DISTRIBUTION AND DEGREE OF OCCURRENCE OF AFLATOXIN IN GROUNDNUTS AND GROUNDNUT PRODUCTS

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Under appropriate conditions, certain strains of the fungus *Aspergillus flavus* produce a toxin when they grow on cereals, legumes or other organic matter. The name 'aflatoxin' conveys that it is the toxin of *A. flavus*. Toxin production is not peculiar to *A. flavus*. Other species of Aspergillus as well as other genera of fungi also produce toxins under required conditions.

When a problem assumes abnormal proportions efforts are generally made to determine the extent to which different localities are affected. Hence, when certain classes of livestock died during 1963 as the result of ingesting aflatoxin-contaminated groundnuts, it became necessary to determine where harmful nuts had been delivered to the agents of the Oilseeds Control Board. The Jansen Commission on aflatoxin therefore recommended at its first meeting that emergency facilities for aflatoxin determinations be provided in the laboratories of the Oilseeds Control Board. The purpose of this contribution to the Symposium on Mycotoxins in Foodstuffs is to report on:

(a) the nature and distribution of the aflatoxin-contaminated groundnuts and groundnut products examined in the crisis laboratory during 1963;

(b) the aflatoxin survey carried out by the officers of the Oilseeds Control Board during 1964, and

(c) the steps taken to reduce the acceptance of aflatoxin-contaminated groundnuts to an absolute minimum.

PROCEDURE

Sampling

Representative samples of approximately 2 lb. each, and subsequently 5 lb. samples, were drawn by members of the grading staff of the Oilseeds Control Board from individual stacks of bagged, shelled groundnuts held on the premises of the 18 agents of the Board. Other samples of groundnuts, oilcake and peanut butter were obtained for examination from various sources. Over 1,000 samples of groundnuts and groundnut products were examined during 1963 and another 1,000 samples of producers' grades were dealt with during 1964.

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Aflatoxin Determinations

Appropriate sub-samples were employed for the aflatoxin determinations. At the outset the method of Broadbent, Cornelius and Shone[2] with a sensitivity of 0·1 ppm aflatoxin was followed. Subsequently the method of De Iongh, Beerthuis and Van Pelt,[3] which can detect 0·025 ppm of aflatoxin, was introduced in a slightly modified way.

The Analysts

(*a*) The crisis laboratory, 1963—The analysts who took part in assessing the aflatoxin content of the groundnut materials while the crisis laboratory functioned, were from:

(i) The Council for Scientific and Industrial Research: Mr. L. J. Vorster, leader of the team

(ii) The Oilseeds Control Board: Messrs. J. C. G. du Preez and I. de Klerk

(iii) S.A. Bureau of Standards: Mr. J. W. Steyn and Miss S. van Coller

(iv) University of Pretoria: Mr. F. Booyse and Miss G. Mackay

(v) Department of Agricultural Technical Services: (*a*) Soils Research Institute: Miss M. Esterhuizen, and (*b*) Veterinary Research Division: Mr. W. Ross.

(*b*) 1964—The 943 samples reported on in connection with the 1964 survey were examined by Messrs. J. C. G. du Preez and W. P. Pelser of the Oilseeds Control Board.

RESULTS AND DISCUSSION

The results of the aflatoxin determinations are given, by way of intensity symbols, on a geographical basis, on the two accompanying maps (Figs. 1 and 2).

Distribution and Degree of Occurrence of Aflatoxin, 1963

Altogether 501 samples are reported on.

The distribution of the different types of symbols in Fig. 1 suggests that the samples obtained from the North-Western Transvaal, or so-called Bushveld areas, were those most seriously contaminated with aflatoxin. Almost all of the 75 samples found to contain more than 2 ppm of aflatoxin emanated from the receiving depots in the North-Western Transvaal.

The samples from the Northern Cape Province, Orange Free State and Natal, except for 5 samples with 0·5 - 2·0

ppm and 11 samples with 0·1 - 0·5 ppm, were free from aflatoxin.

Distribution and Degree of Occurrence of Aflatoxin, 1964

Altogether 943 samples are reported on.

