% were *Torula sacchari, A. candidus, Cladosporium* sp., *Trichothecium roseum,* and *Mucorales,* which were predominantly species of *Rhizopus* and *Mucor. Aspergillus ruber, A. repens,* and *A. restrictus* were the predominants of five species of the *A. glaucus* group as determined by mold counts of composite samples from each of the 26 bins. *A. chevalieri* and *A. amstelodami* occurred frequently, but at relative low densities.

Welty and Cooper (121) determined the number and kind of fungi associated with kernels plated after being stored for 1-6 months. Peanuts were field cured for 6 days and then mechanically dried to 8% moisture in about 4 days. Pods were shelled and storage of the seeds subsequently reduced kernel moisture content to 4.5%. Seeds were stored in desiccators at varying relative humidities. Only data from the controls stored in dry air (below 70% RH) is pertinent here. Initially, *A. repens* was isolated from 54% of the kernels, *Penicillium* spp. from 80%, and *A. flavus* from 7%. After 2-3 months of storage, the percentage of kernels with *A. repens* and *Penicillium* dropped to about 10% and then gradually increased back to about 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 kernels. *A. ruber* and *A. amstelodami* were isolated from a low percentage of the kernels (4.4%) throughout the 6 months storage.

Borut and Joffe (10) found *A. niger* to be the most common species of 71 fungi occurring in 114 stored samples of 1963- and 1964-crop peanuts in Israel. *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 total mycoflora on stored kernels. Most of the species found on stored kernels had earlier been reported in fresh kernels (68). *Rhizopus* spp., *P. rubrum, P. funiculosum, Fusarium* spp., *Rhizoctonia* spp., and other *Aspergillus* spp. were both prevalent and of significant density in this study.

Joffe and Lisker (70) reported the distribution of the fungi in Israel as to frequency and prevalence (density) in the 1965 and 1966 crops stored for 210 days. *Aspergillus* spp. and *Penicillium* spp. had frequencies of 100% throughout the study, although the prevalence varied tremendously during storage of each year's crop. *A. niger* was the dominant fungus during the first 6 months of storage. *Penicillium* spp. were the next most important with only 10-20% as many colonies as *A. niger.* The *A. glaucus* group *(A. repens* and *A. ruber)* increased in frequency and prevalence after 60-97 days of storage. Joffe (63) considered the high incidence of *A. niger* in stored kernels that were disinfected with mercuric chloride to indicate a selective removal of antagonists. This could be due to mercury tolerance of the species, whereas the disinfectant was quite toxic to *A. flavus, Fusarium solani, P. rubrum,* and *P. funiculosum.*

Joffe (67) reported *A. niger* as the dominant species in the mycoflora of 419 samples of stored kernels examined over a 5-year period in Israel. *A. niger* was even more predominant in stored kernels than on fresh ones. *P. funiculosum* and *P. rubrum* were also prevalent. *Rhizopus nigricans, Rhizoctonia* spp., *Sclerotium* spp., *F. solani,* and *A. flavus* were also present but in relatively small quantities in comparison to *A. niger.* There was no regularity to the variations in total mycoflora or to the individual species during the 2-7 months storage of kernels. *A. niger* made up about 47% of the total kernel mycoflora, whereas *Penicillium* spp. and *F. solani* made up about 5% each.

During the period 1943-52, about 2000 carloads per year of shelled peanuts (mostly Spanish and Runner) were unloaded in Chicago (46). Samples of damaged peanuts from approximately 100 cars were cultured to determine the fungi associated with discoloration and decay of peanuts on the market. The frequency of fungus species observed was: *Diplodia natalensis* 20.2%, *Penicillium* spp. 15.8%, *Rhizopus* spp. 13.1%, *Aspergillus* spp. 8.8%, *M. phaseoli* 6.0%, *Alternaria* spp. 4.1%, and *T. roseum* 4.0%. *Diplodia* was present as concealed damage, but is no longer a significant problem in American runner peanuts due to the development of resistant varieties.

In evaluating the published data on the mycoflora of peanuts that have been stored after curing, it is generally accepted that the medium used for isolation will determine the range of species that are recovered. It will be even more significant, if any kind of quantitative count of fungal propagules are made. The malt-salt medium used by Diener (18) and Welty and Cooper (121) was selective for low-moisture tolerant storage fungi and reduced the rate of growth of field fungi. Joffe (62, 63, 66-68, 70, 71), Jackson (47, 50, 52, 59), and Hanlin (44, 45) in their investigations used media (Czapeks, RBM-2) that were appropriate for the field fungi found in the soil, on pod surfaces, and in kernels from freshly-dug immature and mature peanuts. However, for isolations of mature peanuts during curing and storage, it is highly probable that these media were inadequate for the isolation of species of the *A. glaucus* group *(A. ruber, A. repens, A. amstelodami, A. chevalieri, A. restrictus).* In evaluations of 11 different media, it has been noted

> "that the standard Czapek-Dox medium is not suitable for determining the mold count of these samples (11). It not only gave one of the lowest total mold counts, but *A. glaucus* did not appear at all, whereas the other media indicated that this fungus was present in considerable numbers".

Christensen (14) noted,

> "it is almost axiomatic in microbiological work that any culture medium used to isolate microoganisms 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 (14) further stated,

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

Garren (34) had made this same point in the isolation of the terrestial mycoflora of peanut pods. Similarly, the influence of surface disinfestation of the pod on results of isolation has been recognized by Joffe (63).

It must be recognized that in isolating fungi from soil, peanut kernels, and pods at periodic intervals during the growing season and through several months or years of storage, the researcher is faced with several problems. It may not be feasible to use two or more media. To change the medium at a given point in time eliminates any

basis of comparisons in time. Likewise incubation temperature, isolation techniques, and the examination date after plating all greatly influence quantitative mycofloral data.

C. *Environments Favoring Invasion of Peanuts by Storage Fungi*

The main factors influencing the growth of storage fungi in peanut pods and kernels 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 Panasenko (96), Scott (111), and Semeniuk (114).

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 that the moisture content rises enabling a new succession of fungi to develop. The RH equilibrium of peanuts was determined by Karon and Hillery (73). Additional data for peanut kernels and meals at 30 C were reported by Austwick and Ayerst (3) and are re-presented in Table 2.

Table 2. The moisture equilibrium of groundnuts[1]

Relative humidity at 30° C	Moisture content Kernels	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

[1]Taken from Austwick and Ayerst (3).

Diener (18) found that high mycofloral counts were associated more often with high initial moistures of peanuts going into storage than any other factor. Austwick and Ayerst (3) reported the growth of *Aspergillus flavus* and *A. chevalieri* of the *A. glaucus* group at different RH (% moisture in peanuts) and temperatures. Other studies with *A. niger, A. tamarii, A. fumigatus,* and *Penicillium martensii* gave results similar to *A. flavus* in that they will not grow below 80-85% RH. They confirmed that the *A. glaucus* group grows rapidly at low RH or moisture contents in peanuts (96). Diener and Davis (24) have reviewed the 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.

