A consideration of the mycotoxin hypothesis with special reference to the mycoflora of maize, sorghum, wheat and groundnuts (G105)

Tropical Products Institute

G105

A consideration of the mycotoxin hypothesis with special reference to the mycoflora of maize, sorghum, wheat and groundnuts

P. M. D. Martin*
in collaboration with G. A. Gilman

July 1976

Tropical Products Institute 56/62 Gray's Inn Road London WC1X 8LU
Ministry of Overseas Development

*Reader in Biology, University of Botswana, Lesotho and Swaziland, Roma, Lesotho, and Research Officer, South African Institute for Medical Research, Johannesburg, Republic of South Africa.

This report was produced by the Tropical Products Institute, a British Government organisation which helps developing countries to derive greater benefit from their renewable resources. It specialises in post-harvest problems and will be pleased to answer requests for information and advice addressed to the Director.

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Tropical Products Institute
ISBN: 0 85954 054 5

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ACKNOWLEDGEMENTS

This paper is the partial outcome of a project on the mycology of Southern African foodstuffs sponsored jointly by the South African Institute for Medical Research and the Tropical Products Institute, London. It is a pleasure to express my appreciation to the South African Institute for Medical Research and to the University of Botswana, Lesotho and Swaziland for the use of their facilities, to Mr. G. A. Gilman for his most helpful collaboration, and to the Tropical Products Institute for the use of their library and for a financial grant during my sabbatical leave in England in the final phase of this study. My thanks are due to the Deferred Pay Interest Fund of the Chamber of Mines, Johannesburg, for financing the Lesotho Cancer Survey on which some of the data in this review is based. My thanks also go to Mr. Anthony Johnstone, Dr. Colin Booth and the staff of the Commonwealth Mycological Institute, Kew, for making it possible for me, during my sabbatical, to examine the Herbarium and Library in detail.

Mr. P. K. C. Austwick of the Nuffield School of Comparative Medicine generously assisted with the literature review, and I am grateful to Dr. Mary Noble, Dr. R. Sherwood and Dr. A. Coady for contributing information. I am also indebted to the librarians of the TPI and CMI, Mr. K. Piper, Miss O. Moran, and Miss S. Daniels for their cooperation. Finally, I am also extremely grateful to Dr. Paul Keen of the Cancer Research Unit, S.A.I.M.R., for reading the script and making many helpful suggestions. Mrs. B. Boyes, U.B.L.S., Roma, helped greatly in the typing and layout, and it is highly probable that without her able assistance this paper would never have been completed.

Summary

A consideration of the mycotoxin hypothesis with special reference to the mycoflora of maize, sorghum, wheat and groundnuts

'And so from hour to hour we ripe and ripe,
And then from hour to hour we rot and rot
And thereby hangs a tale' (Shakespeare)

This review attempts to trace the connection between the mycology of foodstuffs and the onset of disease due to the toxins that various fungi produce within those foodstuffs. The association of fungal activity with the occurrence of various disease syndromes in man and animals has only recently been recognised. Possibly this is because the epidemiology of mycotoxins involves more than one scientific discipline, and the collation of knowledge has inevitably been slow. Also the chronic long term effects of mycotoxin poisoning may have been obviated by better preventive measures in the developed countries; certainly it is the Third World countries which have contributed most to our understanding of this subject. Because of the relative remoteness of these areas from the main areas of research however, it has taken longer to collect sufficient data, especially that pertaining to the human situation.

Studies of the underlying causes of these disease syndromes show that they are due to a combination of synergistic factors, such as the physiology and genetics of crop plants, the preference of fungi for certain substrates for development and toxin production, various geographical factors affecting temperature and humidity, and dietary and food storage habits of human populations.

Most of the recent critical laboratory work has concentrated on defining more precisely those conditions under which toxigenic fungi will grow and produce toxins. In the case of aflatoxin and zearalenone, a divergence in behaviour *in vitro* has been shown to correlate broadly with the geographical distribution of disease outbreaks due to these toxins.

An important characteristic of mycotoxins is that certain parts of the body may be specifically affected by them, namely the liver (aflatoxin), kidneys (ochratoxin and citrinin), uterus (ergotamine and zearalenone), and the nervous system (tremorgen).

The idea that various chronic, as well as acute, diseases of man and animals involving these organs may be at least partly due to mycotoxins, was contained in the *mycotoxin hypothesis,* now ten years old. Four epidemiological studies have served to relate aflatoxin and human hepatoma, and the potential involvement of mycotoxins in other syndromes remains as an exciting future research problem.

The literature on mycotoxicosis has reached enormous proportions, much of it being contributed during the last fifteen years; consequently it is difficult to do justice to all aspects. The writer decided to place particular emphasis on the practical side of the problem, particularly with reference to the physiological

interactions among fungi, the incidence of fungi and their toxins in various substrates, the ecology of mycotoxin formation, and the acute and chronic effects of mycotoxicosis. These topics are logically related, and a proper study of them may provide the answers necessary to eliminate a hazard that, in the opinion of the author, is still far more prevalent than is generally realised.

RESUME

Examen de l'hypothèse des mycotoxines avec référence spéciale à la mycoflore du mais, du sorgho, du blé et des arachides

Dans cette revue, on tente de mettre en évidence la relation entre la mycologie des denrées alimentaires et l'apparition de maladies dues aux toxines que divers champignons produisent dans ces denrées alimentaires. L'association de l'activité des champignons à l'apparition de divers syndromes morbides chez l'homme et les animaux n'a été reconnue que récemment. Il est possible que ce soit dû au fait que l'épidémiologie des mycotoxines implique plus d'une discipline scientifique et que le collationnement des connaissances ait inévitablement été lent. Les effets chroniques à long terme de l'intoxication par les mycotoxines auraient pu être évités par de meilleures mesures préventives dans les pays développés; ce sont certainement les pays du Tiers Monde qui ont contribué le plus à notre connaissance de ce sujet. Mais en raison de l'éloignement relatif de ces régions des principaux centres de recherche, cela a demandé beaucoup de temps pour recueillir suffisamment de données, en particulier en ce qui concerne la situation humaine.

Les études des causes se trouvant à la base de ces syndromes morbides montrent qu'ils sont dûs à une combinaison de facteurs synergiques, tels que la physiologie et la génétique des plantes récoltées, la préférence que montrent les champignons pour certains substrats pour se développer et produire des toxines, divers facteurs géographiques influençant la température et l'humidité et les habitudes alimentaires et de conservation des aliments des population humaines.

La plupart des travaux de laboratoire fondamentaux récents ont été axés sur le problème consistant à définir de façon plus précise les conditions dans lesquelles des champignons toxigénes sont susceptibles de se développer et de produire des toxines. Dans le cas de l'aflatoxine et de la zéaralénone, il a été montré qu'une différence dans le comportement in vitro se trouve en corrélation dans les grandes lignes avec la distribution géographique des manifestations des maladies dues à ces toxines.

Une caractéristique importante des mycotoxines est le fait qu'elles peuvent attaquer spécifiquement certaines parties du corps, à savoir le foie (aflatoxine), reins (ochratoxine et citrinine), utérus (ergotamine et zéaralénone) et système nerveux (trémorgène).

L'idée que diverses maladies chroniques, et également aiguës, de l'homme et des animaux, impliquant ces organes, pourraient être dues au moins en partie aux mycotoxines, était incluse dans l'*hypothèse des mycotoxines,* qui a été émise il y a dix ans. Dans quatre études épidémiologiques, on a établi la relation entre l'aflatoxine et l'hépatome humain, et l'intervention possible des mycotoxines dans d'autres syndromes reste un problème de recherches futures passionnantes.

La littérature sur la mycotoxicose a atteint des proportions énormes et cela essentiellement au cours des quinze dernières années, en conséquence, il est difficile de faire valoir tous les aspects. L'auteur a décidé de mettre spécialement l'accent sur l'aspect pratique du problème, en particulier en ce qui concerne les interactions physiologiques parmi les champignons, l'apparaition de champignons et de leurs toxines dans divers substrats, l'écologie de la formation des mycotoxines et les effets aigus et chroniques de la mycotoxicose. Ces sujets ont

entre eux un lien logique et leur étude appropriée peut fournir les réponses qui sont nécessaires pour éliminer le risque qui, de l'avis de l'auteur, est encore bien plus répandu qu'on ne le pense généralement.

RESUMEN

Una consideración sobre la hipótesis de la micotoxina, con especial referencia a la micoflora del maíz, sorgo, trigo y cacahuete

Esta revisión intenta describir la conexión entre la micología de los productos alimenticios y la aparición de la enfermedad debida a las toxinas producidas por diversos hongos en el interior de dichos productos. Recientemente se ha reconocido la asociación de la actividad de los hongos con la incidencia de diversos síndromes de enfermedad en el hombre y animales. Posiblemente esto se debe al hecho de que en la epidemiología de las micotoxinas están implicadas varias disciplinas científicas, y el avance de los conocimientos ha sido inevitablemente lento. En los países desarrollados los efectos crónicos a largo plazo, producidos por envenenamiento micotóxico, se han podido evitar por medio de mejores medidas preventivas; sin duda son los países del Tercer Mundo los que más han contribuido a la comprensión de este problema. Sin embargo, a causa de la relativa lejanía de estas áreas de las principales áreas de investigación, se ha necesitado más tiempo para recoger datos suficientes, especialmente los relativos a la situación humana.

Los estudios de las causas subyacentes de estos síndromes de enfermedad, muestran que se deben a la combinación de factores sinérgicos, tales como la fisiología y genética de las plantas cultivadas, la preferencia de los hongos por ciertos substratos para el desarrollo y producción de toxinas, diversos factores geográficos relacionados con la temperatura y la humedad, así como los hábitos dietéticos y de almacenamiento de productos alimenticios de las poblaciones humanas.

La mayor parte de los recientes trabajos de laboratorio se han concentrado en definir con más precisión las condiciones bajo las cuales los hongos toxígenos se desarrollan y producen toxinas. En el caso de la aflatoxina y zearalenona, se ha mostrado que la divergencia de su comportamiento "in vitro" tiene una clara relación con la distribución geográfica de los brotes de la enfermedad debidos a estas toxinas.

Una característica importante de las micotoxinas es que pueden afectar específicamente a determinadas partes del cuerpo, tales como el hígado (aflatoxina), los riñones (ochratoxina y citrinina), útero (ergotamina y zearalenona) y el sistema nervioso (tremorgena).

La idea de que varias enfermedades del hombre y de los animales, tanto crónicas como agudas, implicando a los órganos citados, pueden deberse al menos parcialmente a las micotoxinas, estaba contenida en la *hipótesis de las micotoxinas,* propuesta hace ahora diez años. Se han utilizado cuatro estudios epidemiológicos para relacionar la aflatoxina con el hepatoma humano y la implicación potencial de micotoxinas en otros síndromes continúa siendo un problema interesante para futuras investigaciones.

La literatura sobre micotoxicosis ha alcanzado enormes proporciones, gran parte de la cual ha sido aportada durante los últimos quince años, por lo cual es difícil juzgarla en todos sus aspectos. El autor ha decidido conceder especial énfasis al lado práctico del problema, particularmente con referencia a las interacciones fisiológicas entre los hongos, la incidencia de los hongos y sus toxinas en diversos substratos, la ecología de formación de micotoxinas y los efectos, agudos y crónicos, de las micotoxicosis. Estos puntos están lógicamente relacionados, y un estudio más completo de los mismos puede suministrar la respuesta necesaria para evitar un riesgo, que en opinión del autor, es aún mucho más frecuente de lo que en general se cree.

The physiology and incidence of fungi in relation to harvesting and storage

The study of fungi on crops has gained a new dimension with the advent of mycotoxicology. Formerly, interest centred on plant pathology, whereas nowadays the sphere has been widened to include the effects, possibly subtle, which even common saprophytes and pathogens may exert on human and animal physiology through the contribution of small quantities of metabolites to the crop as it develops in the field, and during storage. Physiological and other factors controlling the distribution and metabolism of these fungi have an obvious relevance in mycotoxin formation. The study of these factors has progressed rapidly in the last decade in an effort to counteract the problem.

The first detailed attempt to investigate the physiology of stored grain fungi was that of Snow *et al.,* (1944a & b) who examined mould deterioration of feeding stuffs in relation to the humidity of storage. Observations were made on fungal development on six widely differing commodities in storage for periods extending over some three and a half years. Substrates selected were linseed cake, bone meal, oats, "scotch beans", "bran"* and locust beans. The main factors controlling the growth of fungi were listed as:

(a) The relative humidity (RH) defined as the

$$\frac{\textit{moisture content of the substrate}}{\textit{moisture content required for saturation}} \times 100$$

at a given temperature which, rather than the moisture content of a substrate, is the factor directly controlling growth. The moisture content (mc) of the substrate governs the relative humidity, but the relationship between the time of appearance of the mycelium and the RH is closer and more consistent than that between the appearance of mycelium and mc. The latent period required for fungi to develop and cause a serious storage problem sharply increases as the RH decreases below 80%. Safe RH limits were suggested by Snow for various food stuffs, including wheat and maize, for both long and short storage periods, below which mould growth would not take place.

(b) The length of storage period.

(c) The balance of type of nutrients provided by the various feedingstuffs.

(d) Temperature of storage, the degree of invasion increasing generally with higher temperatures up to 30° C.

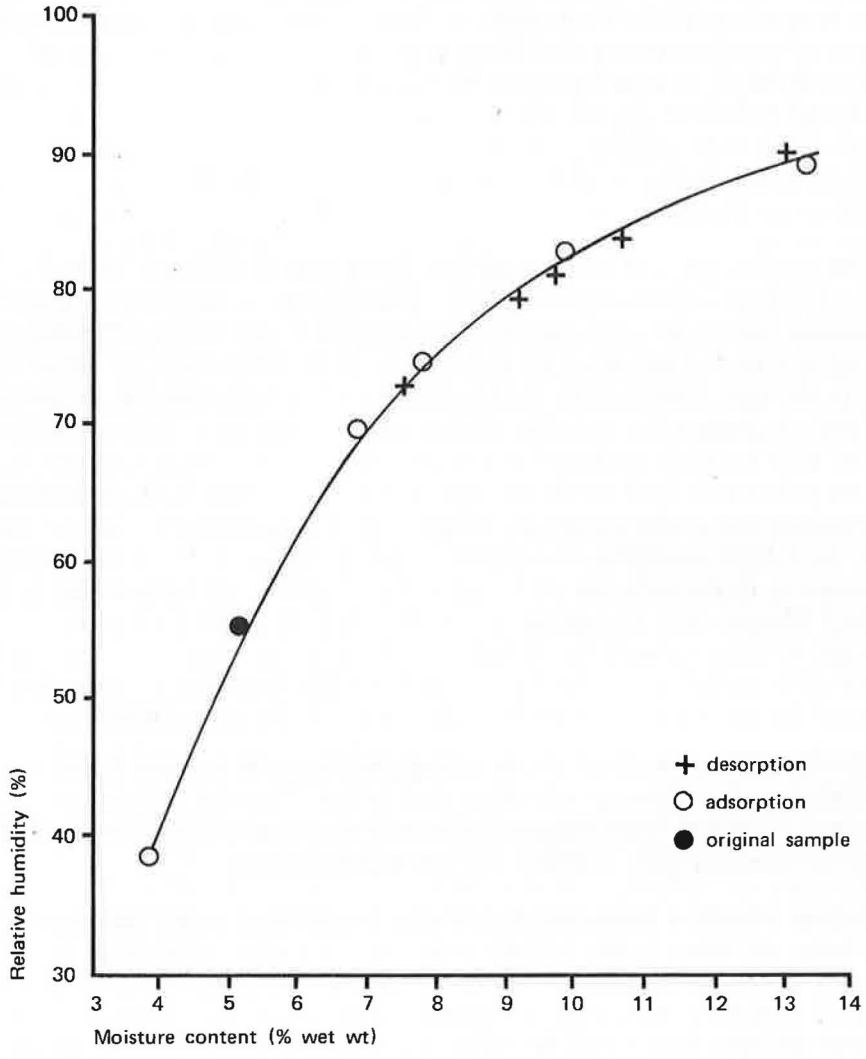
(e) The peculiarities of the fungus, there being specific differences in physiological requirements.

All these factors have been investigated since in greater depth by many workers. The moisture content of a substrate is usually easier to measure than RH and is consequently more commonly cited by recent workers. Brockington, Dorin and Howerton (1949) using an electric hygrometer, determined the RH of air in equilibrium with shelled, yellow corn conditioned to moisture contents between

*Incompletely defined by the authors.

Figure 1

The relationship between moisture content and relative humidity as exemplified by ground nuts



Source: Ayerst and Lee, 1962. Reprinted, with permission, from *Report to the Pest Infestation Laboratory.*

9.4 and 22.6% determined at 80° F ± 6.2° F (26.6° C). The critical moisture content for safe storage of corn in equilibrium with an RH of 75% was found to be 13.8 ± 2% by the Brown-Duvel method and 14.7 ± 0.1% by the two stage vacuum oven method. The figure of 75% was based on work by Milner and Geddes (1945) and by Gilman and Semeniuk (1948), this being the highest moisture content at or below which these authors determined that common storage moulds would not grow. This RH limit has been subsequently reduced to 65% (Christensen, 1973). The critical moisture content can vary widely from one foodstuff to another and is estimated to be 14.5% for sorghum (Christensen 1970), 12.5–13.5% for wheat and maize (Christensen, 1973), but as low as 8% for groundnuts (McDonald, 1968a). Presumably this is a function of the water-holding ability of the plant material. Safe storage moisture content and oil content are closely related; oil seeds always have lower safe storage moisture contents. The RH values, however, are directly comparable between foodstuffs.

The moisture content of the substrate is governed in the first place by its ability to absorb water. Other factors may contribute to the moisture content at the time of harvesting. Lamont (1952) noted a tendency of maize stored in other than airtight chambers to assume a moisture content figure in equilibrium with the air moisture, and this was largely independent of the original moisture content of the grain. Tuite and Foster (1963) determined the equilibrium moisture

content (EMC) and the equilbrium relative humidity (ERH) of shelled corn artificially dried in a pilot dryer and in the laboratory. The ability to absorb water progressively decreased with increased drying temperatures and the effect seemed to be permanent. Increase in ERH with increased temperature was universely proportional to the decrease in EMC. This conclusion confirms the observation by warehousemen that there is greater difficulty in storage of artificially dried corn in that blue mould is commoner. Spread of mould in naturally dried corn is much slower than in grain artificially dried possibly because the latter may undergo surface cracking. According to Koehler (1938) significant differences also exist between strains of corn in the extent to which water is taken up by their seeds.

The general physiology and biology of seed fungi was initially reviewed by Semeniuk (1954) and Christensen (1957). The critical differences amongst fungi governing their behaviour and deterioration potential with respect to crop seeds was first related to the sequence of their invasion, whether prior to harvest or later during storage. Christensen (1951, 1957) and Christensen and Kaufman (1965, 1969) expressed the essential differences in behaviour concisely when they defined the **field fungi** as species that are able to invade or grow on the crop plant up to maturation but which are limited by their relatively high moisture content requirement in the extent to which they can develop in fruits or seeds after they have been collected and stored. **Storage fungi** on the other hand, though common in the soil, are only sporadically present on undamaged fruits and seeds at harvest, and are xerophytic in the sense that they only require relatively low moisture levels for initial growth, although their later development and deteriorative potential is accelerated by high moisture levels. They are also characterized by definable changes brought about in the stored material.

The behaviour and incidence of many pathogenic fungi in storage is often intimately related to the *systemic* role they play within may the plant during its growth cycle. This has been elegantly demonstrated recently by Maude and Presly (1973) working with *Botrytis allii* on stored onions.

With regard to cereals, a harmonious systemic relationship exists between many common fungi and their hosts, disease only resulting when conditions become unfavourable for plant growth, or when the plants are mechanically damaged. Foley (1960) observed from field experience that 'there is no method of obtaining maize plants entirely free from naturally occurring pathogens'. In sorghum plants, *Fusarium* has been demonstrated in the phloem (Futrell, 1971) but it will not proliferate unless the weather is unfavourable: cold weather results in slow growth and seedling blight, hot dry weather in slow growth and stalk rot, and prolonged wet weather in leaf damage and stalk lodging.

Deterioration of maize seedlings by fungi has been related to pericarp injury (Koehler, 1957) and to immaturity (Mendiola, 1930). Valleau (1920) found that virtually no ears of maize in the field were free of infestation by *F. moniliforme* (a potentially pathogenic and toxigenic species) though they were not necessarily diseased. The number of actually pathogenic strains of *F. moniliforme* isolated from maize seedlings was found to be as low as 20/110 (Leonian, 1932). *Diplodia maydis* is another toxigenic species that remains latent in the plant during its growth, and only becomes active at the end of the plant's life, making the stalk weak and brittle and invading the cob.

The pathogenic relationship of fungi to plants is, of course, also profoundly influenced by environmental factors, one of the major ones being nutrition. Increase of calcium in the soil near the fruiting zone of groundnuts has been observed to result in a reduction in podrot (Garren, 1964a). On the other hand, stalk rot and root rot due to *Fusarium* can be materially increased by high nitrogen and low potassium levels (Thayer and Williams, 1960; Abney and Foley, 1971) and by moist calcareous soil (Arya and Jain, 1964). Another factor governing infection with fungi is the age of the plant (Ashworth *et al.*, 1964). Weather conditions may play a part in causing seasonal incidence in pathogenicity from one year to another (Koehler *et al.*, 1924). Also important is the

synergistic effect of fungi acting together to promote disease symptoms of a characteristic type (Ashworth and Langley, 1964), a subject about which little is known.

Last, but not least, are the genetic variations that exist among fungi and their hosts. Wide differences exist among isolates of *F. graminearum* with respect to their ability to cause seedling blight in corn: some isolates are highly virulent and others practically non-pathogenic (Ullstrup, 1935). Hard red winter wheat and white wheats are more susceptible to attack by storage fungi than durum wheats and hard red spring wheats (Christensen, 1955a; Wyllie and Christensen, 1959; Papavizas and Christensen, 1960). Specific differences in host genotype may correlate with susceptibility to infection by certain fungi. The recent discovery of enhanced pathogenicity of *Helminthosporum maydis* in a strain of corn with 'T. cytoplasm' (Anon, 1970; Moore, 1970; Hooker *et al.*, 1970) has led to the demonstration that other fungi may be favoured too, notably *F. moniliforme* (Warmke and Schenck, 1971). Clearly such results have some bearing on the mycotoxin hypothesis of animal disease because crop susceptibility in a given region might be implicated in the local development of a disease syndrome (Ullstrup, 1971).

This variation in susceptibility of a host can be matched by variation in virulence of an invader: different isolates of *Aspergillus candidus* reduced germination of wheat seed at considerably different rates (Papavizas and Christensen, 1957). Moreno-Martinez and Christensen (1971) found widespread differences among races of maize in susceptibility to the combined attack of seven *Aspergillus* test species and to some species of *Penicillium.*

The relevance of this general discussion centres round the correlation between infestation and toxicity already noted. *Any factor that can promote infestation and damage to seeds and fruits can reasonably be expected to enhance toxin production.* Furthermore if the production of toxins in nature is linked to certain environmental factors, a convincing explanation can be offered for outbreaks of disease in animals in specific places at specific times. The difficulty is to discover the process in retrospect.

CHARACTERISTICS OF FIELD AND STORAGE FUNGI IN CROPS FRUITING ABOVE GROUND

The main work on field and storage fungi was first done on cereals. Several criteria useful in identifying these groups were developed in addition to the original definition, based chiefly on their appearance in the life cycle of the plant.

Persistence in the seed

Hyde (1950) and Hyde and Galleymore (1951) have described the field flora of wheat grains at harvest. Dematiaceous subepidermal mycelium was usually found in 'clean' wheat grains in samples from all over the world. The quantity of mycelium varied widely but there were indications that the degree of infection, rated over a ten-point scale, was proportional to the atmospheric humidity present during the ripening of the grain. The grain itself on examination was dry, with a moisture content of less than 14%, supporting the view that the spread of subepidermal mycelium is restricted by desiccation beneath the epidermal layer during ripening. Wheat samples from temperate climates developed mycelium over the greater part of the inner surface of the epidermis, being particularly abundant where the epidermis was loose at the two ends of the grain. Wheat samples from dry climates had a less heavy infestation, the mycelium being restricted to the beard hairs and to a lesser extent at the embryo end of the grain. The method of entry was not established, possibly taking place through stomata at the beard end of the grain or from the flora parts. Comparison of the fungi isolated from sections of wheat stem and from wheat seeds suggested a systemic origin. Study of wheat grains at different stages of maturity showed

that infection by *Alternaria* and *Cladosporium* was restricted to the endosperm and that the embryos were free of field microflora (Lenkov & Khanumova, 1971).

Hyde (1950) found a significantly inverse correlation between the quantity of field fungi and the environmental temperature. It might be expected that fungal growth would be increased by high temperature, but as these were often associated with low humidities, retardation occurred instead.

Christensen (1951, 1965) and Christensen and Kaufman (1969) have also related the dormant mycelium from assorted dry wheat pericarps, in order of frequency, to the genera *Alternaria, Aureobasidium, Cladosporium, Helminthosporium* (*Drechslera* fide M. B. Ellis) and *Fusarium. Alternaria* was found to persist in the dry state for some years, but if the moisture content of the seed was high enough to permit storage fungi to grow, it disappeared relatively rapidly. The fungi isolated from low grade wheat stored under poor (moist) conditions belonged to the *Aspergillus glaucus* group of species, and also included *A. candidus, A. versicolor, A. niger, A. ochraceus* and several *Penicillium* spp. Whereas the field fungi in culture were shown to require high moisture contents to germinate and invade, the storage fungi were essentially xerophytic, though their metabolism increased the moisture content of the substrate with time. In addition Christensen defined a third group: the *advanced decay* fungi, comprising *Fusarium graminearum* and species of *Papulaspora, Chaetomium* and *Sordaria.* These resembled field fungi in that they have high moisture requirements, but they did not commonly invade the grain extensively before harvest. Various yeasts, comprising *Candida, Endomycopsis, Pichia* and *Torulopsis,* are characteristic of ensiled high moisture corn (Burmeister & Hartman, 1966) and also belong in this group.

As primary invaders, field fungi will not readily reinvade seeds once they have been dried and remoistened, or when they have been invaded by other fungi. Moisture contents of 22 to 25% on a wet weight basis or of 28 to 33% on a dry weight basis, have been cited by Christensen (1965) and Christensen and Kaufman (1965) as the lower limit for growth and development for field fungi, and varying moisture contents, according to species, below this for the development of storage fungi.

Various attempts to determine specific critical levels have been made, including those of Koehler (1932, 1938) Bottomley *et al.* (1952), Kaufman (1959) and Christensen (1965, 1973) for maize; Tuite & Christensen (1965) for barley; Aldrick (1971) for sorghum; Milner, Christensen & Geddes (1947) and Tuite & Christensen (1957b) for wheat. Although these levels vary somewhat from one worker to another, they demonstrate the ecological differences between species convincingly, as can be seen from Table 1.

Table 1

Critical moisture content levels for development of microflora on cereals

% Moisture content requirement	Microflora development
10.00–17.00	*Aspergillus glaucus* group (osmophilic) *A. candidus, A. ochraceus, A. restrictus*
15.6–21.0	*Penicillium* spp, including *P. notatum, P. palitans, P. oxalicum, P. viridicatum*
18.0–20.0	*Aspergillus flavus* Bacteria
18.4	*Fusarium moniliforme*
22.2	*F. graminearum*
21.2–33.0	*Alternaria, Cephalosporium acremonium, Diplodia zeae, Fusarium* spp, *Helminthosporium, Nigrospora sphaerica*

The relatively high moisture content requirements have been explained by several authors as the reason for the relative infrequency of active proliferation of field fungi in storage, as opposed to mere persistence, which as we have seen, can still take place under dry conditions.

Further interesting correlations of species of microflora with moisture content and temperatures have been observed in stored maize, as the following table shows:—

Table 2

Variations in the microflora of maize stored at different temperatures and moisture content levels

Temperature °C.	Prevalent species*	% Moisture	Prevalent species†
25	*Penicillium* spp.	18–20	*Penicillium* initially *Aspergillus flavus* with time, *A. glaucus*
30	*Aspergillus flavus*	24	*A. glaucus* group
35	*A. glaucus* group	27–28	*A. glaucus* less abundant, *A. candidus* + *A. flavus* predominant
45	*Mucor* spp. *Penicillium* spp.	31–32	*A. glaucus* + *A. candidus* virtually absent, *A. flavus* + *A. tamarii* most prominent.