The symbols in Fig. 2 show that the samples with the higher degree of aflatoxin contamination again came principally from the North-Western Transvaal. A comparison of Figs. 1 and 2 shows that whereas only 23 samples

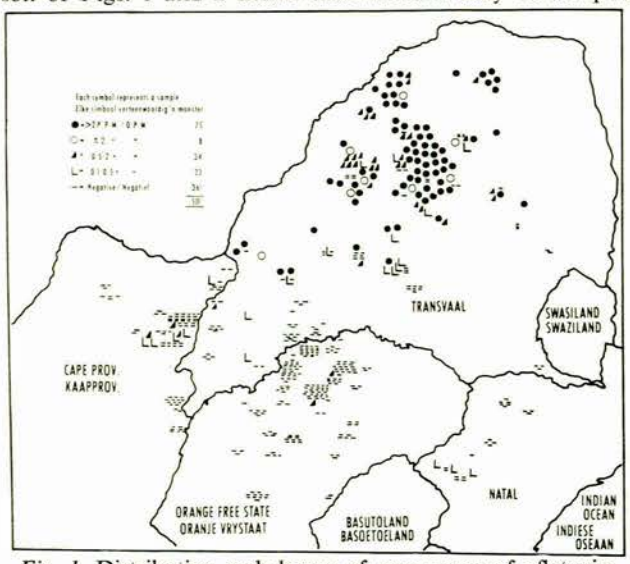

Fig. 1. Distribution and degree of occurrence of aflatoxin in groundnuts in 1963. (Determinations by Oilseeds Control Board and Crisis Laboratory personnel.)

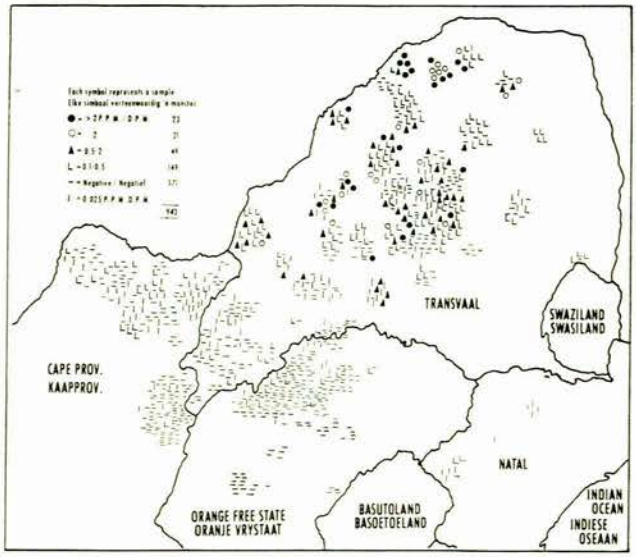

Fig. 2. Distribution and degree of occurrence of aflatoxin in groundnuts in 1964. (Determinations by Oilseeds Control Board Personnel.)

of the 1964 crop were contaminated to the extent of 2 ppm of aflatoxin, that of the previous year had 75 such samples. This improvement is ascribed largely to the stricter measures enforced in respect of the acceptance of groundnuts containing excessive numbers of mouldy kernels. The earnestness with which producers in the worst affected areas set out to eliminate unsound kernels from their deliveries also contributed to an easing of the aflatoxin problem.

That the groundnuts in the North-Western Transvaal should have been so seriously affected is ascribed mainly to (1) the damage caused to the maturing groundnut pods and kernels by certain species of termites; and (2) protracted droughts followed by late rains.

Even without the discovery by Mr. N. P. J. Kriek of the relationship between termites and aflatoxin production, the concentration of the >2 ppm symbols over the Potgietersrus, Waterberg and Brits districts would certainly have provided a clue to an area meriting immediate attention.

Distribution and Degree of Occurrence of Aflatoxin in the Commercial Grades of Groundnuts

Grading requirements. The bulk of each year's groundnut crop consists of the grades S2 and S3. Together they comprise about 66% of the total production. These 2 grades are made up of whole and split kernels of varying sizes and maturity. The SS and S1 grades with their predominantly large kernels and negligible proportions of shrivelled, damaged and unsound kernels, account for 25 - 30% of each year's deliveries. The inferior grades S4 and S5 represent about 5 - 8% of the annual production. Considering that one area is so much plagued by *A. flavus*, and the others far less so, the extent to which aflatoxin may be present in the respective grades can best be ascertained from the samples from the North-Western Transvaal Bushveld area during 1963 before the grading requirements were amended.

A classification of 148 consecutive samples from the North-Western Transvaal's 1963 crop has been set out in Table I. The data show that the lower or inferior grades are likely to contain greater concentrations of aflatoxin than the higher or superior ones.

