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Fungi and Mycotoxins in Stored Products

Proceedings of an international conference held at Bangkok, Thailand, 23–26 April 1991

Editors: **B.R. Champ, E. Highley, A.D. Hocking, and J.I. Pitt**

Sponsors:
Group for Assistance on Systems relating to Grain After-harvest (GASGA)
Australian Centre for International Research (ACIAR)
Department of Agriculture, Thailand

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Address of Welcome

IT is a great honour and pleasure for me to welcome you, on behalf of the Department of Agriculture, to this International Conference on Fungi and Mycotoxins in Stored Products.

I welcome particularly Dr Yookti Sarikahputi, the Permanent Secretary of the Ministry of Agriculture and Cooperatives, and our joint sponsors, GASGA and ACIAR, and their respective heads, Mr Weber and Dr Rothschild. Equally, I welcome all of you who have gathered here this week because of your concern to better understand and to see positive action to solve a major problem threatening the well-being of humanity.

The storage of grain and other staple food- and feedstuffs free from contamination by moulds is of the utmost importance to the consumers of all nations. It has extra importance to those nations which earn significant income from the export of agricultural commodities. Thailand, which is currently the world's largest rice exporter, is of course such a nation.

Most of us already know something of the serious problems represented by stored products fungi to human and animal health, and to the economies of countries with strong agricultural sectors. The program for the next four days indicates that we will all know very much more by the end of the conference.

We have been able to assemble a formidable array of invited speakers on all aspects of the problem, and the large number of poster papers will give us extra insight into activities and progress in this field in the Asian region.

The strength of the program has ensured that we have a large number of participants drawn from countries throughout the world. They come from not only academic institutions, research organisations, and government agencies, but also milling and trading groups in a number of ASEAN countries. That we have representatives of the private sector here is gratifying because it indicates that they see the potential for innovation and progress possible by adoption of the results of research and development work.

I am pleased to note too the strong presence of personnel from my own Department. Their presentations will give you a clear picture of our fungi and mycotoxin survey and research activities. They in turn will, I am sure, gain much from discussions with specialists from around the world.

Ladies and gentlemen, though you have a very full conference program this week, I hope that you will be able to find some time to sample some of Thailand's unique culture and food, and to avail yourselves of the splendid shopping facilities of our capital.

Welcome to Thailand. I hope your stay will be enjoyable, and the conference stimulating and productive for you. Thank you.

Mr Somrit Chaiwanakupt
Acting Director General
Department of Agriculture
Ministry of Agriculture and Cooperatives
Thailand

Objectives of Conference

LADIES and gentlemen, it is a pleasure for me, on behalf of the Group for Assistance on Systems relating to Grain After-harvest, to outline the objectives of this conference to you before you begin what I am sure will be four extremely useful and stimulating days of formal and informal discussion.

GASGA — the Group for Assistance on Systems relating to Grain After-harvest — is a voluntary association of organisations primarily linked with donor operations. Currently, GASGA has the following seven members:

- Australian Centre for International Agricultural Research, Canberra, Australia (ACIAR)
- Centre de Cooperation International en Recherche Agronomique pour le Developpement, Montpellier, France (CEEMAT/CIRAD)
- Deutsche Gesellschaft für Technische Zusammenarbeit, Eschborn, Federal Republic of Germany (GTZ)
- Food and Agriculture Organization of the United Nations, Rome, Italy (FAO)
- Food and Feed Grain Institute, Kansas State University, Manhattan, Kansas, USA (KSU)
- International Development Research Centre, Ottawa, Canada (IDRC)
- Overseas Development Administration, Natural Resources Institute, Chatham, England (NRI)

These organisations, of which four are represented at this meeting, all have major involvement in most, if not all, of the following activities:

- the provision of professional advice;
- the conduct of field projects;
- the training of developing country personnel; and
- the conduct of research and its application in relation to the problems of the postharvest sector of the production of grain and other major food commodities in developing countries.

GASGA's central objectives are:

- to stimulate technical cooperation in all aspects of grains postharvest research and development in both developing and developed countries:
- to identify and seek ways of meeting R&D, training, and information needs in the postharvest subsector; and
- to serve in an advisory role and provide a forum for exchange of technical information, experience, and ideas.

In seeking to fulfill the last-mentioned of these objectives, GASGA sponsors and organises workshops, conferences, and other types of meetings to bring together specialists and others with common interest in a particular topic. It is thus pleased to cosponsor, with the Department of Agriculture, Thailand, and ACIAR, this International Conference on Fungi and Mycotoxins in Stored Products.

As I am sure you all know — indeed, it is no doubt why you are here — the topic is an important one. It is an issue on which there has been growing concern over the past few years as more is learned and understood about the serious implications of fungal infection of grains for both human health and the economics of grain and livestock production.

This conference has been at least two years in the planning and, during that period, we have thought hard and long about what topics the meeting should cover and who should be invited to speak. As it turns out, we have been able to arrange something approaching the best of all possible worlds regarding speakers: we have been able enlist not only key international specialists, but also, through a strong program of poster presentations, those working on most aspects of the fungi and mycotoxin problem in the Asian region, which is, of course, the prime target area for our meeting. Whether or not our planning has been fully successful only time will tell but, looking at the program and participants, I have every confidence that it will be.

However, to return to the detailed objectives of our meeting. These are:

1. To provide for participants an up-to-date presentation, discussion, and synthesis of what is currently known on the problems caused by fungi and mycotoxins in stored products.
2. To define and clarify the major problems, their context, and significance for human health and nutrition.
3. To provide some preliminary assessment of the economic implications of the fungi and mycotoxin problem in terms of lost food and reduced animal production.
4. To identify those research areas with potential to yield highest returns.
5. To formulate recommendations for concrete action both in R&D, and in activities and adjustments in the processing–handling–marketing system.
6. To encourage and facilitate professional contacts between researchers in this field.
7. To disseminate, through the published proceedings of the conference, a synthesis of the information gathered and discussed during the meeting.

Ladies and gentlemen, while these are wide-ranging objectives, I am sure that we will all be working hard to achieve them over the next few days. I am personally looking forward to a lively and productive meeting, and I take this opportunity to thank in advance all those who will be making presentations. Thank you.

Mr E.J. Weber
Executive Chairman
GASGA

Collaborative Research and Development

LADIES and gentlemen, I would like to begin by thanking the organisers of this conference for the opportunity to attend and to say something on what I see as the very important benefits that flow from a collaborative approach to research in agriculture, in particular those areas we are targeting in this meeting. These thanks go in particular to Dr Yookti Sarikahputi, Permanent Secretary of the Ministry of Agriculture and Cooperatives, and Mr Somrit Chaiwanakupt, Acting Director General of the Department of Agriculture, Thailand, and to Mr Ed Weber, Executive Chairman of the Group for Assistance on Systems relating to Grain After-harvest (GASGA).

There can be no question about the importance of the topic of this conference: infection of food and feedstuffs by fungi and their toxic metabolites is clearly a serious problem with both health and economic impacts. However, it would be wrong to try to treat each of these two facets of the problem in isolation, because health itself — or more correctly poor health or illness brought on by consumption of contaminated food — has social costs that may impact on economies in no small way. This is an issue that authorities and planners in many developed countries are only just beginning to acknowledge.

Work on fungi and mycotoxins can thus be justified through consideration of a whole spectrum of effects, from the more obvious impacts of losses or downgrading of commodities, through to broad public health implications. International trade in food and feedstuffs creates yet another dimension to the problem. In some respects, indeed, it creates a 'solution' through regulation in international trade of permissible amounts of contaminants. This 'solution' fails, however, to consider the impact of mycotoxins in domestic markets, where poor quality and hence low-priced commodities are often the only foodstuffs that can be purchased by the urban or rural poor, who may thus bear the brunt of health problems caused by mycotoxins.

The occurrence of fungi and mycotoxins in stored products thus creates something of a complex of problems that needs to be approached on a broad front in a collaborative spirit. This is an approach fostered by GASGA and FAO among others.

As far as research is concerned, ACIAR is committed to supporting partnerships and other linkages between groups in Australia and other countries in our region. A project of the Centre surveying the extent of the mycotoxin problem in the Asian region currently involves research teams in Australia, Indonesia, the Philippines, and Thailand. An important component of this and other ACIAR projects is technical training. Wherever possible, ACIAR projects seek to transfer skills and experience that will contribute to future self-sufficiency in research groups.

ACIAR is also supporting production of the quarterly *Australian Mycotoxin Newsletter*. I should point out that the presence of 'Australian' in the title is to do with the source of the newsletter rather than its content, which seeks to provide a continuing overview of the world literature on mycotoxins.

Just as important as linkages between research groups, are those between research and industry, and in that regard it is gratifying to note the very broad representation of public and private sector groups at this conference. Linkages between the private and public sectors enhance the probability that research will be relevant to industry and the community, and that research results will be transferred to where they are needed. In summary then, I see the problems caused by fungi and mycotoxins in food and feedstuffs as being particularly amenable to solution by the collaborative mode favoured by ACIAR, and the Centre will continue to support research in this area. Australia has special competence in mycology which, freely shared with knowledge of specialists in other areas, will surely contribute to progress in overcoming all aspects of the problem.

Dr G.H.L. Rothschild
Director
ACIAR

Keynote Address

LET me begin by again welcoming, on behalf of the Ministry of Agriculture and Cooperatives of Thailand, all participants in this International Conference on Fungi and Mycotoxins in Stored Products.

There can be no doubt about the importance of the topic of this conference not only to Thailand but indeed to the region and the rest of the world, because fungi and the mycotoxins they produce are a global problem with serious health and economic implications. It is very appropriate that this conference has the Group for Assistance on Systems relating to Grain After-harvest as one of its sponsors. GASGA is a worldwide association of sponsors of technical assistance to developing countries in the grains sector, thus setting the scene for a truly global collaborative approach to the fungi and mycotoxins problem.

Mycotoxicosis, or poisoning by fungal toxins, is not a new phenomenon. Poisoning by ergot, the hyphae of *Claviceps purpurea*, a hard, thick-walled, blackish fungus that attacks particularly rye grain, is known to have occurred since at least the Middle Ages in Europe, though it was not until relatively recent times that the causative agent was identified.

Like ergotism, nearly all of the other major human mycotoxicoses have occurred in temperate or cool temperate zones of Europe, the USSR, or Japan. These include alimentary toxic aleukia, ochratoxicosis, and acute cardiac beriberi.

We can only guess at the number of human deaths so far caused by the ingestion of mycotoxins, but the figure must run to tens of millions. In addition, the impact of use of contaminated feedstuffs on animal productivity must also be substantial.

Although I have so far been talking about other parts of the world, I cannot, unfortunately, say that the problem has bypassed us in Asia. On the contrary, there is no cause for complacency here because survey work has shown the occurrence of the fungus *Aspergillus flavus* to be widespread throughout the tropics. *Aspergillus flavus* is a species best known for the production in food and feedstuffs of potent mycotoxins called aflatoxins and there is good evidence for believing that one of these toxins, aflatoxin B_1, is carcinogenic. Available evidence suggests it is confined almost entirely to peanuts and maize — with minor problems in other commodities in some areas. Peanuts and maize, however, are of great importance both as basic foodstuffs and animal feeds — and of course maize is much traded internationally.

The role of *Fusarium*, another fungal genus invading foodstuffs, remains uncertain in Southeast Asia, though in other warm climate regions of the world its toxins are believed to be important carcinogens.

Clearly then, the availability of clean, healthy food is at risk, but unfortunately there is still much basic survey and research work to be done to determine the true extent of fungi and myxotoxin problems, particularly in Asia. Progress in this regard is to some extent dependent on the availability of adequately trained personnel. This is currently being addressed in collaborative research programs, such as those sponsored by ACIAR, IDRC, and other GASGA members but it is as well to point out that there is, at the moment, a worldwide shortage of trained fungal taxonomists and mycotoxicologists. Unfortunately, despite having deleterious implications for health, fungi are not seen as a modern, 'high tech' research area, and there are no throngs of recruits to the field.

Food- and feed-borne fungi and mycotoxins impact not only on health, but also on trade and therefore on the economies of trading nations. For quite acceptable and understandable reasons, there is, in international trade, an increasing drive for 'pure' commodities, free of pesticide residues, insects, moulds, mycotoxins, or other contaminants. It is therefore essential for nations trading in grain and other staple commodities to implement systems and regulations to ensure that shipments are accepted on arrival at importing countries. The economic consequences of rejection of a shipment because of, say, unacceptable levels of mycotoxin contamination can be profound. Not only is that particular sale lost, or at least the grade and value of the commodity considerably reduced, but the exporting country's credentials as a supplier of high grade products may be permanently jeopardised.

The problems associated with international trade are more complex than they might appear at first sight, and involve more than simply 'cleaning up' the commodity. Many trading nations have enacted regulations to cover maximum levels of contaminants, such as mycotoxins, acceptable in foodstuffs. However, these levels vary and tend to fall as analytical sophistication increases and it becomes possible to detect smaller and smaller amounts of contaminants. The situation can thus arise where an exporting country ships grain that meets all of its own quality standards and regulations, but when the shipment reaches its destination it is rejected because the importing country's regulations are more stringent.

This situation represents a clear impediment to international trade in food commodities, and one which tends to work against the interests of the less developed trading nations using less sophisticated detection and analytical technologies.

Just as with pesticide usage, this is thus an area where there is a strong need for harmonisation between nations of approaches and legislation relating to acceptable levels of the common mycotoxins in foodstuffs. This is an issue that is already being addressed by international bodies such as the Food and Agriculture Organization (FAO) of the United Nations and various regional groupings, and which will be covered extensively in papers and discussion at the conference. The problems involved are not going to be easy to solve, but the fact is that they must be solved if real progress is to be made in reducing mortality due to mycotoxicoses on the one hand, and in freeing up international trade on the other.

An integral part of the issue of harmonisation of legislation — indeed it is probably an essential precondition — is agreement on appropriate standardised methodology for the identification of food spoilage fungi, and the detection and measurement of the mycotoxins they produce. Logical first steps to achieving this might be the establishment of collaborative monitoring programs in the region, organised and pursued under the aegis of appropriate international commissions and agencies. There is a need for establishment of at least one regional reference collection of fungi for identification and research purposes. The reference collection must be properly maintained and be able to supply reliable cultures to all groups working in this area. There is a need also for a collaborative analytical program in the mycotoxin area with ready availability of reference samples of the mycotoxins for analytical purposes as well as regular interchange of test samples.

My pleas for a collaborative approach to the problem presuppose adequate networking for contact among the various government authorities, institutions, and workers involved. The success of any moves towards harmonisation will depend on the strengths of the networks operating and the interchange of information and personnel. Strengthening of these networks is of prime importance and must be a top priority.

As I implied earlier, there is also a shortage of trained personnel in stored products mycology, so there is a need for training programs. Most of the specialist short courses offered at present are generally held in Europe and North America and are therefore relatively inaccessible to personnel in Southeast Asia. Perhaps international and regional bodies could focus on this. Another means of providing training in in collaborative research programs, such as those supported by ACIAR and I commend this as a useful approach. I am sure that training will also be a key issue discussed at this conference.

What I have wished to stress in the short time I have had today is the global nature of the problems to be addressed by this conference and the need for a truly collaborative approach to them. Looking at the papers to be presented, their authors, and the list of participants gives me great confidence that this conference will make a considerable contribution to progress in dealing with stored products fungal problems. I certainly hope so, because all producers, consumers, and food trading nations will derive great benefits from such progress.

I wish you a successful conference. The full program indicates that it will certainly be a very busy one and for the region a very fruitful one.

Dr Yookti Sarikahputi
Permanent Secretary
Ministry of Agriculture and Cooperatives
Thailand

Overview of the Fungi and Mycotoxin Problem

Significance of Fungi in Stored Products

J. I. Pitt and Ailsa D. Hocking*

Abstract

Although insects cause the more obvious damage, the role of fungi in the spoilage of stored products should not be underestimated. The primary reason that commodities must be dried and stored dry is to prevent the growth of xerophilic storage fungi. Such fungi cause very large losses in grains, nuts, and other commodities which have been improperly dried, or are stored under humid conditions without adequate protection. Fungi have a second, equally important, role in stored products: the production of mycotoxins.

The detection of spoilage and toxigenic fungi of significance in stored products requires the use of specialised media and techniques developed for the purpose by internationally collaborating food mycologists. This paper will discuss the methods and media currently being promoted.

Many earlier studies have suggested that very wide ranges of fungi are associated with particular commodities. However, the use of modern enumeration techniques has shown that most are present merely as contaminants, and that the range of fungi capable of invasion of a particular commodity, or genuinely associated with it, is quite narrow. Most fungi associated with a particular commodity are 'field' fungi, which invade grains or nuts before or at harvest. All have high water activity requirements, and cease growth soon after the crop is harvested. The majority play little role in spoilage and most are nontoxigenic. The principal exceptions are *Fusarium* and *Alternaria* species. After harvest and during drying, 'storage' fungi take over: however the distinctions are increasingly blurred, as some storage fungi, including *Aspergillus flavus* and related species, are now known to invade nuts and maize before harvest.

Significant spoilage fungi in the tropics are mostly related to those occurring in temperate zones. In the tropics, *Eurotium* species and other Aspergilli are dominant, with *Penicillium* species playing only a minor role. Significant mycotoxin producers in the tropics are relatively few. Growth of *Aspergillus flavus* and the consequent production of aflatoxins is the dominant problem. Among field fungi, *Alternaria* species are the most common. The toxigenicity of tropical *Fusarium* species remains to be elucidated.

INSECTS and fungi compete for the right to live on stored products before these products are consumed by humans and domestic animals. Insects cause the more obvious damage, but the role of fungi in the loss of stored products cannot be ignored. The fundamental reason why commodities must be stored dry is to prevent the growth of storage fungi. If commodities are stored incorrectly, i.e. in an improperly dried state, or under high humidities with inadequate protection, fungi will inevitably grow.

In general terms, fungal growth has two consequences: first, deterioration—in appearance, in quality for processing, and often in food value; and second, the production of mycotoxins.

This overview is concerned not with these considerations, which are covered by other papers in these proceedings, but with the conditions under which fungi grow, and methods for their detection in foods. Specifically, this paper addresses three aspects: first, the conditions governing growth of fungi in stored products; second, the detection and quantification of fungal growth in stored products; and third, in broad terms, the major fungi of significance in stored products.

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Factors Governing Growth of Fungi in Stored Products

Eight factors govern the growth of fungi in foods: (1) water activity; (2) hydrogen ion concentration; (3) temperature; (4) gas tensions, specifically of oxygen and carbon dioxide; (5) consistency, i.e. liquid or solid state; (6) nutrient status; (7) specific solute effects; and (8) preservatives (Pitt and Hocking 1985). In stored products, several of these factors are either fixed or stable. Stored products are solids, of more or less neutral pH, and of

adequate nutrient status for fungal growth. Compounds such as NaCl, which produce specific solute effects, are usually absent. The principal factors governing fungal growth are thus water activity, temperature, gas tension, and preservatives (including fungicides and fumigants). Consideration of each of these follows.

Water activity

Water activity (a_w) is a chemical concept, introduced to microbiologists by Scott (1957), who showed that a_w effectively quantified the relationship between moisture in foods and the ability of microorganisms to grow on them. Water activity is defined as a ratio:

$$a_w = p/p_o$$

where p is the partial pressure of water vapour in the test material and p_o is the saturation vapour pressure of pure water under the same conditions. Water activity is numerically equal to equilibrium relative humidity (ERH) expressed as a decimal. If a sample of food is held at constant temperature in a sealed enclosure until the water in the sample reaches equilibrium with the water vapour in the surrounding air, then

$$a_w \text{ (food)} = \text{ERH (air)}/100$$

For further information on a_w, its measurement, influence on fungal growth, and significance in foods, see Duckworth (1975), Pitt (1975), or Troller and Christian (1978).

In many practical situations, a_w is the dominant environmental factor controlling growth of fungi and hence determining the stability of stored products. A knowledge of fungal water relations will then enable prediction of storage life of commodities, and a knowledge of the water relations of mycotoxin production will assist in understanding the potential for mycotoxins to form.