Source: *(Bottomley *et al.*, 1950) †(Bottomley *et al.*, 1952)

These differences in moisture and temperature requirements by storage fungi have since been confirmed by Ayerst (1969).

The dominance of common species of *Aspergillus, Penicillium, Fusarium* and *Alternaria* on stored grain has been found to vary with the substrate, length of storage and treatment (Moubasher *et al.*, 1972). *A. niger* was dominant in maize, wheat and sorghum at a m.c. below 15% in storage. Above this figure, this species was replaced in time by *P. citrinum* and *A. sydowi* on wheat; by *P. citrinum* and *A. terreus* on corn; and by *A. terreus* on sorghum. When grain was stored at a low temperature (8°C) but at a high m.c., Penicillia tended to predominate. The authors also noted an apparent preference of *A. ochraceus* for wheat. The severe deterioration caused by storage fungi such as *P. citrinum, P. variabile* and *A. niger* was contrasted with the relative lack of injury caused by *Cochliobolus spicifer,* a common field fungus.

Lagrandeur and Poisson (1968) and Lutey and Christensen (1963) have outlined a definite succession among field and storage fungi comparable to that observed in the development of various seres toward a climax comprising plants and animals. Periodic examination of grain from the time of harvest through a long period of storage revealed that the field fungi died off and gave place to storage fungi. Lagrandeur and Poisson (1968) found only *P. cyclopium, P. chrysogenum* and *A. versicolor* on maize at the end of the storage period.

In a further study, on sorghum stored aerobically for seven weeks at different moisture contents above 18% (Burroughs and Sauer, 1971) it was shown that the degree of infestation of *Alternaria* decreased with increasing m.c. and time while *Fusarium, Penicillium* and *Trichothecium* proliferated, depending on the temperature.

When grain has been stored dry, the number of storage species developing has usually been found to be relatively few, and the field flora persists, eventually dying away, some species at a faster rate than others (Machacek and Wallace 1952). When grain has been stored wet, even at a low temperature, succession culminates in the predominance of a further group of organisms. In the study of microbiology of ensiled high moisture corn by Burmeister *et al.*, (1966) filamentous fungi rapidly increased, and then decreased sharply at the end of 30 days, being replaced by yeasts and bacteria. The high incidence of yeasts is interesting because they do not normally occur commonly in stored grain. Christensen and Gordon (1948) have also associated them with high moisture content. A similar progression was found in stored sorghum (Gonen and Calderone, 1968) where

species of *Penicillium* and *Aspergillus* eventually gave place over several months to *Hansenula* and *Trichosporon.* Two series of bins were sampled, one at the beginning and end of the experiment and the other at various intervals. The oxygen concentration in the sealed bin was low (0.0 to 0.4%) and the m.c. of the grain was 19.3% or higher.

A somewhat different approach of the classification of seed fungi is represented by the French school of workers. Pelhâte (1968b, 1969) subdivided the mycoflora of grain into three groups, based on an ability to persist: ephemeral, mesobiontic and persistent. The first two groups comprise the field flora and the latter the storage flora, *sensu* Christensen, but perhaps Pelhâte's classification is more flexible. Christensen (1965) and other workers have maintained that there is virtually no overlap between field and storage fungi in terms of distribution, but in practice the writer has isolated both from stored products, though the former exist in more limited quantity. Christensen *et al.,* (1971) implicitly recognized the problem of too rigid a classification when they observed the prevalence of storage fungi in harvested maize that had been damaged.

The life span of fungi on stored grains according to Pelhâte (1969) is influenced by moisture content, temperature, proportion of atmospheric gas constituents and mutual specific interactions. Each species has a humidity optimum for survival and generally speaking low humidity prolongs the life of the field flora and high humidity results in speedy invasion of storage fungi (Table 3).

Sometimes the rapid invasion of grain by particular species of fungi, accompanied by loss in germination and rise in fatty acid content, can be used to characterize them as storage fungi as Martinez *et al.,* (1970) showed when harvested maize with a high m.c. became contaminated with *Penicillium* spp. (44% of seeds), *Fusarium moniliforme* (31%), *Aspergillus echinulatus* (10%) and *A. versicolor* (7%). The high incidence of *F. moniliforme* is interesting since it shows that this species is not restricted as a field fungus. Detailed physiological work, however, establishing tolerance limits under wet and dry conditions, is available for relatively few species, so that only a proportion of the total flora known in storage can be rigorously defined. Mislivec and Tuite (1970 a & b) have investigated temperature and moisture requirements for germination and growth of 14 species of *Penicillium* isolated from corn kernels. Species mainly isolated from *unharvested* corn grow on agar from 8 to 35°C (optimally at 30 to 35°C) and could germinate and sporulate only at an RH of 86% and above. Species isolated mainly from *stored* corn grew on agar from –2° to 30°C (optimally at 23°C) and could germinate and sporulate at an RH of 81 to 83%. On this basis *P. oxalicum, P. funiculosum, P. variable* and *P. purpurogenum* could be identified tentatively as field species and *P. puberulum, P. palitans, P. frequentans, P. chrysogenum, P. urticae, P. cyclopium, P. viridicatum* and *P. breyicompactum* as storage species, while *P. expansum* was intermediate in behaviour.

Further *in vitro* studies have demonstrated a preference by field fungi (*Alternaria tenuis, Cladosporium herbarum*) for oxygen without carbon dioxide as a medium for growth (Hellberg and Kolk, 1972). Storage fungi as represented by *Aspergillus flavus, A. fumigatus* and various Penicillia, however, will tolerate various admixtures of CO_2, up to as high as 43%. At present, studies of this kind in depth are not available for other species isolated commonly from seeds.

The moisture content of stored maize is markedly increased by the activity of insect pests (Joffe, 1958). This in turn promotes general deterioration and the spread of fungi. The growth of storage fungi increases the moisture content of the commodity so that other fungi with higher moisture requirements can invade subsequently. In this way the invasion by fungi once begun, self-accelerates and leads to progressive deterioration even though the original moisture content of the grain may not have greatly exceeded the minimum threshold value. Pockets of grain of higher moisture content than the surrounding bulk, especially inside an enclosed bin or other vessel, can cause severe deterioration. Contrary to

Table 3

Ranking of fungal species in terms of physiological criteria

Fungus development in feedstuffs at different humidities (Snow, 1945)

RH %	Species
75–85	*Penicillium:* minimum level for growth
85–100	Penicillia flourish
90–100	A large variety: *Mucorales* + Fungi Imperfecti

Germination of spores in relation to moisture content (Armolik & Dickson, 1956)

RH %	Species
70	*Aspergillus repens*
	A. ruber
70.3	*A. amstelodami*
79–81	*Penicillium cyclopium*
	P. chrysogenum
87.3	*Fusarium moniliforme*

Minimum humidities below which species were not isolated (Snow, 1945)

RH %	Species
65	*Aspergillus echinulatus*
67	*A. repens*
70	*A. ruber, A. candidus*
75	*A. penicillioides, Paecilomyces varioti, Penicillium spinulosum*
80	*Aspergillus chevalieri, A. amstelodami*
85	*A. versicolor, A. sydowi*
90	*A. niger, Penicillium luteum, P. cyclopium, Sporotrichum, Mucor spinosus*
100	*Penicillium rugulosum, Trichoderma* sp. *Rhizopus stolonifera, Verticillium cinnabarinum, Alternaria tenuis.*

Moisture content requirements for growth (Pelhâte, 1968a)

Species	RH %
Xerophilic,	Optimum 95
Aspergillus conicus	
A. echinulatus	
A. repens	
A. restrictus	
A. versicolor	
Mesophilic,	Optimum 95–100
Alternaria tenuissima	
Cladosporium cladosporioides	
Penicillium cyclopium	
P. spinulosum	
Hygrophilic,	Optimum at 100
Epicoccum nigrum	
Mucor circinelloides	
Trichothecium roseum	

Classification in terms of persistence in storage (Pelhate, 1968b)

Species
Persistent – Storage Fungi.
Aspergillus amstelodami
A. candidus
A. echinulatus
A. flavus
A. niger
A. repens
A. versicolor
Penicillium cyclopium
P. spinulosum
P. stoloniferum
Limited Persistence
Aureobasidium pullulans
Chaetomium globosum
Cladosporium cladosporioide
No Persistence – Field Fungi
Alternaria tenuissima
Epicoccum nigrum
Fusarium culmorum
F. graminearum
F. tricinctum

expectation, the moisture content does not even out through the grain as a whole (Christensen and Drescher, 1954), and this results in the localized growth of storage fungi and the eventual spread of these throughout the stored material. A temperature gradient in the grain may also distribute the moisture present unevenly and result in deterioration (Christensen, 1970).

The source of most of the storage fungi is an interesting puzzle. Tuite and Christensen (1957a) showed that only a small percentage of wheat seeds collected from ripe plants in the field were infested by storage species, and experimental inoculation of the standing, ripe grains, prior to sampling did not materially increase this. Naturally occurring inoculum of storage fungi was uncommon in the air in wheat fields, moderately abundant in elevators and much more abundant in the air of terminal elevators. The percentage of wheat seeds yielding storage fungi increased considerably between the harvest and the arrival of grain at the terminal. There is fairly good evidence, however, that infestation of the seeds by storage fungi does take place prior to harvest, though this tends to be slight (Tuite, 1959). 732 samples of soft red winter wheat yielded only 3 with as much as 3–5% of the seeds infested by storage fungi. The degree of susceptibility varies widely according to the species of fungus (Caldwell and Tuite, 1971). Experimental infection of ears and silks with a spore suspension of 12 *Penicillium* species caused ear rot by one of them, *P. oxalicum,* while the other species were later found to be present in the harvested seed: up to 50% seed infection by *P. cyclopium, P. citrinum, P. expansum, P. viridicatum,* but less than 10% by *P. brevicompactum, P. frequentans, P. palitans, P. purpurogenum* and *P. urticae.* Delaying harvesting of the seed by two months increased seed infestation in four cases by 10 to 33%.

Deterioration

Although many of the field fungi are pathogenic and can invade seeds and cause discolouration (*Alternaria, Fusarium*) they do not specifically attack the germ or contribute to various other characteristic sorts of deterioration. Usually there is a decrease in the processing quality of the crop, "sick" grain resulting in failure of germination due to invasion of the embryo, heating and mustiness and/or poor taste. Storage fungi may or may not be the same as the characteristically seedborne pathogenic fungi that, under suitable conditions during the life of the growing plant, can be responsible for failure of germination, rotting of the stem and root system, and yellowing and blight of the foliage. Because of the intimate relationship of seedborne fungi with the plant, however, most of the seedborne fungi do become active in storage.

A considerable quantity of detailed work has been done on the activity of storage fungi in relation to germination of moist seed. *Fusarium moniliforme* appeared to reduce the germinability of barley more rapidly than did species of *Aspergillus* and *Penicillium* (Armolik *et al.,* 1956). Christensen (1964) showed conclusively that viability of mould free corn, wheat and barley was marginally or not at all affected by length of storage time, temperature and m.c. (within relatively dry limits). In comparing lots of "sick" wheat with reasonably sound and very sound seed samples, he demonstrated an inverse relationship between mould invasion and discolouration and viability of seed (1955a, 1955b). Invasion of wheat was due particularly to *Aspergillus restrictus, A. repens, A. candidus* and *A. flavus.* Detailed studies on invasion of wheat by *A. ochraceus, A. halophilicus* and *A. restrictus* (Christensen 1962, 1967; Christensen and Linko 1963) and of sorghum by *A. glaucus* group (Christensen, 1970, 1971; Lopez and Christensen, 1963) showed that a difference of less than 1.0% in m.c. of the grain had a disproportionate effect on the rate at which the fungus invaded the grain. This causes a decrease of seed viability and an increase in fat acidity values.

Uninoculated maize (which was free from storage fungi) stored at 17% m.c. and at 25°C retained a germination capacity of 98% after twelve weeks, whereas only 6% germinated when inoculated with storage fungi and stored under identical conditions. (Moreno *et al.,* 1965). Inoculation of stored wheat (Papavizas and

Christensen 1957, 1960) and stored corn (Qasem and Christensen, 1958, 1960) by *A. candidus, A. repens, A. restrictus, A. amstelodami, A. ruber, A. flavus* and *A. ochraceus,* alone and in mixtures, also produced at the end of the storage period, varying amounts of discoloured grain in comparison with control samples, associated with loss of viability. Injuries to the pericarp of maize seeds over the germ facilitated the entry of storage fungi (Hurd, 1921). Qasem and Christensen (1958) illustrated the prevalence of different experimentally isolated fungi in corn with ascending moisture contents: at 12% m.c. storage fungi were infrequent, while *A. repens* was almost exclusively present at 14% m.c. and the percentage of seeds invaded increased with increasing temperatures. At 16 to 18% m.c. rapid invasion by all fungi took place. At a given degree of invasion, the authors concluded that *A. candidus* and *A. flavus* were more injurious than members of the *A. glaucus* group. These findings were corroborated later by Christensen (1970), Welty, Qasem and Christensen (1963) and by Qasem (1959).

Although a large number of fungi, particularly belonging to the genus *Aspergillus,* have been designated storage fungi on the basis of their ability to reduce germination, relatively few comparative tests have been carried out using normally accepted field and storage fungi. The effect of *Helminthosporium, Gloeocercospora, Curvularia, Aspergillus* and *Rhizopus* on stored sorghum was interesting in that the first three genera eventually caused seed rot and seedling blight, but only the last two affected seed germination while the seeds were in storage (Mishra *et al.,* 1969). The proven difference in susceptibility among various genetic races of maize and wheat to storage fungi is particularly interesting (Moreno-Martinez and Christensen 1971; Ullstrup, 1971; Wyllie and Christensen, 1959). This may well be an important clue in helping us to understand why outbreaks of mycotoxicosis are particularly prevalent in certain areas.

Biochemical changes

Storage fungi also produce changes in stored material. McDonald and Milner (1954) noted that mould growth on wheat was invariably preceded by elevated temperature and browning of the fresh, unprocessed wheat germ. This led to increased fluorescence, absorbance of acid extracts, fall in pH, decrease in protein content and lowering of the respiratory quotient. Protein condensation in the germ was considered to be the basic cause of brown discolouration. Bottomley, Christensen and Geddes (1950, 1952) concluded that spoilage of corn was affected at first by variation in RH, and then by changes in the atmospheric composition of the storage container. With increase in RH and associated fungal flora, both fat acidity and water soluble nitrogen increased, the latter, however, only slightly. Reducing sugars increased while non-reducing sugars, total dry matter and viability of seed decreased. The decrease in non-reducing sugar content provided the best index of deterioration.

Increase in fat acidity and the other changes noted above have been confirmed by McHargue (1920), Goodman and Christensen (1952), Hummel *et al.,* (1954), Sorger-Domenigg *et al.,* (1955), and Peterson *et al.,* (1956). De Vay (1952) has also reported alteration of free amino-acids and presence of a ninhydrin reacting substance in mouldy wheat. Gamma-butyric acid was found in mouldy wheat with high moisture content.

Reduction of oxygen content, as might well occur in sealed storage conditions, was reported by Peterson *et al.,* (1956) to decrease mould growth, germ damage, fat acidity and respiratory rate, while the seed itself maintained its viability. Increase of the CO_2 level in the presence of 21% O_2 however, had little effect until the concentration had risen between 13.8 and 18.6%, when a marked and sharp inhibition of respiration, mould growth and development of fatty acids took place. Viability of wheat remained high at high levels of CO_2 concentration (50 to 79%). Under conditions of good aeration Bottomley, Christensen and Geddes (1952) showed that for yellow dent corn stored at 30°C at four moisture levels, the mould count and fat acidity generally increased, while viability and concentration of non-reducing sugars decreased with increasing moisture content between

19 to 31% and time of storage. Different species predominated at various intervals of storage and moisture contents. Decreases in non-reducing sugars were related more closely to the mould count than increases in fat acidity, presumably because of the varying ability of different species to form fatty acids. It was found that fat acidity increased rapidly with *A. candidus, A. flavus* and with species of *Penicillium* and *Fusarium* but not with *Aspergillus glaucus.* In non-aerated samples there was little change in fat acidity or non-reducing sugars but there was a decrease in viability. *Candida pseudotropicalis* and a species of *Cephalosporium* and *Penicillium* were found to be especially tolerant of poor oxygen and carbon-dioxide levels. This indicated that spoilage of high moisture grain could not be completely prevented by exclusion of oxygen.

The value of tests for fatty acids as an assay test for the presence of storage fungi is still debated. McGee and Christensen (1970) have reckoned that the action of storage fungi has to become visibly obvious before fatty acids are measurable, thus precluding them as a test criterion. On the other hand Eldridge *et al.,* (1965) and Pattee and Sessoms (1967) have found a high correlation between fat acid levels and aflatoxin in groundnuts. A rapid method of determining fat acidity might be developed to screen samples for aflatoxin. Visible discolouration of groundnut kernels does not necessarily parallel rise in acid content (Halliday 1966).

Heating

Microbiological heating of maize is again related to the relative humidity and the type of organism which will thus proliferate. According to Milton and Jarrett (1970) it is possible to predict the likelihood of mould growth at a given temperature knowing the RH and vice versa:—

Growth of fungi occurred at	when m.c. exceeded
15°C	15%
20	14.5%
25	14.0%
30	13.4%
35	12.6%

The specific microbiological population increases exponentially with time under favourable circumstances. Growth rate of microorganisms is also accelerated by increasing temperature so that for a mixed population the rate of heat production doubles for every 10°C rise in temperature.

Okafor (1966, 1968) found that a thermophilic flora evolved, producing high temperatures in a self heating maize stack that had been accidentally wettened in Nigeria. Mesophilic fungi isolated at 25°C were *Fusarium moniliforme, Aspergillus flavus* and *Rhizopus arrhizus;* thermophiles isolated at temperatures up to 58°C included *Thermomyces lanuginosus, Mucor pusillus, Rhizomucor* sp., *Bacillus licheniformis* (Bacteria) and *Thermoactinomyces thermophilus* (Actinomycetes).

Wallace and Sinha (1962) found that "hot spots" in stored Canadian wheat and oats could develop anywhere in a storage bin. Temperatures of up to 53°C in winter were obtained, usually highest at the base of the bulk of the grain. The heating of the grain was accompanied by killing off of the field fungi (*Alternaria*) and loss of germinability of the grain. The heated grain was eventually relatively dry (m.c. <11% and was predominantly infested by psychrophilic Penicillia and other storage fungi. A further study (Sinha and Wallace, 1965) showed that heating of the grain to a maximum of 64°C was initiated by *P. cyclopium* and *P. funiculosum,* after which the following organisms continued the succession as the grain cooled: *A. flavus, A. versicolor, Absidia* spp. *Streptomyces* spp. Interestingly a hot spot does not infect an entire grain bulk even though the grain immediately affected is rendered unpalatable.

CHARACTERISTICS OF FIELD AND STORAGE FUNGI IN SUBTERRANEAN CROPS AS EXEMPLIFIED BY GROUNDNUTS

So far it appears that the general picture understood for the 'aerial' crops described above broadly obtains for fruits developed below the ground. The division into field and storage species is not as precise however, and there is more opportunity for pathogenic fungi to play a synergistic role in toxin formation.

The groundnut is unique in that the flower is fertilized above ground but the developing fruit or 'peg' bends down and develops in the soil. Contrary to what might be expected, fungi are present from a very early stage of development (McDonald, 1970a). Tests on developing groundnut fruits have shown that 84% of the pegs contained fungi before they entered the ground (Hanlin, 1969). While the fruits matured, shell invasion remained high (90 to 100%) throughout, and seed invasion rose to 82%, declining at harvest time. This high initial infestation is undoubtedly responsible for the prevalence of moulds in stored groundnuts, as observed by Broadbent (1967).

Hanlin (1969) classified groundnut fungi into three ecological groups, an aerial flora, a terrestrial flora and an intermediate one which colonizes groundnuts both above and below ground. The following are the characteristic species:—

Aerial flora: *Alternaria alternata*
Cladosporium herbarum
Colletotrichum dematium
Leptosphaerulina arachidicola

Terrestrial flora: *Corticium* (*Rhizoctonia*) *solani*
Macrophomina phaseolina (*Sclerotium bactaticola*)
Periconia macrospinosa
Trichoderma spp.

Intermediate flora: *Aspergillus* spp.
Fusarium spp.
Gliocladium spp.
Penicillium funiculosum series
Phoma spp.
Rhizopus spp.

Miscellaneous and rare species: These species comprise an assorted number of species that have no clear identification with the groups above.

Garren, Christensen and Porter (1969) have essentially corroborated these conclusions, noting that each microbial community may be compared with an ecological sere as originally described by Tansley (1935) for higher plants. When the fruit approached maturity the microflora of sound pods being more or less quiescent fungi, could be regarded as the climax (allogenic sere). If pod rot sets in before harvest or if the fruits are poorly harvested and stored, then an alternative, deflected climax is reached, at least partly composed of pathogenic invaders. Jackson (1968) has modified this viewpoint, maintaining that since a climax community should be a final or stable community that is self-perpetuating and in equilibrium with the physical environment, this concept cannot strictly be applied to a fruit, since the mycoflora is not in equilibrium. He delimited three terrestrial communities, the first occurring on the outside of pods during the preharvest period (geocarposphere community), comprising *Penicillium rubrum, P. purpurogenum* and *P. citrinum* as dominant species, accompanied by *Aspergillus niger, A. terreus* and *A. flavus. Fusarium* was a subdominant genus. *Rhizopus* became prominent after maturity. The second type of community was characteristic of immature kernels from pods in the soil (endocarposphere community) and was dominated by aspergilli and penicilli as above, but also included *Chaetomium* and *Thielavia. Fusarium* was subdominant and *Rhizoctonia* became prominent at maturity. A post-maturity community could likewise be characterized by dominance of aspergilli, penicilli, *Corticium* and *Rhizopus.*

Finally a community belonging to the shells of mature groundnuts in soil and windrows after harvesting could be distinguished. The dominant genera in soil were found to be *Fusarium* and *Penicillium* with subdominant genera including *Thielavia, Chaetomium, Rhizoctonia, Corticium* and *Macrophomina.* In windrows, *Fusarium, Rhizopus* and *Macrophomina* predominated and *Rhizoctonia, Trichoderma, Nigrospora* and *Curvularia* were less common. In cool weather, invasion by *A. flavus* was negligible while invasion by fungi as a whole was much less than under warm conditions (Jackson 1965c). Typical kernel invaders comprised *M. phaseolina* and species of *Fusarium, Rhizopus, Curvularia, Nigrospora, Chaetomium* and *Rhizoctonia.*

McDonald (1970a) likewise found that *Macrophomina* and *Fusarium* spp. were dominant in the shell flora, and *Aspergillus, Botryodiplodia theobromae, Penicillium* and *Rhizopus* were also common. Seed fungi which developed later, comprised *Fusarium, Aspergillus* and *Penicillium* only.

Jackson did not continue his study further with an investigation of storage fungi, but we can see that his aerial and terrestrial communities and other species discussed above correspond roughly with the "field flora" "storage flora" and "intermediate flora" of cereal grains. The pathogenic seed fungi described by Garren *et al.,* (1947), Wilson (1947b) and by Ward and Diener (1961) are directly homologous in behaviour with the storage fungi of aerial crops.

Hanlin (1970) obtained results with developing groundnuts that were similar to those of Jackson. There was, however, a change in species composition as the season progressed. The percentage invasion by the total number of species of fungi rose gradually during the last three months prior to maturation to nearly 100% in shells and about 80% in kernels. Species of *Aspergillus* and *Penicillium* however, decreased while the *Fusaria* increased, balancing each other out. Garren and Porter (1970) and Porter and Garren (1970) examining mature groundnuts from Virginia, USA and from Puerto Rico, defined 8 endocarpic communities on the basis of high degree of association between various fungal species under different conditions in the field. The ecological significance of these communities however, needs further clarification. The dominant species, defined as having greater than 5% incidence, comprised the *Aspergilli, Colletotrichum, Nigrospora, Phoma* and *Thielavia* spp., and *Trichoderma viride.*

Joffe (1969a, 1969c) showed that the flora of shells and kernels had a wide distribution also in the rhizosphere and accompanying soil. Species of *Aspergillus* were more numerous in heavy soil whereas *Penicillium* favoured light soil and *Fusarium* soil of medium consistency. Soil inoculation with *A. flavus* depressed the number of species in the rhizosphere and soil associated with the groundnut plant. *A. flavus* was, however, isolated in relatively small quantities from fresh and stored groundnut kernels in comparison to *A. niger* which was the species found in greatest numbers, and which continued as the dominant during the first few months of storage (Joffe and Lisker, 1969). *P. funiculosum* and *P. rubrum* were also found commonly both in soils and on kernels. It appears that the living groundnut seed has some resistance to invasion by weakly pathogenic fungi or saprophytes. When Lindsey (1970) grew two varieties of groundnuts under gnotobiotic conditions and inoculated them with *A. flavus* spores, he could demonstrate no evidence of pathogenicity or podrot symptoms. *A. flavus* penetrated the shell tissue consistently but was limited in its seed invasion to the testa. The high rate of shell invasion *in vitro* is also at variance with that observed in nature. Either the normal endogeocarpic mycoflora associated with shells is antagonistic to *A. flavus* (contrary to the experimental work cited earlier) or the faster growing species of the mycoflora mask *A. flavus* when harvested shells and kernels are cultured.

In contrast to the large quantity of work done on cereals, the physiology of groundnut infestation in storage with respect to humidity and temperature variations does not appear to have been as closely studied. In general it appears that

the microflora varies with temperature and moisture content of the substrate as in aerial crops, though sometimes no consistent pattern in microfloral incidences may be obtained (Joffe, 1968). The limiting moisture content for most fungi seems to occur around 10%, although *A. flavus* can grow at moisture content levels down to 4%. Growth of *Botryodiplodia theobromae, Macrophomina phaseolina* and species of *Fusarium* and *Penicillium* could take place rapidly at an m.c. level of 10% (Wilson, 1947b). Welty and Cooper (1968) showed that different species predominated at different moisture contents (see Table 4).

Table 4

Variations in microflora of groundnuts stored at different moisture content levels

Moisture content	Species predominant
4.5%	None: infestation scanty
8–18%	*Aspergillus glaucus group* *Aspergillus flavus* present
9–11%	*Aspergillus ruber*
20%	*A. flavus and Penicillium*
>20%	*Fusarium* spp.

The degree of specific infestation depends on the temperature and probably on mutual antagonisms (Jackson, 1965a). When surface disinfested pods were selectively reinfested and allowed to hydrate during incubation at various temperatures, the empiric relationship between kernel infestation and temperature shown in Table 5 was obtained.

Table 5

Relationship between infestation of groundnut kernels and storage temperature

Optimum range for growth and optimum temperature	Species	Optimum range for sporulation
16–32°C (32°C)	*Rhizopus stolonifer*	16–26°C
21–32°C (26°C)	*Macrophomina phaseolina*	26–32°C
26–38°C (38°C)	*Aspergillus flavus*	21–38°C
	Aspergillus niger	26–32°C

When cross-infection in groundnuts was induced using the four species above and *Penicillium citrinum,* it was clear that *A. flavus* had the greatest general success, and *P. citrinum* the least success in suppressing competitors.

Storage fungi cause essentially the same damage in stored groundnuts as with cereals, resulting in discolouration (Garren *et al.,* 1947), rancidity and increase in free fatty acid content (Wilson, 1947b). When *Aspergillus chevalieri, A. repens, A. restrictus, A. ruber, A. tamarii* and *Penicillium citrinum* were regrown on autoclaved shelled peanuts, the principal biochemical changes observed were: loss in organic matter, degradation of sucrose, decrease in total oil, increase in free fatty acids, and increase in % unsaturated fatty acids from the oil (Ward and Diener, 1961). *P. citrinum* caused the least damage.

After maturation and harvesting the groundnut is definitely more susceptible to visible and concealed damage than before (Wilson, 1947 a & b; Gilman, 1969a). Nine species have been implicated in addition to those above:— *Macrophomina phaseolina, A. flavus, A. niger, Botryodiplodia theobromae, Fusarium solani, F. semitectum, F. oxysporum, Corticium rolfsii* and *Trichothecium roseum.* The discolouration of groundnuts is sometimes due to metabolites produced by the

invader: *Corticium rolfsii,* for example, causing "blue black damage" produces oxalic acid that reacts with pigments of the seed coat similar to an indicator reaction. Jackson (1964) has stressed the importance of the thin seed coat inside the shell as a barrier to infection.

Garren (1964b, 1966) compared the floras of rotten and sound groundnuts, and demonstrated the absence of *Corticium solani* and *Pythium myriotylum* in sound pods as compared with the rotten ones. The presence of these two species is antagonistic to *Penicillia* and *Fusaria.* The pod rotting complex is an essentially synergistic one: a significant relationship between *Pythium myriotylum* and *Fusarium solani* in the increasing incidence of pod rot has been demonstrated by Frank (1972).

