FIELD OBSERVATIONS ON CONDITIONS CONDUCIVE TO THE CONTAMINATION OF GROUNDNUTS WITH THE MOULD *ASPERGILLUS FLAVUS* Link ex Fries

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During the past 5 years the fungus *Aspergillus flavus* has caused concern to groundnut producers, processors and health authorities in different parts of the world including South Africa. It flourishes in the soil, and can occur in groundnut (*Arachis hypogaea* L.) pods and kernels before and after they come out of the ground. By means of their mycelial threads some genera of fungi may invade the forming groundnut pods and kernels as they develop in the soil. Nearly all the groundnut flowers are formed and pollinated cleistogamously above ground, produce long peduncles or fruiting stalks which direct the young pods at their tips one to three inches deep into the soil. Norton *et al.*[12] have shown that by allowing the fungi to grow out of unblemished, surface-sterilized groundnuts by the culture media plating method, an average of about 22·2% of the pods became invaded by fungi over a 6-week period before they had been taken out of the ground. McDonald and Harkness[9] and others, have shown that *A. flavus* and other fungi could be cultured from aseptically-handled groundnut kernels. The extent to which the kernels become infected with one or more genera, species or strains of fungi depends largely on temperature, humidity and other conditions. As long as the fungus threads (mycelia) and spores in and on the pods and kernels remain dormant, and adequate conditions for their growth do not set in, the groundnuts remain free from moulds.

It should not be assumed that the unseen harbouring of mycelia of *Aspergillus* or any other fungus in the immature, undug seeds is a peculiarity of the groundnut. It is common in cereals too. Christensen[3] showed that the *living* mycelium beneath the pericarp (bran) of low-grade wheat was that of *Aspergillus* and *Penicillium* species known to be involved in the deterioration of stored grain.

The fungi most frequently associated with groundnuts before and after they come out of the ground, are common saprophytes. Uniformity between fields in respect of fungus flora is not to be expected (Garren and Higgins[7]). *Diplodia theobromae* was reported as universally present in peanut soils in southern Alabama.[13] *A. flavus* and species of *Fusarium* and *Alternaria* were freely isolated from groundnuts in Texas (Norton *et al.*[12]). The predominant fungi of South African groundnuts are *A. niger*, *A. flavus* and *Penicillium citrinum*.[15]

As with cereals, once the kernels are out of their shells, aerial contamination by means of fungus spores is also possible, and particularly so if the kernels are nicked, bruised or wrinkled to the extent of readily retaining the spores. McDonald and Harkness[9] found that increasing numbers of *A. flavus* and other fungi could be cultured from groundnut kernels from (*a*) unblemished, (*b*) termite-damaged and (*c*) broken pods.

OBSERVATIONS AND DISCUSSIONS

The sudden emergence of the aflatoxin trouble in a part of South Africa during 1963 was generally ascribed to unseasonal winter rains on the inadequately protected stacks, stooks or cocks in which the groundnut plants have to dry out sufficiently before the pods can be separated from the haulms by mechanical means. Producers who had experienced late rains in isolated former seasons were loath to accept that untimely rains had encouraged *A. flavus* growth to the extent that an aflatoxin problem ensued. Nevertheless, long periods of intermittent rain on carelessly built cocks, and open windrows, do stimulate an abundant mould growth. As a contribution to the solving of the aflatoxin difficulty Blatchford and Hall[2] summarized the various natural and artificial methods of drying groundnuts in order to indicate possible ways of preventing the development of *A. flavus* from the time the nuts are lifted until they are shelled.

From the time the 1963 - 64 crop was planted, all parties involved kept an ever-vigilant eye on any condition that might be a precursor or abettor in aflatoxin production.

Termites

(a) *The Large Fungus Growers.* Mr. N. P. J. Kriek, Chief Inspector of the Oilseeds Control Board, found in the vicinity of Marken, in the Potgietersrus district of the Northern Transvaal, that Large Fungus Grower Termites (*Odontotermes latericius* Hav.) played havoc with maturing groundnut pods and kernels in the ground on plants of which the haulms remained lush and green. The destruction caused by *O. latericius* and also *O. badius* varied from the puncturing of one pod per plant at one point, with light gouging of the kernels, to the sloughing away of pods, with whole kernels or portions of kernels remaining as fungal masses. Identifications by specialists confirmed that the termites and *A. flavus* were the principal invaders of the affected groundnuts in the areas mentioned.