Like all other organisms, fungi require water for growth, and the tolerance of low a_w by different classes of fungi is often sharply defined. Certain storage fungi which occur in dried foods have the ability to grow at lower a_w levels than any other organisms.

The degree of tolerance to low a_w is most simply expressed in terms of the minimum a_w at which fungal spore germination and hyphal growth can occur. Fungi able to grow at low a_w are termed xerophiles (literally 'dryness loving') : one operational definition of a xerophile is a fungus able to grow below 0.85 a_w under at least one set of environmental conditions (Pitt 1975). At the lower end of the scale, only a few xerophilic fungi are able to grow at or below 0.75a_w within 6 months (Pitt and Hocking 1985). Put another way, commodities stored at humidities between 75% and 85% ERH are susceptible to attack by xerophilic fungi within normal storage times, that is, a few months.

Temperature

Although temperature is a very important factor in the growth of fungi, the fact is that commodities are usually stored under conditions suitable for fungal growth. Temperatures below 20°C tend to favour cold-tolerant fungi, such as *Penicillium* and *Cladosporium*, while higher storage temperatures favour *Aspergillus* species. Under tropical conditions, stored products are more susceptible to *Aspergillus* species than other fungi, as many Aspergilli are favoured by the combination of low a_w and relatively high storage temperatures.

Gas tension

Both reduction in oxygen tension and increase in carbon dioxide concentrations can have profound effects on the growth of fungi. These factors are important in the storage of commodities, where such conditions are often generated primarily for the control of insects [see Hocking (1991) for a detailed discussion].

Preservatives

Generally, stored products are free of preservatives when destined for human consumption. Insecticides and fumigants may be present under some circumstances, but information about their effect on storage fungi is relatively meagre (Hocking 1991). Commodities for use as animal feeds may sometimes contain weak acid preservatives, such as propionates, usually sold as proprietary chemicals. In general, such preparations are barely cost effective. Maintaining low a_w is usually a more certain means of inhibiting fungal growth.

Combinations of factors

The various factors just discussed do not act independently, and indeed often are synergistic. If two or more factors act simultaneously, it may be feasible to store commodities for longer than would otherwise be expected. For stored products, the combination of low a_w, low temperature, reduced oxygen and/or increased carbon dioxide levels may profoundly influence storage life.

Under natural storage conditions, a_w is the dominant factor determining the storage life of commodities. 'Dry a product quickly and keep it dry' remains the most effective method for ensuring fungi do not invade stored products.

Methods for Mycological Examination of Foods

Methodology in food mycology originally developed from that used in food bacteriology and plant pathology, where the media and methods developed were suitable for bacteria and plant pathogenic fungi, which require high a_w for growth, but were quite unsuited to the xerophilic filamentous fungi important in stored products.

Improved methodology was needed. At a specialist workshop on 'Methods for the Mycological Examination of Foods' held in Boston, USA in 1984, new approaches to methodology were examined in detail, with a view to

agreement and eventual international standardisation. The proceedings of that workshop (King et al 1986) had a big impact. A second workshop, held in Baarn, The Netherlands in 1990 continued development of these ideas, especially by setting up collaborative studies to prove the value of the methods being advocated. An international working group, the International Commission on Food Mycology (ICFM), developed from that workshop. ICFM will continue the study of methods in food mycology, as well as the moves towards standardisation that will allow effective comparisons of studies carried out in different laboratories. The methods described here for the examination of stored products are taken from the recommendations of those workshops.

Sample preparation

Direct plating. For particulate foods, including stored products, direct rather than dilution plating of samples is considered to be effective. In direct plating, grains are placed on suitable agar plates after surface sterilisation in a 10% dilution of household chlorine bleach for 2 minutes (Andrews 1986). Surface sterilisation is essential to remove surface contaminants and allow development of fungi which are actually growing, or have already grown, in the grains. By such means it is possible to establish the real associations of particular fungi and commodities.

Protocol. The following is a suitable protocol for sample preparation and direct plating:

1. Surface sterilise a minimum of 50 food particles (grains, nuts, etc.) in 0.4% chlorine (10% dilution of commercial household bleach) for 2 minutes. Stir occasionally, preferably with forceps (see step 4).
2. Pour off the chlorine and discard.
3. Rinse once in sterile water. This is optional, as rinsing has not been shown to be necessary, but is usually carried out.
4. Using sterile forceps (usually sterilised by immersion in the chlorine, step 1), place 6–10 food pieces (depending on particle size) per plate on suitable agar.
5. Incubate upright for 5 days at 25°C. These are standard temperate zone conditions. Incubation in tropical areas may be at 30°C. Incubation time may vary with temperature and medium used.
6. Count numbers of infected grains and express as percentage invaded. Note that this result has no direct relationship to counts obtained by dilution plating, but is a more effective method for expressing contamination of particulate foods.
7. Preferably, attempt to identify the fungi growing on the grains and surrounding agar, either in situ or after isolation and examination on identification media.

Dilution plating. Dilution plating techniques, which are effective for enumerating bacteria, are still widely used for stored products. However, they select for surface contaminants rather than the fungi growing within the grains. The latter are not readily released by standard homogenising methods such as shaking or pummelling in a Stomacher. Use of a Stomacher and dilution plating should be reserved for powders and liquids, where direct plating cannot be applied effectively.

Media

As noted previously, media for fungal enumeration in foods originally developed from those used in food bacteriology or plant pathology. Such media were universally of high a_w, and thus suited to enumeration of bacteria or fungi associated with growing plants. They were of little value for detecting or enumerating the xerophilic fungi associated with stored products.

A major problem in the enumeration of fungi from stored products is the common presence of rapidly spreading fungi — species of *Mucor*, *Rhizopus*, and other Zygo-mycetes — which quickly overgrow Petri dishes, rendering counting and isolation of important fungi difficult or impossible. An approach to this problem, often used in the past, was to employ a weak medium such as Potato Dextrose Agar (PDA), which limited growth of such fungi. However, PDA is simply not suited to the growth of storage fungi.

Dichloran rose bengal chloramphenicol agar

A breakthrough in medium formulation came with the introduction of dichloran as a means of inhibiting the growth of spreading fungi. Dichloran rose bengal chloramphenicol agar (DRBC; King et al. 1979) is an effective medium for enumeration and isolation of fungi from foods. It has a high a_w, but sufficient nutrient to permit growth of many normal storage fungi, including *Penicillium* species, most *Aspergillus* species, and common genera associated with developing grains, such as *Fusarium*, *Alternaria*, and *Curvularia*. It is recommended by ICFM as a general-purpose isolation medium. It is also highly effective for detection and enumeration of the major mycotoxin-producing fungi, including *Aspergillus flavus*, *A. ochraceus*, and some *Fusarium* species.

The formula for DRBC is as follows:

Glucose	10 g
Peptone, bacteriological	5 g
KH_2PO_4	1.0 g
$MgSO_4.7H_2O$	0.5 g
Rose bengal	25 mg (5% in water, 0.5 ml)
Dichloran	2 mg (0.2% in ethanol, 1.0 ml)
Chloramphenicol	0.1 g
Agar	15 g
Water, distilled	1 litre

After the addition of all ingredients, sterilise by autoclaving at 121°C for 15 minutes. Store prepared media away from light, which causes slow decomposition of rose bengal. The medium will keep for months in darkness. The stock solutions of rose bengal and dichloran need no sterilisation, and are also stable for very long periods. The original formulation of King et al. (1979) contained chlortetracycline as the antibiotic. The reformulation with chloramphenicol, an effective antibiotic originally recommended for mycological media by Put (1974), is easier to prepare, is not affected by autoclaving, and has greater long term stability.

Dichloran 18% glycerol agar

Effective isolation of xerophilic fungi, that is, those usually causing deterioration of stored products, requires a medium of much lower water activity than DRBC. Dichloran 18% glycerol agar (DG18; Hocking and Pitt 1980) was developed specifically for the isolation and enumeration of xerophiles, and is currently accepted as the most satisfactory medium for this purpose. DG18 provides good growth of fungi such as *Aspergillus penicillioides, Wallemia sebi*, and *Eurotium* species, all of which may be overlooked on PDA or DRBC, but which are of great significance in stored products.

The formula for DG18 is as follows:

Glucose	10 g
Peptone, bacteriological	5 g
KH_2PO_4	1.0 g
$MgSO_4.7H_2O$	0.5 g
Glycerol, A.R.	220 g
Dichloran	2 mg (0.2% in ethanol, 1.0 ml)
Chloramphenicol	0.1 g
Agar	15 g
Water, distilled	1 litre.

To produce this medium, add minor ingredients and agar to about 800 ml distilled water. Steam to dissolve agar, then make up to 1 litre with distilled water. Add glycerol (which gives a final concentration of 18% w/w). Sterilise by autoclaving at 121°C for 15 minutes. Note that the glycerol is 18% weight in weight, not weight in volume. The final a_w of this medium is 0.955.

Aspergillus flavus and parasiticus agar

More selective media are also of value for detecting specific fungi in stored products. Aspergillus flavus and parasiticus agar (AFPA; Pitt et al. 1983) is recommended for the detection and enumeration of *A. flavus* and other potentially aflatoxigenic fungi in nuts, maize, spices, and other commodities (Hocking 1982). When incubated at 30°C for 48 to 60 hours, colonies of *Aspergillus flavus* and *A. parasiticus* are distinguished by bright orange–yellow reverse colours.

The advantages of AFPA for routine use include: rapidity, as 48 hour incubation is usually sufficient; specificity; and simplicity, as little skill is required in interpreting results. In consequence, it can be a simple, routine guide to possible aflatoxin contamination (Pitt 1984).

Few other fungi produce colourations similar to *Aspergillus flavus* and closely related species on AFPA. Only *A. niger* can be a source of error: it grows as rapidly as *A. flavus* and sometimes produces a yellow, but not orange, reverse colour. After 48 hours *A. niger* colonies begin production of their diagnostic black or dark-brown heads, providing a ready distinction from *A. flavus*. After prolonged incubation, 4–5 days, *A. ochraceus* colonies may also produce a yellow reverse, but this species grows slowly at 30°C, and the colour reaction does not appear within 48 hours.

The formula for AFPA is as follows:

Peptone, bacteriological	10 g
Yeast extract	20 g
Ferric ammonium citrate	0.5 g
Chloramphenicol	0.1 g
Dichloran	2 mg (0.2% in ethanol, 1.0 ml).
Agar	15 g
Water, distilled	1 litre

After addition of all ingredients, sterilise by autoclaving at 121°C for 15 minutes. The final pH of this medium is around 6.2.

Dichloran peptone chloramphenicol agar

For enumeration of *Fusarium* species from stored products, Dichloran peptone chloramphenicol agar (DCPA; Andrews and Pitt 1986) is of value. We have also found DCPA to be effective as an isolation medium for other genera of field fungi such as *Alternaria, Drechslera,* and *Curvularia*, which usually sporulate well on it. In addition, DCPA is a very useful medium for the identification of *Fusarium* species because it induces the abundant formation of *Fusarium* macroconidia.

Note that isolates should not be maintained or stored on DCPA for more than two weeks as ammonia may be produced in aging cultures.

The formula for DCPA as follows:

Peptone	15 g
KH_2PO_4	1.0 g
$MgSO_4.7H_2O$	0.5 g
Chloramphenicol	0.1 g
Dichloran	2 mg (0.2% in ethanol, 1 ml)
Agar	15 g
Water, distilled	1 litre

After addition of all ingredients, sterilise by autoclaving at 121°C for 15 minutes.

Table 1 summarises the media recommended here for direct plating of stored products.

Table 1. Media for direct plating

Medium	Purpose	Incubation
DRBC	General; suitable for most Aspergilli, Penicillia and mycotoxigenic fungi	5 days at 25°C
DG18	Xerophiles, best for *Eurotium* spp., *Wallemia, A. penicillioides*	5–7 days at 25°C
AFPA	*A. flavus* and other aflatoxin producers	2–3 days at 30°C
DCPA	*Fusarium, Alternaria, Drechslera,* and other dematiaceous hyphomycetes	5 days at 25°C

Fungi Significant in Stored Products

In collaboration with the Department of Agriculture, Bangkok, Thailand, the Southeast Asian Centre for Tropical Biology (BIOTROP), Bogor, Indonesia, and the National Postharvest Institute for Research and Extension(NAPHIRE), Muñoz, the Philippines, we are currently undertaking extensive studies on the mycological flora of Southeast Asian commodities during drying and storage, using the modern techniques described in this paper. A very limited range of fungi has been consistently encountered. The main results to date are given in Table 2.

Table 2. Significant fungi in Southeast Asian commodities

Commodity	Field fungi	Spoilage fungi	Mycotoxigenic fungi
Cashews	*Cladosporium cladosporioides* *Nigrospora oryzae*	*Chaetomium* spp	*Aspergillus flavus*
Cassava	*Lasiodiplodia theobromae* *Nigrospora oryzae* *Phoma* sp.		
Copra	*Nigrospora oryzae*	*Chaetomium* spp *A. tamarii* *A. niger*	*Aspergillus flavus*
Maize	*Nigrospora oryzae* *Curvularia pallescens* *C. lunata, C. clavata* *Lasiodiplodia theobromae* *Bipolaris maydis* *Arthrinium phaeospermum* *Rhizopus oryzae* *Phoma herbarum* *Rhizoctonia solani*	*Aspergillus niger* *Chaetomium* spp. *Penicillium citrinum* *P. funiculosum* *A. wentii*	*Aspergillus flavus* *Fusarium moniliforme* *F. semitectum*
Peanuts	*Cladosporium cladosporioides* *Lasiodiplodia theobromae* *Pestalotiopsis guepinii*	*Aspergillus niger* *Penicillium pinophilum* *Chaetomium* spp.	*A. flavus*
Rice	*Bipolaris maydis* *Fusarium semitectum* *Cladosporium cladosporioides* *Nigrospora oryzae* *Curvularia lunata* *C. genticulatus, C. oryzae* *C. eragrostidis, C. pallescens* *Phoma* sp. *Colletotrichum* sp.		*Alternaria padwickii* *A. alternata* *A. longissima*
Sorghum	*Bipolaris maydis* *Cladosporium cladosporioides* *Fusarium semitectum* *Nigrospora oryzae* *Curvularia lunata* *C. pallescens* *Phoma* sp. *Setosphaeria rostrata*	*Aspergillus niger* *Eurotium chevalieri* *E. rubrum* *Chaetomium* sp.	*A. flavus* *Fusarium moniliforme* *Penicillium citrinum* *Alternaria longissima* *A. alternata*

Table 2. (contd.)

Commodity	Field fungi	Spoilage fungi	Mycotoxigenic fungi
Soybeans	*Arthrinium phaeospermum* *Lasiodiplodia theobromae* *Fusarium semitectum* *Cladosporium cladosporioides* *Nigrospora oryzae* *Curvularia lunata* *C. pallescens* *Phoma* sp. *Epicoccum nigrum* *Pestalotiopsis guepinii*	*Aspergillus niger* *A. wentii, A. restrictus* *A. penicillioides* *Eurotium rubrum* *Eupen. cinnamopurpureum* *Chaetomium* sp.	*Aspergillus flavus* *Fusarium moniliforme* *Penicillium citrinum* *Alternaria alternata*

It can be seen from Table 2 that the majority of the fungi isolated from the commodities examined were field fungi, a total of about 20 species. Such fungi commonly require high a_w levels for growth. In many cases growth must have occurred before harvest. This implies a close relationship with the growing grain. As a result of the use of this technique, it is increasingly clear that close associations between particular fungi and developing grains are more widespread that earlier suspected, and not confined to true pathogens. Most of the fungi encountered this way are of doubtful significance, as they cause little deterioration and are not mycotoxigenic. *Fusarium* and *Alternaria* are exceptions.

Spoilage fungi were represented by *Aspergillus* and *Eurotium* species, which are capable of growing and causing deterioration of products stored at moisture contents only a little above safe levels. Several *Chaetomium* species were also isolated, but their role in stored products is less certain.

Penicillium species, common in temperate zone commodities, were quite rarely encountered. Only three species, *P. citrinum, P. funiculosum* , and *P. pinophilum,* were at all common.

Only a few toxigenic fungi were isolated. However, *A. flavus* was very common in maize and peanuts, and also was isolated quite frequently from copra and sorghum. A clear implication is that aflatoxins are a major problem in these commodities in Southeast Asia. *Fusarium moniliforme* was found at high levels in maize.

F. moniliforme is the source of the fumonisins, toxins known to be responsible for severe diseases in some animals, and suspected to be involved in human oesophageal cancer in parts of China and southern Africa. The importance of *F. moniliforme* in the tropics remains to be elucidated. *Alternaria* species were found at moderate levels in sorghum and rice. That the presence of *Alternaria* toxins in feed leads to poor performance by domestic animals is well established.

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A Global View of the Mycotoxin Problem

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Abstract

Global concern is given to the mycotoxin contamination of food products. Numerous activities at national, regional and international levels have been carried out in order to prevent mycotoxin development, to monitor and control mycotoxin contamination and to train various staff involved in research, prevention, surveillance and control activities in both public and private sectors. Strategies to prevent and control mycotoxin contamination of food have been proposed and implemented around the world. International cooperation and assistance have helped in addressing this global problem.

MYCOTOXINS are natural substances produced by certain moulds which grow on agricultural commodities under particular conditions. Mycotoxin-producing moulds are widely distributed in nature. Agricultural commodities such as grains, seeds, and nuts are vulnerable to mould damage during pre- as well as postharvest stages of production. Storage is often identified as a major site of contamination. Insufficient ventilation may lead to temperature-driven moisture migration creating pockets of wet grain conducive to mould development.

Mycotoxins have been attracting global attention in view of their adverse health effects and negative economic impact.

Health Concerns

It has long been recognised that consumption of foods and feeds contaminated with moulds may cause serious health problems which could ultimately result in death. For many centuries, poisoning due to consumption of bread made from rye infected with ergot has been recognised. In the 1960s, the toxic metabolites of *Aspergillus flavus* and *A. parasiticus* were identified as the causal agent of 'turkey X disease', further stimulating global concern about mycotoxins.

Mycotoxins have been demonstrated to induce adverse health effects in laboratory and farm animals. A variety of tissues and organs, such as the liver, kidneys, and nervous and gastrointestinal systems, can be affected, depending on the mycotoxin. Mycotoxins may also weaken the immune system of the body. Some of the compounds such as the aflatoxins, have been shown to be mutagenic, carcinogenic, and teratogenic. In addition, mycotoxins have been reported as responsible for imbalances in feed-live weight conversion ratio in farm animals and for lowering their productivity.

Nevertheless, vital statistics on mycotoxin-induced diseases are generally lacking. Many gaps remain in our knowledge, resulting in global concern and the need for more descriptive and analytical epidemiological studies.

Trade and Economic Aspects of Mycotoxins

Mycotoxin contaminated products cause significant economic and trade problems at almost every stage of marketing between the producer and consumers.

Despite this, reliable published information on the economic and trade aspects of contamination by mycotoxins is rather limited and, when it is available, it has often not been expressed in quantitative terms. The studies and statistics available are often incomplete and restricted to aflatoxin only. Hence, it is difficult to provide an estimate of economic losses due to mycotoxin contamination. Outright losses of food and feed could be very large, but again are not quantifiable at present. Numerous incidences of losses of livestock have been reported, including deaths of large numbers of animals. Other losses, such as lowered meat, egg, or poultry productivity, deterioration in quality, and poor work performance could be greater in aggregate, though much less obvious.

The significance of mycotoxins in international trade is being increasingly recognised by both developed and developing countries.

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During the past few years, the export of agricultural commodities such as peanuts, cottonseed, pistachio nuts, and copra, and their derivatives, have all been affected. Often, and especially in developing countries, the best quality batches of these commodities (which are free from mycotoxins) are exported while the substandard products, not acceptable to foreign buyers, are distributed and sold within the country with potential consequences for the health of the local population or productivity of the animals fed with contaminated or substandard feed.

