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THE FUNGUS FLORA OF STOCK FEEDS IN SOUTH AFRICA

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Introduction

The discovery of aflatoxin, produced by *Aspergillus flavus* Link, in groundnut meal (Sargeant, Sheridan, O'Kelly & Carnaghan, 1961) helped to focus attention on the problem of mycotoxins and mycotoxicoses. This problem is by no means new as several fungi had been known to form toxins. One of the oldest known toxigenic fungi is *Claviceps purpurea*, causal organism of ergot on wheat (Ramsbottom, 1953). Other known examples are cited by Scott (1965).

In 1918 *Diplodia zeae* was suspected of causing nervous disorders in cattle and sheep (Mitchell, 1918; Theiler, 1927). Steyn (1934) mentioned the toxicity of *Fusarium moniliforme* whilst, according to Forgacs (1965), the toxicity of *Stachybotrys atra* (*S. alternans*) has been known for a considerable period.

Samples of stock feeds suspected of toxicity have been examined over a considerable period of time for toxic fungi, mostly *Diplodia zeae* and *Fusarium moniliforme*. The results of more recent investigations showed, however, that a more complete survey of the fungal flora of stock feeds was necessary. Most investigations on fungal flora were done on cereal grains and legume seeds. A wide variety of materials besides grains and seeds is, however, used as animal feeds, for example cereal and legume hays, silages, maize cobs and stalks and similar waste material as well as compounded and concentrated rations. Virtually no information was available on the fungal flora of these substrata.

The present investigation was thus undertaken to determine and compare the fungal flora of various kinds of stock feed, including samples of feeds suspected of toxicity as well as others known to be harmless, and to investigate the correlation between the incidence of toxicity and the occurrence of certain fungal species.

Literature Review

It has been known for many years that cereal and leguminous seeds in storage support an extensive fungal flora, which may be active or dormant, depending on the conditions of storage (Semeniuk & Gilman, 1944; Hyde & Galleymore, 1951; Diener, 1960; Christensen, 1965). The fungal flora of stored grains and the various conditions which influence its development have been the subject of intensive studies (Thom & Le Fevre, 1921; James, Wilson & Stark, 1946; Semeniuk, Nagel & Gilman, 1947; Lichtwardt, Barron & Tiffany, 1958; Austwick & Ayerst, 1963). It is known that temperature, aeration and kernel moisture have marked effects on the growth of the fungi present on and in the stored grains (Semeniuk & Gilman, 1944; Christensen & Gordon, 1948; Christensen & Kaufmann, 1965). Growth

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of the so-called "storage fungi" results in deterioration and spoilage of seeds (Bottomley, Christensen & Geddes, 1950; Christensen, 1957; Christensen & Kaufmann, 1965; Golumbic, 1965). Under these conditions these fungi may form metabolites which could be toxic to animals. The presence of such toxic metabolites in seeds has been demonstrated by many investigators (Tuite & Christensen, 1955; Aust, Albright, Olsen, Byers & Broquist, 1963; Albright, Aust, Byers, Fritz, Brodie, Olsen, Link, Simon, Rhoades & Brewer, 1964; Forgacs, 1965; Majumder, Narasimhan & Parpia, 1965; Scott, 1965; Wilson, 1965; Boller & Schroeder, 1966). It is noteworthy that while these authors associated fungal growth and toxin formation with reasonably high temperatures, Joffe (1962) found that several species of *Fusarium* and other toxigenic genera could grow and produce toxins at 0°C. Non-toxigenic strains of the same species failed to grow at this temperature.

A large number of genera have been found, by many investigators, to contain toxigenic strains. These include:—

Absidia (Majumder *et al.*, 1965)

Alternaria (Forgacs & Carll, 1964; Majumder *et al.*, 1965)

Aspergillus (Wooley, Berger, Peterson & Steenbock, 1938; Florey, Jennings & Philpot, 1944; Carll, Forgacs & Herring, 1954; Forgacs, Carll, Herring & Mahlandt, 1954; Iizuka & Iida, 1962; Bampton, 1963; Forgacs & Carll, 1964; Rabie, De Klerk & Terblanche, 1964; Wilson & Wilson, 1964; Majumder *et al.*, 1965; Rabie, Terblanche, Smit & De Klerk, 1965; Scott, 1965; Borker, Insalata, Levi & Witzeman, 1966)

Cephalosporium (Majumder *et al.*, 1965)

Ceratostomella (Borker *et al.*, 1966)

Cladosporium (Joffe, 1962; Borker *et al.*, 1966)

Claviceps (Ramsbottom, 1953; Borker *et al.*, 1966)