The changes in frequency of six species of fungi isolated from kernels stored at controlled humidities of 75, 85, and 99% for 6 months at 22-28 C were investigated by Welty and Cooper (121). After 4 months at 75% RH and kernel moisture content (KMC) of 8.8%, the percentage of kernels invaded by *A ruber* had risen from 0 to nearly 80%, by *A. amstelodami* from 0 to 25% and by *A. repens* it had decreased from 80% to 4%. At 85% RH (KMC of 10.5%), the percentage of kernels infested by *A. ruber*

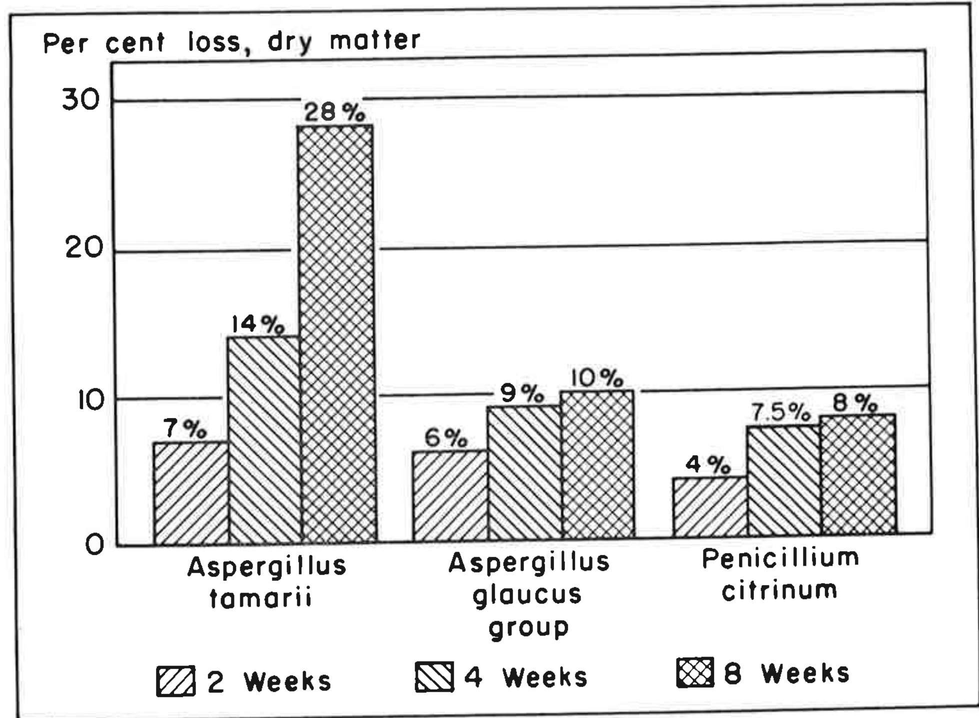

Figure 1. The fungus *Aspergillus tamarii* of the *A. flavus* group caused the most serious loss in dry weight of the six fungi tested.

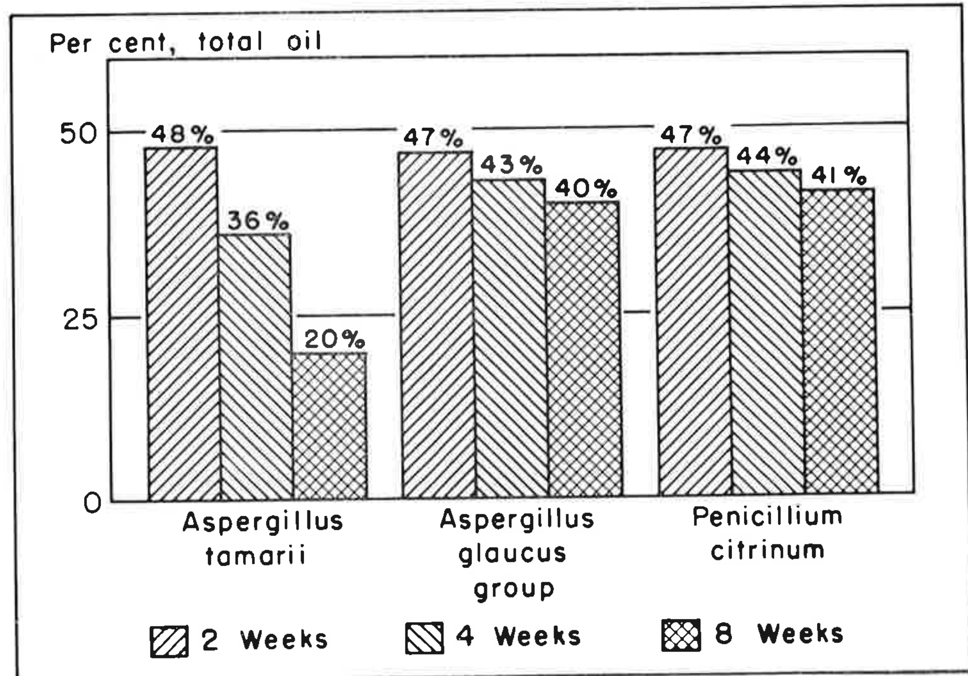

Figure 2. All six fungi seriously reduced the percentage of oil of the stored peanuts in the experiment.

went from 0 after one month's storage to 97-100% in the 3-4 months. *Penicillium* spp., *A. repens,* and *A. flavus* dropped from 80%, 57% and 7%, respectively to 10%, 10%, and 1%, respectively, after 2 months. All decreased to negligible levels in 4 months. At 99% RH, the KMC increased slowly to 28% with monthly variations in the dominant fungus. Under the circumstances of competition between these storage fungi, Welty and Cooper (121) concluded that *A. ruber* grew best at 12-15% KMC, *A. repens* at 18.5%, *A. flavus* and *Penicillium* spp., at 20% and *A. amstelodami* at 20-28%. *Fusarium* was detected in the 4th month and became the dominant fungus as KMC 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 (114). However, a few grow well at temperatures of 35-45 C. Growth rate is reduced at lower temperatures for most, but some may continue to grow slowly at 5-15 C. The cardinal temperatures of several species of storage fungi have been summarized elsewhere (114). Jackson (51) found kernel invasion of intact, surface-sterilized pods by *A. flavus, A. niger, S. bataticola,* and *Rhizopus stolonifera* was favored by temperatures of 26-38 C. Temperature is significant in its relation to time. 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 determine the rapidity of spore germination. In general, the higher the temperature at a given RH the less time is required for germination (96). 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 kernel 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 development depends on the presence of the oxygen in the air. Landers, *et al.* (78) 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 kernels. Growth was also measured by the increase in free fatty acids (FFA) in fungus contaminated kernels as compared to control peanuts. Striking reductions in fungal growth and sporulation resulted when O_2 concentrations were reduced from 5% to 1% in combination with 0, 20, 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.* (108) evaluated the combination of RH and temperature in reducing fungal growth with CO_2. Visible growth and FFA formation by *A. flavus* was 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%.

Jackson and Press (61) 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. Composition of pod surface mycoflora did not change. A significant reduction in number of fungus propagules occurred after 27 C storage treatments regardless of gaseous environment. Mycoflora consisted primarily of *A. flavus, A. niger, Chaetomium* spp., *Cladosporium* spp., *Fusarium* spp., *Penicillium* spp., *Rhizopus stolonifer,* and *T. viride.* At 4 C the number of pod surface fungi remained relatively unchanged through the 12 months.

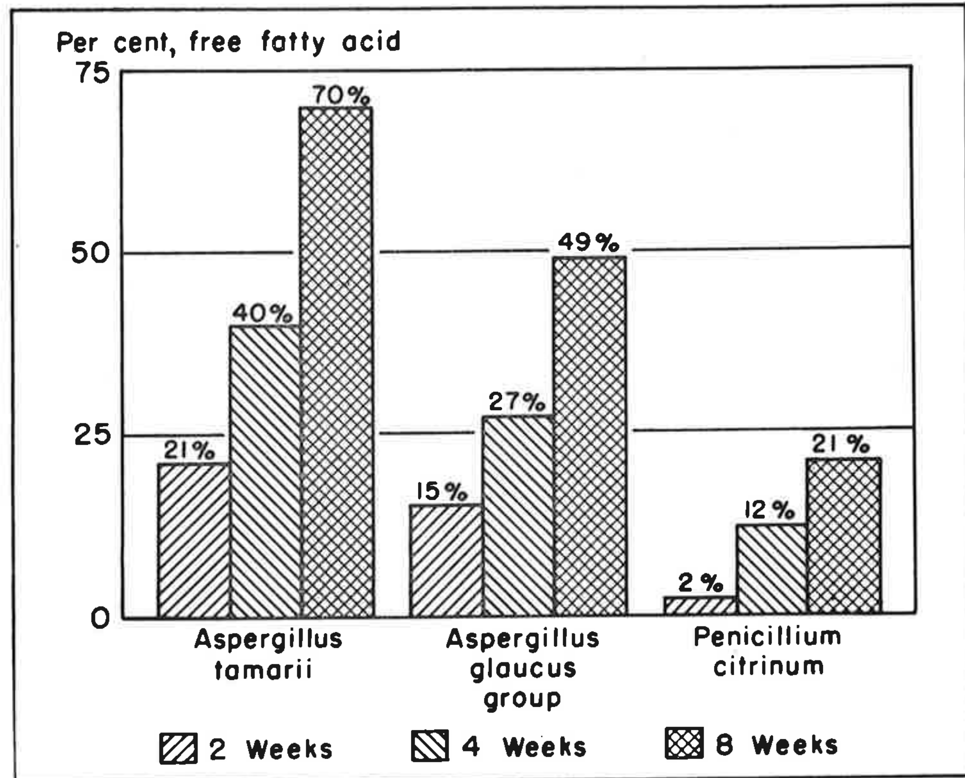

Figure 3. *Aspergillus tamarii* caused the greatest increase in free fatty acids, greater hydrolytic rancidity.