In addition to what has already been mentioned, there are further losses caused by mycotoxins along the food chain. The losses to the primary producer on account of mould damage and mycotoxin contamination in terms of outright food/feed loss, reduced sale prices for contaminated crops, potential loss of outlets, reduced productivity of livestock leading to less income, and so on, are considerable.

Because of the problems faced by farmers, they are now being organised into unions in many countries, to press for subsidy provisions for mycotoxin-contaminated crops. Such actions would be great financial burdens on national economies.

Traders of poor quality products lose both income and reputation. Trade frictions are also created between exporters and importers as to whether the contamination occurred before exporting or during shipping. Also there have been disputes and disagreements between importing and exporting countries regarding the methodology of sampling shipments for analysis, the analytical methodology for estimation of aflatoxins, and the establishment of tolerance limits. The risk of rejection of commodities after they have reached their destination in countries where there are better facilities for the control of aflatoxin, including analytical capabilities, also causes problems. Countries which do not have stringent quality specifications and control, and countries which agree on barter trade, obtain substantially lower prices and payments often have to be accepted in local rather than convertible currency.

The loss to the national exchequer in terms of reduced foreign exchange earnings and increased costs of inspection, sampling, analysis, research, surveillance, compliance procedures, training and extension programs, detoxification, and subsidies could also be considerable.

Prevention and Control of Mycotoxins

Recognition of the problems due to mycotoxins in various countries is the first step to implementing appropriate programs for their prevention and control. These include not only the prevention of mycotoxin formation in agricultural commodities but possibly also their removal through detoxification or decontamination. In addition, routine surveillance, regulatory measures to control the flow of mycotoxin contaminated commodities in national and international trade, as well as information, education, and communication activities are required. Each of these approaches forms a part of overall strategies to minimise the problems of mycotoxin contamination. These include:

- The prevention of aflatoxin formation in agricultural commodities at farm levels through better farm management practices, including the use of fungus- and toxin-resistant varieties of plants, proper irrigation techniques and fertiliser practices, pest control, and crop rotation.
- The improvement of postharvest techniques, including drying practices and storage conditions, in order to ensure safe levels of moisture content for food commodities, and adequate use of chemicals (either industrial or natural plant extracts) to help in preventing fungal contamination.
- The use of detoxification, decontamination, or segregation to avoid high loss of contaminated products.
- The development of internationally harmonised, regulatory control measures on mycotoxins in order to protect public health and promote fair trade at national and international levels.

Various countries around the world have been involved in the development of these actions. The role of international cooperation in ensuring the identification and implementation of national, regional, and international strategies cannot be overemphasised.

FAO is greatly concerned with the problem of mycotoxins in food, including their effects on food trade, since the mycotoxin contamination of food has a negative impact on food availability and consumption.

FAO work to assist its member countries in the prevention and control of mycotoxins has been directed in three main areas:

- an advisory role for the identification of mycotoxin prevention and control strategies at national, regional, and/or international levels;
- the provision of technical assistance to developing countries through national project activities, including the training of all staff involved in mycotoxin prevention and control; and
- the international harmonisation of mycotoxin tolerance levels in food and feeds.

These areas are addressing the concerns of most countries.

Advisory Role

In dealing with mycotoxin problems, FAO has organised, in collaboration with other international organisations

such as WHO and UNEP, international meetings where experts from member countries are invited to participate and exchange their views on the problem, identify priorities at regional or international level and propose strategies for further action around the world. Hence in 1977 and 1987, joint FAO/WHO/UNEP International Conferences on Mycotoxins were held (FAO 1977, 1988b). In recent years, various meetings of the Joint FAO/WHO Expert Committee on Food Additives and Contaminants evaluated the hazards of specific mycotoxins. Reports or technical documents are issued after such meetings and widely distributed around the world. These publications are informative in nature, providing information on the latest developments in various matters related to mycotoxin occurrence, including research, prevention and control. They may also be of an advisory nature, proposing to the countries concerned, strategies to prevent and control mycotoxin contamination of foods.

The report on the 1977 FAO/WHO/UNEP International Conference on Mycotoxins was published as an FAO Food and Nutrition Paper (FAO 1977). It provided up-to-date information on the world situation regarding mycotoxin contamination of foods and the research under way to improve understanding of the problem. It also provided countries with detailed recommendations of the conference concerning prevention of mycotoxin development at field, harvest, storage, transport and marketing levels, including decontamination aspects and training needs in mycotoxin prevention and control.

A Second Joint FAO/WHO/UNEP International Conference on Mycotoxins was organised and held in Bangkok, Thailand in 1987 (FAO 1988b) to review world developments and activities undertaken by international agencies and countries regarding the mycotoxin situation since the first meeting. This second conference again viewed with a great concern the health and socioeconomic consequences of mycotoxin contamination of human food and animal feedstuffs and urged United Nations organisations, national governments, and interested bodies to provide assistance in programs designed to reduce or eliminate the problem of mycotoxin contamination. The conference identified the following activities as having high priority:

- strengthening of existing centres dealing with mycotoxin control and, where necessary, the establishment of new facilities by the provision of equipment and training of laboratory personnel and extension staff;
- the development, application, and harmonisation of reliable, inexpensive, and internationally accepted methods of sampling, sample preparation, and analysis, and standardisation of legislation, including the establishment of permissible levels of mycotoxins in food;
- the development of feasible and practical means of preventing preharvest contamination of food crops;
- the development of feasible and practical methods to improve postharvest practices at both the local and large warehouse level so as to prevent fungal infection and insect damage;
- the extension and coordination of research into significant problems of mycotoxin toxicity incidence, prevention, and elimination; and
- the development of feasible, economical, and safe means of decontamination of mycotoxin-contaminated food commodities and products.

Specific recommendations of the conference centred on prevention of mycotoxin development, monitoring of mycotoxin contamination, and training, information, and research related to mycotoxins. The conference report has been published and widely distributed to the member countries of FAO, WHO, and UNEP involved in mycotoxin prevention and control. Recommendations have been implemented in a number of cases and training activities at regional or national levels in aflatoxin detection, in particular, can be mentioned.

Another important international advisory activity carried out by EAO on mycotoxins is the work done by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) regarding the evaluation of various mycotoxins, possible level of exposure, and tolerance levels. This aspect will be detailed later in this paper.

FAO, in response to the concerns of its member countries, has also organised various meetings in order to review and adapt, at regional levels, the international strategies proposed (FAO 1990b, 1991b). Recent activities include FAO's organisation of regional workshops on aflatoxins in Asia (Chiang Mai, Thailand, February 1990), Africa (Cairo, Egypt, December 1990), and Latin America (San Jose, Costa Rica, February 1991). These workshops assisted in identifying specific regional needs and priorities. For instance, the Asia workshop (FAO 1990b) referred to the need for a bold Asian initiative for observing the 1990s as the 'decade of prevention and control' with activities designed to minimise mycotoxin contamination in food. A discussion paper provided the workshop with an overview of the problem both at the global and Asian levels, and proposed strategies for the region. In particular, it identified the need for strengthening national capabilities for inspecting, sampling, and monitoring for aflatoxin levels in food. It recommended regional networking activity on training and dissemination of information, including a data base for documenting prevention and control measures. The international need for harmonised tolerance levels was reaffirmed.

In Africa (FAO 1991b), the regional workshop recommendations included the establishment or strengthening and expansion of food control infrastructure, and the establishment of an African network for the transfer of appropriate food contaminant (i.e. aflatoxins) control technologies. Similar recommendations were issued by the Latin American workshop.

Technical Assistance

FAO has provided for review missions to study food control systems in general and, in many instances, those for dealing with problems caused by mycotoxins in particular.

The aim of such review missions is to identify food control problems and weaknesses of infrastructure, such as lack of organisational structures, inspectors, analysts, technical specialists, rules and regulations, and adequate import/export control services. Recommendations for improvement and strengthening of these activities, including the provision of further technical assistance, are also made.

FAO has provided expertise and consultants to a number of countries regarding various mycotoxin-related problems including many of the Asian countries (FAO 1980–1990). Existing systems for monitoring food for contaminants including aflatoxins, have been studied and strengthened in countries of Asia including Bhutan, China, India, Indonesia, Nepal, Pakistan, the Philippines, Sri Lanka, and Thailand, as well as in Cuba, Dominica, Guatemala, and Chile in Latin America and the Caribbean region, and in Rwanda, Tanzania, and Malawi in Africa.

A project was implemented in Thailand in 1985–86 to strengthen facilities for food analysis and to carry out a pilot study for food additives and contamination monitoring and intake. Aflatoxins were one of the contaminant groups studied.

In the Pacific Island state of Vanuatu, a project was implemented in 1986 to control aflatoxin in copra. Activities undertaken included the assessment of aflatoxin levels in copra both for domestic consumption and for export to Europe. Additionally, the project identified measures to improve the quality of the domestic product.

In the African country Rwanda, the aflatoxin contamination of food products is being assessed and steps taken to monitor it. Similar activities commenced in Tanzania in 1988–90 (Centre for International Projects 1989).

Mycotoxins received attention in the FAO Prevention of Food Losses (PFL) program, many projects of which seek to control mycotoxin contamination so as to reduce qualitative and quantitative losses. Of some 195 projects which have been implemented recently, at least 52 include a mycotoxin component.

Projects have been implemented for the development and introduction of quality standards and grading systems that encourage farmers to prepare and sell crops adequately dried and kept at low moisture contents. They have also related to the improvement of storage facilities around the world, including the training of warehouse staff. A regional project covering 13 Asian countries introduced and suggested appropriate postharvest technologies related to mycotoxin prevention in rice and maize with an emphasis on drying facilities (FAO 1991a). A project undertaken in Bhutan to prevent postharvest losses in maize included control of aflatoxin contamination. In Senegal, West Africa, a postharvest project is considering the problem of rice quality, including possible mycotoxin contamination.

Training is an important area of FAO assistance. Training activities have included not only programs for farmers, warehouse managers, traders, and laboratory staff, but also overseas study tours for extension workers, laboratory technicians, and managers.

At the Asian regional level, a joint FAO/UNEP/USSR project to strengthen the capability of food laboratory personnel and extension staff in mycotoxin detection and prevention is being developed. A preliminary mission, in June 1989, identified institutions in the region (India, the Philippines, and Thailand) interested and technically capable of participating in such a project and which could act as regional reference centres in selected fields of expertise.

Regional Training Courses on Aflatoxin Analysis were held in Malawi in 1988, in Trinidad and Tobago in 1989, and in Senegal in 1990. About 25 analysts from food control laboratories participated in each session. A national training program organised in Mexico in 1990 emphasised criteria for selecting and standardising analytical methodology, especially simple methods for rapid screening, as well as the implementation of a national food contamination monitoring program for aflatoxins in food and feedstuffs.

Publications and training aids on various mycotoxins control matters have been prepared by FAO and widely distributed. In addition to the two reports of the International Conferences on Mycotoxins (1977, 1987), three FAO Food and Nutrition Papers (FAO 1979a,b, 1982) have been prepared which are directly related to mycotoxin surveillance, as well as various manuals on food quality control that contain information on biological aspects of mycotoxigenic fungi and methods of analysis for mycotoxins (FAO 1979c, 1986, 1988a). A training syllabus (FAO 1990a) has been developed for use in short training courses on aflatoxin analysis in Africa. It includes general information on mycotoxin problems, and practical aspects of sampling, analytical procedure performance characteristics, and quality assurance, as well as prevention and decontamination. Prevention of postharvest losses, including those attributable to mycotoxins, has also been addressed in a number of FAO technical publications (FAO 1983, 1989a,b, 1991a).

International Tolerance Levels

As has often been stressed by experts and government representatives in various meetings, the setting of internationally agreed tolerance levels for mycotoxins in food and feed is of global importance.

International agencies such as FAO encourage the compilation of information from various countries regarding maximum limits for aflatoxins levels in foods and feeds. The most recent compilation (1987) contains information obtained on mycotoxin regulations, tolerance levels, and methods of sampling and analysis (van Egmond 1987).

Aflatoxin as a contaminant was evaluated for the first time by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at its 31st meeting in 1987 (WHO 1987). JECFA considered aflatoxin to be a potential human carcinogen. It felt that sufficient information was not available to establish a figure for a tolerable level of exposure. The JECFA committee urged that the intake of dietary aflatoxin be reduced to the lowest practicable level, so as to minimise, as far as possible the potential risk. It reiterated the view that 'a health hazard can be determined only by taking into account the toxicological knowledge and information about potential exposure'. However, in the case of potent carcinogens, for example certain mycotoxins, the committee believed that efforts should be made to limit their presence in food to irreducible levels. It defined an irreducible level as 'that concentration of a substance which cannot be eliminated from a food without involving the discarding of that food altogether, severely compromising the ultimate availability of major food supplies'.

JECFA has also taken preliminary steps to assess hazards from other mycotoxins. During its 35th session (1989) it considered data available on patulin, and set a provisional tolerable weekly intake (PTWI) for this compound (WHO 1990). The committee considered that adherence to good manufacturing practices (GMP) in apple juice production should avoid unnecessary exposure to sources of patulin such as mouldy fruit and reduce levels to below the PTWI.

In its 37th Session (1990), JECFA evaluated ochratoxin and set a provisional tolerable weekly intake (PTWI) of 112 ng/kg body weight (WHO 1991). The committee considered that the occurrence of elevated ochratoxin A levels in foodstuffs was associated with poor grain storage conditions, and recommended that efforts be made to highlight the improvements needed.

Reports of JECFA meetings are referred for further consideration to the Codex Alimentarius Commission Committees concerned, such as the Codex Committee on Food Additives and Contaminants.

The Codex Committee on Food Additives and Contaminants (CCFAC), during its 21st session in March 1989 (FAO 1989c), considered methods of analysis for aflatoxins in food and feed, including aflatoxin M_1 in milk, methods of sampling for aflatoxin, and guideline levels for aflatoxins in foods. The committee agreed to accept for immediate guidance a sampling protocol that should be able to determine aflatoxins at levels low enough to assure a low probability of acceptance of lots that contain aflatoxins exceeding whatever tolerance is established.

The committee (FAO 1989c) noted that insufficient data were available for the establishment of maximum limits for aflatoxins in foods and feeds. Taking into consideration the interrelationship of different aspects of guideline levels, and methods of analysis and sampling, it decided to establish a working group on mycotoxins to address the problem and report back to it.

While discussing the working group report, the 22nd session of the CCFAC (1990) agreed that aflatoxins in food should be expressed as total aflatoxins (FAO 1990c). In addition, while noting that certain developing countries wanted to establish higher tolerances for aflatoxins in food, the committee expressed the view that 10 µg/kg total aflatoxins was a reasonable compromise, though the views of governments should be sought on the proposed level. The committee also agreed that AOAC (Association of Official Analytical Chemists) methods based on thin-layer chromatography were fully validated methods and could be accepted as reference methods for aflatoxin in maize, cottonseed products, coconut, copra and copra meal, pistachio nuts, peanuts and peanut products, and soybeans.

The chairman of the working group on aflatoxins reported to the 23rd session of the CCFAC (1991). He noted that the proposed level of 10 µg/kg total aflatoxins for all foods had been extensively discussed and that the importing and exporting countries were moving further apart on the acceptability of a maximum level for all food. As a result of these discussions, the committee (FAO 1991c) decided not to establish a level for total aflatoxins in foods at the present time, but rather to solicit information and comment from governments on the following issues:

- specific foodstuffs that could be contaminated with aflatoxins and which moved in, or caused problems with international trade (e.g. figs, tree nuts, dried fruits, peanuts, and maize);
- technological and intake data on individual commodities, and information on stage and effects of processing;
- information on the consumer of the commodity (human or animal);
- national regulations concerning aflatoxins; and
- suggestions for suitable sampling plans.

The Committee reinstated the Working Group on Mycotoxins.

The 22nd Session of the CCFAC (1990) also discussed the establishment of guideline levels for animal feeds. The CCFAC has circulated for government comment the proposed guideline levels of 10 ppb aflatoxin B_1 in dairy cattle feed. The committee also agreed to seek advice from the Joint FAO/WHO Committee of Government Experts on the Code of Principles Concerning Milk and Milk Products (CCMDS). The CCMDS, which met in November 1990, considered practical levels of aflatoxin M_1 in milk, the relation of level of aflatoxin M_1 in milk to aflatoxin B_1 level in feed, and the availability and sensitivity of analytical methods for determining aflatoxin M_1 in milk. The results of these discussions were provided to the 23rd session of the CCFAC (FAO 1990e, 1991c) which noted that several delegations supported the levels (0.05 µg/kg for bulk milk; 0.01 µg/kg for milk for baby food) for aflatoxin M_1 and that it had decided to seek government comments on these levels. Regarding maximum levels for aflatoxin in feed, the committee decided to send the proposed draft level of 5 µg/kg aflatoxin B_1 in supplementary feed for milk-producing animals to governments for comments at step 3 based on the information submitted by the Milk Committee concerning the relationship between aflatoxin M_1 in milk and aflatoxin B_1 in feed.

In addition, the 7th Session (1990) of the Codex Committee on Cereals, Pulses and Legumes (CCCPL), while noting discussions held at the 22nd CCFAC, reaffirmed its decision that any proposed guideline levels for aflatoxin in cereals, pulses, and legumes would need to be linked to a sampling protocol. A draft guideline level and sampling plans for total aflatoxins in peanuts were proposed for circulation to governments for comments. The CCCPL report was sent for consideration to the 23rd session of the CCFAC (FAO 1990d, 1991c) which decided to submit to the CCCPL the different opinions expressed in the session concerning requests for information on the data underlying the levels as well as the stage of processing. The CCFAC (1991) also decided to circulate a letter seeking comment and information on sampling plans and confidence limits for those commodities which are items of concern to governments. The committee also agreed in principle with the CCCPL to request the commission to consider holding an expert consultation to examine those issues regarding sampling plans for aflatoxins.

Strategies for the Future

An Asian workshop held in February 1990 in Thailand called for making the 1990s, the fourth decade since the discovery of aflatoxins, the decade of 'prevention of mycotoxins'. The first decade since the discovery of aflatoxin was the decade of 'biological effects', while the second was the decade of 'surveillance', and the third the decade of 'regulatory controls'. Such an approach calls for a clear definition of the goals for achieving the prevention and control of aflatoxins and minimising their hazards. The work carried out could be oriented in such a way that the benefits are indeed derived at the grass-roots level, that is, by the farmers and traders, and consumers at national and international levels. To achieve this goal priorities need to be set so as to ensure best utilisation of scarce resources.

The Second Joint FAO/WHO/UNEP International Conference on Mycotoxins identified priority actions that are being followed and implemented by countries around the world, in particular at field and storage levels in the production sectors, to prevent mycotoxin contamination of food products. Much extension work is also required in order to promote prevention activities at farm, storage, and distribution levels of the food susceptible to mycotoxin contamination.

The development of safe and economically feasible means of decontamination of mycotoxin-contaminated commodities and products is one of the measures of prevention of mycotoxins being assessed. Segregation of contaminated commodities such as maize and peanuts appears to be feasible. The process of detoxification using ammonia on both small and large scales appears promising, although reservations have been expresses as to its widespread acceptance, particularly in terms of loss in the nutritional quality and reduced acceptability of the final product.

The need for networking for both dissemination of information and training of personnel on a regional basis has been identified many times as an activity to be developed in the future. As a component of such a network, a data base for documenting and disseminating information could be created. Inter- and intra-laboratory collaborative studies on aflatoxins as part of an analytical quality assurance system should be continued in order to ensure confidence not only among the international buyers of agricultural commodities likely to be contaminated with mycotoxins but also among laboratory personnel engaged in the analysis of mycotoxins. Training of control staff, and analysts in particular, should be continued at national and regional levels.

A decade-long drive aimed at prevention and control of mycotoxins should result in minimising the problem to ensure relatively mycotoxin free commodities for human and animal consumption by the year 2000.

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Overview of the Fungi and Mycotoxin Problem Session Summary

Chairman: Mrs Dara Buangsuwon, Department of Agriculture, Thailand
Rapporteur: Professor U. Samarajeewa, University of Peradinya, Sri Lanka

THE presentations and discussions in this first conference session highlighted the need to standardise methods for both isolation and identification of fungi, and chemical analysis of mycotoxins.

