Aflatoxins: Environmental Factors Governing Occurrence in Spanish Peanuts

Abstract. *Aflatoxins are absent from freshly harvested peanuts although* Aspergillus flavus *infest most of the kernels from pods having visible openings. Microbial competition, governed by kernel moisture, limits aflatoxin content of kernels. The toxins are subject to microbial breakdown but the amount broken down is governed by initial aflatoxin concentration.*

The common saprophytic mold *Aspergillus flavus* Link produces four similar toxic substances, called aflatoxins, during its normal course of development. Aflatoxins are important because of their extreme toxicity to mammals and their carcinogenic action (*1*). They present a hazard to livestock and are recognized as a potential threat to human life. Under ordinary field conditions, aflatoxins of peanut (*Arachis hypogaea* L.) are associated with kernels from pods having visible openings (*2–4*). Visibly damaged kernels from these broken pods contain a large amount of aflatoxin (*4, 5*). Undamaged kernels from broken pods contain less toxin than damaged kernels, and no toxin occurs in kernels from undamaged pods (*4*). Schroeder and Ashworth found that kernels from parasite-damaged pods had smaller amounts of aflatoxins than kernels from broken pods (*4*) and concluded that microbial competition or microbial breakdown of toxins might be responsible for these observations. We report here experiments on the role of microbial competition, as influenced by kernel moisture, on development of aflatoxins, and on the role of the microflora in the peanut pod and kernels on the breakdown of aflatoxins.

Peanuts used in these experiments were collected in Erath and Waller counties, Texas. The general procedures have been described (*2, 4*) for determining damage in pods and kernels, molds in kernels, and aflatoxins. To determine aflatoxins at the time of harvest, the peanuts were shelled as they were removed from the soil, and kernels were placed directly into the extracting solvent, methanol. To facilitate aseptic transfer of kernels from peanuts of maximum moisture content (38 to 43 percent) for determination of the fungal flora, intact pods were dried (4 hours at 45°C). In these cases malt-salt agar (*6*) a selective medium for culturing *Aspergillus* and *Penicillium* species, and acidified (*p*H 5.5) potato dextrose agar, selective for other soil fungi, were used. Only malt-salt agar was used for the other samples. Samples were also dried to various moisture contents and incubated for 10 days at 30°C in polyethylene bags plugged with glass wool, and aflatoxin analyses were made. The moisture contents are expressed on a wet-weight basis and were determined at the time samples were incubated by drying 30- to 50-g samples for 6 hours at 130°C. Duplicate samples were analyzed for aflatoxins, and mold counts were based on isolations from 100 seeds.

Common fungi that invade peanut pods were tested for their ability to grow in competition with *Aspergillus flavus* on peanuts sterilized in moist steam. Fifty-gram samples (air-dry weight) were inoculated with 2 ml of a suspension of spores and mycelial fragments. Single and double (two species) inoculations were made at the start, and, when appropriate, challenge inoculations were made 48 hours later. The cultures were analyzed for aflatoxins after 10 days at 30°C. Several fungi commonly found in peanut pods and kernels also were tested for their ability to destroy aflatoxins. In one test, peanuts infested with *A. flavus* were sterilized, and 5-g samples which were placed in liquid medium (*7*) were inoculated with each of several fungi. In another test, 50 ml of the liquid medium, containing crystalline aflatoxin B1, were inoculated with test fungi.

The parasitic fungus *Rhizoctonia solani* Kuhn was responsible for most pod damage whereas a lesser amount of damage was caused by mechanical breaks. Few kernels from unbroken pods were discolored (2 to 6 percent), and no *Aspergillus flavus* and few other fungi (1 to 4 percent) were isolated at time of harvest. On the other hand, all kernels from broken pods were discolored, and *A. flavus* was common, being isolated from 58 to 74 percent of the kernels. Eleven kernel samples from

Table 1. Influence of competition between *Aspergillus flavus* and other fungi upon development of aflatoxins in inoculated peanut kernels.