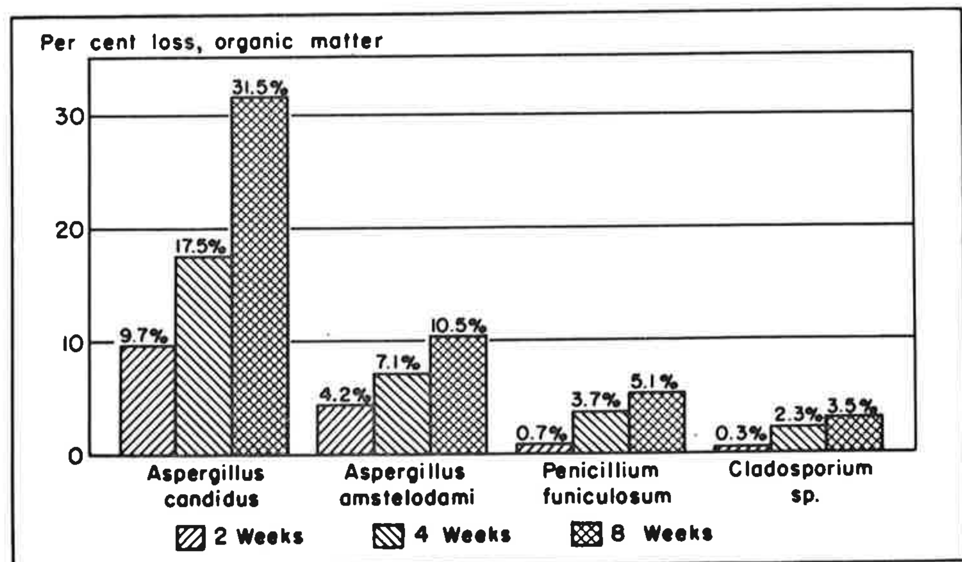

Figure 4. *Aspergillus candidus* caused the greatest loss in organic matter of the four other storage fungi tested.

D. *Changes in Peanut Quality Caused by Storage Fungi*

Seed microbiological research, reviewed and summarized by Semeniuk (114) and Christensen (14), attributed much of the deterioration in stored wheat and other grains to fungi. Fungal activity was associated with decreases in seed viability, processing and storage qualities, nutritive value, edibility and industrial usefulness of grains and grain products. During storage, Johnson and Gilliland (72) found that farmers stock peanuts (in-shell) undergo changes that reduce their value, such as loss of quality and weight, damage to kernels, changes in moisture content, and losses due to insects and rodents. Moisture content of peanut kernels, when they were first placed in storage, was probably the most important factor affecting storability and changes in quality. Quality of peanut kernels is usually measured by the free fatty acid (FFA) content of the oil. The quality of the oil, which makes up about 47-50% of the peanut, is judged from determinations of not only FFA, but also peroxide value, carbonyls, iodine number, and the kind of fatty acids making up the oil. An increase in FFA indicates a lowering of quality (hydrolytic rancidity). An increase in peroxide value, carbonyls, and iodine number is associated with a lowering of quality (oxidative rancidity). In peanut oil, natural antitoxidants such as tocopherols help prevent oxidative rancidity. Other changes in oil quality are based on changes in color, odor, and flavor. Peanut quality is also determined from analyses of sugar and proteins. A decrease in sugars appears to be correlated with poor flavor, whereas a loss in protein is indicative of a less nutritious peanut.

Diener (18) found that high populations of fungi were associated with the kernels of farmers' stock peanuts stored at high initial moistures; similarity of species with those reported by Christenson (14) suggested that fungi might be an important cause of deterioration of quality usually in peanuts. Six of the dominant fungi from that study were selected for determinations of the role of individual fungi in causing biochemical changes in peanuts by procedures (120) similar to those of Nagel and Semeniuk (93). Each fungus was grown separately on sterilized peanut kernels at 30 C. After 2, 4, and 8 weeks, samples of peanuts were removed and weighed to determine loss in dry matter and analyzed chemically for several quality factors.

The growth of six fungi on sterile peanuts resulted in a loss of dry weight, a decrease in percent oil, and an increase in FFA (27, 120). The data for four species of the *A. glaucus* group were averaged together in the results illustrated in Figures 1, 2, and 3, (27). *A. tamarii (A. flavus* group) caused a large decrease in dry matter and total oil and an increase in FFA that far exceeded those caused by *Penicillium citrinum* and the *A. glaucus* group. Other data (120) showed that fungal growth resulted in an almost complete loss of sugars (sucrose) in 2 weeks. On the other hand, the fungi did not appreciably affect the total quantity of proteins. Fungi caused the oil to have a deep yellow color *(A. tamarii)* to dark reddish-orange or amber color *(A. glaucus* species). The oil also had a strong musty to acrid-burnt odor. However, *P. citrinum* did not discolor peanut oil and the odor was flat rather than "peanutty". No increase in peroxide value, carbonyls, and iodine number in the oil was recorded, nor was there a decrease in tocopherols from the growth of these storage fungi on peanut kernels. Fatty acid composition of the oil also was not greatly affected by the fungi.

Deterioration in peanuts caused by four other storage fungi was similarly investigated (20). Results (unpublished) are presented in Figures 4, 5, and 6. *A. candidus* reduced organic matter about 30% in 8 weeks with losses by the other three fungi

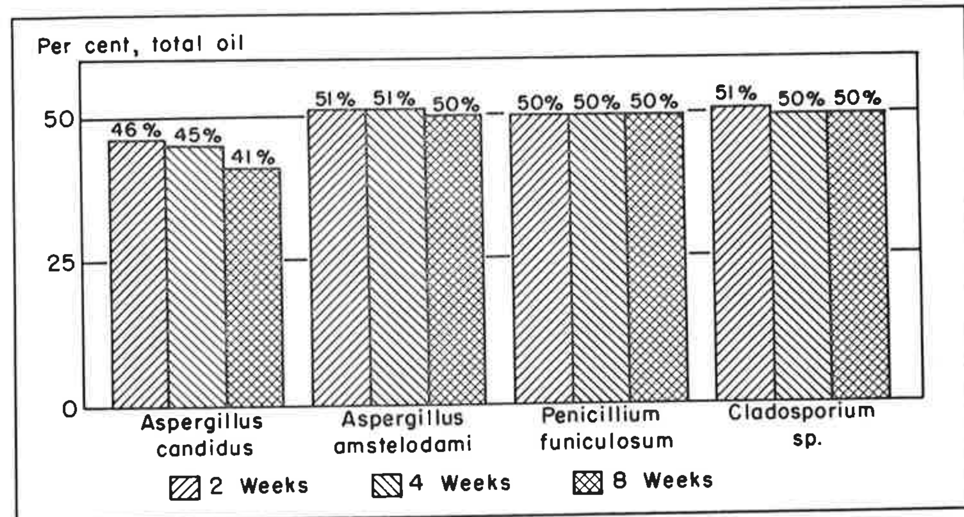

Figure 5. *Aspergillus candidus* was the only fungus of the four to cause a serious reduction in total oil.