The need for surface sterilisation of samples before isolation of fungi was stressed. Also, it was pointed out that selection of appropriate microbiological media, in particular use of media with similar characteristics to contaminated commodities, yields better results. Further, research aimed at developing such media was deemed necessary.

It was recognised that, for chemical analyses, there is a need for standardisation of sampling procedures and analytical methods. However, in considering standardisation, the types of facilities that can be maintained in developing countries must be taken into account (e.g. thin-layer chromatography versus more sophisticated techniques). Also in this regard, a plea was made for further development of training facilities, especially in those countries where such facilities currently do not exist. Expansion of FAO activities in this area was suggested.

There are indications that importing and exporting countries have moved further apart in fixing maximum tolerance levels for mycotoxins in foods. It is essential that regulatory authorities take cognisance of the levels of toxins that can be detected using the analytical technology available in the less developed countries. These are generally somewhat higher than the limits of detection of more sophisticated methods used in the developed countries. There is also room for rational assessment of the need to reduce tolerance levels from present established levels, which are still widely regarded as 'safe' upper limits.

The maintenance of national and international standards at different levels appears a possibility. Nevertheless, the need to reduce mycotoxin levels in foods and feeds as far as possible should be stressed.

In general, the extent and levels of fungal infection and mycotoxin contamination in foodstuffs in the Southeast Asian region is not well understood, and there a need for further extensive survey work.

Suggestions by conference participants for future activities in this field included:

- collection of information on actual monetary losses to foods caused by fungi and mycotoxins;
- development of preventative methods based on locally available technology;
- greater exchange of information;
- expansion of training facilities; and
- persuasion of governments to implement regulatory measures.

The Fungi

Advances in the Taxonomy of Food Spoilage Fungi

J.I. Pitt*

Abstract

Fungal classification has traditionally been based on the appearance (morphology) of colonies and microscopic structures. Because this information is qualitative, decisions on the relationships among species (taxonomy) was often of necessity based on personal opinion. Moreover, correct procedures for naming fungi (nomenclature) were often ignored. A further problem has been that the literature is diffuse, requiring extensive background knowledge to locate information. Clarifying and stabilising both nomenclature and taxonomy of food spoilage and mycotoxigenic fungi has become imperative. Improvements to our understanding must inevitably involve the taxonomy of three genera: *Penicillium*, *Aspergillus*, and *Fusarium*.

Great advances have been made in recent years, especially in *Penicillium*. The difficulties in naming fungi which possess both sexual (teleomorphic) and asexual (anamorphic) states have been addressed and clarified. The principles of priority, typification, and type specimens are increasingly being applied to both teleomorphs and anamorphs, bringing order to nomenclature. New taxonomic approaches are providing criteria independent of traditional methods. The most important advances have come from studies of secondary metabolites and mycotoxins, electrophoretic patterns of certain isoenzymes, and DNA restriction enzyme fragment polymorphism studies.

As a result of these initiatives, the taxonomy of most *Penicillium* subgenera has become firmly established, and distinctions among aflatoxin producing *Aspergillus* species have been clarified. Using techniques developed from *Penicillium* studies, the taxonomy of the Ascomycete genus *Monascus* has been revised.

Two textbooks on food spoilage fungi, based on the results of these modern initiatives, have been published recently. They bring together the common food borne fungi in single volumes, greatly facilitating identifications.

Fusarium, however, remains difficult. Few of the techniques applied to *Aspergillus* and *Penicillium* have been effective, and specialist taxonomists remain divided over fundamental issues.

International collaboration, rather than activity by individual taxonomists, is increasingly seen as the way of the future. The recently established International Commission on *Penicillium* and *Aspergillus* will play a key role in continued development and stabilisation of names in these genera. Improved agreement on *Fusarium* taxonomy can be expected as the result of formation of a broadly based international subcommission. Further international developments can be expected to result in stabilisation of the names of most of the common food spoilage fungi within the next few years.

FUNGAL classification has traditionally been based on morphology; that is, on the appearance of colonies and microscopic structures. The information obtained is qualitative and often subjective, being dependent on the media used, on the conditions of growth, and the observer's interpretation. Decisions on the relationships among species was, and still is, often a matter of opinion, Moreover, correct procedures for naming fungi (nomenclature) have often been ignored. All too often the end result has been confusion.

A further problem for the scientist interested in fungi in foods has been that the literature is diffuse, requiring extensive background knowledge to select the books containing effective taxonomies of the species of interest.

Improvements to our understanding of the taxonomy of food borne fungi are inevitably tied up with the taxonomy of the three major food spoilage and mycotoxigenic genera: *Penicillium*, *Aspergillus*, and *Fusarium*. Much of this paper will be devoted to the first two of these genera, in which significant advances have been made in recent years.

Simple, accurate, and reproducible classification of *Penicillium*, *Aspergillus*, and *Fusarium* species is highly

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desirable, because they are three of the most important genera of food spoilage fungi. Ideally, identification of species in these genera should be unequivocal, accurate, simple, and immutable. The majority of the significant recent work has dealt with *Penicillium*, the largest and most complex of these genera, so it will be emphasised in this paper. *Aspergillus* and *Fusarium* will be considered where appropriate.

The taxonomy of *Penicillium* has always been difficult, as the genus has many species that are often difficult to differentiate. The first workable taxonomy was produced comparatively recently, by Raper and Thom (1949). In its day a masterpiece, it was in almost exclusive use for nearly 30 years. However, like all fungal taxonomies based exclusively on morphology, it had shortcomings. The taxonomic base, that is, the range of characters used to distinguish species, was narrow. Too much reliance was placed on colony texture, especially in delimiting subgeneric divisions. Even the expert had difficulty distinguishing many of the species accepted by Raper and Thom (1949).

The discovery of penicillin launched an enormous upsurge of interest in secondary metabolite production by common fungi, while the later realisation that many of these metabolites were toxic added a further incentive for studies in this area. The literature on metabolite production, biochemistry, and biodeterioration by these genera grew rapidly. However, the consequences of the shortcomings in taxonomy were inevitable. *Penicillium* taxonomy in the 1960s and 1970s became uncertain, indeed close to chaotic, with inaccurate identifications widespread in the literature, and consequent confusion about which species were present in foods or produced mycotoxins. Many of these inaccuracies remain to the present time, both in the review literature and in major culture collections, where uncritical accessioning has too often occurred.

Aspergillus taxonomy was assisted by the publication of a new monograph by Raper and Fennell (1965), though that publication also had weaknesses. Like the previous *Penicillium* monograph, the taxonomic base was narrow, and the work suffered from severe nomenclatural problems due to Raper and Fennell's failure to adhere to the International Code of Botanical Nomenclature (ICBN).

Booth (1971) revised the taxonomy of *Fusarium*, attempting to provide a rational alternative to the taxonomy of Snyder and Hansen (1945). The Snyder and Hansen system included only nine species, and was quite inadequate to account for the diversity seen in the genus. However, Booth (1971) failed to find widespread support, and the Snyder and Hansen system continued in widespread use.

New Approaches: Nomenclature

As a consequence of the Raper taxonomies of *Penicillium* and *Aspergillus*, nomenclatural problems with priority, typification, teleomorph names, and infrageneric taxa all had to be addressed by later systematists.

Priority

Raper and his coworkers sometimes failed to accept names which had priority, as required by the ICBN, on the grounds that are unacceptable now. Problems of priority in *Penicillium* were largely corrected by Samson et al. (1976) and Pitt (1979), but at the expense of confusion caused by name changes. Problems over priority in *Aspergillus* and *Fusarium* are equally severe, but have not yet been addressed adequately. It is clear that some important names, such as *P. chrysogenum*, *A. niger*, and *A. nidulans*, are threatened by older species names and hence are being proposed as *nomina conservanda* (Frisvad et al. 1990). Controversy over priority for names of some *Fusarium* species continues unabated (Nirenberg 1990).

Typification

Raper and Thom (1949), Raper and Fennell (1965) and recent *Fusarium* taxonomists (Booth 1971; Gerlach and Nirenberg 1982; Nelson et al. 1983) have failed to specify dried types for any of the species they accepted, though in their defense it must be said that many other contemporary taxonomists have been equally negligent. More seriously, however, even living type material has not been identified in descriptions, and sometimes existing type material has been ignored altogether.

To provide a basis for resolution of such typification problems, Pitt (1979) typified names he accepted in *Penicillium*, while Samson and Gams (1985) typified the majority of the *Aspergillus* names accepted by Raper and Fennell (1965). Kozakiewicz (1989) corrected this list and added further names. *Fusarium* species have not been typified by recent authors (Booth 1971; Gerlach and Nirenberg 1982; Nelson et al. 1983)

Teleomorph names

Raper and Thom maintained that teleomorph species with anamorphs in *Aspergillus* or *Penicillium* should be named in the anamorph genera, and made new combinations as necessary (Thom and Raper 1946; Thom 1954; Raper 1957). For example, *Eurotium amstelodami* Mangin was renamed *Aspergillus amstelodami* (Mangin) Thom and Church (Thom and Church 1926) and maintained this way in later taxonomies. However, the ICBN, and the weight of nomenclaturalists' opinions, dictate that teleomorph names have priority over anamorph names.

A second problem concerns species described in an anamorph genus but which included the teleomorph as is the case, for example, for *Penicillium javanicum* van Beyma. Raper and Thom (1949) accepted such species

as being correctly placed in *Penicillium*, contrary to the ICBN.

Hawksworth and Sutton (1974) showed that the ICBN then current was ambiguous in the naming of species such as *Penicillium javanicum*. As a consequence, the International Botanical Congress in Sydney altered articles in the new ICBN (Voss et al 1983) to make naming of such species unequivocal. The price was that some well established names, especially in *Aspergillus* and *Penicillium*, had to be changed. The nomenclatural problem was overcome, but at the expense of introducing new and unfamiliar names. Pitt (1979) made the necessary changes to *Penicillium*. For example, '*Penicillium javanicum* van Beyma' is now known correctly as *Eupenicillium javanicum* (van Beyma) Stolk and Scott when the teleomorph, or the whole fungus, is under consideration, and as *Penicillium indonesiae* Pitt when the anamorph alone is being considered. Samson and Gams (1985) carried out the necessary alterations in *Aspergillus*: '*Aspergillus amstelodami* (Mangin) Thom and Church' is correctly called *Eurotium amstelodami* Mangin when the teleomorph is present, and *Aspergillus hollandicus* Samson and W. Gams for the *Aspergillus* state alone. For a more detailed discussion of this point see Pitt (1988).

Infrageneric taxa

Thom and his coworkers arranged clusters of species in *Aspergillus* and *Penicillium* into 'Groups', a subgeneric classification without nomenclatural status under the ICBN. Pitt (1979) replaced the 'Groups' structure in *Penicillium* by a nomenclaturally correct sectional structure. Gams et al. (1985) carried out similar changes to *Aspergillus*.

In *Fusarium*, the use of teleomorph names remains a dilemma. Teleomorphs are seen in culture for only a few food borne species, and most taxonomists prefer to continue identifying *Fusarium* species by the *Fusarium* names.

New Approaches: Taxonomy

Cultural methods: new media and incubation conditions

The development of new selective media and incubation conditions to improve the basis for *Penicillium* identifications was pioneered by Abe (1956). He made two proposals: the use of a gross physiological character, growth at 37°C, and a discriminatory medium containing nitrite, as selective agents for differentiating species. Pitt (1973) followed this lead, using both 5°C and 37°C, and introduced 25% glycerol nitrate agar (G25N, 0.935 a_w) as a medium that provided some measure of physiological adaptation to low water activity. To make effective use of these concepts, Pitt (1973) also introduced a carefully standardised set of media and incubation conditions. He quantified the influences of temperature and water activity by measurements of colony diameters after a set incubation time of 7 days. Pitt (1979), the first complete *Penicillium* taxonomy for 30 years, was based primarily on morphological characters and colony diameters.

Further developments of taxonomically valuable selective media followed. Pitt et al. (1983) introduced Aspergillus flavus and parasiticus agar (AFPA) for the recognition of *Aspergillus flavus* and *A. parasiticus*. Frisvad (1983) developed Dichloran Yeast Extract Sucrose agar (DRYS) for distinguishing *Penicillium verrucosum* and *P. viridicatum* from other related species. Frisvad (1985a) also introduced Creatine Sucrose Agar (CSA) which permits separation of species in *Penicillium* subgenus *Penicillium* into two natural groups related to preferential utilisation of protein or carbohydrate substrates. A selective medium for isolation and enumeration of *Fusarium* species has recently been described (Abildgren et al. 1987). Czapek Iprodione Dichloran Agar is selective against a wide range of other common food-borne fungi. Each of these media has proven to be of considerable taxonomic value.

Using the media and plating regimes developed for *Penicillium* by Pitt (1979), Hawksworth and Pitt (1983) revised the taxonomy of *Monascus*, a genus important in foods, though more from the point of view of fermentation than spoilage. They reduced 16 species and varieties to 3 species, on the basis of colony diameters and ascospore morphology.

Gerlach and Nirenberg (1982) published an atlas of *Fusarium* species, based on microscopic morphology. Cultural morphology was not included, and no keys were provided. Nelson et al. (1983) produced a new taxonomy of *Fusarium* based on growth of isolates in culture under carefully defined conditions, allowing effective identifications of fresh isolates from food-borne sources for the first time.

Secondary metabolites

A very wide variety of secondary metabolites has long been known to be produced by fungal species (Turner 1971), but their potential in taxonomy was ignored until Ciegler et al. (1973) suggested dividing *Penicillium viridicatum* into 'subgroups', partly on the basis of secondary metabolite production. This approach became of practical value with the development of a simple technique for distinguishing mycotoxins and other secondary metabolites directly in Petri dish cultures. Small samples cut with a cork borer were directly spotted onto a thin layer plate, with or without the aid of a drop of solvent, and chromatographed (Filtenborg and Frisvad 1980; Filtenborg et al. 1983). Mycotoxin and secondary metabolite production can be assessed qualitatively much faster by this method than by conventional extraction, clean up, and concentration techniques.

Frisvad's approaches met with opposition because his concepts were not clear (Frisvad and Filtenborg 1983; Frisvad 1985b, 1986). However, difficulties with misidentified cultures, and the relationship of secondary metabolite profiles to morphological taxonomy have now been clarified, especially as correlations between metabolite profiles and electrophoretic isoenzyme patterns have been excellent (see next section). Patterns of secondary metabolites have now become an effective taxonomic tool.

Frisvad and Filtenborg (1983), Paterson (1986), and El-Banna et al. (1987) have published detailed thin-layer chromatographic solvent systems and R_f values for a wide variety of *Penicillium* metabolites. Frisvad and Thrane (1987) introduced high performance liquid chromatography with diode array detected UV spectra as a sophisticated method for comparing unknown secondary metabolite profiles against standard isolates. Metabolite profiles can now be used by the determinative taxonomist.

Frisvad and Filtenborg (1989) have recently published a new classification of species in *Penicillium* subgenus *Penicillium*, based on the examination of more than 4000 isolates. Although the authors introduced 4 new species and 10 new varieties, a very high overall agreement exists with the more traditional classification of Pitt (1979, 1988). On the basis on secondary metabolite profiles, Frisvad (1989) reidentified more than 150 *Penicillium* isolates incorrectly reported in the literature.

Secondary metabolite profiles have also been applied to the taxonomy of *Aspergillus* teleomorphs. Frisvad (1985c) examined secondary metabolite production by 30 species of *Emericella* and related *Aspergillus* species. Samson et al. (1990) distinguished nine taxa in *Neosartorya* by ascospore morphology and secondary metabolites.

The picture with secondary metabolites in *Fusarium* is more complex. A clear relationship between *Fusarium* species and secondary metabolite production has not yet been established, and indeed may not exist (ApSimon et al. 1990).

Isoenzyme electrophoresis

Cruickshank (Cruickshank and Wade 1980; Cruickshank 1983) developed effective methods for separation of species of *Sclerotinia*, *Botrytis*, and other genera by examining patterns of pectic enzymes after separation by gel electrophoresis. Small samples of culture fluid were subjected to electrophoresis at low temperatures, then the enzymes allowed to act on methoxy pectin incorporated into the gel, and the sites of enzyme action visualised by ruthenium red staining.

To enable the differentiation of the many species in subgenus *Penicillium*, Cruickshank's technique was broadened by including the examination of amylase and ribonuclease isoenzymes. For amylases, soluble starch was added to gels as a substrate, and for ribonucleases, ribosomal RNA. Fungi were cultured on wheat grains for the production of both these sets of enzymes (Cruickshank and Pitt 1987a).

A study of the isoenzyme patterns (zymograms) for all species accepted by Pitt (1979) in *Penicillium* subgenus *Penicillium* has shown a high correlation with classical taxonomic methods (Cruickshank and Pitt 1987b). All isolates examined from the great majority of species accepted by Pitt (1979, 1988) showed an absolute correlation with specific zymogram patterns, greatly reinforcing our confidence in current species concepts in this subgenus. The correlation between this technique and secondary metabolite profiles (Frisvad and Filtenborg 1989) is also very high. Speciation in subgenus *Penicillium* can now be confidently stated to be well understood. Isoenzyme patterns have been of great value as a research tool for investigating speciation in *Penicillium*.

Isoenzyme profiles have not yet been used effectively in studies of speciation in *Aspergillus* or *Fusarium*.

Genetic studies

Until recently, the powerful genetic tools of DNA hybridisation and analysis of DNA and RNA sequences have been little used in the systematics of these genera.

On the basis of studies of DNA homology among *A. flavus* and related species, Kurtzman et al. (1986) reduced several well known species to subspecies or varietal status. Specifically, *A. parasiticus* Speare became *A. flavus* subspecies *parasiticus* (Speare) Kurtzman et al.; *A. oryzae* (Ahlburg) Cohn became *A. flavus* subspecies *flavus* variety *oryzae* (Ahlburg) Kurtzman et al.; and *A. sojae* Sakaguchi and Umada became *A. flavus* subspecies *parasiticus* variety *sojae* (Sakaguchi and Umada) Kurtzman et al.

Klich and Mullaney (1987) showed that DNA restriction enzyme fragment polymorphism indicated differences between the DNA of *A. flavus* and *A. oryzae*, and disagreed with the varieties proposed by Kurtzman et al. (1986). Klich and Pitt (1988) also argued against these changes, on the grounds that morphological differences existed between these two species. They also considered it important, on practical grounds, that species used for food fermentations be known by different species names from those which are mycotoxigenic.

Logrieco et al. (1990) determined ribosomal RNA sequences for a number of terverticillate Penicillia. Their results indicated that *P. aurantiogriseum, P. viridicatum, P. verrucosum, P. expansum, P. echinulatum, P. hirsutum, P. granulatum* and *P. puberulum* were distinct species. With the exception of *P. puberulum*, this is in accordance with the new taxonomic schemes of Pitt and Cruickshank (1990) and Stolk et al. (1990).

Genetic studies of the types described above have great potential for aiding understanding of phylogenetic rela-

tionships among genera and subgeneric taxa (Taylor et al 1990). It must be emphasised, however, that genetic studies are only one of many tools for defining species, and need to be used in conjunction with other techniques, such as those outlined in this paper.

Texts on Food Borne Fungi

Recent years have seen the publication of two books designed specifically to facilitate the identification of food-borne fungi. Using the methods and media established for *Penicillium* taxonomy by Pitt (1979), Pitt and Hocking (1985) produced 'Fungi and Food Spoilage', a text which provides keys and descriptions to 200 commonly occurring food-borne fungi. 'An Introduction to Food-borne Fungi' by Samson and van-Reenen Hoekstra (1988) is a guide to 100 species, primarily based on microscopic morphology. Both books have taken advantage of recent advances in knowledge. The taxonomies provided are soundly based, and similar to each other.

Collaborative Studies

An important development from the increasing emphasis on modern techniques in *Penicillium* and *Aspergillus* taxonomy has been the emergence of collaboration, effectively spelling the end of the solitary, monographic approach to the systematics of these genera (Samson and Pitt 1985, 1990).