Podrotting fungi such as *Corticium solani* and *Macrophomina phaseolina* are of importance in that they eventually lay the way open for further invasion by saprophytes (Ashworth and Langley, 1964). *Corticium solani* alone and in combination with insect larvae caused 87% of the preharvest pod damage in Texas grown Spanish groundnuts. On the other hand *Macrophomina phaseolina* was found to be active both before and after harvest (Gilman, 1969a). Infection with this species was markedly reduced by *A. flavus* (Jackson, 1965b), but not by *A. niger.* The action of *A. flavus* against other fungi deserves further study.

The natural incidence of fungi in foodstuffs

Although there has been a great deal of work done on the compilation of lists of mycofloras from various products, relatively little has been done on the relative incidence of the various species. This is at least partly due to the dilemma arising from the difficulty of standardisation of incidence; species can be enumerated in terms of their occurrence in a given number of seeds, or in a number of samples of seeds or products from them, or in terms of the number of colonies plated out per gram of material as estimated by a simple dilution method or by means of the Anderson air spore sampler (Clarke, 1968). Each method involves certain errors. If the incidence in seeds is measured, then there is a good chance that a species may be recorded at a higher incidence than it actually occurs due to the spread of that species within the sample en route to the laboratory. If the incidence per sample is counted, then the actual original incidence among the individual seeds is obscured, and so-called 'faithful' or 'indicator' species which have a constant though rare occurrence in seed will get a rating equivalent to that of fungi whose incidence in seeds is much higher. Counting colonies may lead to error because the number of viable bodies (spores, mycelial portions, etc.) produced by a fungus differs widely from one species to another, and heavily sporulating species will be grossly over-represented. Interpretation of the figures in any case is difficult, since the relationship between sporulation and growth cannot be precisely defined (Barron and Lichtwardt, 1959; Broadbent 1966a and b). Although the determination of relative incidences is a valuable exercise, the results ought probably to be interpreted only qualitatively. Figures available for sample incidence are usually higher than those of seed incidence, the latter are often unexpectedly low, especially when harvest material is being examined (vide Jackson, 1963; Tuite, 1961). Tables 6, 7 and 8 further illustrate this and give details of the fungal floras of maize, wheat and groundnuts as determined by several authors.

Notwithstanding the above difficulties, studies of fungal incidence in foodstuffs in Southern Africa (Keen and Martin 1971a; Martin, Gilman and Keen 1971) have shown that the species composition of each foodstuff is often characteristic. This work has also assisted with the recognition of field and storage fungi as well as revealing changes in mycoflora during processing and with storage container.

Table 6

Incidence of fungi in maize seeds (%)

Author	Tuite (1961)			Christensen *et al.*, (1971)	Hoppe (1943)	Manns & Adams (1923)	Melchers (1956)	Tuite & Caldwell (1971)	Warmke & Schenck (1971)		Levenberg (1966)	Martinez *et al.*, (1970)
Remarks	Pre-harvest 1956	Pre-harvest 1957	Pre-harvest 1958	Damaged, in field	Harvest	Harvest	Harvest	Harvest	Harvest normal	Harvest T. cytoplasm	storage in silos	shops and storehouses
Species												
Acremoniella	0.3	3.3	0.8					0.3				
Alternaria								0.2			8–18	
Aspergillus							0.7–20.0(4.0)					
A. candidus				8								0.02
A. chevalieri												4.9
A. echinulatus												10.1
A. flavus				9				0.4			2–23	2.5
A. fumigatus											4–98	
A. glaucus group				54				0.1				
A. nidulans											4–10	
A. niger											2–68	1.8
A. ochraceus				2–3							2–10	
A. restrictus				trace								
A. ruber												4.3
A. terreus											2	0.04
A. umbrosus												0.02
A. versicolor												7.4
A. wentii												4.1
Botryodiplodia												1.0
Cephalosporium											2–70	0.1
C. acremonium	15.5	10.5	7.9					7.4				
C. sacchari						39.5						
Chaetomium							0.0–4.0(0.4)					
Cladosporium	0.1	2.0	0.2									0.6
C. herbarum									1.3	0.0		
Diplodia zeae	0.4	0.3	0.3		0.0–70.5	5.7	0.0–6.0(1.1)					0.2
Fusarium graminearum	0.2	4.5	2.7		0.0–15.0	6.0		2.7				0.7
F. moniliforme	9.0	13.3	2.2		1.0–60.0	19.9	4.0–94.0(40.1)	44.6	31	58		30.6
F. oxysporum												0.3
F. spp. exclud. *moniliforme*							0.0–5.3(0.8)					
Helminthosporium maydis								9.7	2.5	17.5		
Mucor	0.3	0.9	0.7		0.0– 5.2						6–96	
Mycosphaerella												0.1
Nigrospora												0.8
N. oryzae	0.8	1.5	3.6		0.0–34.0		0.0–10.9(0.6)	31.3				
Penicillium	1.9	3.3	0.2	22			0.0–10.7(1.5)	3.0			2–70	43.8
P. canescens									3.8	2.5		
Phoma											2–8	
Rhizopus							0.0–4.0(0.6)	0.2				
Rh. stolonifera									1.3	1.3		
Trichoderma viride							0.0–3.0(0.2)	0.4			7–44	

Table 7

Incidence of fungi in wheat seeds (%)

Author	Hewett (1965, 1967)	Hyde & Galleymore (1951)	Machacek *et al* (1951)	Pixton *et al.*, (1964)
Remarks	Harvest, England	World-wide	Harvest, Canada	Harvest, Canada
Species				
Absidia			0.01	7–8
Acremoniella			<0.01	
Alternaria			55.14	31–88
A. tenuis		64.4		
Aspergillus				10–32
Aureobasidium pullulans		4.8		
Botrytis			0.03	
B. cinerea		1.9		
Camarosporium			<0.01	
Cephalosporium			<0.01	0–48
Cephalothecium			0.02	
Chaetomium			0.05	
Circinella			<0.01	
Cladosporium			0.30	1–2
C. herbarum		5.8		
Cochliobolus sativus			2.45	
Constantinella			<0.01	
Curvularia			0.06	
Delitschia			<0.01	
Drechslera avenaceum			<0.01	
Epicoccum			0.51	0–7
Fusarium		1.9	0.65	
F. avenaceum	1–2			
F. culmorum	0–3(0.3)			
F. nivale	0.3			
F. poae	1–1.5			
Gonatobotrys			<0.01	0–2
Helminthosporium			0.21	
Leptosphaeria nodorum	1.0–7.4			
Monotospora			<0.01	
Mucor			0.03	1
Mycogone		7.7		
Nigrospora			0.80	
Papularia			0.01	0–5
Penicillium			0.21	8–46
Phoma			0.02	
Pullularia			0.56	
Rhizoctonia			<0.01	
Rhizopus			0.02	5–7
Sclerotinia			<0.01	
Sclerotium			<0.01	
Scopulariopsis			<0.01	
Septoria			0.70	
Sordaria			<0.01	
Stemphylium			0.02	0–4
S. botryosum		0.9		
Syncephalastrum				0–1
Torula			0.02	
Trichoderma			0.01	
Yeasts			<0.01	

Table 8

Incidence of fungi in groundnut shells and kernels (%)

Author and date	Gilman 1969a	McDonald 1970b				Hanlin 1972		Norton *et al.* 1956	Borut & Joffe 1966	Diener 1960
Stage	Pre+Post Harvest	lifting		in windrows		Post Harvest		Post Harvest	In storage	In storage
Material	kernels	shells	kernels	shells	kernels	shells	kernels	kernels	kernels	kernels
Species										
Alternaria								12.6		
Aspergillus	4			0.0–1.0	0					
A. candidus										26.9
A. flavipes		0.0–0.5	0					0.3		
A. flavus	14	0.0–0.17	0.0–1.0					24.7	63.5–78.4	
A. fumigatus		0.0–0.5	0.0–0.5							
A. glaucus grp.										100.0
A. nidulans		0.0–1.5	0.0–0.5							
A. niger		0.0–15.5	0.0–5.5					7.2	91.4	
A. tamarii										84.6
A. terreus								0.7		
A. versicolor		0.0–1.0	0							
Botryodiplodia theobromae	3	0.5–8.0	0.0–3.5	3.0–6.0	1.0–7.0					
Botrytis sp.						33.0	15.0			
Cladosporium										100.0
C. herbarum		0.0–1.0	0.0–1.0	0.0–2.0	0.0–1.0					
C. oxysporum	3									
Corticium rolfsii	3	0.0–2.0	0	0	0.0–1.5			0.6		
C. solani		0.0–5.0	0.0–2.0	0.0–4.0	0.0–6.0					
Cunninghamella elegans		0	0	0.0–1.0	0					
Curvularia		0	0	0.0–1.0	0					
C. lunata								0.3		
Cylindrocarpon		0.0–0.5	0	0	0					
Epicoccum						8.0	0.0			
Fusarium	11	25.0–53.0	1.0–18.5	38.0–79.0	13.0–34.5	32.9	11.0	10.8		
Gliocladium						6.4	0.0			
Helminthosporium								0.9		
Macrophomina phaseolina	57	14.0–69.0	0.0–31.5	58.0–73.0	10.5–45.0			2.4		
Monilia		0.0–0.5	0	0	0					
Mucor						10.4	4.5	0.4		
Mucorales										73.1
Myrothecium verrucaria		0.0–3.0	0.0–0.5	0.0–1.0	0					
Neocosmopara vasinfecta		0.0–1.0	0.0–1.5	0.0–2.0	0.0–1.5					
Penicillium		0.0–2.0	0.0–2.0	0.0–7.0	1.0–4.5	17.4	26.6	7.8		
P. citrinum		0.0–1.5	0	0	0					100.0
P. funiculosum		0.0–2.5	0.0–8.0	0	0					38.5
P. janthinellum										26.9
Phoma		0.0–1.5	0	0	0	26.3	2.1			
Rhizoctonia								8.1		
Rhizopus		2.0–36.0	0.0–9.0	3.0–23.0	0.0–10.5					
Rh. stolonifer								8.9		
Torula sacchari										80.8
Trichoderma viride								0.4		
Trichothecium roseum										26.9

The ecology of mycotoxin formation

Since mycotoxins were realised to be a potential hazard to human health, considerable work has been done on the factors influencing the formation of toxins in the field and *in vitro.* The greatest amount of attention has naturally been given to aflatoxin.

Much of the work on natural formation of aflatoxin has centred on harvest conditions, post-harvest conditions, relationship to moisture content and interaction with other species, physiology of toxin production *in vitro* and substrate preference.

Environmental conditions during the preharvest and harvest period, and during storage play the major role in mycotoxin formation:—

MOISTURE

Studies of invasion of groundnut kernels in relation to moisture suggest that there is both a minimum limit and a maximum limit of moisture content below and above which growth of *A. flavus* does not occur. At 48% m.c. only 1% infestation of kernels was observed, 29.5% m.c. resulted in 9% infestation and 27.1% m.c. gave 55% infestation (Diener *et al.,* 1965). On the other hand no growth of *A. flavus* was observed at an m.c. of less than 8%, equivalent to a relative humidity of 75% (McDonald, 1968a). Moisture contents in excess of 9% accompanied by temperatures of 10—45°C. provided conditions suitable for the growth of *A. flavus* (Burrell, Grundey and Harkness, 1964). McDonald, Harkness and Stonebridge (1964) suggested that undamaged kernels at lifting, normally with an m.c. of 30—45%, may not be invaded due to some innate resistance, because contamination and aflatoxin formation did not occur in their crop until at least 6 days after lifting. Thereafter, contamination increased until the pods dried to a safe moisture content level of 8—10%.

It has been generally assumed that high levels of *A. flavus* incidence predispose material to aflatoxin formation. In fact various experimental studies have shown that this may not be necessarily true. The temperature range of growth for *A. flavus* was determined by Ayerst (1964) as 15—45°C, and the m.c. required decreased from 12% at 20°C to 9% at 30—35°C. The optimum moisture content range for *A. flavus* contamination was determined as 15—25%, whereas toxin formation could occur at a much higher m.c. (39.0%) if there was damage to the pod (Austwick and Ayerst, 1963). Rabie and Smalley (1965) determined the growth optimum for a strain of *A. flavus* at 18°C whereas the optimum for toxin production was 24°C. Diener and Davis (1967) found experimentally that aflatoxin in general was produced at high levels of RH (87—98%) and at moderate temperatures. No aflatoxin occurred at 15°C and 45°C. Dickens and Pattee (1966) determined that when the temperature was high (90°F or 32.8°C) optimum aflatoxin formation occurred within the m.c. range of 28—38%, and that this range was lowered to 20—26% when the temperature was moderate (70°F or

21.1°C). Much less overall production of aflatoxin was obtained at the lower temperature, and conversely, reduction of the RH to 50% while maintaining the temperature at 90°F completely inhibited production. These results are consistent with those of Eldridge *et al.*, (1965) who obtained good production of aflatoxin at 30°C and 95% RH. The general conclusion was that storage at cool temperatures and low m.c. would lead to inhibition of toxin formation whereas the reverse would encourage it.

The results from various field observations have not consistently supported the experimental conclusions.

In the classic series on the growth of *Aspergillus flavus* and production of aflatoxin on groundnuts, a number of authors (Bampton, 1963; McDonald and Harkness, 1963, 1964; McDonald and A'Brook, 1963; Burrell, Grundey and Harkness, 1964; McDonald, Harkness and Stonebridge, 1964; Harkness *et al.*, 1966; McDonald, 1969), working in Nigeria, demonstrated that humidity governed by the extent of rainfall was the major factor affecting the development of fungi in groundnuts, but not necessarily aflatoxin.

Groundnuts are produced in two distinct zones in Nigeria; a dry northern zone containing 95% of the crop and a wetter riverain zone accounting for the remainder. A much higher degree of fungal infection occurred in the riverain zone than in the northern, but toxin formation appeared with equal frequency in both northern and riverain provinces, and none of the varieties sampled were apparently more resistent to toxin formation than another.

Increase in moisture leading to fungal activity was brought about by a sharp rise in atmospheric humidity at the onset of the rainy season, followed by moisture movement within stored material along temperature gradients set up by insect or other biological or physical activities, and also by direct penetration of rain or soil water into the storage chamber. The latter is likely to be more serious when underground pits are used as the conventional method of storage.

The only unequivocal evidence where a difference in toxin production according to rainfall conditions has been shown is that of Habish and Abdulla (1971). Two main regions in the Sudan were demarcated: the rainlands and the dry area which required irrigation for the growth of groundnuts. The concentration of aflatoxin in harvested groundnuts was much higher in the former region. The aflatoxin content of the kernels here was also broadly correlated with the extent of contamination by *A. flavus* and with pod damage.

In other work with stored groundnuts, the quantity of aflatoxin B has not been found consistently associated with m.c. or with prevalence of *A. flavus,* but has increased with length of storage time (Welty and Cooper, 1968). When the distribution of aflatoxin in the groundnut crops in South Africa between 1963 and 1966 was examined in conjunction with meteorological data (Purchase, 1967b) no correlation with high temperature and humidity could be made. Groundnuts stored at 4.5% m.c. at a temperature range of 22–28°C are deemed to be relatively safe from storage fungi including *A. flavus.*

The converse supposition that mould growth should not be significant in dry conditions was disproved by the discovery of aflatoxin in significant quantities in foodstuffs in the driest area in Uganda (Alpert *et al.*, 1971). It is the microenvironment of the foodstuff, whether during harvesting, drying or storage, that is the major determining factor in the degree of mould growth and ultimately toxin production. If, in spite of a low mean annual value, the rainfall is concentrated into a short rainy season turning a semi-desert region into a mud field for a short period, then the threat of toxin formation is as great as if the rainfall was quantitatively greater and spread over a longer time. This idea has been supported by Shank (1973) who found that aflatoxin contamination of foodstuffs in Thailand was better correlated with local flooding than with overall rainfall or humidity.

In the case of substrates other than groundnuts, various workers have obtained similar results. There is no essential relationship between moisture content and aflatoxin formation. The m.c. of flour required for growth of *A. flavus* lay between 14% and 16%, i.e. the fungus was mesophilic in its growth requirements (Seeder *et al.*, 1969), but subsequent work showed that more aflatoxin was produced on whole seed of maize than on maize flour at suitable moisture contents (Jemmali *et al.*, 1969). The latter authors also determined that products produced by dry methods (e.g. semolina) were more heavily contaminated by aflatoxin than products by wet milling (starch, corn steep, dry protein). This ambivalent conclusion as regards moisture content is consistent with the apparently puzzling natural occurrence of aflatoxin in field crops under dry conditions.

Experimental work has shown that when there is plenty of water available, aflatoxin can be formed on maize just as plentifully as on groundnuts. Maize contaminated experimentally with *A. flavus* together with a naturally contaminated sample, were steeped and wet milled. Aflatoxin was found primarily in the steep water and in the fibre, with the remainder in the gluten (14.7%) and the germ (6–10%) (Yahl *et al.*, 1971). In field conditions the formation of aflatoxin in maize is probably restricted by more stringent moisture and temperature limits. Broadbent (1966b) found detectable quantities of aflatoxin in two of six sacks of maize from poultry and livestock farms where the moisture content ranged from 16.2 to 24.4%, much higher than the minimum limit for mycelial growth. Periodic aflatoxin determinations have also been made on fresh and remoistened samples of maize at various moisture contents stored at different temperatures (Trenk and Hartman, 1970). Aflatoxin B_1 and B_2 were formed at levels about 17.5% m.c. and 24°C. Greater aflatoxin production was observed in remoistened dried maize than in freshly harvested maize. Further experimental work on the formation of aflatoxin in maize samples kept in storage 12 months and naturally infected with *A. flavus* and then conditioned at moisture contents of 17–18% or 18–19% and incubated at 20°, 25° and 30° for 4 weeks, showed that aflatoxin accumulated only in those samples with an m.c. of 18–19% at the two higher temperatures (van Warmelo, van der Westhuizen and Minne, 1968). These conclusions are also in agreement with various field observations.

TRAUMA

Both the degree of fungal contamination and aflatoxin formation appear to be enhanced by trauma to the groundnut pods. Kernels from broken or termite damaged pods are more heavily infested by *A. flavus* than those from undamaged pods (McDonald and Harkness, 1963, 1967; Sellschop, 1965). Overmature and aborted pods also have been found susceptible to infestation (McDonald, 1969; Porter and Wright, 1971). Field trials indicate that *A. flavus* has a preference for moribund or dead matter rather than living tissue, a view substantiated by finding that preharvest development of aflatoxin only took place in pods cracked due to irregularity of growth (Schroeder and Ashworth, 1965; Ashworth, Schroeder and Langley, 1965). Aflatoxin was not found in imperforate pods even though *A. flavus* may have been present.

The incidence of contamination by *A. flavus* and of aflatoxin in relation to damage is expressed in tables 9–12.

Table 9

Contamination of groundnuts by *A. flavus* in Alabama (Diener, 1965)

	Kernels	Hulls
Immature	–	1%
Mature	2%	9%
Overmature – damaged	8%	55%
Stored for 3 days	–	6%

Table 10

Contamination of groundnuts by *A. flavus* in Nigeria (McDonald and Harkness, 1963)

	Unbroken pods	Broken pods
Discolouration due to Microbial growth	2–6%	100%
A. flavus incidence	0%	58–74%

Table 11

Aflatoxin content of damaged and normal groundnut pods in Nigeria (McDonald *et al.*, 1964)

Samples	Pickings			Gleanings		
	Undamaged	Termite damage	Broken	Undamaged	Termite damage	Broken
No. tested	69	25	25	9	5	5
No. toxic	0	4	4	0	3	4
% toxic	0	16.0	16.0	0	60.0	60.0

Table 12

Contamination of groundnuts and aflatoxin formation in the Sudan (Habish and Abdulla, 1971)

Sample	% damaged pods	% kernels infested by *A. flavus*	% kernels with aflatoxin	Toxicity
1	67.4	81.2	35.6	very high
2	69.6	68.0	33.6	high
3	69.2	59.6	22.0	medium
4	69.4	72.8	27.6	medium
5	58.7	4.5	2.0	low
6	59.5	15.2	5.5	low

The infestation of groundnuts by *Aspergillus flavus* is also encouraged by nematode galling of pods and pegs (Minton and Jackson, 1967) and by mites and termites acting as vectors (Aucamp, 1969; Sellschop, 1965) but the aflatoxin producing potential of these processes has not been assessed.

HARVESTING METHOD

The method of harvesting presumably affecting rates of breakage and drying, may also influence the degree of aflatoxin formation. *A. flavus* was observed to be more abundant in kernels from pods gathered by a combine harvester than from hand harvested pods, and the respective aflatoxin B concentrations were 1 780 ppb and 140 ppb. Inverted windrow drying leads to less contamination by *A. flavus* than random windrow drying of the harvest (Porter and Wright, 1971).

PROCESSING TREATMENT

Rapid drying of groundnut kernels resulted in low fungal infestation and little or no toxicity – slow drying the reverse, (McDonald and Harkness, 1966). Protecting the groundnuts with covers during showers and during the night led to uninterrupted drying and less susceptibility to aflatoxin production than leaving the groundnuts exposed (Burrell, Grundey and Harkness, 1964). These authors also showed that artificially dried pods were free of toxin whereas groundnuts

sundried after wet weather yielded 0.1–0.5 ppm. Paradoxically the former showed a higher degree of kernel contamination but this may have merely reflected surface contamination induced by treatment with the drier. The degree of toxin production has been reduced by use of artificial drying equipment (Pettit and Taber, 1968) in Texas, and fast drying rather than slow has also been recommended (Jackson, 1967b, Troeger, Williams and Holaday, 1970).

The combined effect of moisture, storage and various harvesting treatments was studied by Keen and Martin (1971a) in Swaziland. One hundred and thirty random samples of groundnuts, collected from various rural areas, which had been stored under mainly unsatisfactory conditions were compared with 34 samples stored under shelter and in dry conditions. The former yielded 3–25 fungal species per sample with an average of 14.4; 77 samples (59.2%) yielded *A. flavus* and 34 (26.1%) were aflatoxin positive. The latter yielded 5–12 species with an average of 7.9; *A. flavus* occurred in only 13 samples (38.2%) and no aflatoxin was found.

When five methods of harvesting and storage were compared experimentally, it became clear that groundnuts dried under cover did not develop aflatoxin in storage except when moistened deliberately. Groundnuts stored in jute bags were on the whole less liable to aflatoxin formation than when stored in metal containers, possibly due to better aeration mitigating against the accumulation of moisture.

These observations show that while the various factors affecting aflatoxin development in the field may not have been completely elucidated, there is little doubt that from a practical point of view, adequate harvesting and storage can reduce fungal infestation and toxicity. In concluding this brief review of the development of aflatoxin in the field it is worth citing Bushnell's directives (1964) designed to reduce mould incidence and aflatoxin formation:

(i) groundnut plants should only be lifted when the bulk (>70%) of pods are clearly mature, as shown by brownish-black markings on the pod lining;

(ii) curing should achieve rapid drying out of nuts without undue exposure to direct sunlight or rain;

(iii) pods should be picked when the m.c. is 10–15% (i.e. when the kernels rattle);

(iv) shelling should separate kernels from pods with minimum kernel damage.

SOIL

The soil may also exert an influence on the incidence of *Aspergillus flavus*. Groundnuts harvested from land on which groundnuts had been planted the previous year were more highly infested and contained more aflatoxin than groundnuts grown on land previously planted with rye, oats, melons or potatoes (Pettit and Taber, 1968). Likewise, previous fungicide treatment of the soil has been known to reduce incidence of *A. flavus* from pod, shell and seed of groundnut fruits to very low levels (Barnes, 1971). Accordingly it might also be useful to add recommendations on appropriate crop rotation programmes and soil treatment in order to reduce the hazards from mycotoxins.

FUNGAL SYNERGISM

A fascinating though incomplete line of research is the synergistic effect of competition by other species of incidence of *A. flavus* and aflatoxin production. Wells, Kreutzer and Lindsey (1972) grew groundnuts gnotobiotically in the presence of *Trichoderma viride, Penicillium funiculosum* and *A. flavus.* Immature pods, mature pericarps and testae were all susceptible to invasion. Colonization by *A. flavus* was reduced by *T. viride* whereas *P. funiculosum* appeared to

stimulate it. Mature of moribund tissue was colonized heavily whereas immature tissues were apparently invaded non-pathologically. Other work is somewhat contradictory in terms of expectation. Choudhary and Manjrekar (1967) found a 26–25% incidence of *A. flavus* in field kernels discoloured presumably by the activity of other fungi, whereas lesser contaminated white nuts were free of *A. flavus;* however pods infected by *Corticum solani* investigated by Schroeder and Ashworth (1965) showed minimal aflatoxin concentrations, in keeping with results obtained for 'clear and sound' pods. When comparisons were made between the floras of blight and non-blight-damaged maize seed (Doupnik, 1972) there were significantly higher infestations of other fungi (*Fusarium moniliforme, A. flavus, Penicillium* spp., *Aspergillus* spp. etc.) associated with the presence of *Helminthosporium maydis* than with its absence. One quarter of the blighted seed samples were positive for aflatoxin, whereas only 5% of the non-blighted samples were. A similar conclusion was made from the results of Keen and Martin (1971a) discussed above. Detailed analysis of the total fungal population in 107 samples of the 130 field samples of groundnuts referred to, revealed that the incidence of *A. flavus* was higher among those samples from which a large number of other species were also isolated. The same was broadly true of the distribution of aflatoxin in these samples, suggesting that perhaps the interaction of other species had stimulated its formation.

PHYSIOLOGY OF THE FUNGUS

In vitro studies of the physiology of *A. flavus* have yielded interesting information about the biology of the species but more work needs to be done in order that a clear relationship can be demonstrated between the various physiological characteristics and its behaviour as observed in nature. No clear correlation can be drawn at present between the morphology of the species – colour, sclerotial production, conidiophores and size of conidia etc. – and the ability to produce toxin (Anon, 1963; Vanderhoven, Remâcle and Ramaut, 1970). Numerous studies have shown that toxin production in this and other toxigenic species is not a universal feature (Codner, Sargeant and Yeo, 1963). The proportion of toxic strains varies from one fungal species to another throughout many fungi (Martin, Gilman and Keen, 1971): In *Aspergillus flavus* and in species of *Fusarium* the proportion is fairly high. Joffe (1969d) found that only 10.4% of isolates tested from groundnuts and soil of groundnut fields in Israel failed to produce aflatoxin in culture. The number of aflatoxins produced and the quantity, however, varied tremendously.

The optimum temperature for aflatoxin production on rice by *A. flavus* has been determined as 28–32°C for aflatoxin B_1 and 28°C for aflatoxin G_1 (Sorenson, Hesseltine and Shotwell, 1967). No aflatoxin was produced at temperatures lower than 8°C. Van Walbeek, Clademenos and Thatcher (1969) however found that significant concentrations of toxin could nevertheless be formed under conditions resembling household refrigeration (7.5–10°C). Aflatoxin production was not related to the growth rate of the fungus (Schindler, Palmer and Eisenberg, 1967). One isolate at 41°C, still at almost maximum growth rate, produced no aflatoxin. In many isolates the optimum growth temperature varied between 29–35°C. The ratio of the four aflatoxins to each other also varied with temperature (Schindler, Palmer and Eisenberg, 1967) and with concentrations of sugar (Schroeder, 1966). Short periods of high temperatures (40–50°C) in each 24 hour diurnal temperature cycle (average 25°C) reduced growth of *A. parasiticus* and production of aflatoxin. The reverse with cold periods of as low as 10°C did not appreciably affect growth or toxin production (Schroeder, 1968); under cool conditions (60°F/15.6°C) however, lack of oxygen can be a crucial factor in toxin synthesis and below 54°F (12.2°C) both toxin formation and fungal growth are inhibited (Epstein *et al.,* 1970). At higher temperatures and with otherwise optimum conditions, oxygen reduction still decreases sporulation and toxin concentration.

Toxin production was also found to be proportional to concentration of substrate (Schroeder, 1966) and to aeration by agitation (Hayes, Davis and Diener, 1966).

Yeast extraction was a stimulatory additive, glucose, sucrose or fructose were preferred as carbon sources and casamino acid as a source of nitrogen. Zinc was an essential element in laboratory production (Davis, Diener and Eldridge, 1966; Mateles and Adye, 1965). A strong connection also exists between nutrient supply and ochratoxin formation (Lai *et al.*, 1968). Ochratoxin depends on magnesium, is stimulated by sucrose and glucose, and the concentration is higher on wheat than on soybeans as a natural substrate.