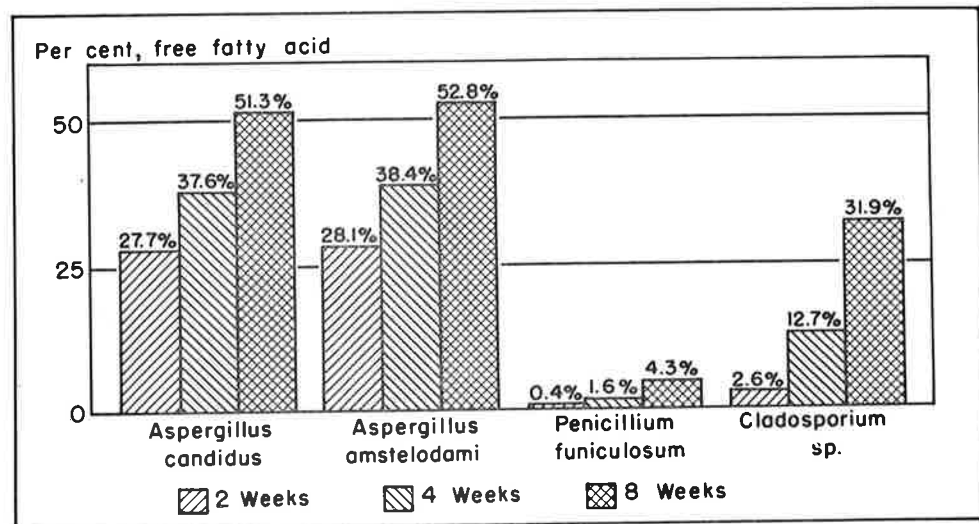

Figure 6. Three fungi caused large increases in free fatty acids, increased hydrolytic rancidity.

of about 5-10%. *A. candidus* was the only fungus of the four that caused a significant decrease (20%) in total oil. This fungus and *A. amstelodami,* another species of the *A. glaucus* group (107), increased FFA to over 50%, while *Cladosporium* sp. increased FFA to about 32% and *P. funiculosum* to only 4%. High organic (carboxylic) acid production was noted for *A. candidus* and *A. amstelodami.*

The relationship of rapid increase in FFA to growth of *A. flavus* in peanut kernels has been reported by Pattee and Sessoms (98). They found that FFA was highly correlated with visible fungal growth.

Results of these investigations (20, 27, 98, 120) revealed that several storage fungi lowered peanut quality by causing hydrolytic rancidity of the oil. Decreases in

oil and sugar content were associated with corresponding drops in dry weight in peanuts. Loss in dry weight demonstrated that fungi as well as other factors can be a significant cause of "shrinkage" in commercial warehouses under conditions that favor fungal growth (124). The decrease in oil content accompanied by hydrolytic rancidity (high FFA) results in peanuts of low quality, which are unacceptable for processing into edible products.

Deterioration in peanuts by storage fungi is very similar to deterioration in stored small grains, corn, soybeans, and cottonseed (15). The species of fungi and the environmental factors affecting their development in peanuts have been worked out for *A. flavus* and some species of the *A. glaucus* group. These results correspond closely to those with stored grains. The only significant research on biochemical changes in peanuts caused by storage fungi has been published by the Auburn group (18, 20, 27, 120). Some details (20) are presented here for the first time. Extension of research to the effect of storage fungi on seed viability and other quality factors, when peanuts have been stored at suboptimal conditions for long periods of time, would be of great value to the industry.

IV. *Production of Fungal Metabolites Toxic to Animals*

Fungi and their toxic metabolites (mycotoxins) in grain, seeds, and feeds have been a problem for man and domesticated animals for hundreds of years. Mycotoxicoses are diseases caused by the ingestion of foods made toxic by fungi and 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 (8). The literature on mycotoxicoses has been recently reviewed (15, 19, 32, 122, 123); diseases included were stachybotryotoxicosis that killed horses in Russia in 1931, swine losses in U.S.A. from barley and corn infected by *Fusarium* blight or scab, moldy corn toxicoses in swine and dogs, bovine hyperkeratosis in cattle, estrogenic syndrome in swine, hemorrhagic syndrome in poultry, facial eczema in ruminants in New Zealand and Australia, and moldy bermuda-grass toxicosis that killed cattle in southeastern United States as recently as 1971. Species of *Aspergillus, Penicillium, Fusarium, Alternaria, Chaetomium, Rhizoctonia, Trichothecium, Claviceps, Pithomyces,* and *Stachybotrys* produced the mycotoxins involved in most of these mycotoxicoses of animals. Seven of these genera are common field and storage fungi associated with corn, small grains, cottonseed, peanuts, and soybeans.

The surge of present day interest in mycotoxins resulted from the death of 100,000 turkey poults on 500 farms in England in 1960. Investigation revealed the presence of a toxic fungal metabolite (aflatoxin) of *A. flavus* in the Brazilian peanut meal fraction of the feed (77, 109). *A. flavus* is distributed world-wide in soil and air, and is a seed-inhabiting storage-fungus of most of the important food crops of man such as corn, wheat, rice, soybeans, and peanuts. 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, seeds, and feeds (39, 122). Research on aflatoxin was extensively reviewed by Goldblatt (39) in 1969 and the relationship of aflatoxin and other mycotoxins to the role of fungi in quality loss in "Grain Storage" has been evaluated by Christensen and Kaufman (15).

Aflatoxin B_1 is the most potent, natural occurring carcinogenic substance known and has induced cancer and caused mortality in most domesticated and experimental animals. Kraybill and Shapiro (75) concluded that "suggestive evidence from animal

experimentation produces a strong case for cause and effect relationships between aflatoxins and the incidence of diseases in man". They also relate significant direct evidence from India wherein aflatoxin was incriminated as a probable cause of infantile liver cirrhosis.

Of 173 species of field and storage fungi associated with peanuts in Israel (67), only *A. flavus* and *A. parasiticus* Speare were shown to produce aflatoxins. A review of literature (24) has shown that over 50% of *A. flavus* and *A. parasiticus* isolates may be toxigenic. In Israel (65), only 10.4% of 1,626 isolates of *A. flavus* from peanut kernels and soils produced no toxin. Rice, wheat, corn, and other high carbohydrate seeds support much higher levels of aflatoxin than oilseeds such as cottonseed, peanuts, and soybeans. Most seeds, feeds, and foodstuffs will support the growth of *A. flavus* in warm, humid environments and aflatoxin will be formed. Literature on the fungi that produce aflatoxin and the factors influencing toxin production in natural substrates has been reviewed (24).

A. *Moisture*

The most important factor in growth and aflatoxin production by *A. flavus* is the moisture or relative humidity (RH) surrounding the substrate (3). It is difficult and probably arbitrary to separate the interrelationships of temperature and time from that of RH or moisture in this review. Under precise controlled conditions in the laboratory, the limiting RH for aflatoxin production in heat-killed Early Runner peanuts was 85 $\pm$ 1% at 30 C for 21 days (22). Aflatoxin production in living kernels from freshly-dug, surface sterilized Early Runner peanuts was limited at a similar RH (23). In stored, unsterile living peanuts, low levels of aflatoxin were formed in immature and broken mature (damaged) kernels after 84 days at 84% RH and 30 C, but not in sound mature and unshelled peanuts (25). No aflatoxin occurred at 83% RH in any treatment. This limiting relative humidity is in equilibrium with 10.5-11.0% kernel moisture content (KMC). In pure culture studies, Austwick and Ayerst (3) reported that *A. flavus* was limited by 80% RH (9% KMC) and the growth rate on culture media between 80-85% RH (9-11% KMC) was very slow (0.1-1 mm/day).

In the field, *A. flavus* appears to develop most rapidly in peanuts at KMC of 12-20%. Most workers (2-5, 26, 59, 84, 88, 103) agree that there is little invasion of kernels in sound immature or mature peanut pods in the ground by *A. flavus,* and little or no aflatoxin present in kernels before lifting at the normal harvest time under typical conditions. However, results of Norton, *et al.* (94) and Joffe (67) are to the contrary. Joffe (68) had earlier attributed his results in part to a richer laboratory medium and longer incubation period (3 wk vs. 1 wk); other factors (67) proposed were: (a) peanut after peanut rotation, (b) advanced maturity of the crop at harvest in Israel, and (c) longer period (3 days) from actual lifting of pods to their examination. Norton found *A. flavus* to be present in 16% of the unblemished nuts.