To clarify the taxonomy of *Aspergillus* species producing aflatoxins, Klich and Pitt (1985, 1988) carried out a collaborative study involving the detailed morphological examination of more than 200 isolates of *Aspergillus flavus* and related species. Cultures were examined in two different locations, with isolates identified only by code. Features distinguishing *A. flavus* from *A. parasiticus* and from the closely related food fermentation species, *A. oryzae* and *A. sojae*, were documented.

International Commission

As a direct result of the study by Klich and Pitt (1985), an international working group on *Penicillium* taxonomy was formed at the First International *Penicillium* and *Aspergillus* Workshop held in Baarn, The Netherlands in 1985. This soon became a subcommission of the International Commission on Taxonomy of Fungi (ICTF). Known as the Subcommission on *Penicillium* and *Aspergillus* Systematics (SPAS), this group began collaborative studies on the taxonomy of difficult species clusters in *Penicillium* and *Aspergillus*. The first such study, on species related to *Penicillium glabrum*, has been published (Pitt et al. 1990). More recently, the Subcommission has been upgraded to full IUMS Commission status as the International Commission on *Penicillium* and *Aspergillus* (ICPA). ICPA has twelve members from seven countries, and comprises both morphological taxonomists and those skilled in the newer techniques described in this paper. ICPA plans to carry out collaborative taxonomic studies, revisions of culture collection names, development of taxonomic techniques and other activities. A significant impact is expected.

A Subcommision to study *Fusarium* systematics has recently been established under the ICTF. It is hoped that substantial agreement on names for *Fusarium* species will result.

Recently, a new approach to the stabilisation of fungal species names has emerged. A strong international effort is now being directed towards drawing up lists of 'Names in Current Use', and the establishment of protected status for them. In the next few years, this approach promises to bring much-needed stability to names of *Penicillium* and *Aspergillus* species. The production of a similar list for *Fusarium* species is equally important, but its path to establishment is not so clear.

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Identification of Food-Borne *Penicillium*, *Aspergillus*, and *Fusarium* Species

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Abstract

Among the fungi occurring in food, feedstuffs, and stored products, members of the genera *Penicillium*, *Aspergillus*, and *Fusarium* are often common. As well as being spoilage agents, the production of mycotoxins by these fungi has a significant effect on the quality of the commodity. For quality control and the prevention of contamination, identification to species level is therefore crucial. Until recently the identification of members of these three genera proved to be extremely difficult. This was caused by different species concepts, problems in nomenclature, and lack of objective methods.

The taxonomy of *Penicillium* and *Aspergillus*, in particular, has been greatly improved by the introduction of new approaches, including the application of profiles of secondary metabolites and isozymes. Reliable identification is now possible, because in several important groups of these genera a general consensus and stable taxonomy have been established. International collaboration has contributed to taxonomic stability. While progress in *Fusarium* identification is not as far advanced, practical identification manuals are available. For correct identification, media and cultural conditions are important and these aspects are briefly discussed. For rapid recognition of important mycotoxin-producing species, selective media have been developed and have proven to be valuable for screening. However, serological and molecular biological techniques have recently been proposed and these will have a significant impact on quality control of food and stored products.

MEMBERS of the genera *Penicillium*, *Aspergillus* and *Fusarium* are organisms well-known for the spoilage of food and stored products and production of harmful mycotoxins. To advance our knowledge of the role and significance of mycotoxigenic species, correct identification is crucial, but hundreds of species have been described and, in the past, identification based on subjective criteria was extremely difficult. The identification of species belonging to the three genera is still primarily based on morphological criteria, which are often not stable. Several classification schemes with quite different species concepts were proposed, resulting in a confusion of names (Samson and Gams 1984).

In recent years the systematics of the genera *Penicillium* and *Aspergillus* has progressed significantly. Besides the conventional morphological criteria, biochemical characters have been used to define the species. In most groups, species concepts have been clarified and a more stable taxonomy is now available. With *Fusarium*, however, there are still deviating opinions about species concepts and the introduction of new techniques has been limited. Nevertheless, for mycotoxigenic Fusaria there are practical manuals that can be used for identification. This paper reviews the current status and developments in the identification of these important microorganisms.

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Identification of *Penicillium* and Its Teleomorphs

Raper and Thom (1949) published an authoritative monograph for the identification of *Penicillium* which has been used for several decades. As pointed out by Pitt (1979), Pitt (1989), and Pitt and Samson (1990b) , however, Raper and Thom's work had several shortcomings, resulting in a species concept open to variable interpretation. In the 1970s and 1980s, new classifications (e.g. Samson et al. 1976; Pitt 1979; Ramirez 1982; Stolk and Samson 1983) with different species circumscriptions were proposed, but none of them was generally accepted.

New approaches to improve the taxonomy of the

difficult genus *Penicillium* have been introduced since the First International Workshop on *Penicillium* and *Aspergillus* (Samson and Pitt 1985a). The use of isoenzyme patterns (Cruickshank and Pitt 1987), the profiles of mycotoxins (Filtenborg et al. 1983; Frisvad 1981, 1983, 1985b, 1986, 1989; El-Banna et al. 1987; Frisvad and Thrane 1987) and other secondary metabolites, are effective tools and have led, for example, to clarification of the taxonomy of the subgenus *Penicillium* containing many toxigenic species. Pitt and Cruickshank (1990), Stolk et al. (1990) and Frisvad and Filtenborg (1990 a) have reached close agreement on taxonomy, resulting in well defined species. Table 1 gives a checklist of common *Penicillium*, *Eupenicillium* , and *Talaromyces* species compiled from Stolk and Samson (1972), Samson and Pitt (1985 b), Pitt and Cruickshank (1990), and Stolk et al. (1990)

In addition to the taxonomy of the subgenus *Penicillium,* other groups in *Penicillium* have been studied recently. Frisvad and Filtenborg (1990b) examined the subgenus *Furcatum*, while Frisvad et al. (1990a, b) and Van Reenen-Hoekstra et al. (1990) revised, respectively, the genus *Talaromyces*, the complexes of *Eupenicillium javanicum*, and *Penicillium funiculosum*. Samson et al. (1989) and Frisvad et al. (1990c, d) described new taxa and discussed the disposition of 122 species, varieties, and new combinations of *Penicillium* and its teleomorphs described since 1977 on the basis of morphological structures and production of secondary metabolites.

For the identification of the common food-borne *Penicillium* taxa, two laboratory guides are available (Pitt 1988; Samson and van Reenen-Hoekstra 1988). Identification procedures are still based on morphological and colony characters, but in some cases special media such as creatine sucrose agar (Frisvad 1985a) and profiles of known mycotoxins can be used for important toxigenic strains (Frisvad and Filtenborg 1989, 1990a).

Identification of *Aspergillus* and Its Teleomorphs

The monograph by Raper and Fennell (1965) contains a description of 132 species subdivided into 18 groups. Generic and species concepts are well circumscribed. Nowadays, most of the keys and descriptions are generally accepted and this monograph is still a standard reference book. However, many taxa have been described since its publication and Samson (1979, 1991) provides a compilation of the species and varieties described since 1965, with a critical review of the validity of the taxa described. Several groups, including *A. niger* (Al-Mussalam 1980; Kusters-van Someren 1990, 1991; Kozakiewics 1989), *A. ochraceus* (Christensen 1982), *A. nidulans* (Christensen and States 1982; Horie 1980), *A. flavus* (Murakami 1971; Murakami et al. 1982; Christensen 40U‰; Klich and Pitt 1985, 1988a,b), *A. fumigatus* (Frisvad and Samson 1990; Samson et al. 1990), and *A. restrictus* (Pitt and Samson 1990a), which remained problematic for identification, have been reinvestigated. As for *Penicillium* , the taxonomy of *Aspergillus* has been improved by the introduction of new techniques and multidisciplinary studies. Accounts of advances in *Aspergillus* systematics are given by Samson and Pitt (1985a, 1990).

Kozakiewicz (1989) gives an account of *Aspergillus* species on stored products, treating 13 groups and including two new ones. However, important groups such as the *A. clavatus* and *A. nidulans* group are omitted, and the species concept and identification are based primarily on conidial ornamentation as seen by scanning electron microscopy. Observations were made on conidia from cultures at least 2 weeks old, while for members of the *A. niger* and *A. ochraceus* (= *A. alutaceus*) group cultivation for up to 4 –6 weeks is required. Because rapid determination of toxigenic Aspergilli in stored products is extremely important, the practical value of this methodology is debatable.

Samson (1991) reviews current taxonomic concepts in *Aspergillus* and its teleomorphs, and discusses the accepted species. Keys to common Aspergilli are provided by Pitt and Hocking (1985), Samson and Van Reenen-Hoekstra (1988), Klich and Pitt (1988a), Tzean et al. (1990), and Singh et al. (1991). Bilai and Koval (1988) produced a Russian translation of Raper and Fennell's monograph, updated with the species accepted by Samson (1979).

Species of subgenus *Aspergillus* (= the *A. glaucus* group), with their *Eurotium* teleomorphs, are common on stored products and are characterised by their xerophilic nature. About 20 species are known, but most common are *E. chevalieri*, *E. amstelodami* , *E. repens* and *E. herbariorum* . The fungi grow optimally on low water activity media (MEA or Czapek agar with additional sucrose). Three classifications modified from Raper and Fennell's (1965) taxonomy were proposed by Blaser (1975), Pitt (1985), and Kozakiewicz (1989).

Identification of the important species in the section Flavi (*A. flavus* group) has been problematic due to the variability of the isolates and the morphological similarities of species producing mycotoxins (*A. flavus* and *A. parasiticus*) and those used in the food fermentation industry (*A. oryzae* and *A. sojae*). Much confusion was caused by genetic studies showing that the species concerned are closely related (Kurtzman et al. 1986). However, Klich and Pitt (1988b), Liljegren et al. (1988), and Cruickshank and Pitt (1990) showed that there is a good correlation between morphological, mycotoxin, and isoenzyme profiles and that identifications of species in this group can be made with confidence.

While *A. nomius* (Kurtzman et al. 1987) is so far known only from a few isolates it is probably more common. It resembles *A. flavus*, but differs in having smaller and elongate sclerotia and by producing aflatoxin G_1 and G_2 and nominine (Klich and Pitt 1988 a; Liljegren et al. 1988; Samson and Frisvad 1991).

Table 1. Checklist of common *Penicillium* species and their teleomorphs

Genus *EUPENICILLIUM*

E. cinnamopurpureum Scott and Stolk
E. ochrosalmoneum Scott and Stolk
E. pinetorum Stolk
E. javanicum (van Beyma) Stolk and Scott
E. lapidosum Scott and Stolk
E. crustaceum Ludwig
E. shearii Stolk and Scott
E. hirayamae Scott and Stolk

Genus *PENICILLIUM*

Section Aspergilloides

P. glabrum (Wehmer) Westling (= *P. frequentans* Westling)
P. spinulosum Thom
P. lividum Westling
P. thomii Maire
P. sclerotiorum van Beyma (= ***P. multicolor*** Grigorieva-Manilova and Poratielova)
P. citreonigrum Dierckx (= *P. citreoviride* Biourge = *P. toxicarum* Miyake)
P. decumbens Thom

Section Divaricata

P. simplicissimum sensu Stolk and Samson (= *P. janthinellum* Biourge)
P. janczewskii Zaleski (= *P. nigricans* Bainier)
P. canescens Sopp

Section Furcatum

P. oxalicum Currie and Thom
P. raciborskii G. Smith
P. citrinum Thom
P. corylophilum Dierckx
P. simplicissimum (Oudem.) Thom
P. herquei Bainier and Sartory

Section Penicillium

P. arenicola Chalabuda
P. atramentosum Thom
P. aurantiogriseum Dierckx (= *P. cyclopium* Westling = *P. aurantiovirens* Biourge = *P. martensii* Biourge = *P. puberulum* Bainier)
P. aurantiogriseum var. *viridicatum* (Westling) Frisvad and Filtenborg (= *P. viridicatum* Westling)
P. brevicompactum Dierckx
P. camemberti Thom (= *P. candidum* Roger)
P. chrysogenum Thom (= *P. griseoroseum* Dierckx = *P. meleagrinum* Biourge = *P. cyaneofulvum* Biourge = *P. notatum* Westling)
P. echinulatum Raper and Thom ex Fassatiov
P. expansum Link
P. coprophilum (Berk. and Curt.) Seifert and Samson (= *P. concentricum* Samson et al.)
P. crustosum Thom
P. solitum Westling
P. digitatum (Pers.:Fr.) Sacc.
P. glandicola (Oud.) Seifert and Samson (= *P. granulatum* Bain.
P. griseofulvum Dierckx (= *P. patulum* Bainier = *P. urticae* Bainier)
P. hirsutum Dierckx
P. hordei Stolk
P. italicum Wehmer
P. olsonii Bain. and Sartory
P. roqueforti Thom
P. verrucosum Dierckx (very often cited as *P. viridicatum*)
P. vulpinum (Cooke and Massee) Seifert and Samson (= *P. claviforme* Bain.)

Subgenus Biverticillium

P. duclauxii Delacr
P. minioluteum Dierckx
P. pinophilum Hedgcock
P. funiculosum Thom
P. purpurogenum Stoll
P. verruculosum Peyronel
P. islandicum Sopp
P. variabile Sopp
P. rugulosum Thom
P. piceum Raper and Fennell
P. rubrum Stoll
P. tardum Thom (= *P. phialisporum* Udagawa)

Genus *TALAROMYCES*

T. flavus (Kloecker) Stolk and Samson
T. stipitatus C.R. Benjamin
T. luteus (Sacc.) Stolk and Samson
T. wortmanii C.R. Benjamin
T. trachyspermus (Shear) Stolk and Samson
T. thermophilus Stolk

Genus *HAMIGERA*

Hamigera striata (Raper and Fennell) Stolk and Samson (= *T. striatus* sensu Pitt)

Sources: Stolk and Samson (1972); Samson and Pitt (1985b); Pitt and Cruickshank (1990); Stolk et al. (1990)

Identification of *Fusarium*

Various species concepts exist for *Fusarium*: Wollenweber and Reinking (1935) list 142 taxa while Snyder and Hansen (1940, 1941, 1945) list only nine species. Booth (1971) lists 146 taxa, while Gerlach and Nirenberg (1988) accept 100 species and varieties. Nelson et al. (1983) and Marasas et al. (1985) propose 53 species, including many toxigenic taxa. Although the taxonomic situation in *Fusarium* is still in a state of flux, and reliable identification is difficult, mycotoxin researchers often consult the laboratory guides published by Nelson et al. (1983) and Burgess et al. (1988) for identification of toxigenic isolates. For European isolates on cereals and potatoes, a key was published by Nirenberg (1989). Nirenberg (1990) discusses the current situation of the systematics of the genus and the newly described taxa. For correct identification of Fusaria, culturing methods described in the next section are extremely important.

Recently, profiles of secondary metabolites, including mycotoxins, have also been applied to taxonomic studies of the genus *Fusarium*. Thrane (1989) and Altomare et al. (1989) provide examples suggesting that profiles of secondary metabolites seem to be consistent for species in *Fusarium* ; used in combination with morphological, physiological , and pathological characteristics this can be extremely useful. However, Thrane (1990) finds no qualitative differences between the profiles of chromophore families in *F. culmorum*, *F. graminearum*, and *F. crookwellense* . On the other hand, quantitative data (i.e. the heights of the peaks) are different, supporting the subtle morphological distinctions between the three taxa. In a detailed study of the secondary metabolites of the three species last named, with particular emphasis on the trichothecene biosynthetic family, Miller et al. (1991) also suggest that the use of quantitative differences is the only way to distinguish groups chemotaxonomically.

Media for Cultivation and Identification

For the identification of isolates of *Penicillium* and *Aspergillus* , the First International Workshop on *Penicillium* and *Aspergillus* (Samson and Pitt 1985a) recommended Czapek agar with or without yeast autolysate and Malt extract agar. Isolates should preferably be inoculated in three positions and incubated for at least 5 days at 25°C to allow optimal colony development. Recently, several problems concerning variations in the colour and patterns of colonies of *Penicillium* isolates on Czapek agar were reported. These are due to the presence of insufficient copper and zinc, and the problem can be overcome by the addition of a 0.5 g copper sulphate and 1 g zinc sulphate per 100 mL Czapek concentrate solution (1 mL per litre of media). For isolates producing teleomorphs (e.g. *Talaromyces*, *Neosartorya*, and *Emericella*) oatmeal or cornmeal agar is used to induce the formation of ascoma. Xerophilic species, particularly *Eurotium* spp. and *A. penicillioides,* must be cultivated on low water activity media e.g. Czapek and malt extract agars with additional sucrose. Formulations for these media can be found in Samson and Pitt (1985a), Pitt and Hocking (1985), Samson and Van Reenen-Hoekstra (1988), and these proceedings.

In contrast to *Penicillium* and *Aspergillus* species, which often can be identified directly from the natural substrate, the identification of *Fusarium* isolates requires cultivation on (semi) artificial media. Nutrient-rich media such as Czapek agar, Potato dextrose agar, Oatmeal agar, Malt sucrose agar etc. are not suitable for observation of their characters. The morphological characters of conidia and conidiophores are often distorted by the presence of vacuoles and granular elements within the structures. In addition, the abundant aerial mycelium produced may interfere with the observation of morphological structures. Whereas colony patterns can be best observed on Potato dextrose agar (PDA) or potato sucrose agar (PSA), typical sporulating structures can be best obtained on low nutrient media such as Carnation leaf agar (CLA; Fisher et al. 1982) or Synthetic meagre agar (SNA; Nirenberg 1976). Nirenberg (1990) has discussed the use of both media and recommends SNA agar with filter paper, because it is easier to prepare and provides information on pigments produced by many Fusaria. For the preparation of CLA, gamma-irradiated carnation leaves must be used, and the leaves are not pigmented by the *Fusarium* isolates.

For cultivation of *Fusarium* isolates it is essential to incubate the culture under either UV light or diffuse daylight.

Selective Media and Direct Identification

Several media have been devised for isolation and diagnosis of food-borne fungi. Dichloran 18% Glycerol agar (DG18; Hocking and Pitt 1980) and Dichloran rose bengal chloramphenicol agar (DRBC; King et al. 1979) are good examples of these media, which support the growth of all important species of *Penicillium*, *Aspergillus,* and several other genera. However, the procedures involved are time-consuming, requiring at least 5–7 days for initial growth on the isolation medium and 7 days for identification on diagnostic media. An alternative approach is the development of selective media, of which several have been proposed for *Penicillium* , *Aspergillus,* and *Fusarium* (Engel and Teuber 1978; Pitt et al . 1983; Frisvad 1983; Abildgren et al. 1987; Andrews and Pitt 1986; and see also Samson et al. 1991). However, only two or three media are now used in several laboratories: AFPA for the screening of *Aspergillus flavus* and *A. parasiticus* (Pitt et al . 1983), PRYES for the screening of *P. verrucosum* chemotype II (Frisvad 1983), and CZID (Czapek-Dox Iprodione Dichloran agar) for *Fusarium* (Abildgren et al. 1987).

In comparison with bacteria, the identification of

moulds is a time consuming procedure, which usually requires at least 14 days, including isolation and subculturing. Filtenborg and Frisvad (1990) have investigated the possibility of performing identification directly on the isolation media, using TLC screening of secondary metabolite profiles. They report that a number of food spoilage and mycotoxigenic moulds produce secondary metabolites on the isolation media DRBC and DG18 and that such fungi can be identified directly in mixed culture on isolation media.

Computer-Assisted Keying

For the identification of the many taxa in the genera *Penicillium*, *Aspergillus*, and *Fusarium*, computer-assisted keys can be helpful particularly for those researchers who do not carry out mycological identification on a regular routine basis. Bridge (1990), Williams (1990), and Pitt (1990a,b) have discussed and provided computer-assisted keying for some groups of *Penicillium* and *Aspergillus*. A computer key by Pitt (1990b) called PENNAME is now available. The key is basically a large synoptic key for identifying 70 common taxa. Thrane (1991) developed FUSKEY, a computer key for 17 common *Fusarium* species, based on a data matrix of approx 1200 *Fusarium* isolates using the DELTA System software (Dallwitz 1980: Dallwitz and Paine 1986), and the key-generating program INTKEY (Partridge et al. 1988).