Inocula		Toxin after 10 days (ppm)
Initial	Challenging after 48 hr	
None	None	0
A. flavus	None	133
A. niger	None	0
Rhizoctonia solani	None	0
A. flavus + *A. niger*	None	12
A. flavus	*A. niger*	96
A. niger	*A. flavus*	0
A. flavus + *R. solani*	None	133
A. flavus	*R. solani*	114
R. solani	*A. flavus*	29

Table 2. Aflatoxins present (ppm) in an aflatoxin-containing liquid substrate (0.5 and 1.0 ppm of aflatoxin B1) and in toxin-containing peanuts 10 days after inoculation with fungi that invade peanut pods and kernels.

Fungi *	Liquid		Peanuts
	0.5 ppm	1.0 ppm	
None	0.3	0.6	114
A. niger	.0	.0	68
R. solani	.1	.2	71
M. phaseoli	.0	.1	43
F. roseum	.1	.2	91

* Respectively noninoculated, *A. niger*, *R. solani*, *M. phaseoli*, and *F. roseum*.

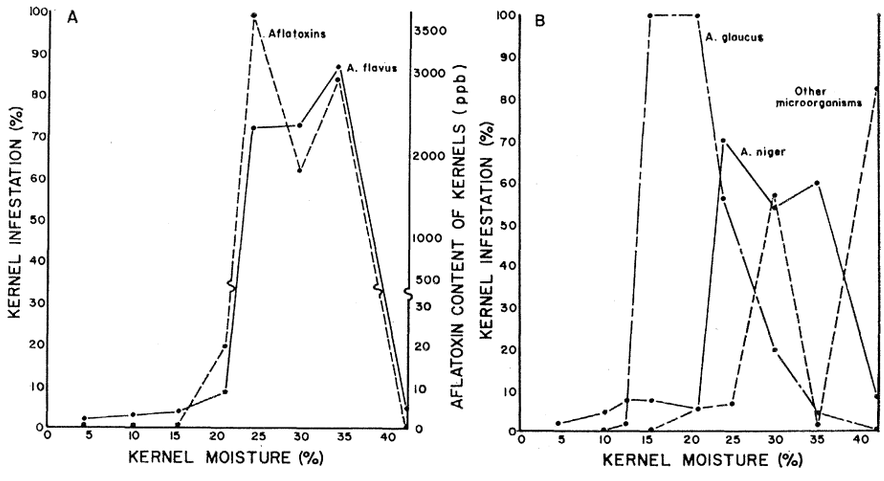

Fig. 1. *A*, Gross influence of kernel moisture on development of *Aspergillus flavus* and aflatoxins; *B*, influence on other microorganisms.

unbroken pods and ten samples from broken pods had no aflatoxins when harvested, an indication that pod and kernel damage are unimportant at that time and that toxins accumulate after harvest.

Determinations were made for the gross influence of kernel moisture on development of *A. flavus* and aflatoxins and on the development of the microorganisms (Fig. 1). Each point of these curves is a mean for two to five samples having similar moisture contents. Samples were collected from three locations. Aflatoxin concentrations were high where *A. flavus* was abundant, that is, when the moisture content was 23 to 34 percent; but at lower or higher moisture contents where *A. flavus* grew scantily there was little aflatoxin. The fungus was visible with the naked eye on 1 to 6 percent of the kernels that had a high aflatoxin concentration. But growth of the fungus was not visible in this way when aflatoxin concentration was low or absent. About 82 percent of the kernels with the highest moisture contents (38 to 43 percent) were invaded within 10 days by microorganisms. Unidentified bacteria and two fungi, *Rhizopus nigricans* Ehr. and *Fusarium oxysporum* (Schl.) Snyd. and Hans., were most prevalent. Two other fungi, *F. roseum* (Lk.) Snyd. and Hans. and *Macrophomina phaseoli* (Maubl.) Ashby, were less prevalent. Few organisms except *Aspergillus flavus* (Fig. 1) and *A. niger* van Tiegh. (Fig. 1) were isolated at 35 percent kernel moisture. Curves for these two fungi were parallel, although *A. flavus* was always more common than *A. niger*. A mucoraceous fungus accounted for nearly all of the "other" group in kernels having 30 percent moisture; species of *Penicillium* accounted for nearly all of the invaders in the "other" group at moisture contents below 30 percent. *Aspergillus glaucus* Link was the most prevalent fungus in kernels having 15 to 21 percent moisture, and kernels with less than 12 percent moisture were nearly free from fungus invasion.