It is generally agreed that invasion of peanut pods and kernels by *A. flavus* and other fungi usually occurs after lifting, during curing when the variety has been dug near maturity (3, 26, 88). After lifting, peanuts are most rapidly invaded by *A. flavus* during drying or dehydration in windrow or stack at 14-30% KMC (16, 88, 91). Apparently *A. flavus* does not become established rapidly in peanuts at higher or lower moisture contents (3, 88, 89). 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. McDonald and Harkness (88) suggested that the drop in moisture content to a more susceptible state is associated with the physiological change from active growth to one of a low metabolic state. Dickens and Pattee (16) found that aflatoxin developed within 2 days at KMC between 15-30% at 32 C and within 4 days at KMC between 20-31% at 21 C in freshly dug peanuts. Pettit, *et al.* (102) found that peanuts grown under dryland conditions, where drought stress occurred, accumulated more aflatoxin before digging than peanuts grown under irrigation. It appeared that when kernel moistures were above 30% or below 10% *A. flavus* activity was restricted. Similar data were obtained by McDonald, *et al.* (91). They found little or no aflatoxin developed in peanut samples harvested from random and inverted windrows after being dried to 15% KMC at 32 C and 50% RH, while the majority of 128 samples dried at 32 C and 85% RH contained aflatoxin B_1 in quantities of 6-960 ppb. It was further demonstrated that moisture percentage decreased more rapidly in inverted windrows than in random windrows; lower *A. flavus* and mycofloral invasion also occurred in inverted windrows (16, 95, 100, 104, 105).

When curing peanuts are in the general range of 14-24% KMC, 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 (3, 4). In northern Nigeria, no aflatoxin was formed in peanuts left windrowed for 6 days after lifting, whereas those left for longer periods were found to contain toxic kernels (86). The longer the crop was in the field before artificial drying, the greater was the amount of aflatoxin. McDonald and Harkness (88) found that both contamination with *A. flavus* and appearance of aflatoxin occurred at least 5-6 days after lifting. Contamination with other fungi at lifting was recorded in nearly all samples. This may be competition or an ecological phenomenon related to the moisture content of the substrate.

It has been pointed out (90) that where kernel moisture is high there is some resistance to invasion by *A. flavus*. Support for this explanation comes from field studies by McDonald (83). At Mokwa, where the 1963 crop matured and was lifted well before the rains had ended, toxicity at lifting was very rare. However, at Kano where the rains ceased before harvest, toxicity increased as the moisture content of the kernel at lifting decreased. The high level of pod invasion by *A. flavus* in the soil has been associated both with overmaturity and with low KMC due to low rain; low vigor of the plant and reduced physiological activity in seed coincided with the drop in KMC and overmaturity. All of which resulted in increased susceptibility of pod and kernel to *A. flavus* invasion and aflatoxin formation (88, 90). Pods collected from dead plants of "Samaru 61", at Kano, Nigeria contained toxic kernels of 5-14% KMC, whereas living plants at harvest had no toxic kernels and 24-34% KMC (83). It was concluded that delayed lifting could have undesirable results.

B. *Temperature*

Aspergillus flavus has been classified (114) as a mesophilic fungus having cardinal growth temperatures 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 (14).

The optimal temperature and time for aflatoxin production by *A. flavus* on sterilized peanuts in culture flasks was 25 C and 7-9 days (21). 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 aflatoxin B_1 was produced at 30-35 C and maximum G_1 at 25-30 C. Large amounts of total aflatoxins were produced at 25-30 C during incubation periods of 7-15 days.

In more critical studies with 2- to 3-lb samples of heat-treated peanuts in environmental cabinets, Diener and Davis (22) 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 (23). The upper limiting temperature was slightly above 40 C, since small amounts of aflatoxin were formed at that temperature. Burrell, *et al.* (13) 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 (16) found that in ten days aflatoxin developed rapidly in peanut samples of 15-30% moisture held at 90 F (32 C) but developed in relatively few samples held at 70 F (21 C). Minimum time for aflatoxin production was 2½ days after inoculation in their investigation.

Research on the relation of time to the formation of aflatoxin 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 (4). Also, kernels with testae damaged during shelling showed sporulating *A. flavus* in 4 days. McDonald and A'Brook (86) found that artificial drying after 4-6 days in the field gave toxin-free kernels, but that samples left for 8-12 days or sun-dried for 10-16 days gave low to medium toxin yields (25-500 μg/kg). McDonald and Harkness (88) found that contamination of kernels with *A. flavus* and aflatoxin did not occur until at least 5-6 days after lifting. They (89) also found more *A. flavus* and other fungi in kernels from slowly-dried pods (attached to plant) than from rapidly-dried pods. Jackson (58) found high levels of aflatoxin as well as high percentages of kernel invasion by *A. flavus* in slowly-dried pods as compared to rapidly-dried pods.

In controlled environment studies with living cured peanuts, Diener and Davis (25) found that the upper limiting temperature for growth and aflatoxin production by *A. flavus* was 40.5 ± 0.5 C at 99% RH in 21 days for sound and broken mature kernels, immature kernels, and kernels of unshelled peanuts. The lower limiting temperature was 13 ± 1 C for sound and broken mature kernels incubated up to 84 days at 99% RH. Some aflatoxin developed in mature kernels 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 kernels 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, living Early Runner peanuts (23) in that some aflatoxin was found at 20 C, but none at 15 C in 21 days. Thus, living peanut kernels stored in intact pods were much less susceptible to invasion and aflatoxin formation by *A. flavus.*

C. *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 two weeks at 30 C and 99% RH, Landers, *et al.* (78) observed that no visible change in fungal growth and sporulation occurred when CO_2 concentration was increased from

0.03% (air) to 20%, although aflatoxin formation was reduced 75%. Fungus growth, sporulation, and aflatoxin were reduced with successive 20% increases in CO_2 from 20 to 100%. No growth or aflatoxin production occurred in 100% CO_2. The effect of CO_2 concentrations from 20-80% in reducing growth of *A. flavus* was similar to that reported for *A. flavus* by Golding (41) and for other fungi by Brown (12). However, CO_2 has also been reported to be one of the essential factors for initiating germination of *Aspergillus* conidia (116). With the same experimental design as Landers, *et al.* (78), Sanders, *et al.* (108) found that aflatoxin production was inhibited for two 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 at 86 and 92% RH and by 40% CO_2 in 86% RH, but not at higher RH (92%) and higher temperatures (30 C).

The inhibitory effect of low O_2 on *A. flavus* growth was reported by Miller and Golding (92) to be in proportion to oxygen solubility in the medium or mycelium. 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 (78). In general, reducing the O_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. Aflatoxin was low in peanuts stored for 6 weeks at 15 C under 20% CO_2 and 5% O_2. No measurable aflatoxin was formed in peanuts stored for 6 weeks at 15 C under 40% CO_2 and 5% O_2. Growth of fungi in oxygen concentrations of less than 1% has been reported (12, 31). However, Landers, *et al.* (78) found that growth and aflatoxin production 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. Thus, the conclusion of Stotsky and Goos (115) that soil microoganisms are generally tolerant to conditions of high carbon dioxide and low oxygen applies to *A. flavus,* which is a soil organism as well as a storage fungus.

D. *Pod and Kernel Damage*

The thesis that kernels of unblemished, intact immature and mature pods are rarely invaded by *A. flavus* and other fungi before digging is widely accepted. Ashworth and Langley (1) found that less than 1% of the kernels of such pods had visible necroses and only a few such kernels from undamaged pods yielded fungi in agar culture. In Texas, *A. flavus* was the dominant fungus isolated from kernels and shells of unblemished Spanish peanuts samples over a 6-week period before and after harvest (94).