Immunoassays

Recently, sensitive enzyme immunoassay systems and production of poly- or monoclonal antibodies which identify unique epitopes produced by *Penicillium* and *Aspergillus* were introduced. Kamphuis et al. (1989, 1991) report practical serological methods for the detection of heat stable extra-cellular polysaccharides and a latex agglutination method for *Penicillium* and *Aspergillus*. Stynen et al. (1991) describe a latex agglutination test for the detection of *Aspergillus* in the serum of patients with invasive aspergillosis . The test has now been modified for the detection of Aspergilli and Penicillia occurring in food.

Both latex tests are commercially available and although identification down to species level is not possible by this assay its practical value for a rapid detection of viable or non-viable propagules of Aspergilli and Penicillia in stored products is evident. It may also indicate presence of possible mycotoxins. Other immunoassays for detecting Mucorales and Fusaria are being developed.

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The Taxonomy and Biology of Dematiaceous Hyphomycetes and Their Mycotoxins

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Abstract

Species of about 32 dematiaceous hyphomycetes produce mycotoxins and metabolites. They include *Alternaria (Pleospora); Bipolaris (Cochliobolus); Botrytis (Botryotinia); Cercospora (Mycosphaerella); Cercosporidium (Mycosphaerella); Cladosporium; Corynespora; Curvularia (Cochliobolus); Dematophora (Rosellinia); Drechslera (Pyrenophora); Epicoccum; Exserohilum (Setosphaeria); Fulvia; Graphium; Helminthosporium; Memnoniella; Mycocentrospora; Nigrospora; Nodulisporium; Periconia; Phaeoramularia; Phymatotrichiopsis; Pithomyces (Leptosphaerulina); Pseudocercospora (Mycosphaerella); Pyricularia (Magnaporthe); Rhynchosporium; Sirosporium; Spilocaea (Venturia); Stachybotrys; Stemphylium (Pleospora); Thedgonia*; and *Zygosporium*. The majority of these toxin-producing hyphomycetes are anamorphs of bitunicate ascomycete genera but this has no relative significance except that it reflects that they have been investigated more than the other dematiaceous hyphomycete anamorph–teleomorph groups. The taxonomy and biology of some important hyphomycete genera and their toxin-producing species are discussed. More species in the genera *Alternaria, Bipolaris, Cercospora, Curvularia, Drechslera*, and *Exserohilum* have been investigated for mycotoxins than those in the other genera

Mycotoxins Producing Genera and Their Species

ALTERNARIA Nees

Teleomorph: *Pleospora* Rabenh. ex Ces. & de Not. (Sivanesan 1984).

SPECIES of *Alternaria* are present universally in a wide variety of habitats. Many of them are common plant pathogens infecting different kinds of food crops and often contaminate grain crops, hay, and silage in the field. Several toxins have been isolated from culture filtrates of many species. The genus is characterised by effuse, usually grey to black colonies consisting of submerged to partly superficial mycelia bearing solitary to fasciculate conidiophores with cicatrised, sympodial, terminal to intercalary, polytretic conidiogenous cells. Conidia are borne singly or in chains, typically obovoid or obclavate, often rostrate, smooth to verrucose, brown, and muriform.

The metabolites produced by *Alternaria* species belong to quinones, dibenzo-α-pyrones, dihydropyrone, polypeptides, tetramic acids, other heterocyclic compounds, pentasubstituted benzene, and perylene derivatives. The mycotoxins may be host-specific or non host-specific. *A. alternata* (Fr.) Keissler, *A. kikuchiana* Tanaka, and *A. mali* Roberts are known to produce host-specific toxins. Reviews on *Alternaria* toxins and their structures are found in Harven and Pero (1976), Schade and King (1984), and Templeton (1972). Most of the species have been described and illustrated by Ellis (1971, 1976) including their distribution and hosts. The species are separated on the basis of conidial morphology and host.

Several metabolites and toxins have been isolated from *A. alternata* and they include AF-toxins I and II (Maekawa et al. 1984), alkaloids (Rizk et al.1985), alternariol (Raistrick et al. 1953; Logrieco et al. 1990), alternariol methyl ether (Pero and Main 1969; Thomas 1961; Tirokata et al. 1969), alternariolide (Ueno et al. 1975a), altenuene (Pero et al. 1971; Rizk et al. 1985), altenuic acid I, II and III, altenusin (Rosett et al. 1957), altenuisol (Pero et al. 1973), altertenuol (Rosett et al. 1957), altertoxins I, II, and III (Chu 1981; Stack et al. 1986), AK-toxins I and II (Tadakazu et al. 1985), AM-toxins I and II (Ueno et al. 1975b), cutinase (Tanabe et al. 1988), dehydro-altenusin (Rosett et al. 1957), dihydrotentoxin (Liebermann et al. 1988), isoaltenuene (Visconti et al. 1988), mannitol (Coombe et al. 1970), HS maculosin (Stierle et al. 1988), tentoxin (Templeton et al. 1967), and tenuazonic acid (Rosett et al. 1957; Janardhanan and Husein 1975).

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Species of *Alternaria* (excluding *A. alternata*) and their metabolites

Species	Metabolites	Reference
A. angustiovoidea Simmons	Phytotoxins	Yang et al. 1990.
A. brassicae (Berk.) Sacc.	Desmethyldestruxin	Ayer and Pena-Rodriguez 1987.
	Destruxin	
	Homodestruxin	
A. brassicicola (Schwein.) Wiltshire	Brassicicolin A	Ciegler and Lindenfelser 1969.
A. carthami Chowdhury	Brefeldin A	Tietjen et al. 1983.
	7-dehydrobrefeldin A	
	Zinniol	
A. cassiae Jurair & Khan	Altertoxin I and II	Hradil et al. 1989.
	Alterperylenol	
	Stemphyperylenol	
	Stemphyltoxin	
A. chrysanthemi Simmons & Crosier	Radicinin	Robeson et al. 1982.
	Radicinol	
A. cinerariae Hori & Enjoji	Curvularin	Robeson and Strobel 1981.
	Dehydrocurvularin	
A. citri Ell. & Pierce	Alternariol	Freeman 1965.
	Alternariol methyl ether	
	α, β-dehydrocurvularin	Starratt and White 1968.
	Host-specific toxins	Kohmoto et al. 1979.
	Tenuazonic acid	Kinoshita et al. 1972.
A. cucumerina (Ell. & Ev.) Elliott	α, β-dehydrocurvularin	Starratt and White 1968.
	Alternariol	Raistrick et al. 1953.
	Alternariol methyl ether	Freeman 1965.
A. dauci (Kühn) Groves & Skolko	Alternariol	Raistrick et al. 1953.
	α, β-dehydrocurvularin	Starrett and White 1968.
	Alternariol methyl ether	Freeman 1965.
	Zinniol	Barash et al. 1981.
A. eichorniae NagRaj & Ponnappa	Alteichin	Robeson et al. 1984;
	Bostrycin	Okuno et al. 1983;
	4-deoxybostrycin	Stevens et al. 1979; Charudattan and Rao 1982.
	unidentified toxin	Maity and Samaddar 1977.
A. helianthi (Hansf.) Tubaki & Nishihara	Deoxyradicinin	Robeson and Strobel 1982b,
	Deoxyradicinol	1985; Tal et al. 1985;
	3-epideoxyradicinol	
	Radicinin, radianthin	
A. japonica Yoshi	Tenuazonic acid	Kinoshita et al. 1972.
A. kikuchiana Tanaka	Altenin	Sugiyama et al. 1965 1966;
	Host-specific AK-toxin	Hiroe and Aoe 1954;
	Host-specific Phyto-alternarin A, B and C	Kameda et al. 1973.
	3, 6, 8-trihydroxy-3-methyl-3, 4-dihydro-isocoumarin, alternariol	
	Alternariol monomethyl ether	Tirokata et al. 1969.
	Succinic acid	Sugiyama et al. 1966.
	Citrinin	Park 1977
	Tenuazonic acid	Kinoshita et al. 1972.

Species of *Alternaria* (excluding *A. alternata*) and their metabolites (cont'd)

Species	Metabolites	Reference
A. longipes (Ell. & Ev.) Mason	Tenuazonic acid	Mikami et al. 1971; Ramm and Lucas 1963.
	Cellulase, polygalacturonase	
	Pectimethylesterase	
A. macrospora Zimm.	α, β-dehydrocurvularin	Robeson et al. 1985.
A. mali Roberts	Host-specific alternariolide	Okuno et al. 1974.
	Host-specific AM-toxin I, II, III	Ueno et al. 1975a,b,c.
	Tentoxin	
	Tenuazonic acid	Kinoshita et al. 1972.
A. oryzae Hara	Tenuazonic acid	Kinoshita et al. 1972.
A. porri (Ell.) Cif.	Altersolanol A	Suemitsu and Nakamara 1981.
	Erythroglaucin	Suemitsu et al. 1977a.
	Macrosporin	Suemitsu et al. 1961.
	Physcion	Suemitsu et al. 1975.
	2-methylxanthopurpurin -7-methyl ether	Suemitsu et al. 1974.
	3, 4, 5-trihydroxy-7-methoxy-2-methylanthraquinone	Suemitsu et al. 1977b.
	Alternaric acid, zinniol	Yu et al. 1983.
A. radicina Meier	(-)-7-hydroxy-4-oxo-oct-2-enoic acid lactone	Aldridge and Grove 1964.
	Radicinin	Grove 1964.
A. scirpicola (Fuckel) Sivan. Teleomorph: *Pleospora scirpicola* (DC.) Karsten	Dehydrocurvularin	Assante et al. 1977b.
	cis-4, 6-dihydroxy-mellein	
	(-)-11-acetaldehydro-curvularin	
A. solani Sorauer	Alternaric acid	Brian et al. 1949.
	Alternarin	Darpoux et al. 1950.
	Altersolanol A	Stoessl 1967; Holenstein and
	Altersolanol B, C	Stoessl 1983.
	Anthraquinone A, B, C	Stoessl 1967, 1969.
	Macrosporin	Stoessl 1969.
	Tenuazonic acid	
	Zinniol	Stoessl et al. 1983.
A. tagetica Shome & Mustafee	Zinniol	Cotty et al. 1983.
A. tenuissima (Kunze : Pers.) Wiltshire	Tenuazonic acid	Davies et al. 1977.
A. tomato (Cke) Jones	Adenosine	Hyeon et al. 1976.
	β-Dehydrocurvularin	
	β-hydroxycurvularin	
A. triticina Prasada & Prabhu	unidentified toxins	Vijay-Kumar and Rao 1979.
A. zinniae M.B. Ellis	Mannitol	White and Starratt 1967.
	Zinniol	Starrett 1968.

BIPOLARIS Shoem.

Teleomorph: *Cochliobolus* Drechsler (Sivanesan 1987).

Conidia acropleurogenous, fusoid, obpyriform, navicular, oblong, cylindrical, obclavate, clavate, ovoid, solitary, curved to straight, mostly smooth, 2-or more distoseptate, septa sometimes thickened and dark, various shades of brown, germinating mainly from one or both polar cells with the basal germ tube originating close to the hilum and growing semiaxially, hilum associated with a slightly protruding, truncate section of the wall or rarely protuberant, borne on terminal or intercalary, sympodially proliferating, cicatrised conidiogenous cells of the conidiophore. First conidial septum is median to submedian, second septum delimiting the basal cell and the third septum is distal. *Bipolaris* species are mainly graminicolous pathogens causing diseases like leaf spots, seedling blights and foot and root rots of economically important cereals. Toxins have been implicated as important causal factors in the development of a number of devastating diseases of economically important cereals. Southern leaf blight of maize caused by *B. maydis* and the Bengal famine in India initiated by *B. oryzae* are two prime examples. The species are separated by their conidial size, shape, and number of septa in the conidium. A monograph of graminicolous *Bipolaris* species by Sivanesan (1987) giving full descriptions, illustrations, geographical distribution, and hosts should be consulted for details of species discussed here. Many species produce phytopathogenic toxins of which the host-specific HS toxin victorin produced by *B. victoriae* is by far the most potent and selective toxin known. Metabolites produced by species of this genus include pigments polyhydroxyanthraquinones and polyhydroxyxanthones, ophiobolins, spiciferone, sterigmatocystin, cytochalasin, host-specific (pathotoxins) and non host-specific toxins (phytotoxins). Ophiobolins were given many different names and their nomenclature has been clarified by Tsuda et al. (1967), and a further elucidation of their structure and nomenclature are also provided by Shotwell and Ellis (1976). Earlier reviews on metabolites and toxins produced by species of this genus have been discussed under the old name of *Helminthosporium* (Luke and Gracen, 1972; Pringle 1964; Shotwell and Ellis 1976).

Species of *Bipolaris* and their metabolites

Species	Metabolites	Reference
B. cynodontis (Marignoni) Shoem.	Bipolaroxin Dihydrobipolaroxin	F. Sugawera et al. 1985
Teleomorph: *C. cynodontis* Nelson	Cynodontin Helminthosporin	Raistrick et al. 1933. Raistrick et al. 1934.
B. ellisii (Danquah) Alcorn	Curvulin	Coombe et al. 1968.
Teleomorph: *C. ellisii* Alcorn	Curvulinic acid O-methylcurvulinic acid	
B. euchlaenae (Zimm.) Shoem.	Cynodontin	Raistrick et al. 1933.
B. hawaiiensis (M.B. Ellis) Uchida & Aragaki	Unidentified toxin	Hirenath et al. 1984.
Teleomorph: *C. hawaiiensis* Alcorn		
B. indica Rai, Wadhwani & Tewari	Curvulin O-methylcurvulinic acid	Kenfield et al. 1989.
B. leersiae (Atk.) Shoem.	Ophiobolin A Alboleersin Luteoleersin	Canonica et al. 1966a. Ashley and Raistrick 1938.
B. maydis (Nisikado & Miyake) Shoem.	Four host-specific toxin of race T	Payne and Yoder 1978; Karr et al. 1974, 1975.
Teleomorph: *C. heterostrophus* (Drechsler) Drechsler	Ophiobolin A Ophiobolin B Ophiobolin C Ophiobolin F Anhydroophiobolin A 6-epiophiobolin A Geranylnerolidol	Canonica et al. 1966a; Tsuda et al. 1967; Nozoe et al. 1965, 1966. Ishibashi 1962c. Cordell 1974. Canonica et al. 1966b. Sugawera et al. 1987. Nozoe et al. 1968.

Species of *Bipolaris* and their metabolites (cont'd)

Species	Metabolites	Reference
B. nodulosa (Berk. & M.A. Curtis) Shoem.	Averufin	Aucamp and Holzapfel 1970.
	Bipolarin, Curvularin	
Teleomorph: *C. nodulosus* Luttr.	Sterigmatocystin	Holzapfel et al. 1966.
	Unidentified toxin	Vidhyasekaran 1977.
B. oryzae (Breda de Hann) Shoem.	Ophiobolin B	Nakamura and Ishibashi 1958; Orsenigo 1957.
Teleomorph: *C. miyabeanus* Ophiobolin A (Ito & Kurib.) Drechsler ex Dastur	Ophiobolin I, 6-epiophiobolin, Anhydroophiobolin, 6-epianhydroophiobolin	Xiao et al. 1991.
	Ophiobolin A	Ohkawa and Tamura 1966.
	Cynodontin	
	Dihydroxy and trihydroxy-methylanthraquinones	Johnson and White 1969.
	Ergosterol	Murty and Subramanian 1959.
B. panici-miliacei (Nisikado) Shoem.	Ophiobolin A	Canonica et al. 1966a; Nozoe et al. 1965; Tsuda et al. 1967.
B. papendorfii (van der Aa) Alcorn	Curvulin	Coombe et al. 1968.
	Curvulinic acid	
	O-methyl-curvulinic acid	
	Curvularin	
B. ravenelii (M.A. Curtis) Shoem. Teleomorph: *C. ravenelii* Alcorn	Ravenelin	Raistrick et al. 1936.
B. sacchari (E. Butler) Shoem.	Helminthosporoside	Beier et al. 1982; Steiner and Strobel 1971.
	3 isomeric host-specific toxins	Macko et al. 1983.
B. setariae (Saw.) Shoem. Teleomorph: *C. setariae* (Ito & Kurib.) Drechsler ex Dastur	Ophiobolin A	Nukina and Marumo 1976.
B. sorghicola (Lefebvre & Sherwin) Alcorn	Ophiobolin	Sugawera et al. 1987.
	Ophiobolin I	
	6-epiophiobolin	
B. sorokiniana (Sacc.) Shoem. Teleomorph: *C. sativus* (Ito & Kurib.) Drechsler ex Dastur	Helminthosporol	Aldridge and Turner 1970a; De Mayo et al. 1961.
	Helminthosporol I	
	Helminthosporal II	White and Taniguchi 1972.
	9-hydroxyhelminthosporal	
	9-hydroxyprehelminthosporal	Aldridge and Turner 1970a.
	Cynodontin	
	Victoxinine	Dorn and Arigoni 1972.
	Prehelminthosporal	Nukina et al. 1975.
	Prehelminthosporal cis-sativenediol	Nukina et al. 1975.
	(-)-sativene	De Mayo and Williams 1965.
B. spicifera (Bainier) Subram. Teleomorph: *C. spicifer* Nelson	Spiciferone A	Nakajima et al. 1989.
	Cynodontin	Coombe et al. 1968.
	Curvularin	
	D-mannitol	
B. victoriae (Meehan & Murphy) Shoem. Teleomorph: *C. victoriae* Nelson	Cynodontin	Pringle 1958.
	Gliovictin	Dorn and Arigoni 1974.
	Victorin (HS)	Wheeler and Luke 1954.
	Victoxinine	Nishimura et al. 1966;
	Victoricine	Pringle and Braun 1960.
	Victorin B, D and E	Wolpert et al. 1986.
B. zeicola (Stout) Shoem.	HC-toxins I, II, III, IV (HS, polypeptide)	Ramussen and Scheffer 1988.

Species of *Bipolaris* and their metabolites (cont'd)

Species	Metabolites	Reference
Teleomorph: *C. carbonum* Nelson	Carbtoxinine	Pringle and Scheffer 1967b.
	Victoxinine	Nishimura et al. 1966. Scheffer and Ullstrup 1965.
B. zizaniae (Nisikado) Shoem.	Ophiobolin A	Canonica et al. 1966a;
	Ophiobolin B	Nozoe et al. 1965, 1966; Ishibashi 1962a.
	Ophiobolin C	Nozoe et al. 1966.
	Anhydroophiobolin A	Cordell 1974.

CERCOSPORA Fres.

Teleomorphs: *Mycosphaerella* Johanson and *Sphaerulina* Sacc. (Sivanesan 1984).

Conidiophores fasciculate, sometimes geniculate above, brown, straight to flexuous, often formed on a stroma. Conidiogenous cells integrated, terminal, polyblastic, sympodial, cicatrised with thickenings extending over the whole of the scar except for a small pore. Conidia dry, solitary, simple acropleurogenous, obclavate to subulate, multiseptate, hyaline to pale brown, smooth.