In contrast to aflatoxin, the physiology of formation of the various fusarial toxins is radically different in that they are stimulated in production by cool temperatures or by exposure to a low temperature for part of their development. Joffe (1971) has shown that *Fusarium poae, F. sporotrichioides, Cladosporium epiphyllum* and *C. fagi,* all involved as agents in alimentary toxic aleukia, preferred low temperatures from −7°C to +25°C for growth and toxin production. A routine involving alternate freezing and thawing for 9–15 days was the best for this purpose (Bashmakova, 1965; Joffe, 1971). Yates *et al.,* (1968) also found that cool temperatures were required for toxin formation by *F. nivale. F. tricinctum* produced toxin maximally at 15°C on white corn grits and declining quantities at higher temperatures, yielding no toxin at 32°C (Burmeister, 1971). On corn, wheat and rice liquid media, production of T_2 toxin was optimal at 8°C.

The laboratory behaviour of these fusaria accords well with known field behaviour, since it is the ingestion of over-wintered grain that was responsible for ATA (Joffe, 1971) and fescue foot is reported to occur in the cold months, particularly in Missouri (Yates, 1971).

With respect to other fusaria, however, the action of cold is not so marked. Loncarevic *et al.,* (1970) reported toxin production from *F. moniliforme* at 26°C but not at −4 to 0°C. Sherwood and Peberdy (1972a & b) found that high quantities of zearalenone could be produced by *F. graminearum* experimentally in wheat, barley, maize and oats at moisture contents of 23–37% wet weight at 25°C. Toxin production increased almost linearly from 4 to 5 000 ppm over the range 14.5–54% m.c. Optimal production occurred on wheat with an m.c. of 37% when held at 25°C for 4 weeks followed by 6 weeks at 12°C. Enhancement of zearalenone production by this period of cooling was considerable. When the temperature was kept at 25°C however, toxin yields were low, being seldom higher than 100 mg/g of grain even though mycelial growth was rapid (Sherwood and Peberdy, 1974). However, the more important overall factor as these authors stress (1973), is moisture content, and this is of particular significance where grain is stored at high moisture contents for milling and preparation of animal feeds.

As with aflatoxin, production of zearalenone is probably influenced by substrate preferences. Zearalenone production by *F. roseum* (=*F. graminearum*) was best on polished rice at 60–65% m.c., and good on maize and wheat at 45% m.c., whereas very little occurred on oats and barley and none on soybeans or peas (Eugenio, Christensen and Mirocha, 1970).

Fusarium spp. are both field and storage fungi, but the factors which govern their prevalence and toxin formation in seeds are still not well known. Little if any increase in grain infestation could be established in mature seeds of maize by *F. moniliforme* merely by incubation in a warm moist atmosphere (Edwards, 1941), whereas field infection experiments showed that a high incidence of internal grain infection could be established by inoculating the developing cobs at all stages of maturity from pollination onwards. The highest incidence was obtained by inoculation between pollination and the dent stage of development. When the pericarp was injured and the seed then kept in a moist atmosphere, kernel rot resulted however, this being rare otherwise.

With regard to other species, the evidence available is still very fragmentary. Several species of *Penicillium, P. chrysogenum, P. palitans* and *P. viridicatum* are able to grow at freezing temperatures and cause 'blue eye' in cribbed corn

over winter, but their toxin potential is unknown (Semeniuk and Barre, 1943). Tremortin production by Penicillia, however, is known to be optimal at low temperatures (4°C) (Hou, Ciegler and Hesseltine, 1971b). *Aspergillus fumigatus* produces toxin optimally at 20° or 30°C, *A. niger* at 10–15°C (Kolesova, 1964; Rutquist, 1965) while *Penicillium rubrum* produces rubratoxin at an ambient temperature (Hayes, Wyatt and King, 1970). The relation of mycotoxin formation in relation to temperature may be complex. More tremortin was found to accumulate at a low temperature (4°C) over a period of time than at a higher one (20°C), but the initial *rate* of toxin formation by *Penicillium cyclopium, P. crustosum, P. palitans* and *P. puberulum* on various cereals was higher at the latter temperature. Toxin formation by *A. fumigatus* was found to be the same initially at 20°C and 37° (Rutquist, 1965) but decreased markedly at 37° with time in contrast to material kept at 20°C. Zinc is needed for production of rubratoxin. Restricted aeration is better than ample aeration provided by agitation of the medium for rubratoxin production and for toxin production by *P. viridicatum* (Hayes and Wilson, 1968; Budiarso, Carlton and Tuite, 1971). On the other hand, an abundant supply of oxygen and high acidity appears to be essential for a good yield of gliotoxin, and ammonium salts were preferred to peptone or nitrates as a source of nitrogen (Weindling, 1941).

The natural occurrence of mycotoxins

The list of products in which mycotoxins have been demonstrated is now very comprehensive. Scott (1965) in his review of mycotoxins in stored grain listed wheat, wheat flour, spaghetti, corn, rice, sorghum, oats, rye, barley, malt sprouts, breakfast cereals and all major grain staples, hay and feedstuffs from a large number of countries in Europe, Africa, Asia, the Far East and America. Although the various toxins occurred only in a small fraction of the samples examined by each worker or research team, and the concentration in absolute terms was small, their universal distribution and known effect on animals in tiny concentrations is sufficient justification for paying serious attention to them.

From the epidemiological viewpoint it is important to know the frequency of isolation and the substrate preference of each mycotoxin, since if this coincides with the dietary preference of any group of people, important circumstantial evidence may be forthcoming about the cause of a disease syndrome that hitherto was obscure.

There is a fair quantity of indirect evidence to show that there is a particular affinity of *A. flavus* for groundnuts, both as a substrate for growth and for aflatoxin formation. Lafont and Lafont (1971) tested 125 isolates of *A. flavus* and characterized two principal groups, one with a high degree of toxigenicity and the other with a weak or zero toxic potential. Isolates in the former group were derived from groundnuts rather than from wheat, maize or animal feeding stuffs.

As regards the field development of aflatoxin, the general consensus is that aflatoxins are either absent from freshly harvested groundnuts (Ashworth, Schroeder and Langley, 1965) or present in minimal concentrations (Barnes and Young, 1971; Joffe, 1972) and that the aflatoxin level rises with late harvesting (McDonald and Harkness, 1967) and with storage. Joffe's work indicates that a small, probably insignificant quantity (<125ppb) of aflatoxin may be formed regularly even under good storage conditions in about one-third of the kernels. As we have seen elsewhere, the quantity of aflatoxin formed is markedly enhanced by trauma and by poor storage conditions. Outbreaks of livestock poisoning are probably linked with the rise in aflatoxin concentration above a threshold level. When an outbreak of livestock poisoning due to aflatoxin occurred in 1963 in South Africa (Sellschop, Kriek and Du Preez, 1965), analysis of 501 groundnut samples from the four provinces showed that 214 (43%) contained more than 0.1 ppm aflatoxin of which 75, all from the groundnut growing areas in the Northwest Transvaal, exceeded 2 ppm. In the following year, when no outbreaks of poisoning were reported, a survey of 943 groundnut samples from the same areas showed that 315 (33%) contained more than 0.1 ppm, and only 23 exceeded 2 ppm. In the USA Dickens and Welty (1968) have reported the occurrence of aflatoxin in 5% of 2 000 samples of farmers' stock groundnuts with an average concentration of 380 ppb. Other surveys quoted by Barnes (1970) registered small but significant quantities in groundnut samples from Uganda, Ethiopia and Senegal. It is reasonable, therefore, to assume that aflatoxin may be produced wherever groundnuts are grown.

Aflatoxin also has a high natural occurrence in groundnut cake and groundnut oil (Boutibonnes and Jacquet, 1969; Bubien *et al.*, 1968; Dwarakanath *et al.*, 1969; Fonseca, 1968; Halliday and Kazaure, 1967; Jacquet *et al.*, 1970; Krogh and Hald, 1969; Ling *et al.*, 1968). Sellschop *et al.*, (1965) obtained concentrations exceeding 2 ppm in 11 out of 16 samples of groundnut oil, cake and meal, and concentrations ranging between 0.025 ppm and 0.5 ppm in 17 out of 101 samples of groundnut butter.

Although the evidence is still scanty, it would appear that aflatoxin has a wide natural occurrence in foodstuffs apart from groundnuts, and may reach extremely high concentrations, as in cassava (Serck-Hansson, 1970) and in cottonseed meal and cottonseed cake (Ashworth *et al.*, 1971). A high concentration of aflatoxin was also found in a dish of cabbage fried with pork and garlic in Thailand (Shank, 1971; Shank *et al.*, 1972). Garlic and fish were named as amongst the commonest components of toxin contaminated foods in that country. Aflatoxin has also been demonstrated in a substantial proportion of samples of coconut products (Arseculeratne & De Silva, 1971); haricot beans and other pulses in the Sudan (Habish, 1972); millet, sorghum-flour, sorghum, wheat and teff in Ethiopia (Coady, 1965); and beans, maize, sorghum, groundnuts, millet, peas, cassava, rice and other grains in Uganda (Alpert *et al.*, 1971; Richard and Cysewski, 1971). It is naturally extremely hard to compare foodstuffs directly in terms of quality loss, so that any comparison between substrates even in the same area will contain a certain degree of error. It is worth noting however, that it was the groundnuts that yielded the highest number of samples with an aflatoxin concentration exceeding 1 ppm.

Other indirect evidence indicates that staple foodstuffs are not equally prone to the formation of aflatoxin. A survey of the natural occurrence of aflatoxins in the USA and Canada (Shotwell *et al.*, 1969a & b) in harvested samples of staple crops revealed marked differences in incidence of the fungus and of the toxin (see Table 13).

Table 13

Incidence of aflatoxin in North American crops

Substrate	No. of samples	No. of toxic samples	Description	% toxic >10 ppb	% infected with *A. flavus*
Maize	1 311	35	10 grades 1–4 5 grade 5 20 sample grade*	2.7	54
Soybeans	866	2	2 sample grade	0.2	50
Sorghum	533	6	2 grade 4 4 sample grade	1.2	43
Wheat	531	2	2 sample grade	0.4	20
Oats	304	3	2 grade 4 1 sample grade	1.0	14

* sample grade = very poor quality

In another survey (Shotwell *et al.*, 1973) 21 out of 60 maize samples were positive, with a mean aflatoxin level at 66 ppb.

Clearly the incidence of the fungus and formation of the toxin were related up to a point but these figures also show that high levels of aflatoxin were not necessarily contingent upon high fungus incidence. The % incidence of aflatoxin was statistically correlated with low grade. Differences in storage conditions may have contributed to the apparent differences between substrates. The same conclusion is apparent from a survey of Southern African foodstuffs (Martin, Gilman and Keen, 1971; Martin, 1974), the results of which are presented in Table 14.

Table 14

Incidence of aflatoxin in crops and prepared foodstuffs from Southern Africa

Substrate	No. of samples	% frequency of *A. flavus*	% frequency of aflatoxin (>10μg/kg)	Comments
Maize	418	37.0	4.3	Mostly good quality, stored above and below ground
Groundnuts	180	49.4	11.1	Mixed quality but representative
Groundnut meal	238	78.2	12.6	Prepared by local methods
Groundnut butter	190	85.1	18.9	Prepared by local methods
Sorghum	39	33.3	7.7	Mostly good quality
Sorghum malt	33	60.6	0.0	Prepared locally for beer
Various pulses	46	54.3	0.0	Mostly good quality

Apart from staple foods, milk, milk powder, and loaves of bread have been incriminated (Kiemaier, 1971; Purchase and Vorster, 1968; Spicher, 1970). The secretion of aflatoxin in milk by cows ingesting contaminated material is a potentially serious hazard (Allcroft and Lewis, 1963; Allcroft and Roberts, 1967).

A survey of retail stores and processing plants in Canada (van Walbeek *et al.*, 1968) linked the discovery of tiny quantities of aflatoxin to reported cases of illness in households and complaints of the reception of mouldy bread by consumers. All these reports are drawn from countries where the standard of hygiene is good, so that the problem is not restricted, as might be supposed, to countries with tropical climates, or where there is relatively little development.

Many reports exist of the experimental production of aflatoxin and other mycotoxins on a variety of foodstuffs but these should be treated with some reserve since the optimum conditions as determined in the laboratory may not occur in the field. Their main value lies in revealing substrate preferences and rates of formation. Aflatoxin can develop on groundnuts experimentally within 48 hours providing that aeration is good (Pattee, Sessoms and Dickens, 1966) whereas the minimum period on wheat is 4 to 5 days (Stubblefield *et al.*, 1967). Rice and pork apparently support toxin formation better than groundnuts, while soybeans are a comparatively weak experimental substrate (Borker *et al.*, 1966). Aflatoxin is also formed abundantly *in vitro* on cassava (Nartey, 1966) and on pawpaw (Bassir and Adekunle, 1972), the latter substrate being much more suitable than sugar cane, coconut, sweet orange, lemon, grapefruit or pineapple. Large quantities of aflatoxin were also formed on 3 month old Cheddar cheeses inoculated with *A. flavus* and *A. parasiticus* and sampled after 10 and 52 days of incubation (Lie and Marth, 1967).

With regard to other mycotoxins, the information concerning natural occurrence is much more scanty. Zearalenone formation obviously takes place over a wide range of conditions, because most of the outbreaks of hyperoestrogenism have been traced to stored material. Mirocha & Schauerhamer (1973) have in fact demonstrated the presence of the toxin in significant quantities in 28 out of 65 samples of feeds contributing to hyperoestrogenism in pigs and cattle. The toxin has also been recently found in dent corn in the field after infection with *Fusarium* spp. (Caldwell and Tuite, 1970 & 1974). The geographic distribution of the outbreaks in those parts of the world where there is a cool temperate but continental climate with considerable and sudden swings of temperature, is consistent with physiological studies showing that toxin production is enhanced by sudden cold. Presumably in nature the attacks of toxicity are precipitated by ingestion of seed which has been exposed to cold after harvest.

In comparison to reports of the incidence of aflatoxin, the relative sparsity of those on other mycotoxins might simply be due to the fact that they have not been looked for as intensively. Nevertheless, they appear to be less frequent if one examines the results of the surveys on harvested maize by Shotwell *et al.*, (1970, 1971) in the USA and Canada (Table 15).

Table 15

Yearly incidence of mycotoxins in American maize

Year	No. of samples	aflatoxin	Number with ochratoxin A	zearalenone
1967	283	6	1 (110–150 ppb)	2
1969	293	8 (6–25 ppb)	3 (83–166 ppb)	5 (450–750 ppb)

As before there was a general association between these samples and poor storage. A survey of the 1972 maize crop (Eppley *et al.*, 1974) in areas where the potential for fusarial contamination was considered to be high or where known fusarial damage had occurred, yielded a zearalenone sample incidence of 17% as against only 2.3% for aflatoxin. The distribution in grade of foodstuff was apparently at random in this survey.

The 1967 sample yielding ochratoxin had a musty odour, 18.1% m.c., 1.8% foreign material and 23.3% total damage (Shotwell *et al.*, 1969c). In another survey (Anon., 1973; Scott *et al.*, 1970, 1972) ochratoxin A was detected in 18 out of 29 samples of treated grain from Saskatchewan farms at concentrations of 0.03 to 27 ppm. 13 samples contained citrinin and one contained sterigmatocystin. Krogh (1973) obtained similar results in Denmark where an outbreak of mycotic nephropathy in pigs was associated with the isolation of ochratoxin A from 54% of samples of feed barley and citrinin from 8% of the samples. Ochratoxin A was also found in meat samples of affected pigs. The general paucity of isolation of these substances, however, indicates that they are not widely distributed in nature. This has a special significance where sterigmatocystin is concerned because the action of stigmatocystin in forming liver turmours in the rat under experimental conditions strongly resembles the observed process in human liver cancers and it is tempting to regard sterigmatocystin as a natural initiator of hepatoma (Purchase and Vorster, 1970). The infrequency of natural isolation, however, mitigates against the likelihood of this being a reality.

One of the exciting recent discoveries has been the demonstration of zearalenone in 4 samples of malted sorghum, 27 pooled samples of 'sour' fermented porridge and beer made from maize and sorghum, and in two samples of mouldy maize off the cob from Swaziland (Martin, 1974).

This study has been extended to Lesotho (Bainton, 1975) where small quantities of zearalenone (up to 50 ppb) have been found in 16 of the 71 beer samples examined so far. Other mycotoxins – aflatoxin, patulin, orchratoxin and sterigmatocystin – were not found. The regular ingestion of zearalenone by the Black African population could perhaps explain the high incidence of certain diseases such as cervical cancer, in a way not hitherto suspected.

The role of fungi in non-invasive pathology

The vast quantity of literature produced on mycotoxins in the previous 12 years has revealed that the role of fungi in the diet of animals and man, instead of being comparatively harmless, as still commonly supposed, may be deleterious under certain conditions, some of which still need to be defined. Since 1961 when aflatoxin was first implicated in the sudden death of 100,000 turkey poults through feeding with contaminated groundnuts, there have a been a number of epizootics mostly involving farm animals but a few affecting man himself, that have been traced to feeding mouldy material. The evidence is now overwhelming that fungi in the diet can present a considerable hazard to public health.

The fungi concerned may exert a direct action on the animal system resulting in death or acute toxicosis, or they may have a chronic sublethal and more long lasting one, of which cancer may be only one manifestation. Moreover the action of fungi is in nature probably synergistic in that either several fungi contribute different toxins which then act together, or the same fungus produces one or more toxins, possibly under different conditions. Since a mycotoxicosis as an entity is difficult to diagnose, the guidelines given by Harrison (1974) are worth quoting:—

(i) the disease is not infectious or contagious, and is isolated in occurrence;

(ii) there is no change with drug or antibiotic treatment;

(iii) there are no bacteria or viruses involved on pathological examination;

(iv) there is a similarity to vitamin deficiency but the disease is unrelieved by vitamin treatment;

(v) there is an association with foodstuffs or a change in foodstuffs;

(vi) moulds can be demonstrated in the foodstuff(s).

The following brief account summarizes present knowledge of toxigenic fungi and their chief manifestations.

ALLERGIC ILLNESS

Farmer's lung is primarily caused by the allergic effect of several field fungi on farmers and farm workers, by direct exposure to mouldy crop residues and occasionally farm animals (Spesivtseva and Moroshkin, 1957). Fuller (1953), Studdert (1953) and later workers have described the clinical picture according to three stages: firstly an acute isolated attack resembling bronchitis; secondly a subacute more chronic condition with a tendency to spontaneous recovery; and lastly a chronic irreversible stage associated with secondary lung changes, emphysema and fibrosis. Repeated exposure to spores and mycelium in mouldy material ultimately induces a response in the bronchial wall simulating the fungal granuloma developing in rabbits inoculated with *Candida albicans.* In spite of the frequent reports of *C. albicans* being isolated from sputum of patients with farmer's lung (Sweeney, 1952; Baldus and Peter, 1960) the role of Candida in this

particular condition is likely to be an allergenic rather than an invasive one. Species of this genus are anthrophilic in character and normally thrive in sputum and on mucosal surfaces, especially that of patients with a pre-established pulmonary complaint.

The evidence for fungal aetiology is derived from reports of the role of various fungal spores in producing allergic symptoms (Hyde, Richard and Williams, 1956), the exacerbation of symptoms due to further exposure to mouldy hay (Baldus and Peter, 1960), and of the formation of specific antibodies against field fungi and extracts of mouldy hay (Horejsi *et al.*, 1960; Kobayashi *et al.*, 1963; Romanski and Tarazkiewicz, 1967). In a study of patients in New York, Merksamer and Sherman (1958) demonstrated a correlation between the patients' seasonal symptoms and the atmosphere spore count of *Alternaria.* Samsonov and Samsonov (1965) showed that when mixtures of loess, dust and spores of *Aspergillus, Mucor, Cladosporium* and *Alternaria* were blown into the lungs of experimental guinea pigs and rabbits, they caused significant alteration in pulmonary parenchyma and degeneration in all internal organs. The main fungi involved in the numerous reports of farmer's lung in the literature belong to the genera *Alternaria, Aspergillus, Candida, Cladosporium, Fusarium, Helminthosporium, Penicillium* and *Verticillium* and to the phycomycete family Mucorales, but the total spectrum is likely to be wider.

Rusts and smuts may be major causes of respiratory allergy (Waldbott and Ascher, 1941). Of 106 consecutive cases of asthma and upper respiratory allergy, 7 had symptoms exclusively during the rust and smut season and 12 had a more or less severe exacerbation at that time. Patients in this category had strong reactions to intradermal skin injections of fungal extracts and reproduction of asthmatic attacks was achieved by inhalation of rust spores.

MYCOTOXICOSIS

Krogh (1969a & b) has defined mycotoxicoses as intoxications of animals and men caused by the intake into the organism of mycotoxins. Mycotoxicoses may be sometimes allied to mycoses when the living mycelium responsible for the mycotoxin is ingested and subsequently invades the host. Endotoxins released by dead mycelium of normally pathogenic fungi may be released into the tissues of the host. Many accounts of stachybotryotoxicosis state that at death fungal flaments were seen in the walls of the rumen and other organs of affected cattle; such reports are, however, rare in the case of other mycotoxicoses.

Numerous outbreaks of mycotoxicosis in farm animals, mainly in the USSR, have been related to the action of diverse fungi, with a wide range of organs and tissues affected. Such attacks come under the heading of 'mouldy corn toxicosis'. In other cases the outbreak can be fairly definitely ascribed to the activity of one species only. Krogh (1969a & b) has proposed that these cases be divided into two groups, one having a primary effect on the liver and/or kidneys, and the other attacking the central nervous system, blood system and reproductive system. In actual fact this division may be one of convenience only because of the wide variety and combination of symptoms involved; nevertheless the various toxicoses do seem to be clustered in groups based on the site(s) of attack that may have obvious significance in understanding the aetiology of fungal disease.

Mycotoxicoses with multiple aetiology (mouldy corn toxicosis; mouldy feed toxicosis).

Some of the main disease syndromes are summarised in Appendix 1 and it will be apparent that these toxicoses present different clinical aspects according to the fungi isolated from the foodstuffs ingested. The severity and wide range of symptoms, however, are due to the multiplicity of toxins involved.

Mycotoxicoses primarily involving the liver or kidney

(a) **Aflatoxin** was discovered as a direct result of an epizootic among turkeys in England after ingesting mouldy groundnuts contaminated by *Aspergillus flavus* (Blount 1961). There have, however, been other epiphytotics, the major ones being listed in Appendix 2.

In these animals direct damage to the liver was done in the form of centrilobular necrosis, proliferation of bile ducts and fibrosis and haemorrhage in the intestine. These symptoms were linked to the ingestion of various foodstuffs shown to contain aflatoxin or to be heavily contaminated by *Aspergillus flavus.* The same symptoms have been observed in poultry, rodents, cattle, swine, sheep, goats, dogs and monkeys under laboratory conditions and under experimental conditions in the field, as reviewed by Detroy, Lillehoj and Ciegler (1971). The main site of attack of aflatoxin is the liver where it inhibits leucine incorporation (Anon. 1964a). Variation in symptoms may occur according to the dosage as Purchase (1967a) demonstrated with one-day-old ducklings; a high dosage produced haemorrhagic necrosis, a medium dosage slight bile duct proliferation and a low dosage extensive degenerative change in liver cells and bile duct proliferation. Acute symptoms in animals as a result of massive absorption comprise haemorrhagic necrosis of the liver, as seen in the cat, pig, chicken, duck, turkey, rabbit, rat and guinea-pig (Butler, 1964, 1966; Payet *et al.,* 1966; Gagné *et al.,* 1968; Joffe 1969b, 1970). Chronic symptoms as a result of prolonged absorption comprise biliary canal proliferation, as seen in the cow and pig. The toxic effect is enhanced by youth and somewhat diminished by age. Reduction in protein nutrition has been found to markedly increase the lethal effect of aflatoxin in monkeys (Madhaven, Suryanarayana and Tulpule, 1965). At the biochemical level, aflatoxin inhibits or significantly decreases incorporation of acetate into the lipids of the liver and adipose tissues (Wei *et al.,* 1968).

In addition to liver injury, there may be impairment of other systems by products of *A. flavus.* Upcott (1970) reported failure of the bloodclotting system in calves fed toxic groundnut meal, relieved partially by administration of vitamin A. Antyukov (1965, 1966) found that liquid culture extracts of *A. flavus, A. niger* and *A. fumigatus* introduced separately into three pigs increased peristalsis at first and then resulted in complete atonia. With the weakening of gastric motor function there was a general reduction in acidity and a decrease in free and combined hydrochloric acid. These results are consistent with the clinical syndrom of decreased weight gains and feed efficiency in toxic treated swine (Keyl *et al.,* 1970). Phagocytosis was also inhibited and there were changes in the blood and urine, and dystrophic changes in the parenchymatous organs, CNS and lesions in the GIT and kidneys. The main clinical symptoms were depression, loss of appetite and paralysis of the hindquarters leading to general paralysis and death. These findings were essentially corroborated by Cysewski *et al.,* (1968) also working with pigs, and by van der Watt and Purchase (1970b) with vervet monkeys. In ducklings treated with aflatoxin, loss of weight of the thymus and alterations in the levels of ascorbic acid, cholesterol, cholinesterase glucose, calcium, sodium, potassium magnesium and phosphorous occurred in various sites (Juskiewicz *et al.,* 1967).

Kidney lesions in the pig comprised necrosis in the proximal tubules, separation of epithelial segments from the basement membrane, foamy or granular exudation and severe congestion of interstitial blood vessels (Madhaven and Rao, 1967). The renal lesions were late in development, suggesting an indirect influence of aflatoxin following extensive damage to the liver.

The multiple effect of *A. flavus* is probably due to the fact that more than one toxin is produced by this species. At first only the four main aflatoxins, B_1, B_2, G_1 and G_2, were generally recognized (Hartley, Nesbitt and O'Kelly, 1963; Wogan, 1966). Other toxic metabolites that have been isolated, however, include kojic acid (Werch, Oester and Friedman, 1957), oxalic acid

(Wilson and Wilson, 1961), an endomycelial nephrotoxin (Tilden *et al.*, 1961), a tremorgen (Wilson and Wilson, 1964), aspergillic acid, β nitropropionic acid and '*A. flavus* endotoxin' (Wilson, 1966), sterigmatocystin (Holzapfel *et al.*, 1966) and finally aspertoxin (Rodricks *et al.*, 1968).

The second and possibly more important influence of aflatoxin and other metabolites produced by *A. flavus* from the public health stand point is due to its sublethal effect. The carcinogenic effect of aflatoxin on the liver was proved relatively early (Lancaster *et al.*, 1961); later Carnaghan (1967) showed that hepatic tumours in the rat followed a single oral administration of 0.5 mg crystalline aflatoxin after 26 months. Shank and Wogan (1966) and Carnaghan (1967) found ducks even more susceptible than rats in terms of dose response. Amongst other rodents, mice (Newberne, 1965), guinea-pigs (Barnes, 1967), hamsters (Elis and Di Paolo, 1967) and ferrets (Lancaster, 1968) also develop hepatomas with sublethal dosages of aflatoxin. Jackson, Wolf and Sinnhuber (1968) also demonstrated hepatoma formation in rainbow trout by feeding them with cottonseed containing aflatoxin, and sheep developed neoplasms of the liver after being consistently fed with small quantities of groundnut meal with 1.0—1.75 ppm aflatoxin (Lewis, Markson and Allcroft, 1967).

The carcinogenic action of aflatoxin still needs further clarification but is probably due to the action of the toxin on the nucleus of animal cells as shown in several *in vitro* studies. Aflatoxin B binds to both native and denatured DNA (Sporn *et al.*, 1966) and interferes in RNA synthesis (Floyd *et al.*, 1968; Rees, 1966). The toxin also inhibits mitosis and may produce chromosomal aberrations (Legator, 1966). Zuckerman *et al.*, (1967a & b, 1968) using human liver cells *in vitro* demonstrated blockage of RNA and DNA synthesis by aflatoxin. Thus there is ample theoretical background for a prima facie case of direct involvement of aflatoxin in carcinogenesis.

A large number of other fungi have been demonstrated at one time or another to produce aflatoxin, though *A. flavus* is still regarded as chiefly responsible. Scott, Van Walbeek and Forgacs (1967) demonstrated aflatoxin production by *A. ostianus* while Kulik and Holaday (1966) have recorded in addition to *A. flavus: A. niger, A. parasiticus, A. ruber, A. wentii, P. citrinum* and *P. variabile,* also *P. frequentans* and *P. puberulum* as responsible for trace amounts. Stubblefield *et al.*, (1970) also established the production of parasiticol by *A. parasiticus,* which was acutely toxic to ducklings. Later it was found to be a derivative of aflatoxin and named aflatoxin B_3. The toxicity of *A. wentii* for ducklings has been described by Rabie *et al.* (1965) and by Rabie and Terblanche (1967) and the liver lesions are consistent with those produced by aflatoxin. The same is true for *P. frequentans* with regard to mice (Carlton and Tuite, 1970a). *Rhizopus stolonifer, R. arrhizus* and *R. oryzae* may produce parasiticol (Cole and Kirksey, 1971) but these species though common are not ordinarily toxigenic.