Rapid invasion of pods by *A. flavus* in the soil has been associated primarily with physical and biological damage to the shell and kernels. McDonald and Harkness (87, 88) and McDonald, *et al.* (91) reported that kernels of broken pods showed extensive fungal contamination in comparison with that of kernels from undamaged pods. McDonald and Harkness (88) also reported that preharvest development of aflatoxin occurred only in kernels of broken pods. Schroeder and Ashworth (110) found aflatoxins in kernels of mechanically damaged pods, but much larger quantities developed when pod openings resulted from growth cracks. Similarly, Sellschop (112) reported visible fungal invasion in growth cracks of pods in the soil. *A. flavus* invasion and aflatoxin formation in kernels have been widely associated with pod damage caused by termites in the ground in South Africa (112, 113) and Nigeria (86, 91), and by lesser cornstalk borer in Texas (1). Pod openings caused by pathogenic fungi

such as *Rhizoctonia solani* and *Sclerotium rolfsii* also provided entrance for *A. flavus* and subsequent aflatoxin formation (1, 110).

It has been widely accepted that most *A. flavus* invasion and subsequent aflatoxin development occur after digging and during the curing period (3, 26). Bampton (4) concluded that damage to the shell is a major factor in *A. flavus* invasion and aflatoxin development in the kernel during drying. It was also shown that kernels from damaged pods were more frequently contaminated with *A. flavus* and aflatoxin than kernels from undamaged pods during sun- and artificial-drying (86, 88, 91).

Bampton (4) observed that shelling (decortication) of peanuts resulted in testa damage and usually increased fungal invasion and the possibilities of toxin development in the kernel, especially before the seed were dried to safe moisture levels. The intact shell serves as a barrier to fungus invasion and some protection may also be afforded by the testa. Jackson (48) showed that the mycoflora of mature peanut kernels from unblemished intact pods was usually in or beneath the dead cells of the testa. Under gnotobiotic conditions, Lindsey (79) demonstrated that *A. flavus* readily penetrated the shell, but was limited in its invasion of the kernel to the testa. Colonization of the embryos by *A. flavus* was relatively limited. Damage to shell or kernel affords increased opportunities for rapid and direct invasion of the kernel, which increases the possibility of aflatoxin formation. Pattee and Dickens (97) 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" . . . Damage also increases nutrient availability. Thus, the extent of fungus growth and toxin formation at minimal temperatures and RH will usually be determined by time and the availability of nutrients resulting from damage.

E. *Seed Maturity*

Bampton (4) noted in Nigeria that peanuts left in the ground 4 weeks after maturity contained aflatoxin. Toxin was also detected 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. Metabolic activity of these peanuts probably had been reduced by a 9-week drought. McDonald and Harkness (88) demonstrated in the laboratory that both pods and kernels from peanuts stored for one year were more rapidly invaded by A. *flavus* than freshly-dug immature and mature pods and kernels. In Alabama, a much higher percentage of *A. flavus* invasion occurred in overmature kernels and pods than in immature and mature kernels and pods from the same plants at harvest (26). McDonald, *et al.* (91) associated toxicity in the field with overmaturity or pods from dead haulms (stalks, stems) with kernels of low moisture content. In 1963 and 1964, peanut crops in Northern Nigeria harvested at or earlier than the normal time were free from aflatoxin, whereas late harvesting usually resulted in some toxicity (90). Where rains ended by harvest time, aflatoxin developed earlier than where rains continued after harvest. Sellschop (112) also noted that pods and kernels of peanuts that had not recovered from drought when rains came were conspicuously invaded by *A. flavus*. Although *A. flavus* apparently does not invade undamaged, immature pods in the ground, Sellschop, *et al.* (113) reported that aflatoxin contamination in commercially screened samples was concentrated in the lower grades that had a preponderance of small immature kernels. Probably these immature pods and kernels were invaded after digging. Diener and Davis (22) found that immature kernels had about the same limiting temperature and relative humidity for aflatoxin production as sound mature kernels. Thus, decreased physiological activity associated with maturity

or from low moisture in the soil environment appears to favor invasion of kernels and pods by *A. flavus* and the production of aflatoxin in kernels of such peanuts.

F. *Microbial Interaction*

A. 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. *A. flavus* or competing fungi might absorb or degrade aflatoxin following its formation in the substrate. Schroeder and Ashworth (110) concluded that microbial competition or microbial breakdown might be responsible for smaller amounts of aflatoxin in the kernels of parasite-damaged pods than in kernels from broken pods. Later, Ashworth, *et al.* (2) demonstrated that several fungi could break down aflatoxin in peanuts and in an aflatoxin-containing liquid medium. They also found that *A. niger* and *R. solani* limited the development of *A. flavus* and aflatoxin production in the substrate.

In Israel, Joffe and Lisker (70) noted that a large number of colonies of *A. niger* occurred in the geocarposphere, while penetration into kernels was proportionately moderate in light and medium soil and more common in heavy soil. There was very little penetration into kernels by *A. flavus, F. solani, P. funiculosum,* and *P. rubrum.* Positive relationship between prevalence in geocarposphere and kernels was noted only for *A. niger.* They concluded ready penetration into kernels by this fungus may have prevented penetration by other fungi. Joffe (67) also concluded that the prevalence and dominance of *A. niger* in peanut shells and kernels may explain the relatively low rate of seed transmission of other pathogens, especially *Fusarium* spp. and *S. bataticola.* In other studies (64), the relationship between the dominant fungi *A. flavus, A. niger, P. funiculosum, P. rubrum,* and *F. solani* was studied in 234 samples and 5850 plates from fresh and stored peanut kernels from 2 groundnut crop years in Israel. Consideration of the number of colonies developing in individual plates showed pronounced 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.* Jackson (51) noted that *A. flavus* invaded pods infested by *S. bataticola,* but that the reverse did not occur. In further studies (53) he showed that *A. flavus* had a pronounced depressing effect on the rate and extent of kernel infection by *S. bataticola.* On malt-extract agar, growth of *S. bataticola* was strongly inhibited by *A. flavus,* but lysis was not observed.

Pettit, *et al.* (101) identified *Bacillus subtilis* as a frequent isolate from peanut kernels from New Mexico, Oklahoma, and Texas. They noted that the growth of the bacterium from a peanut kernel prevented the development of fungal growth from that kernel. Species of *Macrophomina, Chaetomium, Choanephora,* and *Penicillium* growing from adjacent, bacteria-free kernels on the same plate were inhibited by an antifungal substance diffusing into the agar. This inhibition suggests that *B. subtilis* may play a role in the inhibition of potential pathogenic and toxicogenic fungi in the peanut kernel.

Lindsey (79) noted that under gnotobiotic conditions *A. flavus* penetrated and colonized a high percentage of shell tissues of living, attached immature and mature pods. Under natural conditions, Garren (35) and Jackson (59) found *A. flavus* in only a small percentage of shells, and Porter and Garren (103) found less *A. flavus* in the shell portion of freshly-dug pods than in the kernels contained in those shells. Either the normal endogeocarpic mycoflora of the shells is antagonistic to *A. flavus,*

which limits its colonization of shell tissue, or the faster growing components of the mycoflora masks *A. flavus* when shells were placed on agar media. In this study, high percentages of kernels (9-77%) from immature and mature pods were colonized by *A. flavus;* however, under natural conditions kernels are generally free of *A. flavus* at digging (2, 3, 26, 58, 59, 86, 88), although invasion of seeds before harvest has been reported (94, 103). Jackson (51) using surface-disinfested attached pods 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 (26-38 C). In the gnotobiotic study by Lindsey (79), there was no evidence of natural resistance to kernel invasion in living attached pods as suggested by Austwick and Ayerst (3). These results (79) suggest that the presence of normal endogeocarpic mycoflora provides a barrier to the invasion of peanut kernels by *A. flavus.*

The factors and conditions under which aflatoxin is formed by *A. flavus* in peanuts in the field, during curing, and in storage have been reviewed here. Several other genera of field and storage fungi associated with peanuts also produce mycotoxins, but none have been detected in peanut products to date. Research on aflatoxin has pointed out the rapidity and efficiency with which capable, cooperative groups of dedicated scientists in universities, USDA, FDA, and industry can join forces and solve highly complex problems of urgent public concern to ensure a wholesome food supply (74). The knowledge and scientific information reproduced in Goldblatt's book (39) represents 5-6 years of research on aflatoxin. Although an omnipresent hazard, aflatoxin has been effectively controlled in peanuts, and peanut products are the most aflatoxin-free foods available.