Cercospora is a well known plant pathogen with several species responsible for leaf spot diseases of many plants. The delimitation of the species is mainly based on hosts and conidial morphology. Descriptions, illustrations, geographical distribution, and hosts of almost all species producing metabolites are found in Ellis (1976) and Chupp (1954). Many species of *Cercospora* produce the phytotoxic and bacteriostatic pigment, cercosporin, and they include *C. apii* Fres. (Lynch and Geoghegen 1977; Fajola 1978), *C. arachidicola* Hora the anamorph of *Mycosphaerella arachidis* Deighton (Abo-El-Dahab et al. 1986; Melouk and Schuh 1987), *C. ariminensis* Cavara, *C. bertoreae* Hollós (Assante et. al. 1977b), *C. beticola* Sacc. (Daub 1982), *C. bizzozerina* Sacc. (Assante et. al. 1977b), *C. canescens* Ell. & Mart. (Fajola 1978), *C. caricis* Oud. (Blaney et al. 1988), *C. carotae* (Pass.) Solheim, *C. chenopodii* Fresen, *C. cistinearum* Sacc. (Assante et al. 1977b), *C. citrullina* Cke (Fajola 1978), *C. coffeicola* Berk. & Cke (Lynch and Geoghegen 1977), *C. dulcamare* (Peck) Ell., *C. erysimi* Davies (Assante et al. 1977b), *C. hayi* Calpouz (Mumma et al. 1973), *C. kikuchii* Matsumoto & Tomayasi (Kuyama and Tamura 1957; Fajola 1978; Yamazaki et al. 1975), *C. malvacearum* Chiddarwar, *C. malvicola* Ellis, *C. medicaginis* Ell. & Ev., *C. microsora* Sacc. (Assante et al. 1977b), *C. nicotianae* Ell. & Ev. (Daub 1982; Fajola 1978), *C. oryzae* Miyake the anamorph of *Sphaerulina oryzae* Hara, *C. plantaginis* Sacc. (Assante et. al. 1977b), *C. ricinella* Sacc. & Berl. (Fajola 1978), *C. rosicola* Pass. the anamorph of *Mycosphaerella rosicola* Davies ex Deighton, *C. sesami* Zimm. the anamorph of *Mycosphaerella sesami* Sivan., *C. setariae* Atk., *C. violae* Sacc. (Assante et al. 1977b), and *C. zebrina* Pass. (Lynch and Geoghegen 1977).

Cercosporin appears to be involved in the necrosis of sugar beet by *C. beticola* (Balis and Payne 1971) and the worldwide distribution of cercosporin producing species of the genus seems to indicate its global phytopathogenicity.

Species of *Cercospora* producing metabolites excluding Cercosporin

Species	Metabolites	Reference
C. arachidicola Hori	Anthraquinoid	Stoessl and Stothers 1985.
	Long-chain fatty aldehyde	Stoessl 1985.
	Averufin, Dothistromin	Stoessl 1984.
C. bertoreae Hollós	*Cercospora* beticola toxin (CBT)	Assante et al. 1977b
C. beticola Sacc.	*Cercospora* beticola toxin (CBT)	Schlösser 1971.
	Triglycerides	Balis and Payne 1971.
C. microsora Sacc.	Dothistromin	Assante et al. 1977b.
C. oryzae Miyake	Unidentified yellow substance	Lu 1985.

Species of *Cercospora* producing metabolites excluding Cercosporin (cont'd)

Species	Metabolites	Reference
C. rosicola Pass.	Dothistromin	Assante et al. 1977b.
	Absicic acid	Assante et al. 1977c.
C. setariae Atk.	Cercosporin esters	Assante et al. 1977b.
C. smilacis Thüm.	Dothistromin, 2-epidothistromin Averufin, Averythin	Assante et al. 1977a.
C. traversiana Sacc.	Traversianal	Stoessl et al. 1989.

CURVULARIA Boedijn

Teleomorph: *Cochliobolus* Drechsler (Sivanesan 1987).

Conidia solitary, often curved, acropleurogenous, broadly fusoid, obovoid, or obpyriform, elliptical, smooth, sometimes verruculose, echinulate or tuberculate, 3- or more septate, hilum may or may not be protuberant, with or without a disproportionately swollen cell which is usually more pigmented than the other cells, septa sometimes accentuated with dark band in some or all the cells. In conidial germination and septum ontogeny it is similar to *Bipolaris*.

Six species are known to produce metabolites. For descriptions, illustrations, distribution, hosts, and the *Cochliobolus* teleomorphs of these species refer to Sivanesan (1987).

Species of *Curvularia* producing metabolites

Species	Metabolites	Reference
C. geniculata (Tracy & Earle) Boedijn Teleomorph: *C. geniculatus* Nelson	1, 4, 5, 8-tetrahydroxy-2, 6-dimethylanthraquinone	Coombe et al. 1968.
C. inaequalis (Shear) Boedijn	1, 4, 5, 8-tetrahydroxy-2, 6-dimethylanthraquinone	Coombe et al. 1968.
C. lunata (Wakker) Boedijn	Brefeldin A	Coombe et al. 1968.
	D-mannitol	
Teleomorph: *C. lunatus* Nelson & Haasis	Anthraquinone	Bohlmann et al. 1961.
	Cytochalasin B	Wells et al. 1981.
	Cynodontin	Eijk and Roeymans 1977.
	Radicinol	Nukina and Maruma 1977.
C. lunata var. *aeria* (Bat., Lima & Vasconcelos) M.B. Ellis	Thermostable toxin	Bisen 1983.
C. pallescens Boedijn Teleomorph: *C. pallescens* (Tsuda & Ueyama) Sivan.	Unidentified toxin	Olufolaji 1986.
C. trifolii (Kauffm.) Boedijn	1, 4, 5, 8-tetrahydroxy-2, 6-dimethylanthraquinone	Coombe et al. 1968.

DRECHSLERA Ito

Teleomorph: *Pyrenophora* Fr. (Sivanesan 1987).

Conidia commonly more less cylindrical, straight, multi-distoseptate, germinating from all cells or from polar cells with basal germ tube originating clear of the hilum and growing at wide angle to the longitudinal axis, hilum inserted within a rounded basal contour, borne on cylindrical, geniculate, cicatrised, sympodial conidiogenous cells of the conidiophores. First conidium septum delimiting the basal cell, second septum more or less median and the third distal.

Many species are plant pathogens of cereals causing eye spots, pre-emergence and post-emergence seedling blight, leaf stripes, leaf spots, and blotch, and are also seed-borne. Toxin producing species and their *Pyrenophora* teleomorphs are fully described and illustrated with hosts and geographical distribution by Sivanesan (1987).

Species of *Drechslera* producing metabolites

Species	Metabolites	Reference
D. avenae (Eidam) Scharif	Cynodontin	
	(-)-dihydropyrenophorin	Sugawera and Strobel 1986.
Teleomorph:	Pyrenophorin	Ishibashi 1961a; Lerario
P. chaetomioides Speg.	Siccanin	and Graniti 1985.
D. campanulata (Lév.) B. Sutton	Unidentified toxin	Westhuizen et al. 1985.
Teleomorph: *P. semeniperda* (Brittlebank & Adam) Shoem.		
D. catenaria (Drechsler) Ito	Catenarin	Raistrick et al. 1934.
	Chrysophanol, Emodin Polyhydroxyanthraquinones	Eijk 1974.
	Helminthosporin	Raistrick et al. 1934.
	Erythroglaucin	Anslow and Raistrick 1940.
D. dematioidia (Bubák & Wróblewski) Subram. & Jain	Cytochalasin A, B, E, F	Aldridge et al. 1967.
D. gigantea (Heald & Wolf) Ito	Gigantenone	Kenfield et al. 1989.
	Petasol and 11 other eremophilanes	Bunkers et al. 1991.
D. graminea (Rabenh. ex Schlecht.) Shoem.	Catenarin	Raistrick et al. 1934.
	Helminthosporin	Charles et al. 1933.
Teleomorph: *P. graminea* Ito & Kurib.		
D. siccans (Drechsler) Shoem.	De-O-methyldiaporthin	Hallock et al. 1988.
	Helmintin	Inagaki 1962.
Teleomorph: *P. lolii* Dovaston	Siccanin	Ishibashi 1962b.
D. teres (Sacc.) Shoem.	N-aspartic acid	Sugawera and Strobel 1986.
Teleomorph: *P. teres* Drechsler	Anhydroaspergillomarasmine A Aspergillomarasmine A	Bach et al. 1979.
D. tritici-repentis (Died.) Shoem.	Catenarin	Raistrick et al. 1934.
	Helminthosporin	Raistrick et al. 1934.
Teleomorph:	Tritisporin	Raistrick et al. 1934;
P. tritici-repentis (Died.) Drechsler		Neelakantan et al. 1956.
	Ptr. toxin	Tomas et al. 1990.

EXSEROHILUM Leonard & Suggs

Teleomorph: *Setosphaeria* Leonard & Suggs (Sivanesan 1987).

Conidia fusiform, cylindrical or obclavate, straight to curved, multi-distoseptate, germinating from polar cells, basal germ tube semiaxial from near the hilum, hilum strongly protruding, borne on sympodial, polyblastic, cicatrised conidiogenous cells of the conidiophores. First conidial septum submedian, second septum distal and the third median to submedian.

Graminicolous plant pathogens causing leaf spot and stripes. Four species are known to produce metabolites. For descriptions, illustrations, distribution, hosts, and the *Setosphaeria* teleomorphs of these species refer to Sivanesan (1987).

Species of *Exserohilum* producing metabolites

Species	Metabolites	Reference
E. holmii (Luttr.) Leonard & Suggs	Exserohilone	K. Sugawera et al. 1985.
Teleomorph: *S. holmii* (Luttr.) Leonard & Suggs		
E. monoceras (Drechsler) Leonard & Suggs	Monocerin	Aldridge and Turner 1970b.
Teleomorph: *S. monoceras* Alcorn		

Species of *Exserohilum* producing metabolites (cont'd)

Species	Metabolites	Reference
E. rostratum (Drechsler) Leonard & Suggs	Glyceollin	Kumar et al. 1984.
	Cynodontin	Eijk and Roeymans 1977.
Teleomorph: *S. rostrata* Leonard		
E. turcicum (Pass.) Leonard & Suggs	Monocerin	Robeson and Strobel 1982a.
	Ophiobolin A	Canonica et al. 1966a,b; Nozoe et al. 1965; Ishibashi 1961b.
Teleomorph: *S. turcica* (Luttr.) Leonard & Suggs	Ravenelin	Raistrick et al. 1936.

Other genera of dematiaceous hyphomycetes which have been reported to produce toxins are listed below. The generic and specific descriptions, illustrations, distribution, and hosts of most of these fungi are available in Ellis (1971, 1976). In addition, unidentified toxins have been isolated from a number of other species of other genera of dematiaceous hyphomycetes including *Epicoccum* (Schol-Schwarz 1959), *Fulvia* (Lazarovitis et al. 1979; Lazarovitis and Higgins 1979), *Graphium* (Charya and Reddy 1983), *Memnoniella* (Sinha 1979), *Phymatotrichiopsis* (Mabellos and Lyda 1988), and *Spilocaea* (Orsenigo and Zangrandi 1959).

Species of other genera of dematiaceous hyphomycetes producing metabolites

Species	Metabolites	Reference
Botrytis cinerea Pers.	Absicic acid	Morooko et al. 1986.
Teleomorph: *Botryotinia fuckeliana* (de Bary) Whetzel	Botrydial	Fehlhaber et al. 1974
	Botrylacton	Welmer et al. 1979.
	Citric acid	Kamoen and Jamart 1974.
	Thermostable toxin	Lyon 1977.
B. fabae Sardiña	Thermostable toxins	Harrison 1980.
Cercosporidium personatum (Berk. &M.A. Curtis) Deighton	Cercosporin	Abo-El-Dahab et al. 1986; Assante et al. 1977b; Venkataramani 1967.
(*Cercospora personata*)	Dothistromin	Ramanujam and Swamy 1985.
Teleomorph: *Mycosphaerella berkleyi* W.A. Jenkins	3, 4-dihydro-4-deoxydothistromin	Gnanamanickam and Stoessl, 1986.
	Glycopeptide	Rajagopal and Vittal Rai 1983; Ramanujam and Swamy 1984.
Cladosporium cucumerinum Ell. &Arth.	Cladochrome A, B	Arnone et al. 1988; Overeem and Sijpesteijn 1967.
C. herbarum (Pers.) Link	Mycosporins	Boulillant et al. 1981.
C. cladosporioides (Fres.) de Vries	Cladospolide A	Mori and Maemoto 1987.
C. phlei (C.T. Gregory) de Vries	Phleichrome	Yoshihara et al. 1975; Shimanuki and Araki 1983.
	Iso-phleichrome	
	Rubellin	
Corynespora cassiicola (Berk. & M.A. Curtis) Wei (*Cercospora melonis*)	3-methoxy-2, 5, 7 trihydroxymethoxy-1, 4-naphthaquinone	Assante et al. 1977b.
Dematophora necatrix Hartig	Cytochalasin E	Aldridge et al. 1967;
	p-hydroxyphenyl	Sawai et al. 1983;
Teleomorph: *Rosellinia necatrix* (Hartig) Berl. ex Prill.	acetic acid	Chen 1960.
	Dioxypiperazones	
	Gliotoxin	
Helminthosporium velutinum Link	Catenarin	Raistrick et al. 1934.
	Ergosterol	
Mycocentrospora acerina (Hartig) Deighton (*Cercospora cari*)	Cynodontin	Assante et al. 1977b.

Species of other genera of dematiaceous hyphomycetes producing metabolites (cont'd)

Species	Metabolites	Reference
M. cladosporioides (Sacc.) P. Costa ex Deighton (*Cercospora cladosporioides*)	Cercosporin	Assante et al. 1977b.
Mycovellosiella ferruginea (Fuckel) Deighton (*Cercospora ferruginea*)	Dothistromin	Assante et al. 1977b.
Nigrospora sphaerica (Sacc.) Mason	Aphidicolin	Starratt and Loschiavo 1974.
Nodulisporium hinnuleum G. Smith	Demethoxyviridiol	Ueno and Ueno 1978.
Periconia circinata (Mangin) Sacc.	Aspartic acid Multiple host-specific toxins	Wolpert and Dunkle 1980. Scheffer and Pringle 1961 Pringle and Scheffer 1967a.
Phaeoramularia capsicicola (Vassiljevsky) Deighton (*Cercospora unamunoi*)	Cercosporin	Assante et al. 1977b.
Pithomyces chartarum (Berk.& M.A. Curtis) M.B. Ellis Teleomorph: *Leptosphaerulina chartarum* Roux	Sporidesmins Sporidesmolides	Leigh and Taylor 1976; Ueno and Ueno 1978; Done et al.
Pseudocercospora columnaris (Ell. & Ev.) Yen	Cercosporin	Fajola 1978.
P. taiwanensis (Matsumoto & Yamamoto) Yen	Taiwapyrone Mellein 4-hydroxymellein	Assante et al. 1977b. Camarda et al. 1976.
Pseudocercosporella capsellae (Ell. & Ev.) Deighton	Cercosporin	Petri and Vanterpool 1978.
Pyricularia oryzae Cav. Teleomorph: *Magnaporthe grisea* (Hebert) M.E. Barr	Pyriculol Pyricularin α-picolonic acid Tenuazonic acid Terrestric acid 3, 4-dihydro-3, 4-8-trihydroxy-1 (2H)-naphthalenone	Tamari and Kaji 1954. Sridhar and Mahadevan 1979. Lebrun et al. 1988. Nukina 1988. Iwasaki et al. 1972.
Rhynchosporium secalis (Oud.) J.J. Davies	HS Rhynchosporoside Mannose, rhamnose, galactose, glucosamine	Auriol et al. 1978. Mazars et al. 1989.
Sirosporium diffusum (Heald & Wolf) Deighton (*Cercospora fusca*)	Dothistromin	Assante et al. 1977b.
Stachybotrys albipes (Berk. & Br.) Jong & E.E. Davies	Verrucarin J Satratoxin H Trichoverrols A, B	El-Maghraby et al. 1991.
S. atra Corda	Verrucarin J Roridin E, Mellein Satratoxin E, F, G, H Trichoverrols A, B Stachybotryotoxin A, B Verrucarol	Jarvis et al. 1986; El-Maghraby et al. 1991; Eppley et al. 1977 1980; Ueno and Ueno 1978. Bata et al. 1985.

Species of other genera of dematiaceous hyphomycetes producing metabolites (cont'd)

Species	Metabolites	Reference
S. kampalensis Hansf.	Verrucarin J Roridin E Satratoxin F, G, H Trichoverrols A, B	El-Maghraby et al. 1991.
S. microspora (Mathur & Sankhla) Jong & E.E. Davies	Verrucarin J Satratoxin H Trichoverrols A, B	El-Maghraby et al. 1991.
Stemphylium botryosum Wallr.	Stemphyltoxin I, II, III and IV	Barash et al. 1982, Manulis et al. 1984; Arnone et al. 1986; Barash et al. 1975.
Teleomorph: *Pleospora herbarum* (Fr.) Rabenh. ex Ces. & de Not.	Stemphylin (altersolanol A) Stemphyperylenol	Assante and Nasini 1987. Arnone et al. 1986.
S. sarciniforme (Cav.) Wiltshire	Stemphone	Ueno and Ueno 1978.
Thedgonia ligustrina (Boerema) B. Sutton (*Cercospora ligustrina*)	Ligustrone A, B, C Perylenequinones	Assante et al. 1977b; Arnone et al. 1975.
Zygosporium masonii S. Hughes	Zygosporin A (cytochalasin D)	Aldridge and Turner 1969; Hayakawa et al. 1968; Minato and Matsumoto 1970.
	Zygosporin D, E, F, G	Minato and Katayama 1970.

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Isolation and Identification of Xerophilic Fungi in Stored Commodities

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Abstract

Fungi which grow in stored commodities are xerophilic, that is, capable of growing at reduced water activity (a_W). Some xerophilic fungi either will not grow or grow only very slowly at the high a_W values of media traditionally used to enumerate yeasts and moulds in foods and feedstuffs. Populations of xerophiles may therefore be underestimated or go undetected if the media and methods normally used for detecting fungi in foods are not modified.

Because of the wide range of a_W values over which various food spoilage fungi will grow, the choice of a plating medium is very important, as it will determine the types of fungi which will be isolated or enumerated. The characteristics of the commodity being examined should be critically assessed, and a medium chosen which reflects those characteristics. High a_W media are suitable for high a_W foods such as meat, seafood, fruits, vegetables, salads etc., but for stored commodities like grains, nuts , and spices, and dried and intermediate moisture foods such as confectionery, dried fruits, and dried meat and seafood products, high a_W media will enumerate only the surface mycoflora, and will not detect the significant fungi that are capable of growing in the product.

If a medium of reduced a_W is used, then type of solute is the next consideration. For foods which are high in sugar, a glucose or glycerol based medium may be most suitable, while for salty foods, the use of a reduced a_W medium containing some salt (but not necessarily based entirely on salt) may be more appropriate.

For general purpose enumeration of xerophilic fungi in stored commodities, a glycerol based medium, Dichloran 18% glycerol agar (DG18) is recommended. For enumeration of spoilage fungi in dried, salted seafood or meat products, a medium containing some salt is preferable. For this purpose, Malt extract Yeast extract 5% NaCl 12% glucose agar (MY5-12, a_W 0.953) has been developed. For foods which contain high sugar concentrations, and are subject to spoilage by extreme xerophiles, Malt extract Yeast extract 50% Glucose agar (MY50G, a_W 0.89) is recommended.

FUNGAL spoilage of stored commodities is due almost entirely to growth of xerophilic fungi; that is, fungi which are capable of growth at water activity (a_W) 0.85 or below. The growth of microorganisms in foods is controlled by many factors, one of the most important being a_W, but interactions with pH also play a part (Fig. 1). If the a_W of foods or commodities is below 0.90 or the pH is less than 4.5, then few bacteria will grow. If the a_W of a food or commodity is below 0.85, then spoilage will be caused almost exlusively by moulds and yeasts. Foods of reduced a_W are often referred to as intermediate moisture foods, and their a_W is generally between 0.85 and 0.60. Commodities with a_W below 0.60 are microbiologically stable, as there are no microorganisms capable of growth at a_W values so low. Intermediate moisture foods can be separated into several groups on the basis of the types of fungi that are able to grow in them and cause spoilage (Hocking 1991). These are:

1. stored commodities — cereals, oilseeds, nuts, and spices;
2. high sugar foods — confectionery, dried fruits, and jams;
3. dried meats and meat products; and
4. dried seafood products.