According to the examinations made by P. M. le Roux (private communication) the termites of Marken are generally well-covered with spores of *A. flavus* and other fungi, and plating tests of sterilized *O. latericius* showed them to be loaded with spores about their mouth parts. Insects as disseminators of spores of the *A. glaucus* group and other groups have been reported on by Agrawal, Christensen and Hodson[1] and others.

So great is the destruction of the groundnut pods by the *Odontotermes,* and so luxuriant the fungal growth in the groundnuts they attack in the soil, that it negates the conclusion arrived at by McDonald and Fatmi[8] from their investigations that *A. flavus* does not attack the groundnut kernels while the pods are still in the ground.

The Fungus Grower Termites are to be studied assiduously. Interesting information on fungus growing by the *Odontotermes* is already forthcoming from the colonies established at the University of Pretoria.

There are reasons for the fact that the termites have contributed so largely to the aflatoxin problem in one area. Coaton[5,6] has pointed out that in the Large Fungus Grower group of termites there are species which sometimes destroy crops including groundnuts. In the veld they feed on dead wood, leaves and other material containing cellulose, but in cultivated areas they are frequently forced to attack living plant tissues owing to the absence of a sufficient quantity of their natural food material when crop production is undertaken.

It should not be inferred that the *Odontotermes* are in such regular abundance throughout the area they inhabit, and so short of food everywhere, that they are a general menace to groundnut crops. There are indications that they could create difficulties in the Bushveld areas where the rainfall is less than 20 inches per annum. This is deduced from observations in groundnut fields in infested and *Odontotermes*-free areas.

It is fortunate indeed that it is now known where special measures can be applied further to reduce aflatoxin-contamination.

(b) *Other termites.* The termites responsible for the pitting of the pods found under groundnut stooks in the vicinity of Klerksdorp were identified as *Macrotermes natalensis.* A few of their pittings were deep enough to form apertures through which moisture, heat and infectious matter could find their way into the pods to create conditions suitable for mould growth. Moses and Sellschop,[11] McDonald and Harkness[9] and others have reported that termites damage groundnuts. Their illustrations generally showed that mainly the pod surfaces had been rasped to various depths. It can be expected that the abrasions on the pods will predispose them to decay by soil organisms.

To avoid attacks, the termites will have to be appeased with adequate quantities of organic material. Coaton[6] says: 'In lands on which susceptible crops are being grown and where clean cultivation is necessary, every endeavour should be made to increase on and in the soil, the quantity of dead matter containing cellulose. Severe attacks on growing plants have been alleviated almost immediately by mulching with e.g. maize cobs and old hay. Damage by termites can be countered by colony eradication carried out preferably before the soil is broken by cultivation.'

Pod-puncturing Insects

Holed groundnut pods came in for frequent examination.

(i) *Cockchafers.* Except for an isolated kernel fraction, the pods suspected of having been holed by cockchafers (*Schizonycha africana* Cast.)[14] were entirely free from portions of kernels. Such material could be expected of being the seats of fungal infection.

(ii) *Bollworms.* Immediately after the plants of 1964 test plots of the Agricultural Research Institute, Potchefstroom, had been packed into cocks, larvae of the bollworm (*Heliothis armigera* Hübn.) were found to be gnawing their way through the pods to the kernels. In the gnawings on the kernels *Penicillium* and *Aspergillus* growth could be seen. Bollworm larvae usually feed on maize, cotton and groundnut foliage.

Several workers[4] have shown that *H. armigera* Hübn. facilitate the entrance of moulds and ear rots in maize.

Physiological Conditions

(i) *Burst pods.* Readily perceptible fungus growth was found at Potchefstroom to have set in on kernels when their pods had burst in the soil. Bursting of the pods takes place when the moisture content of the soil and other conditions fluctuate considerably as the result of alternating drought and short rainy periods. The pods possibly do not resume their growth fast enough after drought periods to provide sufficient space for the faster developing kernels, hence the bulging out of the kernels. The fungal growth in the sutures on the pods, on the kernels and on the inside surfaces of pods were found to be mainly that of *Penicillium.* In a few instances *Trichoderma* could be found. *A. flavus* was observed in the suture of one pod.