Curvularia (Forgacs & Carll, 1964; Majumder *et al.*, 1965)

Dendrodochium (Borker *et al.*, 1966)

Diplodia (Mitchell, 1918; Theiler, 1927)

Epicoccum (Majumder *et al.*, 1965)

Fusarium (Dounin, 1926; Steyn, 1934; Joffe, 1965; Majumder *et al.*, 1965; Scott, 1965; Borker *et al.*, 1966)

Gibberella (Majumder *et al.*, 1965; Borker *et al.*, 1966)

Gliocladium (Borker *et al.*, 1966)

Gymnoascus (Borker *et al.*, 1966)

Mucor (Joffe, 1962; Majumder *et al.*, 1965)

Paecilomyces (Scott, 1965)

Penicillium (Burnside, Sippel, Forgacs, Carll, Attwood & Doll, 1957; Joffe, 1962; Wilson & Wilson, 1962; Hodges, Zust, Smith, Nelson, Armbrecht & Campbell, 1964; Majumder *et al.*, 1965; Scott, 1965; Borker *et al.*, 1966)

Phoma (Majumder *et al.*, 1965)

Pithomyces (Crawley, Mortimer & Smith, 1961; Clare & Gumbley, 1962; Borker *et al.*, 1966)

Rhizopus (Joffe, 1962; Majumder *et al.*, 1965)

Sclerotinia (Borker *et al.*, 1966)
Sclerotium (Terblanche & Rabie, 1967)
Stachybotrys (Forgacs, 1965; Borker *et al.*, 1966)
Stemphylium (Majumder *et al.*, 1965)
Thamnidium (Joffe, 1962)
Trichoderma (Majumder *et al.*, 1965; Borker *et al.*, 1966)
Trichothecium (Joffe, 1962; Scott, 1965)

This list is inevitably not complete.

The discovery of the production of aflatoxin by strains of *Aspergillus flavus* and *Penicillium puberulum* (Hodges *et al.*, 1964) led to extensive investigations of the occurrence of *Aspergillus flavus* on various substrata and the conditions influencing aflatoxin formation (Hartley, Nesbitt & O'Kelly, 1963; McDonald & A'Brook, 1963; McDonald & Harkness, 1963; Terblanche, Van Rensburg, Adelaar, Naudé & Smit, 1963; Wogan, Wick, Dunn & Scrimshaw, 1963; Austwick, 1965; Sellschop, Kriek & Du Preez, 1965; Van Warmelo, Van der Westhuizen & Minne, 1967).

Forgacs & Carll (1962), Austwick (1965), Christensen & Kaufmann (1965) and Scott (1965) have reviewed the literature on the occurrence of *Aspergillus flavus*, aflatoxin and other toxigenic fungi on cereals. There is, however, a marked paucity of information on other materials used as stock feeds. The investigation in which the fungus flora of such material was determined was that by Bonner & Fergus (1959). These authors compared the fungus flora of harmless feeds with that of feeds suspected of having caused illness or death in animals. Their results show that the 16 suspected and 10 non-suspected feed samples supported a great variety of species. Of the 63 species identified from the suspected feeds, 38 were also found on non-suspected feeds. Of the eight *Aspergillus* species isolated, only *A. terreus* occurred exclusively on suspected feed while of 13 *Penicillium* species found, *P. cyclopium*, *P. duclauxi*, *P. expansum*, *P. herquei* and *P. notatum* were only found on suspected samples. Species which are known to contain toxigenic strains, such as *Aspergillus flavus*, *Fusarium moniliforme*, *Penicillium oxalicum* and *P. purpurogenum*, were isolated from both suspected and non-suspected samples. The only fungi found exclusively on non-suspected samples were *Arachniotus terrestris* and *Penicillium vermiculatum*.

It is therefore evident from the literature that toxin production is fairly widespread among the fungi commonly associated with vegetable material in various stages of deterioration.

Materials and Methods

The feeds studied in this investigation were taken from samples sent to the Onderstepoort Veterinary Research Institute suspected of having caused illness or death in stock due to the presence of fungal toxins. Where a sufficient quantity of suspect feed was available, attempts were made to confirm toxicity by feeding experimental animals.

The silage samples were taken from known toxin free silos situated at different localities. The fungi were isolated in the manner described below (Van Rensburg, unpublished data).

The isolation technique described by Bonner & Fergus (1959) was employed. The media used were Czapek-Dox agar (Raper & Thom, 1949), Littman oxgall agar (Littman, 1947) and malt-salt agar (Christensen, 1946). To inhibit bacterial growth 0·25 to 0·5 mg terramycin was added to each Petri dish immediately before pouring the medium. All cultures were incubated at 25°C.