(b) **Sterigmatocystin.** This toxin has been isolated from *Aspergillus nidulans* and *A. rugulosus* (Ballantine *et al.*, 1965; Holzapfel *et al.*, 1966) *A. versicolor* (Bullock *et al.*, 1962; Davies *et al.*, 1960) *Bipolaris sorokiniana (Drechslera)* (Holzapfel *et al.*, 1966) and *Penicillium luteum* (Dean, 1963). The main damage to albino rats when treated experimentally with the toxin was to the liver and kidneys, causing necrosis and eventual peritonitis. Dickens *et al.*, (1966) found that subcutaneous injection of 0.5 mg sterigmatocystin twice weekly for 24 weeks produced a tumour at the injection site; and a hepatoma in one rat and a cholangiosarcoma in another. Liver changes in the rat following injection of 100 ppm of sterigmatocystin resemble the changes in the human liver during hepatoma development, whereas the course of hepatoma formation due to aflatoxin was dissimilar (Purchase and van der Watt, 1968; van der Watt and Purchase, 1970a; Purchase and Vorster, 1970). However, the capacity for tumour induction of sterigmatocystin is weaker than that of aflatoxin, and there is only one record of the toxin being

isolated in significant quantities in mouldy grain in nature (Scott, 1973) and there are no recorded animal or human outbreaks of mycotoxicosis due to this toxin.

Aspergillus nidulans has also been found recently to produce a second toxin, *nidulotoxin* (Lafont, Lafont and Frayssinet, 1970). The toxin was demonstrated using eggs and chicks, and its mode of action still needs further research. Rabie *et al.,* (1964) found that *A. amstelodami* was the dominant fungus in suspect samples of animal feed, and when used experimentally, caused liver lesions and small petechiae in poultry and lack of co-ordination in rabbits leading to death.

(c) **Luteoskyrin** and a chlorine containing peptide produced by *Penicillium islandicum* (Uraguchi *et al.,* 1961a & b) both cause liver damage in mice in the form of centrilobular necrosis, fatty infiltration, cirrhosis and eventual nephrotoxicity (Kurata *et al.,* 1968b). Lesions due to acute toxicity are somewhat different in their course of development from those of other fungi: Chute *et al.,* (1965) compared the effects of feeding methanol-treated wheat cultures of *A. flavus, A. fumigatus* and *P. islandicum* to chickens and found that the latter induced renal and hepatic degeneration rather than hyperplasia. Rats, rabbits and monkeys are also susceptible to luteoskyrin though tumour formation has not so far been observed (Saito *et al,* 1971). The peptide is known to act by inhibiting incorporation of C-14 glycine into mouse liver (Yamazoe *et al.,* 1963).

(d) **Rugulosin,** produced by *P. rugulosum, P. tardum, P. variabile, P. wortmanni, Endothia parasitica* and *E. wortmanni,* is closely allied in structure and effect to luteoskyrin (Saito *et al.,* 1971).

(e) **Ochratoxin.** The structure and behaviour of this toxin has been recently reviewed by Steyn (1971). It was first isolated and described by Van der Merwe *et al.,* (1965) as a toxin produced by *Aspergillus ochraceus,* a fungus known to be able to grow on stored wheat at a moisture content of >16%, and on katsuobushi and fermented fish preparations. Later it was isolated from black pepper (Nesheim, 1967). Ochratoxin is now known to be formed by *A. sclerotiorum, A. alliaceus, A. ostianus, A. melleus* and *A. sulphureus* (Ciegler, 1972). In short term experiments with ducklings, ochratoxin A caused a mild fatty infiltration of the liver when administered in doses of 100 mg. Weanling male rats given comparable doses of toxin developed widespread hyaline degeneration of liver cells with focal necrosis. The endoplasmic reticulum appeared to be the primary site of toxic action (Theron *et al.,* 1966). Doupnik and Peckham (1970) working on cockerels found haemorrhages in the proventriculi, emaciation, dehydration, and dry hard firm gizzard linings on postmortem examination after feeding ochratoxin in moist corn. There was also hepatic injury with fatty changes or necrotic foci, and suppression of bone marrow activity and depletion of lymphoid elements. Ochratoxin also delays sexual maturity in hens and results in a lower rate of egg production (Choudhury *et al.,* 1971). Scott (1973) lists only four isolations of ochratoxin in nature, and as with sterigmatocystin, there have been no reported outbreaks in nature of poisoning due specifically to this toxin. So far there has also been no demonstration of carcinogencity, but mycotoxic nepthropathy in pigs in Denmark and several Balkan countries has been attributed to this toxin and to citrinin (Krogh, 1973). The disease was first described in 1928 (Krogh, 1972).

Recent work, however, has shown that ochratoxin can be also produced by *Penicillium viridicatum* (Van Walbeek *et al.,* 1969). The toxicity of this species was known beforehand, an early account being that of Marchionnato (1942) who reported toxicity of maize 'mildewed' by this species for pigs and horses. There seem to be three main toxic effects attributable to this species. Firstly there is an acute form giving rise to hepatic and renal lesions in mice comparable to those described above (Budiarso, Carlton and Tuite,

1971a, b; Carlton, Tuite and Mislivec, 1968), hepatic lesions in guinea pigs and rats and erosion of the stomach and necrosis of the scrotal skin in rats (Carlton and Tuite, 1970b). Secondly there is a chronic form resulting in a nephrotic syndrome. Krogh and Hasselager (1968) stated that up to 7% of Danish pigs were victims of mould nephrosis due to intake of contaminated barley. Experimental work on swine with *Penicillium viridicatum* showed that the mycotoxin (unidentified) resulted in damage of the proximal tubules of the kidney followed by diffuse formation of connective tissue and cysts. Carlton and Tuite (1970c) also produced nephropathy and an oedema syndrome in miniature pigs by feeding them cultures of *P. viridicatum* grown on autoclaved maize. The pigs became depressed, developed anorexia, paresis, elevated blood urea N-levels, proteinuria and glycosuria. Gross lesions included subcutaneous oedema, ascites and hydrothorax, hydropericardium and mesenteric oedema. Perineal oedema with accumulation of variable amounts of bloody fluid between the renal capsule and parenchyma was a notable feature. Finally Budiarso, Carlton and Tuite (1970) on feeding rice cultures of *P. viridicatum* to mice demonstrated a phototoxic syndrome similar to sheep with facial eczema, in that erythema of ears, muzzle, paws and tails leading to gangrene and blindness developed on exposure to light following intake of the meal. A wide variety of hepatic, gastric, epidermal and ocular lesions developed in rats fed contaminated grain and cultures of *P. viridicatum* (McCracken, Carlton and Tuite, 1974a, b & c). Further work is required to show whether all these symptoms are due to ochratoxin or to some of the other metabolites described for this species such as viridicatin (Wilson, 1971b), citrinin and oxalic acid (Krogh, Hasselager and Friis, 1970).

(f) **Toxins producing polyuria**

(i) Citrinin. Harrison (1971) suggested that this toxin, produced by *P. citrinum, Aspergillus candidus* and *A. clavatus* may have been responsible for a bout of excessive urination in racing stallions. In animal experiments it has been shown to cause kidney damage and mild liver damage in the form of fatty infiltration (Ramadoss and Shanmugasundaram, 1971). Kidney damage is characterized by glomerulonephrosis, deformation and enlargement of the tubules. Clinically there is depression of growth rate and increased diuresis (Saito *et al,* 1971). Citrinin has been implicated with ochratoxin in mycotoxic nephropathy of pigs (Krogh, 1973).

Krogh, Hasselager and Friis (1970) fed hay infested with *P. citrinum* to pigs and rats, and the corn steep medium on which the fungus was grown yielded citrinin and oxalic acid. The gross histological changes found in the pig kidneys were compatible with those known for citrinin elsewhere.

(ii) Toxin from *Absidia ramosa.* Sivers (1962) investigated four cows with haematuria and three healthy ones and found that the mycoflora of the liquid content of the rumen had an appreciably higher number of mucoraceous fungi, *Absidia ramosa* being predominant. These results were confirmed by Pidoplichko and Bilai (1962) who also determined the toxicity of *A. ramosa* for rabbits experimentally.

(iii) Toxin from *Rhizopus stolonifer.* The late implication of such a common species in a mycotoxicosis is somewhat surprising. In 1967 Narasimhan *et al,* published an account of the Sassoon Hospital syndrome in which 150 patients in the Maharastra province of India suffered from anorexia, weakness and fatigue, and polyuria. *Rhizopus stolonifer (Rh. nigricans)* was repeatedly isolated from the central core of millet grains used as the staple diet in that area and the disease was reproduced in rats fed a pure culture. A second human outbreak of the disease was reported in Poona by Deodhar *et al.,* (1970). Fujiwara, Landau and Newcomer (1970a, b) have extracted a hemolysin from fungus mats of *R. stolonifer* cultured for two weeks on Sabouraud's broth, and have demonstrated hemolysis *in vivo* in sheep, rabbits and

guinea pigs, and also *in vitro* in human erythrocytes. A third epizootic, this time in cattle in Moldavia (Kurmanov, 1968b) was related to brewing germs in the food which yielded *R. stolonifer.* Pathological changes in poisoned animals were mainly observed in the liver and kidneys. The disease lasted 20–25 days and in some cases ended in death.

(g) **Toxins producing haematuria**

(i) Gliotoxin. This metabolite was chemically defined from a filtrate of *Trichoderma viride* by Brian (1944) and of *Aspergillus chevalieri* by Wilkinson and Spilsbury (1965) and its anti-fungal properties were clearly established. Johnson, Bruce and Dutcher (1943) and Taylor (1971) have demonstrated the toxicity of gliotoxin for mice and rats, in which haematuria developed. Clinical symptoms were absent from two calves fed barley contaminated by *T. viride* (Kurmanov, 1969). However, the number of lymphocytes decreased from 79.5 to 66.5% and the number of segmented leucocytes increased from 19.5 to 33.5%. Chickens fed with a fungally contaminated meal developed leucocytosis and catarrhal haemorrhage of the fore limbs and intestine. Mouldy melon stems contaminated by *T. viride* were responsible for the abortion of a foetus in a pregnant ewe and the early death of a lamb (Stankushev *et al.,* 1966). Abortion by this fungus was experimentally reproduced on pigs.

(ii) *Chaetomium globosum* toxin. Maize cultures of this fungus fed to rats resulted in haemoglobinuria, haemorrhagic enteritis, and subdural haemorrhage. This toxin has not apparently been isolated and identified at the present date. (Christensen *et al.,* 1966; Mirocha, Christensen and Nelson, 1968a), but may be due to chaetomin (Taylor, 1971).

(h) *Aspergillus fumigatus* **toxins** – fumagillin, fumigatin, '*Aspergillus fumigatus* endotoxin'. The general structure and biology of these toxins have been reviewed by Wilson (1971a). Carll *et al.,* (1955) first demonstrated the toxicity of ether extracts of *A. fumigatus* cultures for the skin of rabbits, a calf and a horse. When a calf was fed for thirteen days with contaminated maize there was at first lachrymation, depression and subsequently progressive toxaemia, leading to prostration, anorexia, foetid diarrhoea, gross dehydration and death. Postmortem examination revealed extensive internal haemorrhage, congestion in kidneys, lungs, liver, intestines and lymph nodes. Subsequently at least three outbreaks attributed to *A. fumigatus* poisoning have occurred in pigs (Zhuravlev, 1962) and in cattle (Starchenkov *et al.,* 1967; Thornton, Shirley and Salisbury, 1968). The pigs suffered inappetence, depression, diarrhoea, and sometimes vomiting, tremors and paralysis leading to death. In cattle there was general depression, diarrhoea anoreaia, hyperaemia and jaundice of mucus membranes, nasal discharge, painful cough and increased temperature, haemorrhage, oedema of mesenteric lymph nodes, digestive tract, tongue and larynx were found on postmortem. Hepatic hyperplasia and bile duct proliferation and/or renal and cortical necrosis were observed in various experiments with animal feeding (Rutqvist and Persson, 1966; Lee *et al.,* 1965; Chute *et al.,* 1965).

The nephrotoxic effect of *Aspergillus fumigatus* is probably due to two types of endotoxin: a purely toxic factor and a hemolytic factor (Rau *et al.,* 1961; Rutqvist, 1965). After extraction of the endotoxins from well washed mycelia of *A. fumigatus,* Tilden *et al.* (1961) injected these intravenously into a variety of experimental animals and found:

(i) a characteristic necrosis of the kidney,

(ii) a strong haemolytic effect and

(iii) a strong dermonecrotic effect.

It was possible to prepare immune sera in rabbits against both toxins which prevented these effects.

(i) **Patulin.** The structure and properties of this toxin has been reviewed in detail by Ciegler, Detroy and Lillehoj (1971). It is a lactone metabolite of several species, including *Aspergillus clavatus, A. giganteus, A. terreus, Byssochlamys nivea, Penicillium claviforme, P. expansum* and *P. urticae* (Cantine *et al.,* 1970; Hori *et al.,* 1954; Shibata *et al.,* 1964). In addition *A. clavatus* produces a derivative of patulin termed ascladiol (Tanabe and Suzoki, 1968). Forgacs *et al,* (1954) first demonstrated the toxicity of *A. clavatus* when this species, together with *A. chevalieri* was implicated in an outbreak of chronic toxicity in calves in Wisconsin fed contaminated feed. An ether extract of *A. clavatus* grown on bread produced skin inflammation on a calf and acute and chronic symptoms leading to hyperkeratosis and death when a calf was force-fed. Moreau and Moreau (1960a & b) reported a further outbreak when farmers in France fed their cows with forage seedlings instead of normal rations due to a severe drought in 1959. Symptoms included fever, inco-ordination and hepatic degeneration. The seedlings were found to be heavily contaminated with *A. clavatus.* The preference of *A. clavatus* for germinating seeds in one form or another is an interesting biological feature. In Bulgaria and East Germany, poisoning of cattle reported by Schultz (1968) and by Schultz *et al.* (1969) has been described as malt germ intoxication. Tomov (1965) described this syndrome further in 250 cows from seven co-operative farms in Russia. Symptoms were consistent with those above and included unsteadiness of hindlegs, reduction in milk yield and by hypersensitivity to external stimuli. When Jacquet and Boutibonnes (1963) and Jacquet, Boutibonnes and Cicile (1963) fed germinating wheat infested with *A. clavatus* experimentally to cattle and mice, they produced the same reactions. One of these filtrates of the fungus contained an unidentified hemolysin which was thought to be produced secondarily, and blood samples from the mice showed a reduced red cell count. Recently Blyth and Lloyd (1971) produced focal necrosis and granulomata in the liver and pancreas of mice after intramuscular or intraperitoneal injection, and also tubular degeneration and metaplasia in the cortices of the kidney, in addition to the usual nervous symptoms. Dickens (1964) and Dickens and Jones (1961) have reported that patulin administered subcutaneously twice weekly to rats for approximately fifteen months produced malignant tumours at the injection site. There is otherwise little evidence of its practical importance in neoplasia.

(j) **Penicillic acid.** This lactone was first isolated by Abbey and Black in 1913 from *Penicillium patulum* grown on corn, and again by Wilson, Harris and Hayes (1967). Hodges *et al.* (1964) claim to have isolated aflatoxin from this species but this has been subsequently queried (Detroy, Lillehoj and Ciegler, 1971). Kurtzman and Ciegler (1970) and Ciegler and Kurtzman (1970) have reported the formation of penicillic acid by four of the *Penicillium* species forming 'blue-eye' of corn: *P. cyclopium, P. martensii, P. palitans* and *P. puberulum.* Ciegler, Detroy and Lillehoj (1971) quote various authors finding penicillic acid as a metabolite of *P. stoloniferum, P. thomii, P. suaveolens, P. palitans, P. baarnense, P. madriti, Aspergillus ochraceus, A. sulphureus, A. quercinus* and *A. melleus.* Ciegler (1972) lists penicillic acid formation together with ochratoxin in the members of the *A. ochraceus* group mentioned above.

Wilson, Harris and Hayes (1967) have described the toxicity of *P. puberulum* following ingestion of contaminated meal by mice and ducklings. In addition to uncoordinated motion, stiffness and exaggerated movements, mice showed a darkening of eye color and cyanotic colouring of the mouth, feet and tail. Death followed convulsive seizures. These symptoms are not definitely ascribable to penicillic acid because *P. puberulum* also produces a tremorgen (see below), and other acids.

Subcutaneous injection of 1.0 mg per dose in rats produced transportable tumours after 64 weeks (Dickens and Jones, 1961, 1965). Carcinogenesis, however, has not been demonstrated following ingestion of material.

(k) **Sporidesmin** (see Filmer, 1958a, b; Mortimer, Taylor and Shorland, 1962; Thornton and Percival, 1959). This toxin produced by *Pithomyces chartarum* causes liver damage in the form of an acute inflammation of the bile ducts of the liver, leading to biliary obstruction and terminating in fibrous obliteration. Because of the damage, phylloerythin, a normal metabolite of chlorophyll, is retained in the blood instead of being excreted in the bile. It is this compound that causes photosensitization with development of inflammation in the skin of the face and udder of sheep and cattle. Not all sheep with liver lesions develop facial lesions, however. Lesions have also been reported by Mortimer (1963) in the urinary tract and kidney, and the disease terminates in the enlargement of the adrenal cortex. Facial eczema has also been reported as due to *Periconia minutissima* (Gouws, 1965).

Several outbreaks have occurred in South Africa, Australia and New Zealand. In view of its demonstrable involvement in animal mycotoxicosis, it is surprising that no clear evidence of human involvement has emerged.

(l) **Cyclopiazonic acid.** This compound, first isolated by Holzapfel (1968) from *Penicillium cyclopium* has been shown by Purchase (1971) to result in death of rats after oral ingestion. Postmortem examination revealed degenerative changes and necrosis in the liver, spleen, pancreas, kidney, salivary glands, myocardium and skeletal muscle. The same were obtained by Carlton and Tuite (1970a) for mice. Harrison (1971) has reported the death of calves from the production of cyclopiazonic acid by *P. cyclopium* growing naturally on crushed barley.

(m) **Toxin from** *Corticium rolfsii.* Terblanche and Rabie (1967) showed that maize cultures of this species were highly toxic to ducklings, chickens, sheep, a horse and a heifer. Symptoms were: anorexia, ruminal atony, nervous disorders, circulatory collapse and varying degrees of liver, kidney and brain damage.

(n) **Diplodiatoxin from** *Diplodia zeae* in mouldy foodstuffs including maize has been recorded as toxic to cattle and sheep (Mitchell, 1918; Division of Veterinary and Educational Research (South Africa), 1925; Theiler, 1927; Adelaar, 1958; Watt and Breyer-Brandwijk, 1962; Steyn *et al.,* 1972) but not to pigs (Melhus, 1943). Symptoms of the disease comprise salivation, incoordination, paralysis, quivering of muscles, and sometimes death due to nephritis and mucoenteritis. Shone (1965) has recorded an outbreak of poisoning of stock through the presence of this fungus on maize, causing kidney degeneration, catarrhal enteritis and lung hyperaemia. The restriction of such reports to Africa is a notable feature.

(o) **Haemorrhagic toxins.** Rubratoxin, (Moss *et al.,* 1967, 1968) reported the discovery of two toxins named rubratoxin A and B from *Penicillium rubrum,* and Natori *et al.* (1970) subsequently found that the closely allied species *P. purpurogenum* produced rubratoxin B. As is the case with many other fungi, work on the toxicological effect of the speices preceded the isolation and determination of the toxin. Wilson and Wilson (1962) had already found that extracts of *P. rubrum* grown on corn-sucrose medium produced hepatotoxic and haemorrhagic symptoms in mice, guinea pigs, rabbis and dogs. Similar results were obtained when livestock were fed feed infested with prime cultures of the fungus. Extreme liver engorgement also followed intraperitoneal injection of the toxin. The symptoms were extremely rapid, in fact more so than those from a comparable quantity of aflatoxin, occurring 1–2 hours after the injection and followed by death. Intraperitoneal injection of the toxin into mice also caused dilation of subcutaneous bloodvessels, prostration and death, extensive haemorrhage and mottling of the liver. This work was essentially confirmed by Wyatt and Hamilton (1971, 1972) who demonstrated a characteristic red mottling of the liver in experimental chickens fed rubratoxin, followed by hypertrophy of the liver, atrophy of the bursa of Fabricius, anaemia, proteinaemia, increase in serum cholesterol

and capillary fragility. The toxin apparently interferes with leucine incorporation into the liver proteins (Hayes and Wilson, 1970).

The practical significance of rubratoxin is still unknown. Most probably it is involved as one of the main factors of mouldy corn toxicosis described above.

(p) **Miscellaneous liver toxins.** Other toxins, producing liver lesions with or without other disorders, have been reported by Rabie, De Klerk and Terblanche (1964) for *Aspergillus amstelodami* on poultry and rabbits; Woolley *et al.* (1938) for *A. sydowi* on rats; Rabie *et al.* (1965) for *A. wentii* on various animals; Kanohta (1969) for *Penicillium frequentans* on mice, Steyn (1970) and Parthasarathy and Shanmugasundaram (1971) for *P. oxalicum* (oxalic acid and secalonic acid) on chicks, and for *P. ochraceum* on mice (Carlton, Tuite and Caldwell, 1972). The histopathological changes of liver and kidney in *P. ochraceum* were said to be identical to those produced by *P. viridicatum.* In addition, Kinosita *et al.* (1968) have reported liver, pancreas and stomach lesions in mice from experimental ingestion of toxins from fermented foodstuffs commonly used in Japan by humans, comprising *Aspergillus candidus, A. flavus, A. glaucus gp, A. oryzae, A. soyae, A. versicolor, Penicillium chrysogenum, P. cyclopium, P. terrestre, Pestalotia* sp.

Mycotoxicoses with sites of attack other than liver or kidney

(a) **Fusariotoxicoses.** Our knowledge of fusarial toxins has been remarkably slow in development, considering the well-authenticated though sporadic reports linking the contamination of grain by *Fusarium* with various toxic syndromes in cattle and other animals well before the aflatoxin era. The first isolation of a toxin was that of Stob *et al.* (1962), and since then numerous epizootics of fusarial poisoning have served to stimulate interest in the isolation and characterization of the remaining ones.

(i) *Fusarium graminearum toxins.* (N.B. This species is often referred to as *F. roseum* in the literature). The main toxic metabolites produced by this fungus are zearalenone (F2-toxin) and F3-toxin, both of which are oestrogens. There are several reports of hyperoestrogenism and toxicosis occurring naturally in animals, mainly pigs, and in some cases dairy cattle, related to the ingestion of feed invaded by *Fusarium.* These are summarized in Appendix 3.

From some of the feed samples zearalenone was isolated in addition to the fungus.

The hyperoestrogenic syndrome has been repeated experimentally by many workers, including several of those cited above. Mirocha, Christensen and Nelson (1967) determined the oestrogen as a derivative of resorcinylic acid, and found it to be closely related chemically to other fungal metabolites, curvularin, radicicol and monorden. A linear response in weight of the uterus according to increasing dosage was observed. Popovic, Zakula and Nikovic (1970) demonstrated a decrease in daily body weight, gain, and feed consumption in treated piglets and Christensen *et al.* (1972b) confirmed this, also showing that the uterine horn of sows increased in relation to the total body weight, while the corresponding weight of the testes decreased in the boars. Gross changes in pigs fed zearalenone by Nelson (1973) comprised tumefaction of the vulva, enlargement of mammary glands and increased size and weight of the uterus. Microscopic changes comprised oedema and hyperplasia of the uterus due to thickening of the myometrium and endometrium duct proliferation and squamous metaplasia of the cervix and vagina. Kurtz *et al.* (1969) demonstrated squamous cell metaplasia and loss of normal mucosal epithelium of the vagina and cervix in gilts given oestradiol, F2 mycotoxin, or maize inoculated with *F. graminearum,* but so far no report of cancerous development has been made.

The effect of *F. graminearum* on other animals seems to be more acute. Sharda *et al.* (1971) observed jaundice accompanied by histological changes in the liver, often accompanied by myocardial granulomas in mice, rabbits, hamsters and rats. Speers *et al.* (1971) found that hens and chickens fed zearalenone in their basal ration decreased in weight and failed to lay eggs, or laid eggs with poor quality shell, whereas chicks showed an increase in total weight, weight of comb and ovary length. Mice and chicks fed experimentally for two weeks developed nervous symptoms and diarrhoea, finally dying (Ide *et al.*, 1967). Young turkeys and chickens often eating contaminated shelled maize suffered loss of weight, some dying as well (Meronuck *et al.*, 1970). The turkeys exhibited swollen vents, prolapsed cloacae and enlarged bursae of Fabricius. Zearalenone is responsible for reduced litter size and splayleg in piglets (Miller *et al.*, 1973). A drop in fertility has been noticed in dairy cows when fed on poor quality hay and a fall in egg-laying by hens fed with zearalenone contaminated barley (Harrison, 1974). The effect of zearalenone may vary a good deal according to concentration; very small doses (36 mg per 100–230 kg) actually stimulate growth rate and feed conversion of cattle (Bennett, Beaumont and Brown, 1974).

The type of symptom obtained may be a function of the age of the animal. Martin (1968, unpublished) and Korpinen *et al.* (1972) on feeding immature rats with rations containing toxin obtained the characteristic oestrogenic pattern, whereas feeding mature rats produced acute toxic symptoms, loss of weight and lesions in the liver.

Fusarium graminearum is not the only species of the genus to produce zearalenone. Caldwell *et al.* (1970) and Scott *et al.* (1972) have demonstrated this metabolite in strains of *F. culmorum, F. equiseti, F. gibbosum* (probably = *F. equiseti fide* Booth (1971), and *F. tricinctum;* Mirocha, Christensen & Nelson (1969) in strains of *F. moniliforme* which were also held responsible for oestrogenism in swine. *Nectria radicicola* was also found to produce the F2 toxin (Mirrington *et al.*, 1964).

There is at least one other metabolite produced by *F. graminearum:* an emetic substance which was extracted from corn and wheat artificially inoculated with *F. graminearum* and also with other species, *F. moniliforme, F. poae, F. culmorum* and *F. nivale* (Prentice and Dickson, 1968). Curtin and Tuite (1966) have suggested that three active metabolites are produced by *F. graminearum;* zearalenone, an anabolic compound causing hypertrophy of the uterus, another with emetic activity so far not chemically defined, and a third substance causing refusal on the part of the bird or animal to consume infested feed. *F. graminearum* has also been held responsible for the 'drunken bread syndrome' which, like ATA, is indigenous to Russia. Ingestion of contaminated grain has given rise to abdominal pain, nausea, vomiting and ataxia in the human population (Louria *et al.*, 1970).

(ii) *Fusarium sporotrichioides.* The toxic symptoms attributed to this species are more acute than for *F. graminearum.* The aetiology of this fungus is important because it is the only one apart from the mushrooms and ergot fungi to be implicated in an outbreak of human mycotoxicosis on a large scale. The chief disease outbreaks recorded are definitely attributed to the presence of this species in food (usually grain) and these are detailed in Appendix 4.

F. sporotrichioides has also been implicated in an endemic arthritis in certain river valleys of the Transbaikal area of Siberia. Recent tests (Anon. 1964b) suggest that it is caused by ingesting locally grown cereals contaminated by the fungus.

The disease for which *F. sporotrichioides* is responsible has been termed alimentary toxic aleukia (Mayer, 1953) and alimentary septic angina

(Joffe, 1960b). Joffe (1960a, 1962, 1963) found that the toxicity of this fungus and of other species isolated from wheat, millet and barley in the region where the disease had occurred was markedly enhanced by winter cold, so that over-wintered grain left exposed in the field, immediately became suspect. Fungal species of which toxic and highly toxic isolates were determined as most common were *F. poae, F. sporotrichioides* and *Cladosporium epiphyllum.* Joffe assumed that because several toxic fungi were isolated from the same sample of cereals that the disease must be the result of synergism (mycoenose) though *Fusarium* was probably the principal factor. The toxic principle in the cereals was notable for the ability to persist 6 years after initial storage of the grain. It is noteworthy that both sporofusarin, from *F. sporotrichioides,* and poaefusarin from *F. poae,* reproduced symptoms of ATA in cats experimentally, comprising necrosis of the digestive tract, destruction of the bone marrow, and adrenal changes, (Joffe, 1969b).