V. *Control of Fungal Deterioration and Mycotoxins in Peanuts*

Control of mycotoxin-producing fungi, particularly *A. flavus,* has been of vital concern to the peanut industry for the last ten years. Research has demonstrated the effectiveness of coordinated efforts and is illustrated by the rapid progress in control of aflatoxin (74). Other field and storage fungi that occur on peanuts, corn, small grains, and other oilseeds also produce mycotoxins. Procedures in this section on control probably would be equally effective against them should their mycotoxins be found in peanuts. Prevention, removal, and inactivation are three possible approaches to control of fungi and their toxic metabolites (aflatoxin) in peanuts (40).

A. *Prevention*

The best approach to control is that of prevention and the first step is recognition and awareness of the problem of quality loss in peanuts. Suppression of field and storage fungi and associated deterioration in peanuts are dependent on control of factors influencing mold growth. Invasion of the developing fruit by fungi in the soil before harvest through damage by cultivation, insects, nematodes, termites, and pathogenic fruit is presently beyond grower control. Thus, most of the deterioration caused by field fungi is an accomplished fact by digging time. After digging, control of deterioration of sound pods is primarily one of moisture control. During curing it is accomplished by promptly drying pods and kernels to a safe storage moisture level. The use of artificial drying (forced air and supplemental heat) when windrow conditions were unfavorable for rapid drying indicated that this practice reduced the possibility of aflatoxin accumulation (99). Tests for kernel moisture content are essential. Safe moisture content is that for all peanuts in the lot being stored and not the average mois-

ture content of the lot as a whole (14, 40); thus, proper aeration is required. Mechanical damage should be avoided during digging, curing, picking (combining or threshing), and handling. Subsequent storage at 70% relative humidity and moderate or reduced temperatures insures the ultimate quality of the product. Storage in gas-tight structures with controlled atmospheres of low oxygen, high nitrogen, and/or high carbon dioxide has resulted primarily in insect control, but some effects on fungi in peanuts under such conditions have been evaluated (61, 78, 108). Harvesting, drying, and storage conditions for preventing molds in farm commodities have been recommended by the United States Department of Agriculture (117).

Much research on fungal deterioration in peanuts has been stimulated by the aflatoxin problem. One logical approach that has been explored is control of fungi by soil and windrow application of fungicides as measured by the development of *A. flavus* and aflatoxin. Bell and Doupnik (7) evaluated several food and feed preservatives or stabilizers and fungicides for prevention or reduction of *A. flavus* and/or aflatoxin accumulation in laboratory experiments. No fungi were isolated from the potassium azide (10%) treatment and no *A. flavus* was isolated from the boracic acid (10%) treatment. Aflatoxins were not isolated from these and six other treatments. Jackson (54) evaluated 18 fungicides for control of 13 fungi isolated from peanuts. Difolatan, DuTer, sodium propionate, and potassium sorbate gave some control of fungi in pods and kernels when used as dusts on unshelled peanuts. Wales and Somers (118) tested 23 fungicides for fungistatic activity against 3 strains of *A. flavus,* two of which were vigorous aflatoxin producers and one which did not form the toxin. With only 3 exceptions, no difference among the 3 strains in susceptibility to a given fungicide was found. The two most effective fungistats were dichlofluanid and Difolatan; only the latter showed fungicidal activity.

The efficacy of several fungicides was tested by spraying peanuts in the windrow in 1965 and 1966 (57). Difolatan and tribasic copper sulfate reduced populations of pod-surface fungi. Kernels from these treatments also tended to have fewer invading fungi than unsprayed controls. However, aflatoxin levels of kernels from slowly-dried pods from these fungicide treatments were not reduced; thus, development of *A. flavus* was not inhibited. Other attempts by Jackson (55, 56, 57) to reduce soil and pod-surface mycoflora by using volatile and non-volatile fungicides as pre-planting soil treatments in Georgia showed little promise. Similarly, Barnes (5) found no differences in fungal populations between soil fungicide treatments, irrigation, and peanut cultivars. Results obtained by Joffe and Lisker (69) were similar except that they recorded depressions in soil populations of *A. flavus* by tetrachloroisophthalonitrile and potassium sorbate. They found that *A. niger* was relatively frequent and *F. solani* scarce in kernels from soil treated with pentachloronitrobenzene at seedling emergence, while the reverse was true when soil was treated 7 weeks after sowing or 4 weeks before harvest. Pettit, *et al.* (102) evaluated the influence of fungicides and irrigation practices on fungal mycoflora and aflatoxin production from 1967-1969 in Texas. Statistical analyses of their data revealed no significant differences in degree of fungal infestation, peanut yields, and grade factors between six soil fungicides applied at planting in combination with five foliar fungicides as compared to checks.

To date, results of research with soil, foliar, or windrow application of fungicides have offered little promise that populations of peanut pod and kernel fungi can be greatly reduced in the field.

A practical approach to prevention of fungal growth in peanuts and associated

mycotoxin production would be the utilization of the natural genetic resistance of plants. Rao and Tulpule (106) evaluated 60 different varieties of groundnuts in India and reported that one U. S. 26 (P.I. 246388, a variety with white testa), supported no aflatoxin production. Kulkarni, *et al.* (76) reported that a red seeded variety, Asiriya Mwitunde, supported only moderate aflatoxin production. Doupnik (29) tested two sources of U. S. 26 and two Asiriya Mwitunde cultivars with two strains of aflatoxin-producing *A. flavus* cultures and could not confirm the Indian work. All peanut cultivars supported aflatoxin production, although one strain of U. S. 26 was noticeably lower in aflatoxin production than Starr (Spanish) and the other cultivars in the experiment. Research is continuing but to date germplasm imparting clear-cut resistance to specific fungi has not been found.

Another approach to prevention is the elimination of aflatoxin-contaminated commodities from food and feed marketing channels (40). This and related efforts have been described (28, 74). In 1967, Dickens and Welty (17) reported a high correlation between aflatoxin in farmers' stock peanuts and the presence in defective kernels of mold that could be identified visually with a low-power microscope as *A. flavus*. They concluded that this was a simple and effective method for detecting peanuts that might contain large amounts of aflatoxin. The 1968 Peanut Marketing Agreement stipulated that every lot of peanuts be examined at the buying station by an inspector trained to identify the fungus; any lots containing any *A. flavus* would be restricted to non-edible uses.

B. *Removal*

Goldblatt (40) distinguished between removal by separation of contaminated kernels, and removal of aflatoxin by extraction from contaminated kernels. It has been demonstrated that aflatoxin levels are correlated with the proportion of broken shells in the lot and the number of shrivelled, rancid, or discolored kernels. When the latter are discarded, the remaining high quality peanuts are relatively free of aflatoxin. The vast majority of aflatoxin in contaminated seeds resides in a relatively small number of kernels. Culling of these seeds is accomplished by screening for size at shelling plants, by removing discolored kernels manually on picking tables, and by utilizing various mechanical or electronic sorting devices which pass or reject each kernel on the basis of color when scanned by a photoelectric cell (28, 40). Another procedure of potential value is air classification, which is based on the principle that lighter nuts have been invaded by *A. flavus* and are contaminated with aflatoxin. Experimental results indicated that air separation could be a useful tool for reducing aflatoxin contamination in peanuts (40).

Research on removal of aflatoxin from seeds by extraction with solvents has been reviewed by Goldblatt (40) and Dollear (28). Removal of aflatoxin from peanut oil is readily accomplished with sodium hydroxide and bleaching earth. Extraction with solvents offers some possibilities for removing aflatoxins from oilseed meals. Essentially, aflatoxin can be completely removed with little chance of forming other products of high biological activity. After extraction the solvent can be recovered without appreciable reduction in nutritional quality of the protein. However, extraction requires special equipment plus the added cost of additional processing. Furthermore, some of the meal or feed carbohydrates are removed with the aflatoxins.