Individual fungi were transferred from the dilution plates to malt extract agar (Christensen, 1946) or, in the case of *Aspergillus* and *Penicillium* species, to Czapek-Dox agar, for identification.

Aflatoxin content was determined by the method described by De Iong, Van Pelt, Ord & Barrett (1964).

Results

A total of 60 species representing 34 genera was isolated from the 39 samples of feed examined. The species isolated, as well as their frequency of isolation from each feed type, are tabulated in Table 1. Included in this table are lists of fungi isolated from similar material by some other workers (Thom & Le Fevre, 1921; James, Wilson & Stark, 1946; Semeniuk, Nagel & Gilman, 1947; Lichtwardt, Barron & Tiffany, 1958; Bonner & Fergus, 1959; Austwick & Ayerst, 1963; Scott, 1965; Van Rensburg, unpublished data). Fungi isolated by these workers which were not found in this investigation are not included in the table.

Table 1

Of the 60 fungi isolated, 13 had not been reported by the other workers. These are *Actinomucor* sp., *Alternaria tenuissima*, *Aspergillus awamori*, *Colletotrichum* sp., *Diplodina* sp., *Monocillium* sp., *Penicillium biforme*, *P. kapuscinskii*, *P. lanoso-coeruleum*, *P. lilacinum*, *P. roqueforti*, *P. varians* and *Sepedonium* sp. Twenty-four species, among 14 genera, have been found by other authors to contain toxigenic strains.

From this table it can be seen that the fungi most frequently encountered by the previous authors were present on nearly all the substrata examined in this investigation. These fungi are *Aspergillus flavus*, *A. nidulans*, *A. niger*, *Cladosporium* sp., *Fusarium moniliforme*, *Hormodendrum* sp., *Mucor* sp., *Rhizopus stolonifer* and *Trichoderma viride*. Of these only *Hormodendrum* has not been proved to be toxigenic.

Because a chemical method for the determination of aflatoxin was available, attempts were made to correlate the presence of detectable amounts of aflatoxin with the occurrence of *Aspergillus flavus* in the feeds, and symptoms of toxicity in test animals.

The occurrence of *Aspergillus flavus* on, the presence of aflatoxin in, and the results of feeding tests of the different feeds, are tabulated in Table 2.

Table 2

Aspergillus flavus was present in a viable state in 16 of the 39 feed samples examined. Aflatoxin could, however, be detected chemically in only five of these samples. There were thus 11 samples in which the presence of *A. flavus* had not resulted in detectable aflatoxin formation, indicating that the strains present were either non-toxigenic or conditions were unfavourable for aflatoxin accumulation. In three samples in which aflatoxin was detected, *A. flavus* did not appear among the fungi isolated. This indicates that the toxigenic strain had either died during storage or that the aflatoxin had been formed by some other species. It is noteworthy that of the 18 samples fed to experimental animals none produced symptoms of toxicity although aflatoxin was present in two of these.

Discussion

In view of the nature and methods of preparation of stock feeds it is not surprising to find that the majority of species in the fungal flora are those which produce large numbers of dry, air borne spores and which are widespread in nature on plant residues.

From the results it is evident that the fungi isolated in this investigation agree very closely with the fungi isolated by other authors. Only eight from the total of 34 genera isolated, were not reported by other authors. As stated before, these authors did, however, isolate fungi which were not found in this investigation and thus do not appear in Table 1.

From Table 1 it can be seen that at least 27 of the 60 fungi isolated from suspected feed as well as from known toxin-free feed, are known to contain toxigenic strains. Table 2 shows that, despite the presence of these potentially toxigenic species, none of the materials fed to animals produced visible symptoms of toxicity. This shows that the presence of a toxigenic species in a feed sample is not necessarily indicative of the toxicity of that sample. The absence of mycotoxins in feed samples found to contain toxigenic species in viable form could mean that either the fungal strain was non-toxigenic or that the growth conditions, such as temperature, substrate or moisture, were unsuitable for toxin production and accumulation. It could also mean that conditions were favourable for a microbial breakdown of toxin as reported by Ashworth, Schroeder & Langley (1965) in Spanish groundnuts.

Failure to isolate known toxigenic fungi from feed is, on the other hand, no safeguard against the presence of mycotoxins. The fungus responsible for an observed toxin may have died (Christensen, Nelson & Mirocha, 1965) or a toxic effect may be due to a chemically unidentifiable toxin.