Experimentally *F. sporotrichioides* has been shown to be toxic for poultry (Kurmanov, 1960; Birbin, 1966): hens revealing characteristic catarrhal inflammation of the stomach and intestine, leucopenia and reduction in haemoglobin, blood content, and ducks necrotic lesions on the mucosa of mouth, tongue and pharynx, oesophagus and crop. Khmelevskii (1970) also demonstrated reduction in blood cholinesterase activity. Experiments with pigs and sheep (Kurmanov, 1963; Marchenko, 1963; Burdelev and Akulin, 1966) have resulted in a wide variety of symptoms: nervous inco-ordination with degeneration of ganglion cells, diarrhoea and various lesions in the GIT, tachycardia and arrhythmic cardiac activity, hyperaemia and serous oedema of the cerebrum. The toxicity of infested rations fed to calves was enhanced by the addition of pepsin (Kurmanov, 1964). The avoidance of acid feeds such as silage, oil cake and brewers' grains is recommended.

(iii) *Fusarium poae.* This species has been observed in conjunction with *F. sporotrichioides* (see above) and in two further epizootics. A large scale toxicosis of sheep was observed in collective and state farms in the Stavropol region of the USSR (Kurmanov, 1961). A few years later 27 out of 41 horses were killed as a result of feeding mouldy hay in the Kazakh region (Spesivtseva, 1967): symptoms included stiffness, haemorrhagic infiltrate, hyperaemic lymph glands and stomach, inflammation of parts of the stomach, and a visible mucous membrane cyanosis. Experimental reproduction of the disease by feeding infected oats or barley infected with these fungi to animals was obtained giving several clinical phenomena: atony of rumen, gnashing of teeth, paresis, paralysis, and rapid pulse in bullocks, gastro-enteritis, lung oedema, liver hyperaemia and haemorrhagic diathesis in sheep and goats (Dzilavyan and Spesivtseva, 1960), and again with *F. poae* alone, acute depression, disappearance of tactile motor and defence reflexes, motor disorders in the GIT, haemorrhagic diathesis and emphysema in sheep (Kurmanov, 1968a). The neurotoxin of *F. poae* could be inactivated by the rumen as long as the pH was alkaline, but severe toxicosis developed when acid silage or pepsin was given.

(iv) *Fusarium moniliforme.* The production of zearalenone by this fungus means that it is probably partly responsible for some of the reported outbreaks of hyperoestrogenism. In addition, this species is known to have been the cause of an outbreak of leucoencephalomalacia from mouldy corn in Egyptian horses (Badiali *et al.,* 1969), and in donkeys from the same area (Wilson and Maronpot, 1971). Experimental verification was made on donkeys. The most important pathological finding was focal necrosis of the white matter of the central hemisphere. Another epizootic was observed by Marasas *et al.* (1973) in South African horses. Experimentally, widespread lesions were found in the liver and brain.

F. moniliforme is also responsible for a second toxin called moniliformin (Cole *et al.,* 1973). This has both a zootoxic and phytotoxic effect.

(v) *The 'fescue' toxins.* Diacetoscirpenol or T_2 toxin, was first isolated from *Fusarium equiseti* by Brian *et al.* (1961) and later from *F. solani* by Ishii *et al.* (1971). It was also isolated from *F. nivale* and *F. tricinctum* by Gilgan, Smalley and Strong (1966) and by Bamburg *et al.* (1968a & b). *F. nivale* and *F. tricinctum* also produce a butenolide lactone termed T_1 toxin (Yates *et al.,* 1968; Grove *et al.,* 1970). *F. equiseti, F. nivale* and *F. tricinctum* have been isolated from mouldy fescue grass, which, when ingested by cattle, produce lameness of hindquarters, gangrene in tail and hoofs and fever in warm weather (Keyl *et al.,* 1967). When the T_1 toxin was injected intramuscularly into the shoulders and thighs of a heifer, dry gangrene was eventually produced at the end of the tail, exactly resembling the fescue disease symptoms (Grove *et al.,* 1970). This interesting finding was subsequently confirmed by Hoyem and Thorson (1970). Injection of the T_2 toxin into a heifer caused death from internal haemorrhage similar to that found in mouldy corn toxicosis (Grove *et al.,* 1970). As with the toxin produced by *F. sporotrichioides,* the clinical picture due to T_1 and T_2 poisoning is very diverse. Hamilton, Wyatt and Burmeister (1971) found that chickens ingesting T_2 toxin lost weight and developed mouth lesions that became secondarily infected. The pancreas and crop increased in relative size. In a further study (Wyatt *et al.,* 1973) neural disturbances were noticed in chickens strikingly similar to those associated with ATA in humans. Christensen *et al.* (1972a) found that lethality of the toxin for turkey poults was accompanied by decreased feed efficiency and weight gain and moderate development of bilateral necrotic lesions at the angles of the mouth. In rats and cattle, Kosuri, Smalley and Nichols (1971) and Kosuri (1970) found that the toxin had a multiple effect, resulting in loss of body weight, drop in rectal temperature, diarrhoea, paraplegia and anaemia, tetanic spasm of skeletal muscle, liver and kidney necrosis, prothrobinopenia and various metabolic changes.

Fusarium nivale also produces nivalenol (Tatsuno, 1968), originally isolated from mouldy rice. It was found to inhibit protein synthesis of rabbit reticulocytes and DNA synthesis of Hela cells and ascites tumour cells, but did not inhibit RNA synthesis. Pathological changes in rabbits included cell degeneration of the bone marrow, lymph nodes, intestine, testes and thymus. Fusarenon-X is another toxic metabolite, discovered and studied by Ueno *et al.* (1970, 1971a, b). It caused haemorrhage and necrosis of skin of rabbit, mouse and guinea pig. In guinea pigs, cats and ducklings the toxin caused multiple necrosis.

(vi) *Other Fusarium toxins. F. culmorum.* Fisher, Kellock and Wellington (1967) found this species associated with an outbreak of toxicosis in dairy cattle following ingestion of contaminated maize. Symptoms comprised decreased milk production, loss of appetite, scouring and occasionally staggering.

F. martii var. *minus.* Shao-Dyan and Alenkovich (1959) regarded this species as responsible for poisoning of horses, donkeys and mules.

(b) *Penicillium citreo-viride* **toxin (Citreoviridin).** The isolation and acute toxicity of citreoviridin has been described by Uraguchi (1950), Kinosita and Shikata (1965) and Ueno and Ueno (1972). It is primarily a neurotoxin, lethal to mice, rats, guinea pigs and ducklings causing progressive paralysis, vomiting, convulsions and respiratory arrest. The heart and circulatory systems are also affected. The symptoms resemble those of acute beri-beri.

(c) **Stachybotryotoxin.** Numerous outbreaks of this mycotoxicosis attributable to *Stachybotrys alternans* on sugar beet pulp, vetch, and on mouldy hay and straw from wheat, oats, and barley and primarily found in pigs, sheep, cattle and horses, have been reported from Russia and eastern Europe. These are summarized in Appendix 5.

The main symptoms of the disease are extensive haemorrhage, ulceration in musculature, subcutaneous connective tissue, and serous and mucus membranes of the tongue, rumen, intestine, liver and kidney, cessation or reduction of lactation, salivation, fever, atony of rumen and intestines, weakened cardiac activity, muscular tremor, loss of appetite and frequent and painful defaecation (Forgacs, 1972). Cellular changes in the blood and bone marrow alter the blood composition and result in leucopenia (Danko, 1974). In spite of the widespread occurrence of this animal toxicosis, only Drobotko (1946) has reported occasional human involvement, apparently as a result of coming into contact with infected hay.

(d) **Dendrochiotoxin from** *Dendrodochium toxicum.* Karpova-Benoua (1954) found that this toxin caused a serious epidemic in horses in the Ukraine in 1947, traced to infestation of cotton fibres by the fungus. Six other hyphomycetous species in the fibre were also found to be toxic. Experimental work with pigs (Stepushin and Chernoy, 1969), horses (Salikov *et al.,* 1970) and rabbits and rats (Malashenko, 1961; Ponomarenko, Skyrta and Malashenko, 1961) shows that dendrodochin has a general toxic resorptive effect, accompanied by paralysis, and also specific effect on the blood system, resulting in generalized haemorrhage, thrombopenia and leucocytosis. In its acute form the disease has no visible clinical characteristics. The properties of the toxin have been investigated in detail by Bilai (1960, 1961, 1962).

(e) **Ergotism.** This is possibly the best known mycotoxicosis because of the consistent reports of outbreaks involving man, cattle and birds, going back to the Middle Ages. Ergotamine, from *Claviceps purpurea,* is a highly complex alkaloid and has a contractile effect on the uterus and circulatory system, leading to abortion in cows (Mantle and Gunner, 1965) and gangrene of the extremities and at the end of the tail (Ainsworth and Austwick, 1959; Moller, 1965). Administration of small quantities, however, has important practical usage in human gynaecology. Side effects of ergotism in animals include hypersensitivity, muscular tremors, muscle inco-ordination, increased glandular secretion, accelerated pulse rate and digestive derangement (Connole and Johnston, 1967) and vesiculitis in birds (Perek, 1958). With public health control measures, the present day risk of ergot poisoning has been considerably reduced, however, and it is only in the countries where these are not maintained that the danger is real.

Other species of *Claviceps* have been recently involved in poisoning. *Claviceps paspali* causes 'paspalum staggers', or damage to the nervous system resulting in trembling and inco-ordinated movement (Adelaar, 1958; Ehret *et al.,* 1968; Gitman, 1963; Sarkisov, 1954). The toxic principle is alpha oxyethylamide of lysergic acid, an alkaloid powerful at very low concentrations (Bianchi *et al.,* 1965). *Claviceps fusiformis* has been reported to cause agalactia of sows in Rhodesia (Loveless, 1967) when ergots grown on *Pennisetum typhoides* were incorporated into stock feeds. Interestingly enough, other unrelated fungi have been shown to produce an ergot like action, including *Cladosporium herbarum* (Perek, 1958), and species of *Aspergillus* and *Penicillium* (Abe *et al.,* 1967; El-Refai, Sallam and Naim, 1970).

Ergotism has never been implicated in carcinogenesis but it is interesting to note that Nelson *et al.,* (1942) have produced tumours termed neurofibrosarcomas in old rats forming a small proportion of a group experimentally treated by prolonged feeding with crude ergot.

(f) **Tremorgen (Tremortin).** Ciegler and Pitt (1970) in their survey of representative members of the genus *Penicillium* found that the production of this toxin is confined to species of the Fasciculata-Asymmetrica section. The most prolific members were *P. crustosum, P. cyclopium, P. granulatum* and *P. palitans.* Other species capable of production were *P. olivinoviride, P. puberulum* and *P. martensii.* The quantity of toxin varied greatly among strains even of the same species. Wilson and Wilson (1964) also report the isolation of a tremorgen nearly identical to the above from *Aspergillus flavus.* Hou, Ciegler and Hesseltine (1970, 1971a) have defined 3 closely related tremorgenic substances from *P. palitans,* tremortin A, B, and C.

Wilson and Wilson (1964) found that the action of the tremorconvulsant from *Aspergillus flavus* was to cause tremors and convulsions when administered to mice and other experimental animals. Later Wilson, Wilson and Hayes (1968) studied the effect of tremorgen from *P. cyclopium* on mice and rats; as little as 250 mg/kg of the toxin injected intraperitoneally caused perceptible tremors lasting several hours. With doses of 2.5 mg/kg and higher, initial tremors soon progressed to clonic or tetanic convulsions.

The only natural outbreak attributable to this toxin so far has been reported by Ciegler (1969) who isolated *P. palitans* from mouldy feed suspected of causing death of several dairy cows. Since *P. palitans* also produced viridication (Ciegler & Hou, 1970), this toxin could have been involved also.

(g) **Cyclopiazonic acid.** Holzapfel (1968) established the structure and toxic properties of this metabolite from *Penicillium cyclopium*. At low dosage levels it was reported to cause convulsions in mice but these symptoms have not been reported so far by other workers, who describe it as having a direct degenerative effect on the viscera. (See above under Section II). It is distinct from tremorgen just described (Wilson, Wilson and Hayes, 1968).

(h) **Kojic acid.** This toxin is produced by *Aspergillus flavus* and *A. parasiticus* (Parrish *et al.,* 1965) and by a large number of other aspergilli. Wilson (1971a) reviews its various anti-microbial properties and its toxicity to animals under experimental conditions. The action of the toxin seems to be a primarily nervous one, resulting in convulsion, salivation and vomiting. Werch, Oester and Friedmann (1957) showed that symptoms similar to those of epilepsy could be obtained in dogs. The incidence of isolation of this toxin in nature, however, has been insignificant, so that it is probably not of practical importance.

(i) **Haemorrhagic toxin** from *Alternaria* and *Cladosporium* and other genera. Gouws (1965) records the 'haemorrhagic syndrome' in birds due to *Alternaria tenius, Cladosporium epiphyllum* and *C. fagi* after these species had ingested grain fed to chickens. Feeding *A. tenuis* in moulded grain experimentally resulted in death for chickens, ducklings, rats and sheep, causing multiple necrosis. This was an interesting finding in relation to the known common occurrence of this fungus on sorghum grain. Forgacs *et al.,* (1962) has also studied the haemorrhagic syndrome in poultry. Substrates naturally contaminated by various fungi responsible for an outbreak were fed to battery chickens and produced depression, anorexia, diarrhoea and death. Multiple lesions were present in acute cases. Examination of the substrates revealed the presence of *Alternaria* sp., *Aspergillus flavus, A. clavatus, Penicillium citrinum, P. purpurogenum, P. rubrum* and *Paecilomyces varioti.* Forgacs and Carll (1955) found that feed on which species of *Alternaria* had been cultured alone, caused the death of 20 six-week-old chicks with typical haemorrhagic symptoms, but in natural outbreaks the range of fungi responsible is likely to be wider. *A. congipes* (Doupnik and Sobers, 1968) and *A. alternata* (Meronuck *et al.,* 1972) appear to be the chief species responsible. The latter workers determined that the metabolite responsible was tenuazonic acid, but this was unfortunately not detected on sorghum and black-eyed peas which were seen to be heavily attacked by the fungus under natural conditions.

Toxins from *Alternaria* may be of human significance since this genus commonly infests tobacco. Smoke aerosols derived from the fungus have caused emphysema in mice (Forgacs and Carll, 1966).

(j) **Leucogenol.** Rice (1966) isolated this compound from the culture filtrate of *Penicillium gilmani.* When injected into rabbits it caused leucocytosis without concurrent increase in body temperature.

(k) **Miscellaneous haemorrhagic toxins.** *Aspergillus chevalieri* was implicated with *A. clavatus* as described above in causing hyperkeratosis in calves and haemorrhagic and other lesions (Forgacs *et al.,* 1954; Carll and Forgacs, 1954). Schumaier *et al.,* (1961a, b) fed extracts of wheat cultures of *A. chevalieri* and *A. flavus* to chicks, resulting in internal haemorrhage, bone marrow changes and diarrhoea. The toxin does not appear to have been identified.

(l) **Miscellaneous toxins from Hyphomycetes.**

Toxins from *Acrospeira macrosporoides* (Berk.) Wilts. Tentatively identified as thiaminase, this toxin was responsible for an outbreak of polioencephalom in calves (Anon., 1969), and was traced to mouldy straw infested by *Acrospeira macrosporoides.*

Maltorhizine. Produced by *Aspergillus oryzae* var. *microsporus*, this toxin has resulted in two cases of cattle poisoning from contaminated malt sprouts (Iizuka and Illoa, 1962). The symptoms are similar to malt germ toxicosis but the toxin differs from patulin.

Myrothecin. Produced by *Myrothecium verrucaria* (Nespiak, Kocor and Siewinski, 1961), myrothecin has a specific affinity for the gastrointestinal tract, causing severe haemorrhagic symptoms and gastroenterocolitis in sheep and claves (Mortimer *et al.,* 1971; Di Menna and Mortimer, 1971). Martinovich, Mortimer and Di Menna (1972) have stated this syndrome to be indistinguishable from 'Kikuyu poisoning'. Karpova-Benoua (1957) and Vertinskii, Dzhilaviyan and Koroleva (1967) observed natural outbreaks of myriotheciotoxicosis in Russia affecting sheep. Anorexia, excessive salivation and foamy discharge from the nostrils, haemorrhagic necrosis and catarrhal inflammation of the digestive tract were the salient features.

Trichothecin. This toxin, produced by *Trichothecium roseum*, is a strong antifungal metabolite (Richard, Pier and Tiffany, 1970; Freeman, 1955) and is also toxic to ducklings, mice and rats. The detailed pathology does not appear to have been described.

Slaframine, a toxin produced by *Rhizoctonia leguminicola,* causes excessive salivation in animals (Broquist and Snyder, 1971). So far it has been isolated from legume crops only.

(m) **Ustilagotoxicosis.** Reports of mycotoxicosis caused by Basidiomycetes belonging to the rusts and smuts are somewhat contradictory, some authors such as J. J. Christensen (1963) and Neverov (1969) maintaining that most evidence indicates that large quantities of corn smut can be fed to animals without apparent ill-effect. Christensen noted that smut galls are still used as food for humans in some Latin American countries, particularly Mexico. On the other hand, Appendix 6 summarizes some of the known toxic outbreaks. The general syndrome of this disease is fairly varied, without any strikingly consistent features.

(n) **Mycotoxins in foodstuffs not yet identified.** A large number of fungi have been determined as toxigenic by the duckling test (Scott, 1965), the one-day-old duckling being the most sensitive test animal known and relatively easy to handle. A positive result (toxicosis or death) by the duckling test does not, of course, necessarily imply that the material tested is toxic to other experimental animals. The list in Appendix 7 illustrates the degree of toxicity of miscellaneous strains, many belonging to species not discussed above.

The role of all fungi implicated in toxicosis has been well reviewed by Brook and White (1966). Semeniuk *et al.,* (1971) investigated 392 strains of *Aspergillus* belonging to 132 species and found 166 strains and 73 species toxic to a varying degree to chicks and mice. The ability to produce toxins potentially dangerous or lethal to animals is obviously very widespread among the fungi, their effects being limited by the complexity of the organism and the degree to which the toxin is actually formed in nature. Richard, Tiffany and Pier (1969) found that at least one toxigenic isolate was obtained from each of twenty-five mouldy corn samples, 246 fungal isolates being obtained in all. Marasas and Smalley (1972) found that ten of the sixteen species they tested from maize were toxic to ducklings. The difficulty lies in the quantitative assessment of the toxins since chemical assay methods are only available yet for the major fungal toxins. Summaries of the chief mycotoxins with their causal agents and the more important toxigenic fungi are given in Appendices 8 and 9.

It is not yet possible to correlate the geographic incidence of outbreaks of all the different types of mycotoxicosis with what is known of their physiology. This needs to be done if the concept that a relationship exists between various chronic diseases and the availability of specific mycotoxins in a given area is to be substantiated. It is significant that out of the fourteen known outbreaks of aflatoxicosis in man or animals ten were due to ingestion of a foodstuff grown in a tropical or subtropical area. On the other hand thirty-three out of thirty-eight epizootics recorded above due to toxins from *Fusarium graminearum, F. moniliforme, F. poae* and *F. sporotrichioides* have occurred in temperate or cold areas with a continental or mediterranean climate where a warm summer is followed by an extremely cold winter, and short term temperature fluctuations are common. This picture accords well with the temperature preferences of *Aspergillus* and *Fusarium* determined in the laboratory. Looking at the situation in human terms, once it is shown further that mycotoxins are ingested to a significant degree by the human population in any area, then their involvement in diseases such as cancer becomes a plausible hypothesis.

The association of mycotoxins with malignant disease

AFLATOXINS AND CANCER

In 1944 a mysterious outbreak of liver cancer in pigs occurred in Morocco (Ninard and Hintermann, 1945). Although a mycotoxin was not specifically mentioned as the cause, the authors traced the source of the toxic agent to various oil food cakes comprising cocoa, cottonseed, groundnut, karite, palm and touresol seeds. The use of karite butter by the Senegalese was suggested as a possible cause of liver cancer in man. The histopathology of the lesions described in the detailed report is entirely consistent with that observed later in hepatomas known to be due to aflatoxin. The French workers therefore came astonishingly close to appreciating the full significance of their discovery.

The potential involvement of aflatoxin in the aetiology of liver cancer in man was, however, first suggested by another group of French workers, Le Breton, Frayssinet and Boy (1962). Earlier in Japan, Miyake *et al.*, (1960) had shown for the first time that hepatotoxins produced by moulds could cause carcinoma of the liver in animals. The possibility of a link between the natural occurrence of mycotoxins in the diet and the incidence of liver cancer was only later put forward by Oettlé (1964) and by Kraybill and Shimkin (1964). These authors were struck by the geographic differences in incidence of primary hepatoma, some of which can be summarized in the following table:

Table 16

Occurrence of primary hepatoma

Area	Absolute incidence in autopsies	% of all carcinomas	Author
Orient + central Africa	1%	14%	Berman (1951)
USA + Europe	0.3%	$<2.5\%$	Kraybill & Shimkin (1964)
Japan	1.5%	7.6%	Takeda & Aizawa (1956)
Mozambique (Lourenzo Marques)			
Africans	–	47.6%	Oettle (1956)
Indians and Europeans	–	4.9%	Oettle (1956)

Doll's figures (1969) also clearly confirm the high rate of liver cancer in Southern Africa, Nigeria and Uganda. Liver cancer has been recognized as a serious disease of the African for a long time, and its uneven distribution within the African continent was noted by Oettlé. Because of this, the idea of uniform human liability to cancer was reckoned to be false. If environmental factors could be incriminated, then they were potentially preventable.

Although Oettlé's work implied a relationship between the incidence of cancer and dietary factors, the first definite statement of what can be termed the 'mycotoxin hypothesis' can be ascribed to Kraybill and Shimkin (1964): 'The potential associ-

ation of the high concentration of mouldy diets with liver injury and liver carcinoma in man would appear inescapable'.

Oettlé (1965 a, b) subsequently outlined the mycotoxin hypothesis with reference to the high incidence of hepatoma in Southern Africa. He interpreted (as with others) the association with race as an expression of differential environmental exposure and the dramatic variations in the disease was ascribed to differences in tribal eating habits. The usage of spoiled grain and other foodstuffs might explain the six fold differences in incidence of liver cancer between males from tropical Africa and South Africa (Oettlé, 1964) but both he and other workers (e.g. De Wit *et al.,* 1966) were aware of the problem of direct proof. Four postulates were initially outlined by Oettlé (1963) as requirements for proof of causation by an environmental factor, similar to Koch's postulates for identification of a microbe as pathogen, which are worth restating:

(1) In the presence of the etiologic factor there should be an increased risk of contracting the disease.

(2) The risk should vary with the dosage.

(3) The site affected should be shown to have been exposed to the aetiological factor.

(4) The factor should be capable of inducing cancer of the same or comparable site in experimental animals.

Oettlé considered that the mycotoxin hypothesis fitted the facts better than other hypotheses in that it could account for the rarity of liver cancer in dry areas (Egypt, Tunisia and Morocco) where mould spoilage was minimal or in tribes as in Uganda where a predominantly fresh diet was consumed. Hypotheses involving malnutrition, severe poisoning, genetics, infectious hepatitis and bilharzia were unable to explain the low rates in Egypt or the remarkable variations in intensity between neighbouring regions. The known distribution of liver cancer accorded better with areas where high humidities could prevail, thus increasing chances of fungal food spoilage. Oettlé included Mozambique, Zaire, Senegal, Zambia, Portuguese Guinea, Mali, Niger and Togoland in his list. There was circumstantial support for the first specific postulate and of the others, the fourth had previously been met (Butler and Barnes, 1963). The acknowledged difficulties centred on postulates 2 and 3; the demonstration that liver cancer rose in proportion with the degree of previous exposure to mycotoxins, and the isolation of aflatoxin in the liver (whether diseased or not). In addition, Oettlé could not explain the high male:female ratio of cancer incidence in Africans. Neither could he specify which environmental or physiological evidence might be used to *disprove* the thesis. In the absence of critical laboratory work at that time on the limitations of fungal growth and toxin formation, the mycotoxin hypothesis had an unwarranted elasticity that could be used to cover a variety of situations which had little in common. Some of the detailed work on fungal physiology since 1964 has been reviewed earlier in this paper, so that ten years later we are in a much better position to assess the likelihood of fungal aetiology in a given situation. We are also able to judge the value of the mycotoxin hypothesis in the light of the epidemiological research work that has been conducted in various parts of the world since 1966. As far as postulate 3 is concerned, the various demonstrations of aflatoxin M, a metabolite of aflatoxin B, in the urine of both animals and humans furnishes proof that aflatoxin must have passed through the liver. Aflatoxin has also been demonstrated *in situ* in the human liver (Shank *et al.,* 1971). Four intensive field investigations have satisfied postulate 2, serving to relate aflatoxin directly to the incidence of human disease.

In Uganda the distribution of hepatoma was found to correlate well with the incidence of foodstuffs, heavily contaminated with aflatoxin, that were selected at random from various homes (Alpert *et al.,* 1968, Alpert and Davidson, 1969). In a further survey (Alpert *et al.,* 1971), a similar result was obtained where 29.6% of samples of miscellaneous foodstuffs contained detectable levels of aflatoxin. The frequency of aflatoxin contamination was particularly high in the eastern

Karamoja district of the Northern Province, where the incidence of hepatoma was also especially high. The results are summarized in Table 17.

Table 17

Comparison of aflatoxin contamination of foods and hepatoma incidence in Uganda

Hepatoma incidence		Aflatoxin contamination		
Tribe	(crude rate)	Province	Samples assayed	% positive
Bwamba	(no data)	Toro	29	79.3
Karamojang	15.0	Karamoja	105	43.8
Bugandi	2.0	Buganda	149	28.9
(Rwanda immigrants)	3.0			
West Nile Tribes	2.7	West Nile	26	23.1
Acholi	2.7	Acholi	26	15.4
Soja	2.4	Busoja	39	10.3
Ankole	1.4	Ankole	37	10.8

Assuming a daily food consumption of 500 gms staple grains per day, the per capita ingestion of Karamoja could be in the order of 0.02 to 2.0 mg daily, a level known to be hepatotoxic to monkeys (Alpert, Serck-Hanssen and Rajagopolan, 1970). According to Alpert and his co-workers, the wide variation in hepatoma incidence cannot be explained on a simple basis of genetic predisposition. The Hutu and Tutsi immigrants in Buganda Province have a 50% higher incidence of hepatoma than the indigenous, racially related, Buganda tribe. Poverty and food scarcity are both major problems among these migrant peoples and among the inhabitants of Karamoja, so it is a reasonable assumption that they would assume the poorer, mould contaminated, grades of foodstuff and thus be exposed to higher levels of aflatoxin than the rest of the population.

Another striking fact was that Karamoja has a dry semi-desert climate where, despite a low mean annual value, rainfall is concentrated into a short rainy season once or twice a year. This could, however, provide an adequate microenvironment for the production of aflatoxin at harvest time.

An earlier detailed survey of the West Nile District of the Northern Province of Uganda (Korobkin and Williams, 1968) showed a fascinating correlation between the distribution of groundnut cultivation and the village distribution of twenty-five hepatoma patients. The difference in distribution of hepatoma patients compared with all other tumour patients was significant at the 0.05 level. No hepatomas occurred in a large area of the district where no little or no groundnut cultivation had previously taken place. A survey of Uganda markets (Lopez and Crawford, 1967) showed that 15% of groundnut samples contained $>$ 1 ppm aflatoxin B_1 and 2.5% contained $>$ 70 ppm. Therefore, the circumstantial evidence for the involvement of aflatoxin and groundnuts in liver cancer in Uganda is very strong.

Another study was carried out by Peers and Linsell (1973) in Kenya. Here the pattern of liver cancer distribution was determined concurrently with the pattern of aflatoxin incidence, and was not known in advance. The area selected was the Murang'a district on the eastern side of the Aberdare Mountains. It had a high density and rural population living traditionally on food mostly produced within the district. Sociological, geographical and meteorological data indicated that the study area could be divided into three sub areas (high, middle and low altitudes) offering different economic and agricultural conditions. Samples of the main daily meal (principally of cereal origin) and honey beers which also contained grain were examined from representative stations within the sub areas. The following table summarizes the principal results.