TABLE I. AFLATOXIN-CONTAMINATED SAMPLES FROM FOUR GRADES OF SHELLED GROUNDNUTS

Grade	*Number of samples*	*Degree of aflatoxin-contamination in ppm*				
		More than 2	*About 2*	*0·5 - 2·0*	*0·1 - 0·5*	*Negative*
		%	%	%	%	%
S1	20	20	—	35	20	25
S2	53	45·28	7·54	20·7	9·34	16·9
S3	59	54·24	5·08	15·2	10·16	15·2
S4 and 5	16	75·0	—	—	—	25·0

Increasing percentages of samples of the successive grades indicated aflatoxin contamination at the >2 ppm rate. Whereas 20% of the grade S1 samples were affected, those of grades S2, S3 and the combined S4 and S5 material came to 45·28, 54·24 and 75% respectively.

Inferior nuts suspected. There is other evidence to the effect that the inferior groundnuts with their high proportions of small, immature, wizened and unsound kernels may be readily suspected of being contaminated with *A. flavus* and aflatoxin. Scott[5] examined some 100 groundnut samples from the Northern Transvaal and found that the incidence of *A. flavus* counts per 1,000 G of material from grades SS to S5 was as follows:

Grade	Aspergillus flavus counts
SS	2
S1	7
S2	80
S3	160
S4	195
S5	490

Aflatoxin in large, small and mouldy kernels. That the small, immature kernels are likely to be the ones most seriously contaminated with *A. flavus* and aflatoxin (and that they may easily collect toxic spores on their roughened surfaces) can be deduced from the aflatoxin determinations made in the Oilseeds Control Board laboratories on 8 varieties of groundnuts harvested after a protracted drought at Towoomba (Warmbad, Transvaal). The kernels were separated into:

(a) large kernels held above the 17/64 in. × 1/4 in. slotted grading screen;
(b) small kernels that passed through the screen; and
(c) mouldy kernels from both the large and small kernels.

The aflatoxin concentrations in ppm in the respective separates are given in Table II.

TABLE II. AFLATOXIN CONTENT OF GROUNDNUTS IN ppm

Variety	Kernels above the $\frac{17}{64}$ in. × $\frac{1}{4}$ in. screen	Kernels below the $\frac{17}{64}$ in. × $\frac{1}{4}$ in. screen	Mouldy kernels
Selection 5	—	3	± 25
Natal Comm. W. 52 G. 107F	0·05	1	± 64
Harmony X NC	—	1	± 80
Mamboleo	—	—	± 120
Nelson Sp.	—	1	± 160
Harmony	—	3	± 160
Valencia	—	3	± 320
Mixture of all the above varieties	0·5–1·0	2	± 512
		3	± 400

Whereas the aflatoxin concentration in the large kernels came to 0·5 - 1·0 ppm, that in the small kernels ranged, except for one negative sample, from 1 to 3 ppm. The aflatoxin content of the mouldy kernels ranged from 25 to 512 ppm.

Aflatoxin in hand-picked, selected (HPS) kernels. Forty-three samples of sized, hand-picked kernels generally referred to either as NC.56, NC.67 or NC.78 as the case may be, in accordance with the number of kernels weighing one ounce, were subjected to aflatoxin tests during 1963. None of the samples was found to be affected. During 1964 samples of all consignments of HPS groundnuts intended for export were tested. Except for *trace* concentrations of aflatoxin in some parcels from a drought-stricken area, all the consignments were found to be free from aflatoxin. Any consignment of HPS kernels found to contain even a trace of aflatoxin is not exported.

Aflatoxin in culled kernels or tailings. The feeding of mouldy kernels culled from farmers' stocks of shelled groundnuts caused the livestock deaths which aroused interest in the aflatoxin problem. Many groundnut producers testify that they have regularly fed large quantities of cullings without harmful effects. The tailings from processing centres have also been continually fed to livestock. This amply proves that all mouldy groundnut kernels are not necessarily poisonous, but that their toxicity depends on whether the moulds present have produced toxins. Veterinarians recommend that commodities with even 3 ppm of aflatoxin be shunned completely.[3] Mould-enveloped kernels from the termite region showed an aflatoxin content of 1,000 - 1,300 ppm, and those from a drought area, referred to in Table II, had 25 - 512 ppm of aflatoxin. It would appear that surveys could now well be made of the tailings that accumulate at the centres where groundnuts are selected for consumption purposes. The reasons are obvious.