It is obvious from the results that natural substrata used as animal feeds support a large and varied fungal flora. Species of fungi known to include toxigenic strains are distributed indiscriminately among various types of feeds. A general survey of the fungal flora of feeds suspected of causing poisoning of stock is thus not likely to yield results of much value during the early stages of investigations of mycotoxicoses. This is largely due to the complexity of the flora, the variability of the species involved, distribution of toxigenic species and the lack of information on the toxigenicity of many species. It thus appears that investigations of cases of suspected mycotoxicosis should be initiated by controlled feeding tests to identify toxic samples. These should then be examined by means of chemical tests for the presence of known toxic substances of fungal or other origin. Only after known toxins have been excluded and the feed conclusively proved to be still toxic, should a mycological survey be initiated. The fungi isolated from the feed should then be grown under controlled conditions and their toxigenicity determined by biological tests.

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TABLE 1.—*Species of fungi and relative frequencies on different kinds of stock feeds compared with results of previous investigations*

Item	Lucerne hay (Van Warmelo*)	Maize hay (Van Warmelo*)	Maize meal (Van Warmelo*)	Maize silage (Van Warmelo*)	Groundnut seeds (Van Warmelo*)	Groundnut hay (Van Warmelo*)	Lupin seeds (Van Warmelo*)	Grass hay (Van Warmelo*)	Sunflower hay (Van Warmelo*)	Cowpea hay (Van Warmelo*)	Swine meal (Van Warmelo*)	Not reported previously (Van Warmelo*)	Known toxigenic (Borker *et al.*, 1966)	Van Rensburg (unpublished)	Scott, 1965	Austwick & Ayerst, 1963	Bonner & Fergus, 1959	Lichtwardt, Barron & Tiffany, 1958	Semeniuk, Nagel & Gilman, 1947	James, Wilson & Stark, 1946	Thom & Le Fevre, 1921
Number of samples	7	8	7	4	3	3	1	3	1	1	1	39	†	7	†	27	26	†	†	†	†
Actinomucor sp.	1											+	+	+							
Alternaria tenuis	2	3									1	+	+					+			
Alternaria tenuissima	1	5	1								1				+			+	+		
Aspergillus amstelodami	4				1		1	1			1	+	+		+	+	+	+	+		
Aspergillus awamori	2	3				3		2			1				+	+		+	+		
Aspergillus candidus	1	1	1								1			+	+	+		+	+	+	
Aspergillus elegans	1	1	2	3	1	2		1	1			+	+	+	+	+	+	+	+		
Aspergillus flavus	3		2	1	1						1	+	+		+	+	+	+	+	+	
Aspergillus fumigatus	1	2		2	1	1	1	1				+	+		+	+	+	+	+		
Aspergillus nidulans	2	3	6	3	1	1		2		1		+	+	+	+	+	+	+	+		
Aspergillus niger	1							1				+	+		+	+	+	+	+		
Aspergillus niveus												+	+		+	+	+	+	+		
Aspergillus ochraceus	1			1				1				+	+		+	+	+	+	+		
Aspergillus sydowi	2	1	1	4	1							+	+		+	+	+	+	+		
Aspergillus terreus	1	2										+	+		+	+	+	+	+		
Aspergillus ustus	2										1	+	+		+	+	+	+	+	+	
Aspergillus versicolor	1	5	6	1	2	2		2	1	1	1	+	+	+	+	+	+	+	+		
Aspergillus sp.	3											+	+		+	+	+	+	+		
Chaetomium funicolum					1	1		1		1		+	+	+	+	+	+	+	+		
Cladosporium sp.	4	4	1																		
Collectotrichum sp.												+	+								

* See text † Number of samples not given

TABLE 1.—*Species of fungi and relative frequencies on different kinds of stock feeds compared with results of previous investigations* (continued)

Item	Lucerne hay (Van Warmelo*)	Maize hay (Van Warmelo*)	Maize meal (Van Warmelo*)	Maize silage (Van Warmelo*)	Groundnut seeds (Van Warmelo*)	Groundnut hay (Van Warmelo*)	Lupin seeds (Van Warmelo*)	Grass hay (Van Warmelo*)	Sunflower hay (Van Warmelo*)	Cowpea hay (Van Warmelo*)	Swine meal (Van Warmelo*)	Not reported previously (Van Warmelo*)	Known toxigenic (Borker *et al.*, 1966)	Van Rensburg (unpublished)	Scott, 1965	Austwick & Ayerst, 1963	Bonner & Fergus, 1959	Lichtwardt, Barron & Tiffany, 1958	Semeniuk, Nagel & Gilman, 1947	James, Wilson & Stark, 1946	Thom & Le Fevre, 1921
Number of samples	7	8	7	4	3	3	1	3	1	1	1	39	†	7	†	27	26	†	†	†	†
Cunninghamella sp						1								+							
Diplodina sp		1										+									
Fusarium moniliforme	3	3	4	1	2	1		2					+	+	+	+	+	+++	++	+	+
Fusidium sp	1			1	2			1													
Geotrichum sp					1									+			++	+++			
Gliocladium sp								1					+								
Helminthosporium sp		1		1										+				+++		++	
Hormodendrum sp	5	3	5	3		1		3	1									+++			
Monilia sp	1	1	1	1																	
Monocillium sp				1								+									
Mucor sp	5	3	2	4	1	1	1	2	1	1	1		+			+	+	++	++	+	
Nigrospora sp	1																				
Paecilomyces varioti		1	4		2	1				1			+		+		+	++		+	
Penicillium biforme	1				1							+									
Penicillium chrysogenum		1				1							+		++		+++	+++	+	++	
Penicillium frequentans		2		1											+++						
Penicillium herquei				1													+++	+++			
Penicillium implicatum						1															
Penicillium kapuscinskii											1	+++									
Penicillium lanoso-coeruleum	1																				
Penicillium lilacinum											1	+++									