C. *Inactivation*

Treatments for degrading, destroying, or inactivating aflatoxin by heat, chemical, or biological methods must not impair nutritive value of the material nor leave any

deleterious residues (40). Dollear (28) concluded that moisture is required for aflatoxin destruction by heat, but that cooking did not reduce the aflatoxin content of contaminated cottonseed or of peanut meals to levels acceptable for feed use. Prolonged heating or cooking may affect protein quality or lysine availability (28). Waltking (119) reported that pilot-plant roasting of contaminated peanuts reduced aflatoxin B_1 levels by 40-50%. Aflatoxin in peanut butters remained constant after three months of storage at 24 C. These data concur with the findings of others (28, 40). Effects of gamma radiation on aflatoxin have been investigated (30); gamma rays did not significantly degrade aflatoxin in peanut meal.

Research on detoxification of aflatoxins in foods and feeds was first summarized by Feuell (30). Chemical inactivation of aflatoxins by ammonia, methylamine, sodium hydroxide, hydrogen peroxide, ozone and irradiation has also been reviewed (28). Mann, *et al.* (81) later reported that ammonia, methylamine, sodium hydroxide, and formaldehyde reduced aflatoxin levels and appeared practical for large scale treatments. They noted that ammoniation yielded a product of very low aflatoxin content in the shortest time at the lowest temperature and with only moderate alteration in meal properties; the only drawback being the requirement of a pressurized reactor. Methylamine and sodium hydroxide treatments do not require pressurized equipment. Gardner, *et al.* (33) found that ammoniation of aflatoxin-contaminated peanut meal (121 ppb) reduced aflatoxin to undetectable levels. Similar results were obtained with cottonseed meal. Large-scale feeding studies are in progress to evaluate the ammoniated meals.

Technically, it is feasible to reduce the aflatoxin content of contaminated peanut products to well below 30 ppb (28, 40). Separation by hand picking, electronic sorting, and/or air classification can concentrate the vast majority of aflatoxin-contaminated kernels into relatively small fractions. Further improvement in methods of physical separation should be sought. Extraction with certain solvents to achieve essentially complete removal of aflatoxins also is technically feasible (40). Heat, alone, is relatively ineffective, but simple roasting such as for peanut butter results in significant reduction in aflatoxin content and affords a certain margin of safety for such products. Treatment with *Flavobacterium aurantiacum* removes aflatoxin from solution (28) and it and hydrogen peroxide may be useful for elimination of aflatoxin from beverages (40). Chemicals such as ammonia can destroy aflatoxin with relatively little damage to protein quality. Additional research should identify optimum conditions for nearly complete elimination of aflatoxins with minimal damage to protein quality. However, the breakdown products formed from aflatoxin destruction must be evaluated for their toxicity.

VI. Summary

Despite thousands of plant diseases and species of fungi associated with food materials, relatively few have proved to be toxic to man and animals. Many species of fungi contribute to the welfare of mankind in destroying organic waste, formation of mycorhizae, alcohol and organic acid production by fermentation, direct utilization as food (mushrooms), food production such as cheese and bread making, livestock feed as fermentation byproducts, antibiotics, medicinal compounds such as vitamins, and chemical synthesis of enzymes, glycerol, and fats. These benefits are in opposition to harmful activities of fungi such as plant diseases, decay of timber, tropical deterioration of textiles, food spoilage, allergens, and toxin-production (19).

Fungi attack peanut pods and kernels while they are developing in the soil before harvest and after lifting during curing and in storage, whenever environmental

conditions are favorable for their growth and development. The moisture content of the pod and kernel, the physical condition of the pod, the quality, viability, and physiological condition of the kernel, the ambient temperature, the period of time peanut pods remain in the soil beyond maturity, the conditions for drying during curing in windrow or stack, the length of time and aeration in storage, and the activity of insects are the principal factors that determine the degree of proliferation of fungal growth in peanuts. The particular fungus species that develops in a given environment depends on moisture, temperature, presence of competing microorganisms, and the nature and physiological state of the peanut pod and/or kernel. These and other factors still unknown govern the metabolism of a fungus and its capacity for utilization of peanut pod and kernel growth and the production of metabolites.

The nature of deterioration of peanuts by field fungi has not been thoroughly investigated except where pathogenesis is involved before or at digging. What effects (if any) field fungi that do not penetrate the pod and invade the kernel have on pod and kernel development, seed viability, or ultimately on kernel quality for edible uses are unknown.

Research on the deterioration of peanuts by storage fungi has given results that parallel findings with cereals and other oilseeds (14, 15). Activity of storage fungi is closely related to temperature and kernel moisture content and/or the RH of the atmosphere around pod or kernel in storage. Storage fungi are important in stored food materials because they grow at relatively low RH, fairly high temperatures, and are universally present in air and soil. They are not isolated on many common media, but requires a medium of high osmotic tension. Although there is considerable data on factors affecting germination of peanuts (9, 72), the effect of storage fungi on seed viability has not been clearly defined. Johnson and Gilliland (72) noted that the percentage of germination of peanuts after they are removed from storage depends primarily on the kernel moisture of the peanuts when stored, the temperature of the storage environment, and the length of storage period. This is a precise definition of the factors that are most significant in the development of storage fungi. There is little data on their effect (if any) on seed viability when stored at slightly above safe storage moisture levels. Temperature and relative humidity are constantly fluctuating in peanut storage and these changes affect kernel moisture levels that may result in mold proliferation. There is a paucity of data on the effect of storage fungi, individually or collectively, on peanut kernel quality when stored at suboptimal conditions for extended periods. The ability of fungi to metabolize peanut carbohydrates and oil for mold growth and to cause other types of quality deterioration in peanut kernels has been clearly demonstrated (20, 120). The rate and degree of deterioration at less than optimal environments or in competition with other fungi of the mycoflora are open avenues for investigation. Thus, storage fungi adversely affect seed viability, storage quality, nutritive value, and edibility.

The mycotoxin problem has been with us for hundreds of years. Since this chapter deals with fungi in peanuts, the research on aflatoxin in peanuts was also reviewed. Aflatoxin is primarily a mold problem, not just a peanut problem. Practically all agricultural commodities and foods will support the development of aflatoxin, if conditions are favorable for the growth of *A. flavus*. Such environments will also be favorable for the growth of many competing microorganisms. Their interaction or antagonism toward *A. flavus* may be a factor in reducing the level of aflatoxin in any or all natural substrates. This is still an area for fruitful research.

Control of fungal deterioration and mycotoxins of peanuts is primarily an after harvest problem of control of the factors influencing fungal growth. These procedures are not unlike those for controlling fungal spoilage of many crops in storage (42, 117). In the field the grower must harvest peanuts at maturity and follow curing and drying techniques to rapidly reduce kernel moistures to safe levels for storage. Utilize modern drying methods. Store cleaned and sound seed in dry aerated, insect-free storage facilities. Foreign matter, soil or plant debris, damaged kernels, and immature kernels may affect aeration and moisture availability unfavorably. Food and feed processors should determine aflatoxin presence by chemical or other procedures (17) before products are processed. Removal by separation of toxin-contaminated kernels and by aflatoxin extraction from toxic kernels with solvents is considered technically feasible (28, 40). Chemical and inactivation by heat, ammonia, methylamine, sodium hydroxide, and other methods appear near reality. Additional research should accomplish nearly complete elimination of aflatoxins with minimal damage to protein (40). The real effect of fungal toxins in peanuts and peanut products is the economic losses involved with chemical testing, segregation of contaminated lots (17), elimination of contaminated lots for edible uses (17), and additional handling and processing to separate toxic kernels or to extract or inactivate aflatoxin chemically (40).

As a result of the threat of aflatoxin, all segments of the peanut industry united to deal with a common problem. There has been more research on the relation of fungi to peanuts in last 6-8 years than in the previous 30. A portfolio of data has developed on the species and populations of fungi in peanut soils, and associated with pods and kernels before maturity, at digging, and during curing and storage. New developments in windrowing, curing, and artificial drying of peanuts to prevent mold growth have been stimulated. Control with fungicides and by detoxification has been extensive and promises much for the future. As noted by Goldblatt (40),

> "the peanut industry has done an outstandingly effective job in safeguarding from the danger of aflatoxin and, paradoxical as it may seem, the American public is now getting higher quality peanuts and peanut products than ever before".

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