Aflatoxin in groundnut oilcake meal. So much effort had been spent in determining the degree of occurrence of aflatoxin in groundnuts during 1963 that an organized survey of the toxicity of the oilcake meal could not be undertaken as well. Only meal suspected of having been responsible for livestock deaths was submitted for aflatoxin determinations. Eleven of the 16 samples dealt with showed an aflatoxin content of more than 2 ppm of aflatoxin, which was sufficient to incriminate them.

Aflatoxin in roasted groundnuts. Sixteen samples of roasted groundnuts tested during 1963 were found to be free from aflatoxin. It is generally the HPS kernels that are roasted.

Aflatoxin in peanut butter. The extensive use made of peanut butter as human food no doubt justified the avid interest directed towards the aflatoxin content of this food. A total of 101 samples were purchased from factories and all types of purveyors. Twelve samples had an aflatoxin content of between 0·1 - 0·5 ppm and 5 showed approximately 0·025 ppm. There should be no excuse for traces of aflatoxin in peanut butter. This commodity is generally manufactured from HPS kernels. The presence of aflatoxin would suggest that mouldy kernels had not been eliminated from the bulk before roasting was resorted to. Spenseley[7] confirms that selected kernels are used in the manufacture of peanut butter in England. Attempts in defence of peanut butter as a nutritious food, however, received a set-back in 1964. Grigg[4] reported that an aflatoxin-contaminated consignment of peanut butter had reached California from the Philippines. The question can be asked whether the groundnuts used had been hand-picked and were of superior quality. Manufacturers of groundnut butter in South Africa will, of course, be encouraged to eliminate all traces of aflatoxin from their product.

Aflatoxin in other commodities. It could be reiterated that aflatoxin does not occur in groundnuts alone, as shown by Semeniuk[6] and other investigators. Maize, wheat, cotton, sunflower and soybean meal may also at one time or another contain aflatoxin or other harmful fungal metabolites.

AFLATOXIN CONTROL MEASURES

The steps taken to reduce the amount of aflatoxin-contaminated groundnuts coming into commerce need not be dealt with in detail. Suffice it to say that the groundnut grading or acceptance regulations have been amended in such a manner that the definition of unsound kernels is more explicit and lesser percentages of such kernels are now permitted in the various grades than before the aflatoxin problem emerged. Deliveries of groundnuts that contain an unduly large percentage of mouldy kernels, irrespective of the type of mould, will readily find their

way to grade S5. Whereas the price difference between grade S1 and S5 used to be on the level of about R8 per ton, the new difference has been set at R30. Similar measures have been adopted by the United States to safeguard its peanut industry.

Instead of testing its products for their oil content only, the Oilseeds Control Board will, no doubt, ensure that it handles only aflatoxin-free groundnuts and sunflower seed.

Groundnuts should certainly not be synonymous with aflatoxin!

The Oilseeds Control Board had neither aflatoxin-contaminated nor aflatoxin-free groundnuts left for disposal at the end of 1964.

OPSOMMING

Die mate van aflatoksienbesmetting van 501 grondboonmonsters van die oes vir 1963 en 943 van die oes van 1964, is ondersoek om die graad van besoedeling en die verspreiding van die aangedaande voorrade vas te stel. Die resultate word weergegee by wyse van twee kaarte wat die graad van besoedeling en verspreiding van die aangedaande materiaal aandui. Kleiner getalle monsters van gebakte grondbone, grondboontjiebotter, oliekoekmeel, uitskotsel en muf bone is ook ondersoek. Die ergste besoedelde voorrade is in die Noord-westelike gedeelte van die Transvaal of die sogenaamde Bosveldstreek aangetref waar sekere termietsoorte (*Odontotermes latericius* en *O. badius*) die rypwordende grondboonpeule en -bone (pitte) aanval. Die monsters van die suidelike grondboonproduksiegebiede was betreklik vry van aflatoksien. Sewentien van die 101 grondboontjiebottermonsters het geringe tekens van aflatoksien getoon. Heeltemal muf uitskotbone uit die mees geteisterde gebied het van 1,000 tot 1,300 dele per miljoen aflatoksien bevat. Die kommersiële grade met die groter, meer volwasse bone, was in die reël nie besmet nie. Waar dit wel voorgekom het, was die aflatoksien meesal gekonsentreer in die laer grade, wat oorwegend uit klein bone bestaan het. Aflatoksien besoedeling is onder beheer gebring.

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