* See text † Number of samples not given

TABLE 1.—*Species of fungi and relative frequencies on different kinds of stock feeds compared with results of previous investigations* (continued)

Item	Lucerne hay (Van Warmelo*)	Maize hay (Van Warmelo*)	Maize meal (Van Warmelo*)	Maize silage (Van Warmelo*)	Groundnut seeds (Van Warmelo*)	Groundnut hay (Van Warmelo*)	Lupin seeds (Van Warmelo*)	Grass hay (Van Warmelo*)	Sunflower hay (Van Warmelo*)	Cowpea hay (Van Warmelo*)	Swine meal (Van Warmelo*)	Not reported previously (Van Warmelo*)	Known toxigenic (Borker *et al.*, 1966)	Van Rensburg (unpublished)	Scott, 1963	Austwick & Ayerst, 1963	Bonner & Fergus, 1959	Lichtwardt, Barron & Tiffany, 1958	Semeniuk, Nagel & Gilman, 1947	James, Wilson & Stark, 1946	Thom & Le Fevre, 1921
Number of samples	7	8	7	4	3	3	1	3	1	1	1	39	+	7	+	27	26	+	+	+	+
Penicillium nigricans				1									+		+		+				
Penicillium roqueforti		1		1								+	++		++	+					
Penicillium steckii		1		2		1												+			
Penicillium variabile		1				1															
Penicillium varians				1								+			++				+		
Penicillium sp.	7	4	7	2	2	3	1	2	1	1	1		++	++		++	+++	+			
Phoma sp.	2	2				1				1							+	+	+	+	
Pullularia pullulans	1																+				
Rhizopus stolonifer	4	4	6	1	1			2					+	+		+	+	+	+		
Rhodotorula sp.	1				1									+++							
Saccharomyces sp.	3			1	1	1		2					+				+	+	++		
Sclerotium rolfsii					1																
Scopulariopsis brevicaulis		1				1				1											
Sepedonium sp.								1				+									
Stachybotrys sp.								1					++					+			
Stemphylium sp.	2		1							1				+							
Torula sp.	1				1			1										+	+		
Trichoderma viride	1															+				++	
Trichothecium roseum		1		1											++		+	+			
Tritrachium roseum																					

* See text † Number of samples not given

TABLE 2.—*Occurrence of* Aspergillus flavus *and aflatoxin in different kinds of stock feeds and the results of feeding tests*

Substrate	Sample number	Aflatoxin content	Dosing result	Presence of *A. flavus*
Lucern hay	28	Negative	—	—
	2015	Positive-trace	Negative	+
	1565	—	Negative	—
	1456	—	Negative	—
	1372	—	—	+
	1438	—	—	—
	1347	—	Negative	+
Cowpea hay	1614	—	Negative	—
Maize silage	304	0·5–1·0 ppm	—	—
	1040	—	—	—
	1364	—	Negative	+
	25	—	—	+
Lupin seeds	1909	0·5 ppm	Negative	—
Groundnut kernels	1993	0·1–0·5 ppm	—	—
	1177	—	—	+
	1178	—	—	—
Veld hay	243	—	Negative	—
	412	0·1–0·5 ppm	—	+
	1141	—	—	—
Groundnut hay	1639	5 ppm	—	+
	275	4ppm B, 2 ppm G	Negative	+
	488	Negative	—	+
Maize meal	601	—	Negative	—
	1038	—	—	—
	1034	—	—	+
	1035	—	—	+
	1036	—	—	+
	868	Negative	—	+
	1510	Negative	—	+
Sunflower hay	957	0·25 ppm	—	+
Swine meal	3245	—	Negative	—
Maize hay	289	—	Negative	—
	1365	—	Negative	—
	185	—	—	—
	230	—	Negative	—
	84	—	Negative	—
	132	—	Negative	—
	2073	—	Negative	—
	1690	—	Negative	—

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REFERENCES

ALBRIGHT, J. L., AUST, S. D., BYERS, J. H., FRITZ, T. E., BRODIE, B. O., OLSEN, R. E., LINK, R. P., SIMON, J., RHOADES, H. E. & BREWER, R. L., 1964. Moldy corn toxicosis in cattle. *J. Am. vet. med. Ass.*, 144, 1013–1019.