The development of *Aspergillus flavus* and aflatoxin is regulated by competition with other fungi that invade the kernel (Table 1). *Aspergillus niger* was more competitive than *Rhizoctonia solani*, but both fungi limited development of the *A. flavus* when they were allowed to grow on peanut kernels for 48 hours prior to a challenging inoculation of *A. flavus*. Other experiments were made to determine whether the results of this experiment were due solely to competition or whether the common fungi that invade the peanut pods and kernels also might destroy aflatoxins. Results of these experiments (Table 2) indicate that the toxin is subject to fungal breakdown, although the amount broken down is related to initial concentration of aflatoxin.

Our results agree with results of McDonald and Harkness (*3*) which indicate that aflatoxins are rarely found in peanuts at the time of harvest. Our findings indicate that the mold growing on peanut kernel is analogous to the growth of molds in stored grains (*6*). Aflatoxins appear when kernel moisture and competitive factors favor development of *A. flavus* over other microflora on the kernel. The fungus apparently has a competitive advantage in broken pods, and it becomes established more rapidly than in pods with lesions inhabited by several competitors (*2, 4*). The growth of *A. niger*, which resulted in destruction of aflatoxins in culture experiments, and that of *A. flavus* were greatest at the same moisture contents. However, the data indicate (Fig. 1) that *A. flavus* always had a competitive advantage. Large amounts of aflatoxins are most likely to be elaborated when the kernel moisture is between 23 and 34 percent. Our results agree with those of McDonald and Harkness (*3*) who showed that aflatoxins can be essentially eliminated if peanuts are cured in mechanical driers. However, the lower limit of kernel moisture required for aflatoxin development must be determined exactly before recommendations for moisture contents that will prevent the formation of aflatoxin can be made with confidence.

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Lysis of Pleuropneumonia-like Organisms by Staphylococcal and Streptococcal Toxins

Abstract. *Six strains representing three species of* Mycoplasma *were examined for susceptibility to lysis by staphylococcal and streptococcal toxins. All were sensitive to staphylococcal α-toxin, two to streptolysin S, and three to streptolysin O. The results support the concept that the limiting membrane of pleuropneumonia-like organisms is basically similar to those of many other cell types and provide additional evidence for the participation of cholesterol in cytolysis induced by streptolysin O.*

Protoplasts and spheroplasts of certain bacterial species undergo lysis on exposure to staphylococcal α-toxin or streptolysin S, agents which were thought earlier to act principally or exclusively on mammalian cells (*1*). These and other observations indicate that the plasma membrane is the site of action of the lytic staphylococcal and streptococcal proteins. If the membranes that enclose pleuropneumonia-like organisms (PPLO or *Mycoplasma*) are similar to those of bacteria and other cells, and there is substantial evidence that they are (*2*), then it seemed likely that PPLO would also prove susceptible to lysis by α-toxin, streptolysin S, or streptolysin O, or to some combination of them.

Parasitic strains of *Mycoplasma* (*M. gallisepticum* and *M. neurolyticum*) were cultivated in Chanock's medium (*3*) in the absence of agar and antibiotics; saprophytic strains (*M. laidlawii*) were cultivated in modified Edward medium (*4*), usually without addition of serum. The cultures (10 to 50 ml) were centrifuged at 25,000*g*