(ii) *Destructive drought followed by rain.* Groundnuts received from 3 centres where March rains had followed a severe February drought, showed conspicuous *A. flavus* conidiophores on the inside of many slightly cracked and whole pods, and conidiophores and conidiospores on the more shrivelled kernels they contained. At all three centres, namely Warmbaths (Tvl.), Winterton and Tugela Ferry, Natal, much of the haulm growth had succumbed to drought and intense heat during February 1964. The small amounts of plants from the cooperative variety trial conducted at the three centres could be rapidly dried after they had come out of the ground. Hence, it may be accepted that most of the profuse *Aspergillus* contamination had occurred while the plants were still in the ground

during the rains that had followed the extremely low rainfall period. No obvious *Aspergillus* growth could be seen on material received from Vaalharts (Cape), Glen and Theunissen (O.F.S.) and that produced at Potchefstroom, where irrigation was resorted to. Groundnuts are generally planted in November and lifted at the beginning of April.

Towoomba Experiment Station—Warmbaths. (a) The aflatoxin content in the large kernels taken off with a 17/64 in. x 1/4 in. slotted screen varied from 0·5 - 1·0 ppm; (b) that in the kernels which had passed through the foregoing screen came to 1 - 3 ppm, while the aflatoxin content of the mouldy kernels ranged from 25 - 512 ppm. The aflatoxin determinations were made on eight varieties of groundnuts.

The following figures show how the rainfall had suddenly dropped towards the end of the 1963/64 season:

	Rainfall	
Month	*Millimetres*	*Inches*
November 1963	60·50	2·42
December 1963	133·50	5·34
January 1964	159·60	6·98
February 1964	23·00	0·92
March 1964	32·50	1·34

Winterton. The Winterton rainfall was as follows:

	Rainfall	
Month	*Millimetres*	*Inches*
November 1963	140·3	5·6
December 1963	65·7	2·66
January 1964	131·7	5·26
1st 20 days, February 1964	18·4	0·83
5 days during February 1964	51·1	2·04
March 1964	44·2	1·66

Tugela Ferry. At the Tugela Ferry the cooperative groundnut trial became drought-stricken owing to the main irrigation furrow going out of commission.

The comparison made here is between groundnuts dug at (*a*) 4 centres, where drought conditions did not occur, and (*b*) 3 centres, where the crops had to be lifted on account of the ill-effects of drought and heat. It may be concluded that the moribund material was far more prone to fungal attack than the live material lifted at those centres where irrigation could be resorted to. McDonald and Harkness[10] have indicated that *A. flavus* does not readily grow on actively-living tissue, but flourishes on dead or dormant groundnuts.

The experience gained from the groundnuts harvested at the drought-affected centres seem to indicate that the dead material can already be contaminated before harvesting takes place. *A. flavus* growth is, therefore, not necessarily a post-lifting or -digging phenomenon.

OPSOMMING

Waarnemings in verband met toestande wat bevorderlik is vir besoedeling van grondbone met die swam *Aspergillus flavus* Link ex Fries:

Aanvanklik is die ontwikkeling van aflatoksien in 'n gedeelte van die grondbone-oes van 1963 aan oorvloedige, ontydige reëns op onvoldoende-beskermde miedens toegeskryf. Waarnemings wat gedurende die volgende seisoen gemaak is, het aangedui dat die Groot Swamkwekertermiete (*Odontotermes latericius* en *O. badius*) verantwoordelik is vir die vernieling en besoedeling met die *Aspergillus flavus* swam, van die rypwordende peule en bone. Dit is gevind dat larwes van die bolwurm (*Heliothis armigera*) die peule binne boor en die bone vreet onmiddellik nadat die plante uit die grond kom en begin verwelk. As gevolg hiervan vind swambesmetting plaas. Peule wat weens veranderlike vogtoestande in die grond gebars het, het in die barste skuilplek vir skimmelontwikkeling verskaf.

Op die dooie peule en bone van oeste wat nie herstel het van die droogte wat deur reëns gevolg is nie, het opvallende bewyse van *A. flavus*-ontwikkeling getoon.

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