ASHWORTH, J. L., SCHROEDER, H. W. & LANGLEY, B. C., 1965. Aflatoxin: Environmental factors governing occurrence in Spanish peanuts. *Science, N.Y.*, 148, 1228–1229.

AUST, S. D., ALBRIGHT, J. L., OLSEN, R. E., BYERS, J. H. & BROQUIST, H. P., 1963. Observations on moldy corn toxicosis. *J. Anim. Sci.*, 22, 831–832.

AUSTWICK, P. K. C., 1965. *In:* The Genus *Aspergillus*. Raper, K. B. & Fennell, Dorothy I. Chap. 7, Baltimore: Williams & Wilkins Co.

AUSTWICK, P. K. C. & AYERST, G., 1963. Toxic products in groundnuts. Groundnut microflora and toxicity. *Chemy Ind.*, Part 1, 55–61.

BAMPTON, S. S., 1963. Growth of *Aspergillus flavus* and production of aflatoxin in groundnuts—Part I. *Trop. Sci.*, 5, 74–81.

BOLLER, R. A. & SCHROEDER, H. W., 1966. Aflatoxin producing potential of *Aspergillus flavus oryzae* isolates from rice. *Cereal Sci. Today*, 11, 342–344.

BONNER, R. D. & FERGUS, C. L., 1959. The fungus flora of cattle feeds. *Mycologia*, 51, 855–863.

BORKER, E., INSALATA, N. F., LEVI, COLETTE P. & WITZEMAN, J. S., 1966. Mycotoxins in feeds and foods. *Adv. appl. Microbiol.*, 8, 315–351.

BOTTOMLEY, R. A., CHRISTENSEN, C. M. & GEDDES, W. F., 1950. Grain storage studies IX. The influence of various temperatures, humidities, and oxygen concentration on mould growth and biochemical changes in stored yellow corn. *Cereal Chem.*, 27, 271–296.

BURNSIDE, J. E., SIPPEL, W. L., FORGACS, J., CARLL, W. T., ATTWOOD, M. B. & DOLL, E. R., 1957. A disease of swine and cattle caused by eating moldy corn; II. Experimental production with pure cultures of molds. *Am. J. vet. Res.*, 18, 817–824.

CARLL, W. T., FORGACS, J. & HERRING, A. S., 1954. Toxicity of fungi isolated from a food concentrate. *Am. J. Hyg.*, 60, 8–14.

CHRISTENSEN, C. M., 1946. The quantitative determination of moulds in flour. *Cereal Chem.*, 23, 322–329.

CHRISTENSEN, C. M., 1957. Deterioration of stored grains by fungi. *Bot. Rev.*, 23, 108–134.

CHRISTENSEN, C. M., 1965. Fungi in cereal grains and their products. *In:* Symposium on mycotoxins in foodstuffs. Edited by G. N. Wogan, p. 9–14, Cambridge, Mass.: M.I.T. Press.

CHRISTENSEN, C. M. & GORDON, D. R., 1948. The mold flora of stored wheat and corn and its relation to heating of moist grain. *Cereal Chem.*, 25, 40–51.

CHRISTENSEN, C. M. & KAUFMANN, H. H., 1965. Deterioration of stored grains by fungi. *A. Rev. Phytopath.*, 3, 69–84.

CHRISTENSEN, C. M., NELSON, G. H. & MIROCHA, C. J., 1965. Increase in weight of uteri of white rats due to a toxin produced by *Fusarium* and isolation of the toxin. *Appl. Microbiol.*, 13, 653–659.

CLARE, N. T. & GUMBLEY, JANICE M., 1962. Some factors which may affect the toxicity of spores of *Pithomyces chartarum* (Berk. & Curt.) M.B. Ellis collected from pasture. *N.Z. Jl agric. Res.*, 5, 36–42.

CRAWLEY, W. E., MORTIMER, P. H. & SMITH, J. D., 1961. Characteristic lesions of facial eczema produced in sheep by dosing material containing *Pithomyces chartarum* collected from pasture. *N. Z. Jl agric. Res.*, 4, 552–559.