Table 18

Distribution of aflatoxin and hepatoma in Kenya

Altitude	High 6 500′–>1 200′		Middle 5 250′–6 500′		Low 4 000′–5 250′		Total for Muranga District	
	M	F	M	F	M	F	M	F
Total Population (1962)	18 394	20 244	75 138	86 467	68 808	75 803	162 340	182 514
Population ⩾16 yrs.	8 027	10 885	30 105	45 693	30 949	41 375	69 081	97 953
Frequ. diet Aflatoxin Contaminated	39/808		54/808		78/816		171/2 432	
Mean μg/kg	0.121		0.205		0.351		0.226	
Freq. beer Aflatoxin Contaminated	3/101		4/101		9/102		16/304	
Mean μg/l	0.050		0.069		0.167		0.095	
Estimate of mean Aflatoxin ng/kg body wt/day ingested	4.88	3.46	7.46	5.86	14.81	10.03	9.18	6.46
Primary liver cancer cases, age ⩾ 16, 1967–70	1	0	13	6	16	9	30	15
Crude rate	3.11	0.00	10.80	3.28	12.92	5.44	10.86	3.83

The differences in aflatoxin levels and frequencies in the diet from one area to another were found to be statistically significant and so too was the correlation between the estimated degree of exposure to aflatoxin and the distribution of liver cancer cases recorded in a cancer survey over a four year period from 1967 to 1970. The authors point out however, that the liver cancer figures for the high altitude region were to some extent suspect and that a small underestimation in the figures could result in a high difference in the crude rate and hence in the statistical significance.

As with the other surveys, a correlation was sought in Thailand between the distribution of aflatoxin in the three districts and differing rates of liver cancer incidence. The daily consumption of aflatoxins was calculated from estimations based on samples of cooked foods, comprising *inter alia* rice, cabbage, pork and fish collected from families living in three provinces of Thailand, Singburi, Ratburi and Songkla (Shank, 1971). Aflatoxin was found in all these ingredients but the results clearly showed that the Ratburi villages suffered a greater frequency of contamination and those in Singburi the highest levels of contamination, while in Songkla both the frequency of contamination and the quantities isolated were minimal.

Table 19

Extent of aflatoxin contamination of cooked food samples and prevalence of liver cancer in Thailand

	Singburi	Ratburi	Songkla
% samples contaminated	4.4	15.9	1.2
Total aflatoxin concentration ug/kg in sample number			
Trace	22	129	10
<50	4	19	0
50–100	10	7	1
100–200	3	3	0
200–300	1	0	0
300–400	0	1	0
400–500	2	0	0
500–1 000	2	0	0
>1 000	1	0	0
Crude rate of liver cancer	(high but not determined)	6	2

The incidence of liver cancer in two of the three provinces was calculated from thorough screening of available hospital data (Shank *et al.*, 1972). The incidence in Songkla was found to compare favourably with equivalent rates for the eastern United States and Europe; in the Ratburi area, however, it was 3–6 times greater. The occurrence of liver cancer was accompanied by that of Reyes' syndrome (acute encephalopathy and fatty degeneration of the liver, kidney and heart) which led to the death of a 3 year old boy (Bourgeois *et al.*, 1971). Aflatoxin was demonstrated in liver specimens from 22 out of 23 further chidren suffering from the disease (Shank *et al.*, 1971). The highest levels of aflatoxin detected were 93 ug aflatoxin B/kg in a liver specimen, 123 ug/kg in a stool, 127 ug/kg in a stomach and intestinal contents. Aflatoxin M was found in trace amounts in two urine specimens. None of the urine specimens from 39 healthy control chidren contained any aflatoxin and very small quantities of aflatoxin B_1 were demonstrated in some of the autopsy specimens from 11 out of 15 control subjects with diseases other than the above. Other workers (Becroft and Webster, 1972) have not been successful in demonstrating a connection between Reyes' syndrome and aflatoxin even though the evidence for mycotoxin involvement is suggestive (Becroft, 1966; Becroft and Webster, 1972; Reye *et al.*, 1963).

Some evidence is available indicating that ingestion of aflatoxin is related to the incidence of infantile cirrhosis in India (Robinson, 1967). Samples of groundnuts from Bangalore and Hyderabad were found positive for aflatoxin. Forty-three samples of breast milk from mothers of cirrhotic children were likewise examined and three were positive in contrast to the total absence of aflatoxin in the milk of control subjects. A portion of liver from one of the children also was positive for aflatoxin. Correlation between consumption of groundnuts by mothers and presence of liver cirrhosis in infants was difficult to establish due to a failure of communication and/or lack of co-operation.

The association between infantile cirrhosis and aflatoxin is interesting because cirrhosis could well be the precursor of hepatoma. Cirrhosis is fairly common in Southern Africa but the aetiology is not conclusive.

In Swaziland and Southern Africa, as with the work described above, the analysis of various cancer of the liver distributions has been bedevilled by the difficulty of identifying tribal and other differences as genetic or environmental. The main locus of liver cancer has been demonstrated in Southern Africa to be in Mozambique where, although there have been various estimations of its incidence (Prates and Torres, 1965; Doll, 1969; Purchase and Goncalves, 1971), the frequency is indisputably high, reaching a crude rate of 37.0 per 100 000 in the Panda Area. To the south, in the Republic of South Africa, there is a second, lesser focus, in the eastern lowveld of the Transvaal province where an interesting distribution occurs. Barberton in the south has a crude rate in male Africans of 27.0 while in the north at Letaba, near Tzaneen, the rate is only 5.8. Hospitals between these two points register intermediate rates (Robertson, Harington and Bradshaw, 1971).

Analysis of tribe shows further differences of proven liver cancer cases in African males: the Shangaan tribe has the highest proportion (38.9%) closely followed by the Swazi (34.0%), whereas the Sotho only account for 16.7% and the Zulus make up the remainder (10.4%). While the Shangaan are distributed through much of Mozambique, the eastern Transvaal and in the north of Swaziland, the Swazis are much more common in the south and the Sotho are intermediate. Inside Swaziland itself the major part of the territory is occupied by the Swazi tribe. An interesting point therefore has arisen as to whether the decrease in liver cancer northwards from Swaziland is a tribal or geographical feature.

Partial answers to these questions have been obtained by the work of Keen and Martin (1971 a, b) in a study of the epidemiology of liver cancer in Swaziland. From a geographical point of view Swaziland can be divided into four well defined topographical regions. These extend north and south in roughly parallel belts. The highveld in the west has an altitude range of 915 to 1860M, the middleveld adjacent to this is from 335 to 1 070M, and the lowveld is 60 to 335M. The

Lubombo region on the Eastern border is a low range of hills equivalent in height to the Middleveld, 275 to 820M. The Highveld has a humid temperate climate with 100 cm mean annual rainfall, whereas the Lowveld is sub-tropical with an average rainfall of 50 cm. Swaziland therefore presents an interesting range of environmental conditions within a small geographical area.

A survey of hepatoma cases for the years 1964–1968, proved that the incidence of liver cancer cases increased with decreasing altitude, reaching a maximum in the Lowveld. 130 samples of groundnuts collected from Swazi peasant farmers, market places and stores during the same four year period were invaded by *Aspergillus flavus* and contaminated with aflatoxin to a different extent in each topographical region, the quantity also varying in relation to altitude. Highest levels occurred in the Lowveld, and since the groundnut samples were representative of those normally sold to the general population, it was a reasonable assumption that the quantities of aflatoxin ingested would also vary in the same manner according to area. Tables 20 and 21 summarize the information obtained.

Table 20

Geographical distribution of malignant hepatoma, Swaziland, 1964–1968

	Highveld	Middleveld	Lowveld
Number of cases	11	34	44
Crude rate	2.2	4.0	9.7
Population ratio	1.0	1.7	0.9
Risk	1.0	1.8	4.4

Table 21

Geographical distribution of *Aspergillus flavus* and aflatoxin in samples of groundnuts, Swaziland*

	Highveld	Middleveld	Lowveld	TOTAL
Number of samples	37	67	26	130
Presence of *A. flavus*	21	34	20	75
Presence of aflatoxin	3	17	14	34
Negative for aflatoxin and *A. flavus*	16	25	5	46
% samples with aflatoxin	8.1	25.4	53.8	26.2
Ratio of positive samples	1.0	3.1	6.6	–
Average concentration of aflatoxin ug/kg in positive samples	27	51	126	80

A detailed statistical analysis of the various factors – altitude, rainfall, temperature and storage methods – influencing aflatoxin formation has been attempted in order to correlate these with the distribution of hepatoma in Swaziland (Tunstall, unpublished). This confirmed the original observations made by Keen and Martin. Positive correlations were found between the quality of food storage, rainfall and altitude, i.e. decreasing efficiency of method was linked with decreasing altitude and decreasing rainfall. As with the Ugandan study, temperature and relative humidity appeared to take precedence over rainfall in the rapid formation of aflatoxin in foodstuffs.

Two facts were not easy to incorporate into the general Swaziland picture. In the first place the tribal and sexual distribution of hepatoma cases was unexpected.

*The information obtained from this study was confirmed by a recent study in Swaziland by Peers, Gilman and Linsell (1976).

Figure 2

Distribution of malignant hepatoma in relation to altitude, Swaziland 1965–1967

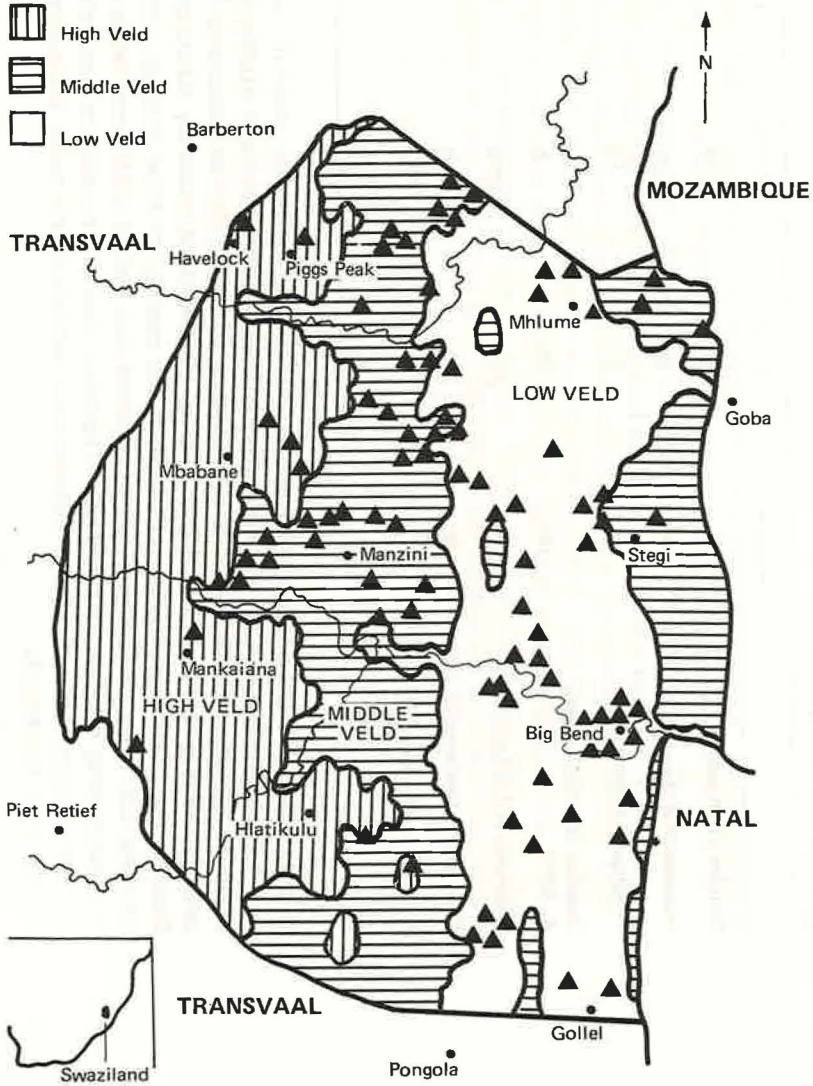
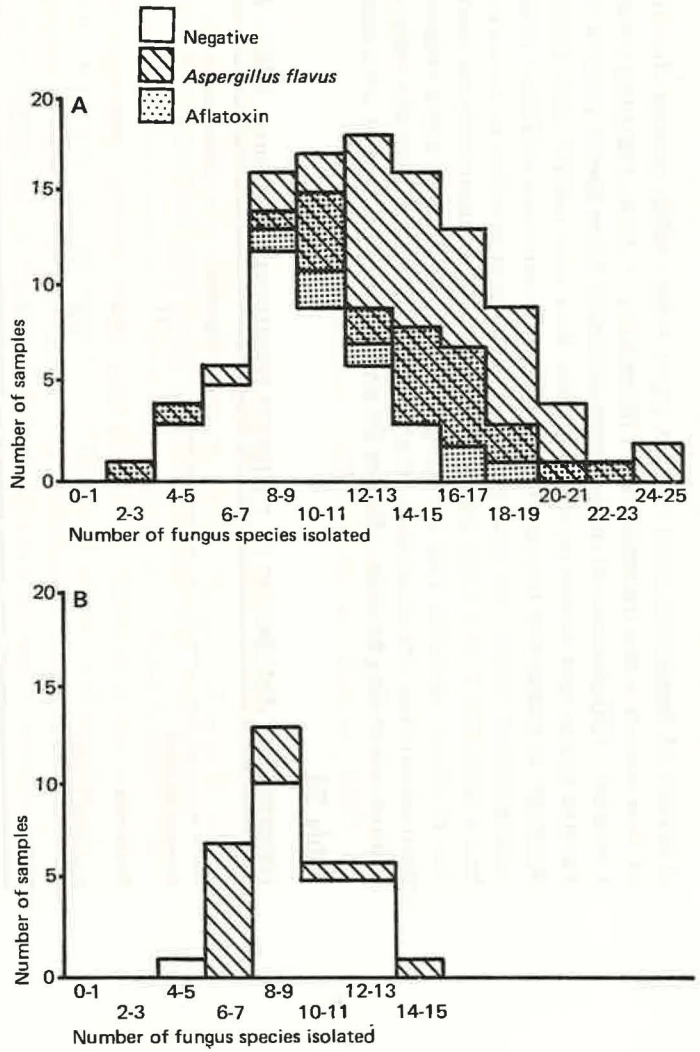


Figure 3

Aflatoxin formation in groundnut samples from miscellaneous indigenous stores (A) and good storage conditions (B) in Swaziland, 1966–1967.

(Note the apparent synergistic effect of the presence of many species upon aflatoxin formation).



Source: Keen and Martin, 1971a. Reprinted, with permission, from *Tropical and Geographical Medicine.*

Figure 4

Etiology of malignant hepatoma: diagram illustrating the hypothesis of synergism in relation to cancer.

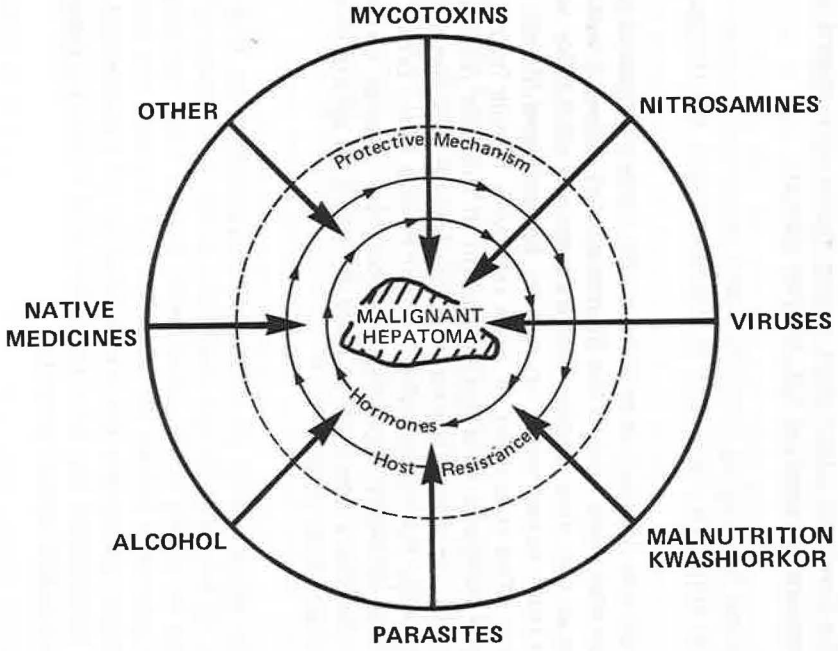


Figure 5

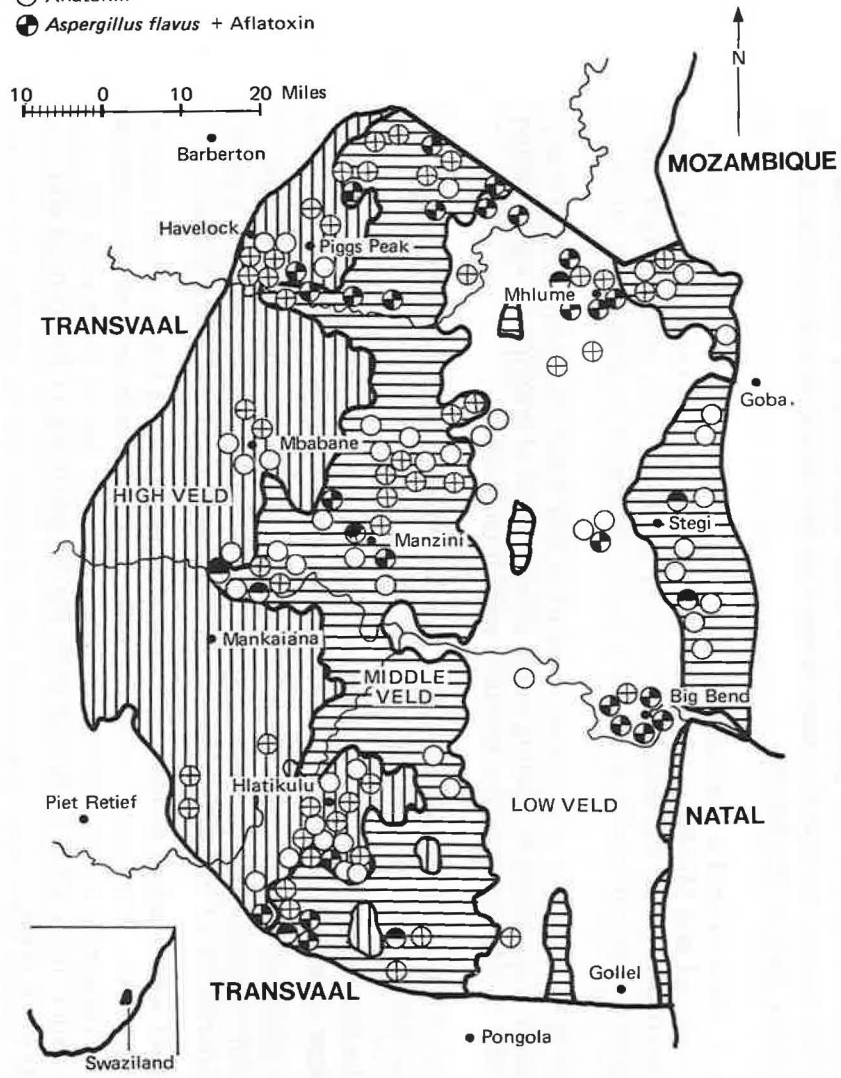
Fungal infestation of 130 groundnut samples in Swaziland.

○ Negative

⊕ *Aspergillus flavus*

◓ Aflatoxin

◓ *Aspergillus flavus* + Aflatoxin



Source: Keen and Martin, 1971b. Reprinted, with permission, from *Tropical and Geographical Medicine.*

Out of the 90 cases of primary liver cancer registered in the cancer survey from 1964 to 1968, there were 64 Swazi males, 7 Shangaan males and 4 Nyasas. There were 14 Swazi females, no Shangaan females and one Nyasa female. The number of Shangaan immigrants into Swaziland is small, approximately 8 600 compared to 350 000 Swazis, so that it is easy to see that the incidence rate in Shangaans is much higher than in the Swazis.

Moreover, there existed a marked difference in distribution of liver cancer cases in the Highveld and Middleveld from north to south, in that there were 26 cases in the northern half of the country compared to seven in the southern half, and this disparity was not accompanied by a major difference in population ratios.

In order to explain this unexpected geographic and tribal distribution of primary liver cancer, a questionaire relating to the consumption of groundnuts was prepared and 1 500 families all over the territory were interviewed.

Analysis of the questionaires and other incidental information allowed certain tentative conclusions to be made.

(1) The Shangaan living in Swaziland ate groundnuts more frequently and in larger quantities than the Swazis living in the same area.

(2) In addition, the Shangaan habit of grinding up groundnuts and using the resulting sticky powder as an additive to their other food gave them greater opportunity of ingesting aflatoxin in larger quantities and more frequently.

(3) There were marked differences in the groundnut eating habits among Swazis themselves. The Swazis living in the Southern Highveld area ate groundnuts less frequently and in smaller quantities and very few of them used the 'powder' form when compared with the Swazis living in the Central and Northern areas.

(4) In areas of Swaziland adjoining Mozambique border where the Shangaan have influenced the Swazi to adopt their eating habits with regard to groundnuts, there is an apparent increased risk of liver cancer.

It would appear that these fascinating intertribal and intratribal variations in eating habits could explain the apparent paradoxes in distribution of the dieases.

The other problem which was difficult to explain on the basis of ingested carcinogens was the difference in sex incidence. From the figures quoted above, it was deduced that for the population at risk they represented a crude rate of 8.6 for males and 1.6 for females. Laboratory experiments on rats by Ratnoff and Mirick (1949) could partly explain this. The treatment of male rats with female hormones considerably reduced the incidence of a toxic reaction in the liver during the ingestion of a hepatotoxin. It would appear that the female liver has a protective mechanism, in all probability hormonal, which might have a fairly broad spectrum since it is well known that amoebic hepatitis, amoebic liver abscess, cirrhosis and bilharzial hepatitis are also more common in male Africans, in approximately the same proportion as primary liver cancer.

Further evidence for the decisive rôle of hormones was provided by Righter *et al.*, (1972). The incidence of liver cancer was markedly lower in prepubertal male rats as compared to that in young adults when both groups were fed sublethal dosages of aflatoxin. Castration before 10 weeks of age obviated the lethal effects of later aflatoxin ingestion; conversely the administration of testosterone to mature aflatoxin-fed castrates reproduced the total mortality that would be normally expected in rats with complete sexual potential.

Various models simulating the role of synergism in the development of cancer have been tested. Domingo, Warren and Stenger (1967) divided 410 female CBA mice into four experimental groups for which the following procedures and observations were made:—

(1) 80 controls were given no treatment. None developed hepatoma.

(2) 95 received monthly injections of 2-amino-5-azotoluene (a carcinogen). One developed hepatoma.

(3) 30 received cercariae of *Schistosoma mansoni*. None developed hepatoma.

(4) 135 received cercariae and the carcinogen as in Group II. 13 developed carcinoma.

The mice in Group III had a far milder and qualitatively different reaction to those in group IV. The two factors together resulted in an early and marked production of hepatoma which either agent alone failed to produce. It is significant that outside actual organic infection, cirrhosis is the only form of liver injury which in conjunction with aflatoxin, gives a higher incidence of hepatoma than aflatoxin alone (Newberne, Harrington and Wogan, 1967; Sun, Wei and Schaffer, 1971).

Before leaving this particular discussion, it is worth noting that a strong correlation in Southern Africa also seems to exist between hepatoma and the cultivation of groundnuts generally. Lesotho, a small country with a cooler climate than the areas surrounding it, lies midway between Swaziland and the Transvaal to the north and the Cape and Transkei to the south. Due to its high altitude which lies between 1 525 and 3 485 M and associated abrupt cold winters, groundnuts cannot be grown in the territory, and the crude rate of hepatoma is remarkably low. A survey of hospital figures for the period 1964 to 1969 indicated a value of only 2.2 for Mosotho males (Martin *et al.*, in press, 1976).

Criticism of the aflatoxin hypothesis

Although the evidence reviewed above is plausible, certain facts mitigate against aflatoxin being a sole or universal etiological factor in development of hepatoma. It is not the purpose of this review to deal thoroughly with these aspects, but two are mentioned below:—

(1) Massive absorption of groundnut cake containing aflatoxin has been known to occur in two children of an early age, and follow up over a number of years indicated that fibrosis but not neoplasm, occurred within the liver as a result (Payet *et al.*, 1966). The children were in good health when last observed. Ziegenfuss (1973) has suggested that viral hepatitis characterized by the production of Australia (AU) antigen (now known as Hepatitis B Antigen) could also be implicated in hepatoma. As the antigen has been found on postmortem examination in more than 60% of hepatocellular cancer cases, this may therefore be of considerable importance (Peers, 1975; personal communication).

(2) Since both kidney and liver are the targets of many mycotoxins, including aflatoxin (Enomoto and Saito, 1972) one would expect a high correlation in incidence of liver and kidney cancers. In fact an inverse correlation is found, kidney cancer being rare where a high incidence of primary liver cancer has been reported. The highest incidence of nephroblastoma is in the industrialized centres of North America and Western Europe, and the regional differences in liver cancer noted above are not accompanied by significant differences in frequency of nephroblastoma (Higginson, 1964). If the mycotoxin hypothesis is true, such a phenomenon would only be explicable on the basis of a different metabolism of the two organs — perhaps the liver retains the carcinogen longer.

OTHER MYCOTOXINS AND CANCER

The similarity in histopathology of primary liver cancer lesions produced artificially in the rat by sterigmatocystin to those of human patients surveyed in Mozambique, has been pointed out by numerous workers in Southern Africa (Purchase and Vorster, 1970; Torres *et al.*, 1970). As we have already observed, however, there is no direct field evidence to support the involvement of sterigmatocystin in human hepatoma.

Aleksandrowicz (1970); Aleksandrowicz *et al.*, (1970, 1971) and Gajda (1970) have established interesting correlations between the occurrence of common fungi (*Alternaria, Cladosporium, Fusarium* and *Penicillium*) and the environment of leukaemics: houses of these patients tended to be damper and mouldier than those of controls. More critical work, however, is needed before the implications become clearer.

Although there is not as yet any direct evidence, a variety of other mycotoxins may have neoplastic potential in humans. The role of luteoskyrin and other compounds produced by *Penicillium islandicum* and of patulin in rat hepatoma and sarcoma has already been discussed. In addition Blank *et al.*, (1968) demonstrated an increase in incidence of leukaemia in mice following injections of extracts of *Candida parapsilosis* and species of *Microsporum, Trichophyton, Epidermophyton* and *Scopulariopsis*. Similar results have been obtained with *Alternaria* spp. and *Aspergillus niger* which commonly contaminate foodstuffs and tobacco (Louria *et al.*, 1970).

The discovery of fungal oestrogens in beer and maize products could have important medical implications, especially in areas with a cold winter, since, as we have seen, zearalenone production is stimulated by near freezing temperatures. It is noteworthy than in Southern Africa, the crude rate of cervical cancer in women in Lesotho is 11.7, while in Swaziland, a much warmer country, it is only 8.7 (Martin *et al.*, 1976, in press). Both these rates, however, are, as elsewhere in Africa, several times as high as those in Europe and North America. The hypothesis of induction of cervical cancer by oestrogens has some support from lab laboratory work. Weekly dosages of only 16.6–50 ppb of oestradial benzoate led to the development of lesions and carcinomas of the cervix in a total of 25 out of 44 mice belonging to two strains with a low susceptibility to mammary cancer (Allen and Gardner, 1941). The levels of oestrogen administered are comparable to those reported earlier as naturally occurring by Shotwell and her associates.

Ten years after the first statement of the mycotoxin hypothesis, there is now substantial evidence available that satisfies all four of Oettlé's original postulates. This is a dramatic advance, considering the short period of time, which has already given an added stimulus to the study of the role of other mycotoxins in the epidemiology of chronic disease.

Concluding remarks

The detailed and intensive work done so far on the action of mycotoxins has opened a field still containing great potential. The incrimination of mycotoxins as a health hazard to man and animals may in fact be only the first of several important discoveries. Further knowledge has, and is being, contributed in four important directions:—

(1) The ways in which DNA and RNA may be altered by mycotoxins, notably aflatoxin, should yield important information on cell biochemistry, and of the effects of change of the genetic material.

(2) The specificity of attack by many mycotoxins on particular organs of the body indicates something of these organs' peculiar vulnerabilities, and of their characteristic reactions under stress.

(3) The discovery of fungal oestrogens may have important gynaecological implications, comparable to the use of ergot in childbirth. It has already been suggested that zearalenone could be used in the manufacture of a birth-control pill.

(4) The synergistic interaction of topography, climate, human customs, other human pathological conditions and the availability of a particular mycotoxin has accelerated the development of an important new branch of epidemiological medicine that received its first impetus with Burkitt's discovery of the interaction between malaria and glandular fever resulting in the later development of Burkitt's Lymphoma. The field observations have been confirmed by a fascinating laboratory series of experiments illustrating various synergistic models which have also helped to develop fresh hypotheses.