DE IONG, H., VAN PELT, J. G., ORD, W. O. & BARRETT, C. B., 1964. A semi quantitative determination of aflatoxin B_1 in groundnut meal, groundnuts and peanut butter. *Vet. Rec.*, 76, 901–903.

DIENER, U. L., 1960. The mycoflora of peanuts in storage. *Phytopathology*, 50, 220–223.

DOUNIN, M., 1926. The fusariosis of cereal crops in European Russia in 1923. *Phytopathology*, 16, 305–308.

FLOREY, H. W., JENNINGS, M. A. & PHILPOT, FLORA J., 1944. Claviformin from *Aspergillus giganteus* Wehm. *Nature, Lond.*, 153, 139.

FORGACS, J., 1965. Stachybotryotoxicosis and moldy corn toxicosis. *In:* Symposium on mycotoxins in foodstuffs. Edited by G. N. Wogan, p. 87–104, Cambridge, Mass.: M.I.T. Press.

FORGACS, J. & CARLL, W. T., 1962. Mycotoxicoses. *Adv. vet. Sci.*, 7, 273–382.

FORGACS, J. & CARLL, W. T., 1964. Toxic fungi from the seeds of *Cycas circinalis* L. *Fedn Proc. Fedn Am. Socs exp. Biol.*, 23, 1370–1372.

FORGACS, J., CARLL, W. T., HERRING, A. S. & MAHLANDT, B. G., 1954. A toxic *Aspergillus clavatus* isolated from feed pellets. *Am. J. Hyg.*, 60, 15–26.

GOLUMBIC, C., 1965. Fungal spoilage in stored food crops. *In:* Symposium on mycotoxins in foodstuffs. Edited by G. N. Wogan, p. 49–67, Cambridge, Mass.: M.I.T. Press.

HARTLEY, R. D., NESBITT, BRENDA F. & O'KELLY, J., 1963. Toxic metabolites of *Aspergillus flavus*. *Nature, Lond.*, 198, 1056–1058.

HODGES, F. A., ZUST, J. R., SMITH, H. R., NELSON, A. A., ARMBRECHT, B. H. & CAMPBELL, A. D., 1964. Mycotoxins: Aflatoxin isolated from *Penicillium puberulum*. *Science, N.Y.*, 145, 1439.

HYDE, M. B. & GALLEYMORE, H. B., 1951. The subepidermal fungi of cereal grains. II. The nature, identity and origin of the mycelium in wheat. *Ann. appl. Biol.*, 38, 348–356.

IIZUKA, H. & IIDA, M., 1962. Maltoryzine, a new toxic metabolite produced by a strain of *Aspergillus oryzae* var *microsporus* isolated from the poisonous malt sprout. *Nature, Lond.*, 196, 681–682.

JAMES, N., WILSON, JOYCE & STARK, E., 1946. The microflora of stored wheat. *Can. J. Res. Sect. C.*, 24, 224–233.

JOFFE, A. Z., 1962. Biological properties of some toxic fungi isolated from overwintered cereals. *Mycopath. Mycol. appl.*, 16, 201–221.

JOFFE, A. Z., 1965. Toxin production by cereal fungi causing toxic alimentary aleukia in man. *In:* Symposium on mycotoxins in foodstuffs. Edited by G. N. Wogan. p. 77–85, Cambridge, Mass.: M.I.T. Press.

LICHTWARDT, R. W., BARRON, G. L. & TIFFANY, L. H., 1958. Mold flora associated with shelled corn in Iowa. *Iowa St. Coll. J. Sci.*, 33, 1–11.

LITTMAN, M. L., 1947. A culture medium for the primary isolation of fungi. *Science, N.Y.*, 106, 109–111.

MAJUMDER, S. K., NARASIMHAN, K. S. & PARPIA, H. A. B., 1965. Micro-ecological factors of microbial spoilage and the occurrence of mycotoxins on stored grains. *In:* Symposium on mycotoxins in foodstuffs. Edited by G. N. Wogan. p. 27–47, Cambridge, Mass.: M.I.T. Press.

McDONALD, D. & A'BROOK, J., 1963. Growth of *Aspergillus flavus* and production of aflatoxin in groundnuts—Part III. *Trop. Sci.*, 5, 208–214.

McDONALD, D. & HARKNESS, C., 1963. Growth of *Aspergillus flavus* and production of aflatoxin in groundnuts—Part II. *Trop. Sci.*, 5, 143–154.

MITCHELL, D. T., 1918. A condition produced in cattle feeding on maize infected with *Diplodia zeae*. *Rep. vet. Res. Un. S. Afr.*, 7/8, 425–437.