The above notions provide another example of the range of opportunities in different disciplines brought about by scientific research in one field. To quote T. S. Eliot: 'All our knowledge brings us nearer to our ignorance'.

Appendices

Appendix 1

Mycotoxicosis with multiple aetiology: some of the main syndromes

Country	Author	Symptoms	Fungi responsible in feed
1 PIGS			
USA	Burnside *et al.* (1957)	Toxicosis, death	*Aspergillus flavus, Penicillium rubrum*
USA	Bailey & Groth (1959)	Toxicosis; feeding mouldy maize responsible to dogs reproduced 'Hepatitis X' syndrome	*Aspergillus flavus, Penicillium rubrum*
Russia	Zinchenko (1959)	Fever, fits, stomach distension	*Aspergillus flavus, Scopulariopsis brevicaulis*
Russia	Borisov & Mikhailyukova (1960)	Inflammation, haemorrhage in GIT. Most severe in gilts	*Aspergillus, Mucor, Penicillium* spp.
USA	Forgacs & Carll (1962)	Depression, anorexia, profuse haemorrhage	*Aspergillus flavus, Penicillium rubrum, P. purpurogenum*
Russia	Noskov & Sharapov (1965)	Yawning, thirst, various GIT symptoms, conjunctivitis	*Alternaria, Fusarium Trichothecium, Cladosporium, Mucor, Penicillium* spp.
France	Pelhâte (1966)	Ulcers of cardiac region	*Aspergillus flavus, Mucor circinelloides, Absidia lichtheimii, Aspergillus orchraceus*
Hungary	Doman (1967)	Weakness and diarrhoea	Not specifically named
Roumania	Negru *et al.* (1967)	Necrosis around snout, mouth, eyes	*Fusarium* spp., *Stachybotrys alternans*
USA	Blevins *et al.* (1969)	Bloody diarrhoea, abortion in sows and gilts	*Aspergillus* spp., *Penicillium rubrum, Rhizopus* spp.
2 CATTLE			
Russia	Bloshitsyn (1959)	Laminitis	Mouldy corn cobs gave *Alternaria, Fusarium Mucor, Penicillium, Rhizopus, Trichothecium*
USA	Sippel, Burnside & Atwood (1953)	Hepatitis, haemorrhage with acute/chronic systemic reaction	Mouldy soft corn, *Penicillium rugulosum,* other spp. unidentified
USA	Carll *et al.* (1955)	Hyperkeratosis	Feed concentrate gave *Aspergillus chevalieri, A. flavus, A. tamarii*
Russia	Meshkov (1961)	Sudden onset of fever, cardiac arrhythmia, diarrhoea, leucopenia, lymphocytosis, ulcers on mucus membranes	*Cladosporium, Fusarium* spp., *Mucor, Penicillium, Stachybotrys alternans, Trichoderma*
USA	Aust *et al.* (1963)	Haemorrhage, external, internal	Mouldy corn yielding *Aspergillus* spp.
Sweden	Hallgren *et al.* (1963)	Paretic/tetanic symptoms, and hypocalcaemia/ hypomagnesaemia	*Mucor, Penicillium* in infected hay
USA	Albright *et al.* (1964)	Death or moribund state with haemorrhagic syndrome; experimentally reproduced	Feed gave *Aspergillus flavus, Penicillium cyclopium, P. palitans*
Germany	Abadjieff *et al.* (1966)	Hyperaethesia, rapid/ laboured breathing, salivation, staggered gait, muscular spasms. PM revealed liver lesions	Malt sprouts yielded *Aspergillus clavatus, Mucor, Penicillium, Rhizopus oryzae*, yeast and bacteria
France	Pelhâte (1966)	Failure of digestive system, Haemorrhage, lesions of viscera	*Absidia lichtheimii, Aspergillus flavus, Hemispora stellata, Scopulariopsis brevicaulis, S. candida*
3 HORSES & DONKEYS			
USA	Schwarte *et al.* (1937) Schwarte (1938)	Necrosis, oedema, Haemorrhagic lesions, degeneration and death	Mouldy corn — no specific fungi isolated
USA	Biester *et al.* (1940)	Toxicosis and death: leukoencephalomalacia	Cornstalks + husks; disease reproduced experimentally with mouldy corn. Chief sp. *Gibberella zeae*

Appendix 1 (*continued*)

Mycotoxicosis with multiple aetiology: some of the main syndromes (*continued*)

Country	Author	Symptoms	Fungi responsible in feed
England	Morgan (1940)	Broken wind in horses, abortion in cows and mares	Mouldy hay
Germany	Zeller (1955)	Colic, sometimes with nervous and allergic disorders	Hay heavily contaminated with *Mucor, Aspergillus, Penicillium*
China	Iwanoff, Chang-Kuo & Shih-Chieh (1957)	Acute encephalitis	Mouldy hay
4 DOGS			
Germany	Schultze, Motz & Schafer (1965)	Toxicosis	Oatflakes yielded *Aspergillus flavus, Penicillium meleagrinum, Rhizopus oryzae*
5 BIRDS			
France	Pelhâte (1966)	Haemorrhagic syndrome as for pigs, particularly in chickens	Wheat infected by *Absidia lichtheimii, Aspergillus flavus, Gliocladium roseum, Mucor circinelloides*
South Africa	Gouws (1965)	as above	Grain infected by *Alternaria tenuis, Cladosporium fagi, C. epiphyllum*
USA	Forgacs & Carll (1962)	Depression, diarrhoea and haemorrhage	*Alternaria, Aspergillus clavatus, A. flavus, A. fumigatus, A. glaucus, Paecilomyces variotii, Pencillium citrinum, P. purpurogenum, P. rubrum, Scopulariopsis*

Appendix 2

Mycotoxicosis caused by aflatoxin, primarily involving the liver

Country	Author	Animal	Symptoms	Material responsible
England	Blount (1961)	Turkeys	Toxicosis and death	Brazilian groundnut meal
England	Loosmore & Harding (1961)	Pigs	Toxicosis	Groundnuts with aflatoxin
USA	Clegg & Bryson (1962)	Cattle	Toxicosis	*Aspergillus flavus* in feed
England	Loosmore *et al.* (1964)	Cows	Toxicosis	Aflatoxin in imported cottonseed cake
South Africa	Minne *et al.* (1964)	Pigs, goats	Death	Mouldy groundnuts with *A. flavus*
India	Sastry *et al.* (1965)	Murrah Buffalo	Toxicosis death	Groundnut cake
Madagascar	Raynaud (1963)	Pigs	Acute toxicosis and death; subacute syndrome with liver and kidney lesions; hepatoma	Groundnut cake
Germany	Schultz *et al.* (1966)	Heifer	Fever, circulatory insufficiency, acute lung oedema, etc., leading to death	Malt seeds infested with *A. flavus*
Latvia	Astrakhantsev (1967)	Mink	Death and fatty dystrophy of liver	Groundnut meal with aflatoxin demonstrated
USA	Wilson *et al.* (1967)	Pigs, cattle	Hepatitis	Feeds with aflatoxin and/or *A. flavus*
India	Gopal *et al.* (1968)	Cattle	Toxicosis and death	Groundnut cake + cotton-seed with aflatoxin
Australia	Connole & Hill (1970)	Sows	Abortion	Sorghum grain with *A. flavus*
Denmark	Krogh & Hasselager (1970)	Pigs	Liver damage and toxicosis	Imported feeds with aflatoxin
India	Amla *et al.* (1970, 1971)	Man	Liver cirrhosis	Millet containing aflatoxin
Germany	Bosenberg (1972)	Man	Acute toxicosis	Not known

Appendix 3

Disease syndromes associated with *Fusarium graminearum*

Place	Author	Animal	Symptoms
Iowa, USA	Buxton (1927)	Pigs	Hyperoestrogenism (swelling, reddening and oedema/necrosis of vulva)
Iowa, ISA	Legenhausen (1928)	Pigs	Hyperoestrogenism
Minn. Iowa Ind. Ill. USA	McNutt *et al.* (1928)	Pigs	Hyperoestrogenism
Minn. USA	Christensen & Kernkamp (1936)	Pigs	Vomiting, toxicity, death
Victoria, Australia	Pullar & Lerew (1937)	Pigs	Vomiting, toxicity, death
Iowa, USA	Koen & Smith (1945)	Pigs	Vomiting toxicity, death
Ireland	McErlean (1952)	Pigs	Vomiting toxicity, death
Ind. USA	Stob *et al.* (1962)	Pigs	Vomiting, toxicity, death
Russia	Kyurtov (1962)	Pigs	Vomiting, diarrhoea, death, abortion in ewes
Italy	Paita (1962)	Pigs	Hyperoestrogenism
Yugoslavia	Stamatovic *et al.* (1963)	Pigs	Hyperoestrogenism
Kirov, USSR	Demakov (1964)	Cattle	GIT disturbances, arrhythmia, restlessness
Minn. USA	Christensen, Nelson & Mirocha (1965)	Pigs	Hyperoestrogenism
Rumania	Mitroiu *et al.* (1966)	Cows	Toxicosis
Rumania	Bugeac & Berbinschi (1967)	Pigs	Hyperoestrogenism
Denmark	Eriksen (1968)	Pigs	Hyperoestrogenism
Minn. USA	Mirocha, Christensen & Nelson (1968b)	Cattle	Hyperoestrogenism
England	Mirocha *et al.* (1968)	Cattle	Hyperoestrogenism
Japan	Kurata *et al.* (1968a)	Humans	Toxicosis
Hungary	Danko & Aldasy (1969)	Pigs	Hyperoestrogenism
Rumania	Jivoin *et al.* (1970)	Horses	Loss of appetite, fever, haematological changes
Yugoslavia	Ozegovic (1970) Ozegovic & Vukovic (1971)	Pigs	Hyperoestrogenism
Finland	Roine, Korpinen & Kallela (1971)	Cows	Hyperoestrogenism
Russia	Boltushkin *et al.* (1971)	Cattle	Loss of reflexes, vision, staggering and paresis
Canada	Bristol & Djurickovic (1971)	Pigs	Hyperoestrogenism
Minn. USA	Meronuck *et al.* (1970)	Poultry	Swollen vents, prolapsed cloacae, enlarged bursae
Hungary	Debreczeni & Borda (1972)	Pigs	Death

Appendix 4

Disease syndromes associated with *Fusarium sporotrichioides*

Place	Author	Animal	Symptoms
Orenburg USSR	Mayer (1953)	Humans, Cattle	'Scalding' of mouth and stomach, destruction of bone marrow, haemorrhage, necrosis and drop in leucocyte count
Russia	Loginov (1958)	Pigs	Pyrexia, inflammation of mucous membranes of nose and throat, swelling of inflammation of lymph nodes, inflammation of lungs and intestines
Russia	Marchenko & Renyanskaya (1959)	Pigs	General inflammation, oedema of eyelids, neck and jaw, dyspnoea; several deaths
Western Ukraine	Izmailov *et al.* (1961)	Cattle	Mass disease with high rate of death — loss of appetite, drop in milk yield, nasal discharge, salivation, high temperature
Siberia USSR	Anonymous (1964b)	Humans	Premature generalized osteoarthritis (circumstantial evidence only)
Russia	Kalmykov *et al.* (1967)	Sheep	GIT disorders, functional disturbance of CNS and cardiovascular systems — abortion in ewes, loss of hair, death

Appendix 5

Mycotoxicosis attributable to *Stachybotrys alternans*

Country	Author	Animal	Symptoms
Ukraine	Drobotko (1946)	Horses + Humans	Toxicosis
Russia	Fortushnyi *et al.* (1959)	Cattle	Toxicosis and death
Russia	Matusevich (1961)	Cows	Toxicosis and death
Russia	Zaichenko (1961)	Cattle, Cows + calves	Toxicosis and death
Russia	Levenberg *et al.* (1961)	Cows + calves	Haemorrhagic inflammation of GIT
Ukraine	Izmailov & Moroshkin (1962)	Cattle	Toxicosis and death
Russia	Koshevoi (1962)	Pigs	Toxicosis and death
Russia	Matusevich *et al.* (1962)	Cattle	Toxicosis and death
Russia	Noskov *et al.* (1966) Noskov & Ogryzkov (1967)	Cows	Toxicosis and death
Russia	Avrorov & Mikhailyukov (1967)	Cattle	Toxicosis and death
Russia	Vachev *et al.* (1970)	Sows	Abortion
Yugoslavia	Ozegovic, Pavlovic & Milosev (1971)	Cattle	Toxicosis
Hungary	Danko (1974)	Young Cattle	Toxicosis and death

Appendix 6

Mycotoxicosis caused by rusts and smuts

Country	Author	Organism	Animal	Symptoms
England	Greig (1924)	*Tilletia tritici*	Dog	Epileptiform convulsions and acute cerebral meningitis
England	Dobson (1926) (experimental)	*Tilletia tritici*	Chickens	Lesions on comb, wattles and mucous membrane
			Dogs	No effect
USA	Moore, Russell & Sachs (1946)	*Ustilago zeae*	Humans	Toxicosis
Rumania	Lapcevic, Pribcevic & Kozic (1953)	*Puccinia graminis*	Horses	Fatal intoxication, salivation and stomatitis
Russia	Oksamitnyi & Vlasov (1958)	'Smut'	Pigs	Conjunctivitis, irritation of upper respiratory tract, oedema of lungs
Egypt	Shalash & Moursi (1962) (experimental)	*Puccinia* sp.	Buffalo Sheep rats	Stimulation of uterine muscles
Russia	Malyavin (1963)	*Ustilago avenae*	Cattle	Mass poisoning, salivation, dilation of pupils, coughing, loss of appetite, weakening, paresis of rumen and dysentery
Russia	Ubragimov (1965)	*Ustilago hordei*	Pigs	Conjunctivitis, icterus of mucous membranes + skin, pulmonary oedema (acute form) encephalitis (chronic form) blood alteration
Russia	Ibraginov (1968) (experimental)	*Ustilago hordei*	Cattle	Toxicosis as above
Russia	Ibraginov (1970)	*Tilletia laevia*	Mice	Toxicity and death
			Rats	No effect
		Ustilago hordei	Rats	No effect

Appendix 7

Other toxigenic fungi

Species tested	Toxic strains out of number tested		Total
	Scott (1965)	Martin *et al.* (1971)	
Alternaria tenuis	–	2/4	2/4
Aspergillus			
alliaceus	0/1	–	0/1
amstelodami	0/6	–	0/6
**auricomus*	–	2/2	2/2
**avenaceus*	1/1	–	1/1
**biplanus*	–	1/1	1/1
candidus	0/4	3/6	3/10
**carneus*	2/4	–	2/4
chevalieri	2/6	–	2/6
clavatus	2/2	1/2	3/4
**echinosporus*	–	1/1	1/1
**effusus*	0/3	–	0/3
**flavipes*	3/3	–	3/3
flavus	6/10	12/15	18/25
fumigatus	2/3	–	2/3
**granulatus*	–	1/1	1/1
**mangini*	1/2	–	1/2
**melleus*	–	0/1	0/1
nidulans	3/5	–	3/5
niger	0/10	9/19	9/29
**niveus*	1/1	–	1/1
ochraceus	3/5	5/10	8/15
**oryzae*	0/2	–	0/2
**repens*	0/3	9/19	9/22
**restrictus*	0/2	–	0/2
**ruber*	0/2	13/26	13/28
**sydowi*	0/3	–	0/3
**tamarii*	0/6	1/5	1/11
**terreus*	0/5	1/1	1/6
**ustus*	0/3	1/1	1/4
versicolor	0/5	–	0/5
wentii	0/5	9/16	9/21
**Botryodiplodia theobromae*	–	0/1	0/1
Cladosporium			
cladosporioides	–	3/6	3/6
sphaerospermum	–	6/16	6/16
Curvularia sp.	–	1/3	1/3
Diplodia maydis	–	0/2	0/2
Fusarium			
equiseti	–	25/39	25/39
moniliforme (oxysporum)	2/10	32/63	34/73
graminearum (roseum)	2/2	–	2/2
sporotrichioides	0/3	–	0/3
**Gliocladium*			
**catenatulatum*	–	3/3	3/3
**roseum*	–	0/2	0/2
Macrophoma sorghicola	–	2/5	2/5
Nigrospora oryzae	–	8/17	8/17
Paecilomyces varioti	2/5	–	2/5
Penicillium			
**aculeatum*	0/2	–	0/2
**brevicompactum*	0/5	–	0/5
**charlesii*	0/4	6/11	6/15
**chrysogenum*	0/3	–	0/3
citrinum	0/3	4/6	4/9
crustosum	–	7/17	7/17
cyclopium	0/5	6/21	6/26
expansum	0/4	–	0/4
frequentans	0/3	7/16	7/19
**funiculosum*	0/5	–	0/5
**herquei*	0/5	–	0/5
**implicatum*	0/2	1/3	1/5
islandicum	1/3	1/2	2/5
**janthinellum*	0/3	–	0/3
**jenseni*	–	1/1	1/1
**meleagrinum*	–	5/19	5/19
**multicolor*	0/2	–	0/2
**nigricans*	0/3	–	0/3
**notatum*	0/5	–	0/5
**oxalicum*	5/5	–	5/5
**piceum*	1/1	–	1/1

Footnote: *Species not discussed in the text.

Appendix 7 (*continued*)

Other toxigenic fungi (*continued*)

Species tested	Toxic strains out of number tested		Total
	Scott (1965)	Martin *et al.* (1971)	
**pulvillorum*	0/3	–	0/3
purpurogenum	0/5	1/8	1/13
**raistrickii*	0/3	–	0/3
**roseopurpureum*	–	0/1	0/1
rubrum	2/2	6/9	8/11
rugulosum	–	9/14	9/14
**simplicissimum*	0/3	–	0/3
**steckii*	0/5	–	0/5
thomii	0/1	–	0/1
urticae	2/2	–	2/2
variabile	1/5	3/8	4/13
viridicatum	0/5	4/13	4/18
Rhizopus			
arrhizus	–	7/15	7/15
stolonifer	–	11/38	11/38
Scopulariopsis brevicaulis	–	1/3	1/3
Trichoderma viride	0/5	4/10	4/15
Trichothecium roseum	1/3	2/5	3/8

Footnote: *Species not discussed in the text.

Appendix 8

Summary of the chief mycotoxins and their causal agents

Toxin	Species responsible
Agalactic toxin	Claviceps fusiformis
Allergenic toxin(s)	*Alternaria, Aspergillus, Candida albicans, Cladosporium, Fusarium, Helminthosporium, Mucor, Penicillium, Puccinia, Ustilago, Verticillium*
Aflatoxin	*Aspergillus flavus, A. niger, A. ostianus, A. parasiticus, A. ruber, A. wentii, Penicillium citrinum, P. frequentans, P. puberulum, P. variabile*
Ascladiol	*Aspergillus clavatus*
Aspergillic acid	*Aspergillus flavus*
Aspergillus flavus endotoxin	*Aspergillus flavus*
Aspergillus fumigatus endotoxin	*Aspergillus fumigatus*
Aspertoxin	*Aspergillus flavus*
*Chaetocin	*Chaetomium minutum*
*Chaetomin	*Chaetomium cochlioides, C. globosum*
Chlorine containing peptide	*Penicillium islandicum*
Citreoviridin	*Pencillium citreoviride*
Citrinin	*Aspergillus candidus, A. clavatus, Penicillium citrinum*
Clavacin	See under patulin
Cyclopiazonic acid	*Penicillium cyclopium*
Dendrodochiotoxin	*Dendrodochium toxicum*
Diacetoscirpenol (T_2 toxin)	*Fusarium equiseti, F. nivale, F. solani, F. tricinctum*
Diplodiatoxin	*Diplodia zeae*
Emetic toxin	*Fusarium culmorum, F. graminearum, F. moniliforme, F. nivale, F. poae*
Ergotamine	*Claviceps purpurea*
F_2 toxin	See zearalenone
F_3 toxin	*Fusarium graminearum*
Fumagillin	*Aspergillus fumigatus*
Fumigatin	*Aspergillus fumigatus*
Fusarenon – X	*Fusarium nivale*
*Gliotoxin	*Aspergillus fumigatus, Gliocladium fimbriatum, Penicillium* spp., *Trichoderma viride*
Haemorrhagic toxin	*Alternaria tenuis, Aspergillus chevalieri, A. clavatus, Alternaria, Cladosporium epiphyllum, C. fagi*
Islanditoxin	*Pencillium islandicum*
Kojic acid	*Aspergillus flavus, A. parasiticus, Aspergillus* spp.
Leucogenol	*Penicillium gilmanii*
Luteoskyrin	*Penicillium islandicum*
Lysergic acid derivative	*Claviceps paspali, Claviceps purpurea*†
Moniliformin	*Fusarium moniliforme*
Myrothecin	*Myrothecium roridum, M. verrucaria*
Nephrotoxin, endomycelial	*Aspergillus flavus*
Nidulotoxin	*Aspergillus nidulans*
β-nitropropronic acid	*Aspergillus flavus*
Nivalenol	*Fusarium nivale*
Ochratoxin	*Aspergillus alliaceus, A. melleus, A. ochraceus, A. ostianus, A. sclerotiorum, A. sulphureus, Penicillium viridicatum*
Oxalic acid	*Aspergillus flavus*
Parasiticol (Aflatoxin B_3)	*Aspergillus parasiticus, Rhizopus arrhizus, Rh. oryzae, Rh. stolonifer*
Patulin	*Aspergillus clavatus, A. giganteus, A. terreus, Byssochlamys nivea, Penicillium claviforme, P. expansum, P. urticae*
Penicillic acid	*Aspergillus alliaceus, A. melleus, A. ochraceus, A. ostianus, A. sclerotiorum, A. sulphureus, Penicillium baarnense, P. cyclopium, P. madriti, P. martensii, P. palitans, P. puberulum, P. stoloniferum, P. suaveolens, P. thomii*
Polyuric toxin	*Rhizopus stolonifer*
Rubratoxin	*Penicillium purpurogenum, P. rubrum*

Footnote: *Refer to Taylor, 1971. †Refer to Fuller, 1968.

Appendix 8 (*continued*)

Summary of the chief mycotoxins and their causal agents (*continued*)

Toxin	Species responsible
Rugulosin	*Endothia parasitica, E. wortmanni, P. rugulosum, P. tardum, P. variabile, P. wortmanni*
Slaframine	*Rhizoctonia leguminicola*
Sporidesmin	*Periconia minutissima, Pithomyces chartarum*
Sporotrichin	*Fusarium poae, F. sporotrichioides*
Stachybotryotoxin	*Stachybotrys alternans*
Sterigmatocystin	*Aspergillus flavus, A. nidulans, A. rugulosus, A. versicolor, Bipolaris sorokiniana, Penicillium luteum*
T_1 toxin	*Fusarium nivale, F. tricinctum*
T_2 toxin	See diacetoscirpenol
Tenuazonic acid	*Alternaria longipes*
?Thiaminase	*Acrospeira macrosporoides*
Tremorgen	*Aspergillus flavus, Penicillium crustosum, P. cyclopium, P. granulatum, P. martensii, P. olivinoviride, P. palitans, P. puberulum*
Trichothecin	*Trichothecium roseum*
*Verticillin A	*Verticillium* ap.
Viridicatin	*Penicillium palitans, P. viridicatum*
Zearalenone (F_2 toxin)	*Fusarium graminearum (Giberella zeae), F. culmorum, F. equiseti, F. moniliforme, F. tricinctum, Nectria radicicola*

Footnote: *Refer to Taylor 1971.

Appendix 9

Summary of the chief toxigenic fungi whose metabolites have been named and/or chemically determined

Species	Toxin
Acrospeira macrosporoides	Agalactic toxin
Alternaria spp.	Allergenic toxin, haemorrhagic toxin
Alternaria longipes	Tenuazonic acid
Alternaria tenuis	Haemorrhagic toxin
Aspergillus spp.	Allergenic toxin, Kojic acid
alliaceus	Ochratoxin, penicillic acid
candidus	Citrinin
chevalieri	Haemorrhagic toxin
clavatus	Ascladiol, citrinin, haemorrhagic toxin, patulin
flavus	Aflatoxin, aspergillic acid, *A. flavus* endomycelial nephrotoxin, aspertoxin, Kojic acid, β-nitropropionic acid, oxalic acid, sterigmatocystin, tremorgen
fumigatus	A. fumigatus endotoxin, fumagillin, fumigatin, gliotoxin
giganteus	Patulin
melleus	Ochratoxin, penicillic acid
nidulans	Nidulotoxin, sterigmatocystin
niger	Aflatoxin
ochraceus	Ochratoxin, penicillic acid
ostianus	Aflatoxin, ochratoxin, penicillic acid
parasiticus	Aflatoxin, Kojic acid, parasiticol
quercinus	Penicillic acid
ruber	Aflatoxin
rugulosus	Sterigmatocystin
sclerotiorum	Ochratoxin, penicillic acid
sulphureus	Ochratoxin, penicillic acid
terreus	Patulin
versicolor	Sterigmatocystin
wentii	Aflatoxin
Bipolaris sorokiniana	Sterigmatocystin
Byssochlamys nivea	Patulin
Candida albicans	Allergenic toxin, endotoxin
Chaetomium cochlioides	Chaetomin
Chaetomium globosum	Chaetomin
Chaetomium minutum	Chaetocin
Cladosporium	Allergenic toxin
Cladosporium epiphyllum	Haemorrhagic toxin
Cladosporium fagi	Haemorrhagic toxin
Claviceps paspali	Lysergic acid derivative
Claviceps purpurea	Ergotamine, lysergic acid derivative
Dendrodochium toxicum	Dendrodochium toxicum
Diplodia zeae	Diplodiatoxin
Endothia parasitica	Rugulosin
Endothia wortmanni	Rugulosin
Fusarium spp.	Allergenic toxin
culmorum	Emetic toxin, zearalenone
equiseti	Diacetoscirpenol, zearalenone
graminearum (Gibberella zeae)	Emetic toxin, F_3 toxin, zearalenone
moniliforme	Emetic toxin, moniliformin, zearalenone
nivale	Diacetoscirpenol, emetic toxin, fusarenon X, nivalenol, T_1-toxin
poae	Emetic toxin, sporotrichin
solani	Diacetoscirpenol

Appendix 9 (*continued*)

Summary of the chief toxigenic fungi whose metabolites have been named and/or chemically determined (*continued*)

Species	Toxin
Fusarium spp.—*contd.*	
sporotrichioides	Sporotrichin
tricinctum	Diacetoscirpenol, T_1 toxin, zearalenone
Gliocladium fimbriatum	Gliotoxin
Helminthosporium	Allergenic toxin
Mucor spp.	Allergenic toxin
Myrothecium roridum	Myrothecin
Myrothecium verrucaria	Myrothecin
Nectria radicicola	Zearalenone
Penicillium spp.	Allergenic toxin, gliotoxin
baarnense	Penicillic acid
citreoviride	Citreoviridin
citrinum	Aflatoxin, citrinin
claviforme	Patulin
crustosum	Tremorgen
cyclopium	Cyclopiazonic acid, penicillic acid
expansum	Patulin
frequentans	Aflatoxin
gilmanii	Leucogenol
granulatum	Tremorgen
islandicum	Chlorine containing peptide, luteoskyrin, islanditoxin
luteum	Sterigmatocystin
madriti	Penicillic acid
martensii	Penicillic acid, tremorgen
olivinoviride	Tremorgen
palitans	Penicillic acid, tremorgen, viridicatin
puberulum	Aflatoxin, penicillic acid, tremorgen
purpurogenum	Rubratoxin
rubrum	Rubratoxin
rugulosum	Rugulosin
stoloniferum	Penicillic acid
suaveolens	Penicillic acid
tardum	Rugulosin
thomii	Penicillic acid
urticae	Patulin
variabile	Aflatoxin, rugulosin
viridicatum	Ochratoxin, viridicatin
wortmanni	Rugulosin
Periconia minutissima	Sporidesmin
Pithomyces chartarum	Sporidesmin
Rhizoctonia leguminicola	Slaframine
Rhizopus arrhizus	Parasiticol (Aflatoxin B_3)
Rhizopus oryzae	Parasiticol
Rhizopus stolonifer	Parasiticol, polyuric toxin
Stachybotrys alternans	Stachybotryotoxin
Trichoderma viride	Gliotoxin
Trichothecium roseum	Trichothecin
Ustilago spp.	Allergenic toxin
Verticillium spp.	Allergenic toxin

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Printed in England for Her Majesty's Stationery Office by Hobbs the Printers Ltd., Southampton
(470) Dd113827 3M 7/76 G3313