RABIE, C. J., DE KLERK, W. A. & TERBLANCHE, M., 1964. Toxicity of *Aspergillus amstelodami* to poultry and rabbits. *S. Afr. J. agric. Sci.*, 7, 341–346.

RABIE, C. J., TERBLANCHE, M., SMIT, J. D. & DE KLERK, W. A., 1965. Toxicity of *Aspergillus wentii* Wehmer. *S. Afr. J. agric. Sci.*, 8, 875–880.

RAMSBOTTOM, J., 1953. Mushrooms and toadstools. London: Collins.

RAPER, K. B. & THOM, C., 1949. A manual of the Penicillia. Baltimore: Williams and Wilkins Co.

SARGEANT, K., SHERIDAN, ANN, O'KELLY, J. & CARNAGHAN, R. B. A., 1961. Toxicity associated with certain samples of groundnuts. *Nature, Lond.*, 192, 1096–1097.

SCOTT, DE B., 1965. Toxigenic fungi isolated from cereal and legume products. *Mycopath. Mycol. appl.*, 25, 213–223.

SELLSCHOP, J. P. F., KRIEK, N. P. J. & DU PREEZ, J. C. G., 1965. Distribution and degree of occurrence of aflatoxin in groundnuts and groundnut products. *In:* Symposium on mycotoxins in foodstuffs. *Tech. Commun. Dep. agric. tech. Serv. S. Afr.*, No. 35, 9–17.

SEMENIUK, G. & GILMAN, J. C., 1944. Relation of molds to the deterioration of corn in storage. A review. *Proc. Iowa Acad. Sci.*, 51, 265–280.

SEMENIUK, G., NAGEL, C. M. & GILMAN, J. C., 1947. Observations on mold development and on deterioration in stored yellow dent shelled corn. *Bull. Ia agric. Exp. Stn*, No. 349, 255–284.

STEYN, D. G., 1934. The toxicology of plants in South Africa. *S. Afr. Agric. Ser.* 13. South Africa: Central News Agency Ltd.

TERBLANCHE, M. & RABIE, C. J., 1967. A toxic fungus (*Sclerotium rolfsii*) isolated from groundnuts. *S. Afr. J. agric. Sci.*, 10, 253–262.

TERBLANCHE, M., VAN RENSBURG, J., ADELAAR, T. F., NAUDÉ, T. W. & SMIT, J. D., 1963. A preliminary note on the toxicity of *Aspergillus flavus* Link as grown in pure culture. *Jl S. Afr. vet. med. Ass.*, 34, 645–648.

THEILER, A., 1927. Die Diplodiosis der Rinder und Schafe in Süd-Afrika. *Dt. tierärztl. Wschr.*, 35, 395–399.

THOM, C. & LE FEVRE, E., 1921. Flora of corn meal. *J. agric. Res.*, 22, 179–188.

TUITE, J. F. & CHRISTENSEN, C. M., 1955. Grain storage studies 16. Influence of storage conditions upon the fungus flora of barley seed. *Cereal Chem.*, 32, 1–11.

VAN RENSBURG, J. Unpublished results. Plant Protection Research Institute, Pretoria, Dep. agric. tech. Services, S. Afr.

VAN WARMELO, K. T., VAN DER WESTHUIZEN, G. C. A. & MINNE, J. A., 19—. The production of aflatoxins in naturally infected high quality maize. *Tech. Commun.* Dep. agric. tech. Serv., S. Afr. (To be published).

WILSON, B. J., 1965. Toxic substances formed by filamentous fungi growing on feedstuffs. *In:* Symposium on mycotoxins in foodstuffs. Edited by G. N. Wogan. p. 147–149, Cambridge, Mass.: M.I.T. Press.

WILSON, B. J. & WILSON, CHRISTINA H., 1962. Extraction and preliminary characterization of a hepatotoxic substance from cultures of *Penicillium rubrum*. *J. Bact.*, 84, 283–290.

WILSON, B. J. & WILSON, CHRISTINA H., 1964. Toxin from *Aspergillus flavus*. Production on food materials of a substance causing tremors in mice. *Science, N.Y.*, 144, 177–178.

WOGAN, G. N., WICK, E. L., DUNN, C. G. & SCRIMSHAW, N. S., 1963. Toxic metabolites produced by *Aspergillus flavus* Link ex Fries. Abstr. No. 2696. *Fedn Proc. Fedn Am. Socs exp. Biol.*, 22, 611.

WOOLEY, D. W., BERGER, J., PETERSON, W. H. & STEENBOCK, H., 1938. Toxicity of *A. sydowi* and its correction. *J. Nutr.*, 16, 465–476.