The mycoflora of stored commodities and of dried meats is fairly similar. Usually, *Eurotium* species are dominant, with the *Aspergillus restrictus* series also very

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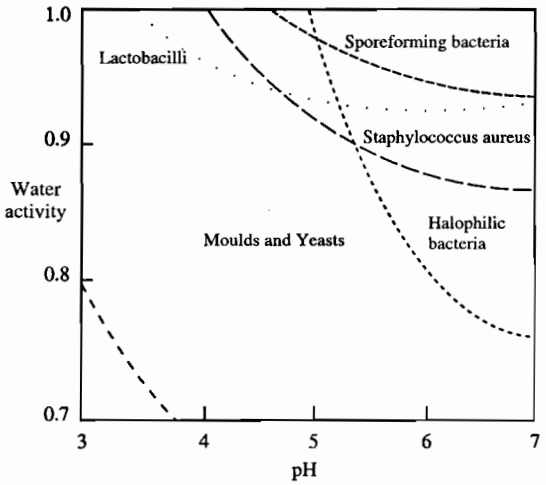

Fig. 1. Diagrammatic representation of the effects of interactions between pH and a_W on microbial growth. In foods of high a_W and neutral pH, bacteria will be the dominant spoilage organisms. As the a_W is decreased, yeasts and moulds become increasingly important as spoilage agents, their only competition below about $0.85a_W$ being halophilic bacteria in salty foods.

common. These two groups are the primary invaders of stored commodities, with minimum a_W for growth near 0.68 for some species. After the establishment of these fungi, other *Aspergillus* species may grow, particularly *A. candidus*. *Wallemia sebi* is also commonly isolated from these types of foods. If the a_W becomes high enough, then *Penicillium* species may also grow, but, in general, Penicillia are not important spoilage fungi at reduced a_W in tropical climates.

High sugar commodities may also be spoiled by all the fungi mentioned above, but in addition, they can be substrates for a highly specialised group of fungi, the extreme, fastidious xerophiles. These include *Xeromyces bisporus*, xerophilic *Chrysosporium* species (*C. inops*, *C. fastidium*, and *C. xerophilum*) and occasionally *Eremascus* species. The xerophilic yeast *Zygosaccharomyces rouxii* is also commonly associated with high sugar, low a_W foods, particularly jams, syrups, and dried fruits (Pitt and Hocking 1985a; Hocking 1991).

Dried seafood products (fish, squid, shellfish, and seaweed) are often salted before drying. This can be selective for salt tolerant or halophilic fungi. In the tropics, two species of white fungi are often found associated with dried seafoods. These are *Polypaecilum pisce* and *Basipetospora halophila*. As well as these specialised fungi, other, more common xerophiles frequently spoil dried seafoods, particularly *Eurotium* and *Aspergillus* species, with *A. wentii* often being isolated (Wheeler et al. 1986).

Methods and Media for Isolation of Xerophilic Fungi

Since grains and nuts are usually contaminated with dust and soil, many fungi can be isolated from these stored commodities. Most of these fungi occur only as surface contaminants, and are not capable of growing in the commodity and therefore spoiling it. Stored commodities always have reduced a_W, and the a_W should be less than 0.60 for them to be microbiologically stable. However, stored commodities are often moister than this, or there are damp pockets due to moisture migration, and xerophilic fungi are able to grow. It is important to use appropriate media and methods for isolating xerophilic fungi from these low moisture foods. If high a_W media are used, then many of the important fungi, such as the *Aspergillus restrictus* series and some *Eurotium* species, will not be able to grow, and will be missed.

Methods

There are two methods commonly used for isolating fungi from foods. The first is dilution plating. This method is familiar to most food microbiologists, and the method for isolating fungi from foods is similar to that used for bacteria, with two basic differences. After homogenisation and dilution, samples are *spread plated*, not pour-plated, with 0.1 mL of dilution being pipetted onto the surface of the agar, then spread with a glass spreader. This allows fungal propagules equal access to oxygen, and a better chance of uniform colony development. The plates are then incubated *upright* rather than inverted, so that fungal spores are not shed onto the lid of the Petri dish.

With reduced a_W commodities, particularly dried seafoods and meats, the sample may need to be rehydrated in the diluent for up to 1 hour, before homogenisation. A suitable diluent for most samples is 0.1% peptone. Rehydration of grain samples may also aid the homogenisation process. Samples can be rehydrated at room temperature. They do not need to be refrigerated during this process, as fungal spores take many hours to germinate, and the counts will not increase during this period. Gradual rehydration can aid resuscitation of yeast cells and fungal conidia, shortening germination times, increasing subsequent growth rates, and also increasing the number of propagules recovered.

The other method that can be used to assess the mycological quality of stored commodities is *direct plating* (Mislivec and Bruce 1977). This involves placing discrete pieces, grains, or kernels directly onto suitable agar, usually after surface disinfection with 0.35–0.4% sodium hypochlorite (Andrews 1986) to remove any contaminating spores from the external surface. Direct plating permits detection of fungal hyphae that have actually penetrated and grown in the commodity; that is, it detects the fungi that are actually *growing* in the grain or nut, not just the spores clinging to the outside.

Direct plating provides an estimate of the extent of infection in a commodity, usually expressed as a percentage. Usually at least 50 and preferably 100 grains or kernels are plated out onto several Petri dishes (about 10–15 per plate, depending on the size), and this makes calculating the percentages very easy. Direct plating is often the best way to study the degree of contamination of a commodity with a particular fungus or group of fungi, when used in conjunction with a selective or differential agar, e.g. Aspergillus flavus and parasiticus agar for aflatoxigenic fungi, DG18 for *Eurotium* species, and MY50G for fastidious xerophiles.

Media

Do we really need specially formulated, reduced a_w media for the detection of spoilage fungi in stored commodities? The answer is an emphatic YES! If high a_w media like MEA, PDA, OGY, or DRBC are used alone, they will often not detect the most common xerophilic fungi, *Eurotium* species and members of the *A. restrictus* series (Hocking and Pitt 1980; Hocking 1981).

Malt salt agar (Christensen 1946) was the first medium developed for detecting storage fungi in grains. Although this medium is better than the high a_w media mentioned above, it is not recommended. Salt is generally considered inhibitory to many fungi (with the exception of halophiles) and it reacts with agar to form a soft, granular gel which makes spread-plating difficult. Salt also reacts with some other ingredients, such as malt extract, giving rise to precipitates that can interfere with counting of fungal colonies.

The medium now recommended internationally (Samson et al. 1991) for general purpose enumeration of xerophilic fungi from stored commodities is Dichloran 18% Glycerol agar (DG18). DG18 (Hocking and Pitt 1980) contains 18% glycerol, which reduces the a_w to 0.95, and dichloran, which restricts spreading growth of *Eurotium* species. It also contains chloramphenicol to prevent bacterial growth, as a number of bacteria, particularly *Bacillus* and *Micrococcus* species, can grow at 0.95 a_w. DG18 permits detection and enumeration of slower growing species like *W. sebi*, *A. restrictus* series, and *A. candidus* in the presence of high numbers of *Eurotium* colonies.

There are two other useful, though more specialised, media for isolation of particular groups of xerophilic fungi.

Malt extract Yeast extract 50% Glucose agar (MY50G; Pitt and Hocking 1985a) is used for isolation of extremely xerophilic fungi from high sugar foods such as confectionery and dried fruits. It is most useful for direct plating of samples, and is used only for dilution plating for detecting xerophilic yeasts in syrups and similar products. Reduced a_w diluents (e.g. 20% glucose) should be used to avoid osmotic shock when enumerating xerophilic yeasts from these concentrated products. MY50G is ideal for isolation of *Xeromyces bisporus*, xerophilic *Chrysosporium* species, *Eremascus* species, and *Eurotium halophilicum*. Because it contains no speading inhibitors, it is not suitable as an enumeration medium if common *Eurotium* species are present, as they will quickly outgrow the slower-growing xerophiles. For isolation of extreme xerophiles in the presence of *Eurotium* species, it may be necessary to use a medium of even lower a_w, such as MY70GF, a_w 0.76, which contains 70% w/w glucose and fructose in equal proportions.

Malt extract Yeast extract 5% salt 12% glucose agar (MY5-12) was developed for isolation of halophilic fungi from salted seafood products (Pitt and Hocking 1985b). This medium is optimal for isolating *Polypaecilum pisce* and *Basipetospora halophila*, two white halophilic fungi which commonly grow on dried seafoods in the tropics. *B. halophila* is the more xerophilic of the two species, and actually grows better if 10% rather than 5% salt is incorporated into the medium. While these fungi do not have an absolute requirement for salt, they grow much faster if some salt is present (Andrews and Pitt 1987).

There are a number of other media for xerophilic fungi (Beuchat and Hocking 1990), many of which are modifications of the media already mentioned. Some are older formulations that are no longer in use in mainstream food mycology, because they are either unnecessarily complicated, or are not as effective as those in current use.

For general purpose enumeration of xerophilic fungi from stored commodities and intermediate and low moisture foods, DG18 is recommended by the International Commision on Food Mycology.

Brief Descriptions of Some Xerophilic Fungi

Eurotium species (Fig. 2a–f)

All *Eurotium* species are xerophilic and grow poorly on high a_w media. They are normally grown on Czapek Yeast extract agar with 20% sucrose (CY20S) for identification purposes (Raper and Fennell 1965; Pitt and Hocking 1985a; Klich and Pitt 1988). This medium has an a_w of about 0.98, and encourages the development of characteristic mycelial colours, while also allowing good development of cleistothecia and ascospores in most species.

The four most common *Eurotium* species, *E. amstelodami*, *E. chevalieri*, *E. repens*, and *E. rubrum*, can be distinguished from each other by the shape and size of their ascospores and the colours of their mycelium. They all produce *Aspergillus* heads (Fig. 2a), bearing spiny or rough conidia (Fig. 2b) on phialides only.

The colonies of *E. amstelodami* are coloured bright yellow from the cleistothecia, with an overlay of dark to dull green *Aspergillus* heads. The ascospores are

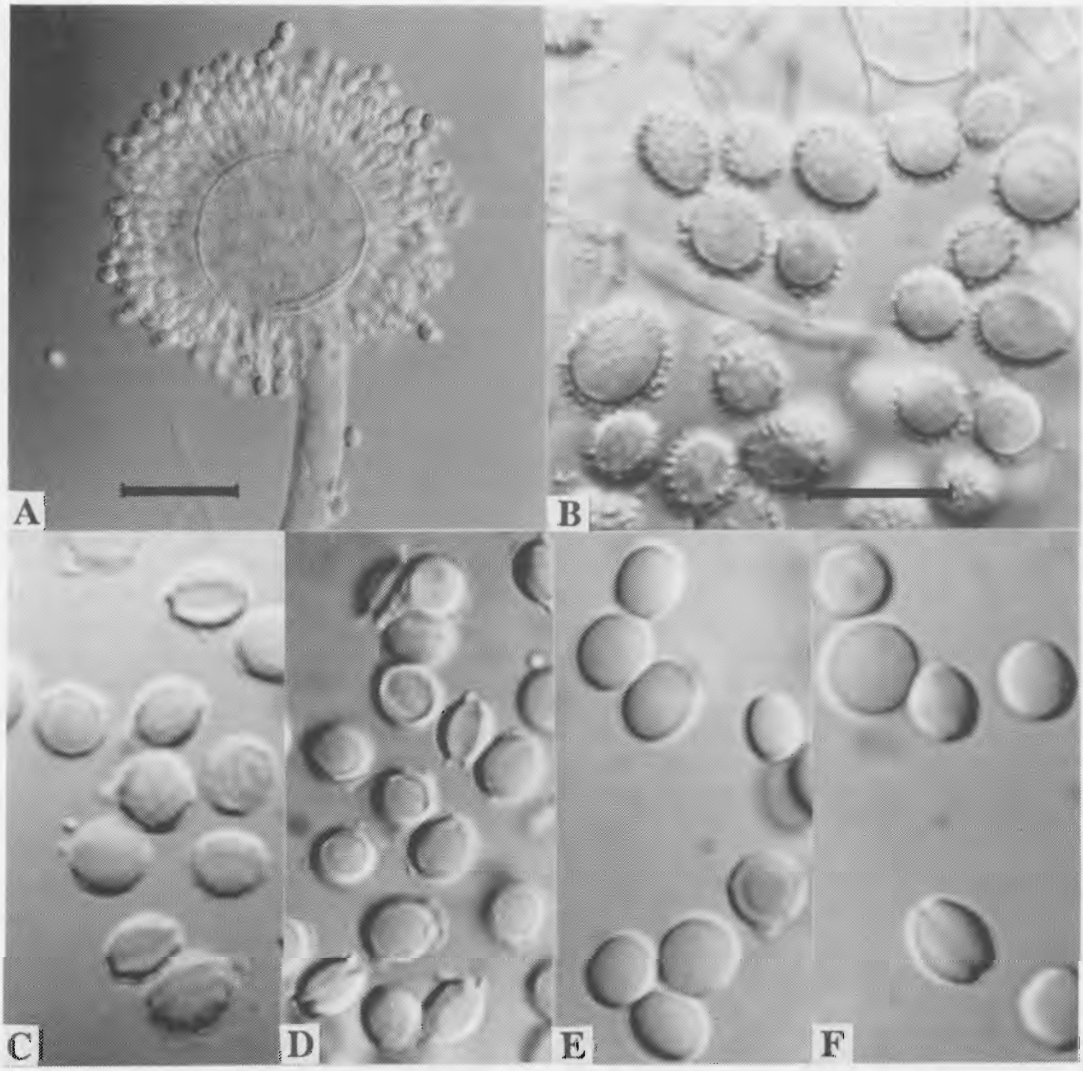

Fig. 2. *Eurotium* species. (a) a head of *Eurotium rubrum*, showing production of conidia from phialides only; (b) spinose conidia of *E. rubrum*; (c) ascospores of *E. amstelodami* are rough walled, and have an obvious furrow; (d) ascospores of *E. chevalieri* are smooth walled with distinct crests; (e) ascospores of *E. repens* are ellipsoidal, smooth walled, with no longitudinal furrow; (f) ascospores of *E. rubrum* are similar to those of *E. repens*, but they have a discernable longitudinal furrow. A, Bar = 20 µm; B–F, Bar = 10 µm.

4.5–5.0 µm long, rough walled, with wide, irregular longitudinal flanges (Fig. 2c).

E. chevalieri colonies are more orange than those of *E. amstelodami* because of the presence of orange hyphae amongst the yellow cleistothecia, with an overlay of grey-green *Aspergillus* heads. The ascospores are very characteristic, shaped like pulley wheels, with two prominent, parallel longitudinal flanges and smooth walls (Fig. 2d).

E. repens colonies are similar to those of *E. chevalieri* but the ascospores are quite different. *E. repens* produces smooth-walled ascospores, 5.0-5.5 µm long, which are almost egg-shaped. They have no ridges or flanges, and there is usually no more than a trace of a longitudinal furrow (Fig. 2e).

E. rubrum colonies are much redder than any of the other common *Eurotium* species, especially in age. The ascospores are similar to those of *E. repens*, except they have a shallow, but distinct, longitudinal furrow (Fig. 2f).

Aspergillus restrictus series (Fig. 3a, b)

The two common species in the *A. restrictus* series, *A. restrictus* and *A. penicillioides*, are both xerophilic, *A. penicillioides* more so than *A. restrictus*. These species often go undetected in stored products because high water activity media are used for fungal enumeration, and *A. penicillioides* in particular, will not grow on normal high a_W media. Both species produce phialides only. They can be distinguished from each other by the shapes of their *Aspergillus* heads, and their conidia. *A. restrictus* has columnar heads because it produces its conidia in long, adherent chains from phialides on the top half of the vesicle (Fig. 3a). The conidia are usually cylindroidal. Growth of *A. restrictus* is faster on CY20S than on MY50G. The heads of *A. penicillioides* are more radiate because the phialides cover more than three quarters of the vesicle, and conidia which are ellipsoidal, are produced in shorter chains and do not adhere to each other (Fig. 3b). *A. penicillioides* grows faster on MY50G than on CY20S.

These two groups of fungi, along with *Wallemia sebi* and other less xerophilic *Aspergillus* species such as *A. candidus*, are the most common spoilage fungi on stored commodities in the tropics. In temperate areas, *Penicillium* species are also important.

Xerophilic *Chrysosporium* species (Fig. 4a-c)

The xerophilic *Chrysosporium* species grow as white or pale yellow-brown colonies on MY50G agar. They produce solitary, hyaline, smooth-walled aleurioconidia either directly (sessile) or on small pedicels on the sides of vegetative hyphae. In some species, the vegetative hyphae may differentiate partially or wholly into conidia: intercalary chlamydoconidia, which are thick-walled and nearly spherical, and unswollen, heavy-walled hyphal segments (arthroconidia). One species, *C. farinicola*, sometimes produces a teleomorph classified in the genus *Bettsia*, which forms black cleistothecia and dark-walled ascospores (Pitt and Hocking 1985a).

Chrysosporium species are differentiated by the size, shape, and proportion of the various types of conidia they produce. *C. fastidium* forms pale yellow-brown colonies which produce mainly aleurioconidia (Fig. 4a) with few intercalary arthroconidia or chlamydoconidia. *C. inops* produces white colonies, and the conidia are predominantly arthroconidia and chlamydoconidia (Fig. 4b). *C. xerophilum* is similar to *C. inops* except that it grow faster (25–30 mm on MY50G after 14 days, compared with 12–20 mm for *C. inops*), produces more aleurioconidia, and in age, the hyphae differentiate almost entirely into arthroconidia and chlamydoconidia (Fig. 4c).

***Eremascus* species**

There are only two *Eremascus* species, *E. albus* and *E. fertilis*. Both are xerophilic, and neither species has been isolated very often (Pitt and Hocking 1985a). Mustard seems to be the most frequent source of *Eremascus* species. Colonies of these species are white and floccose. They form asci which are borne singly without any surrounding

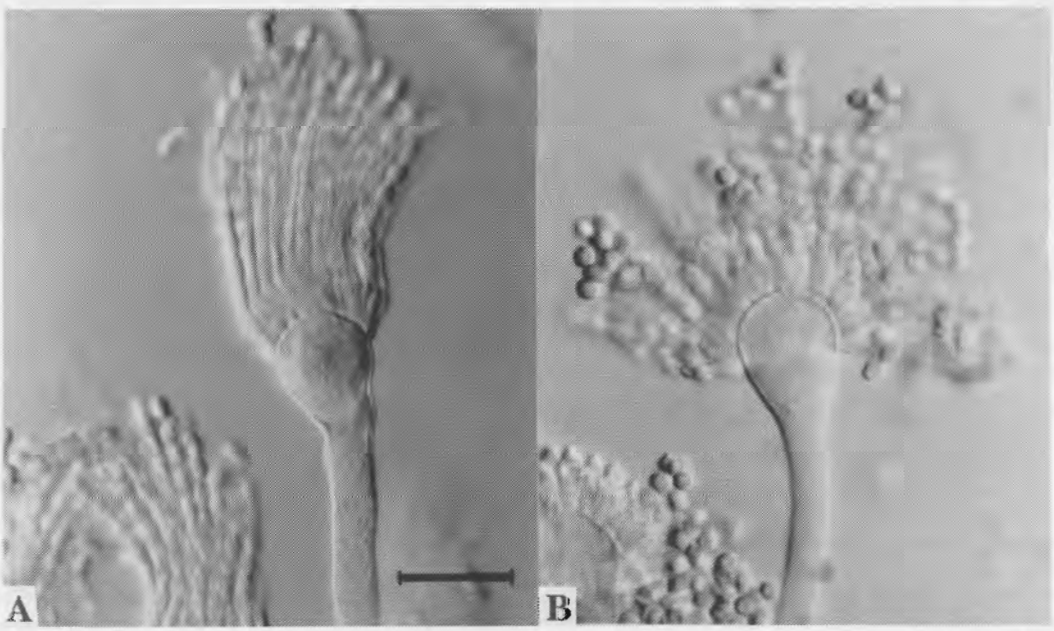

Fig. 3. Heads of (a) *Aspergillus restrictus* and (b) *A. penicillioides*. In *A. restrictus*, the conidia are produced in long, adherent columns; in *A. penicillioides*, the heads are more broadly spreading, and the conidia do not stick together in long chains. Bar = 20 µm.

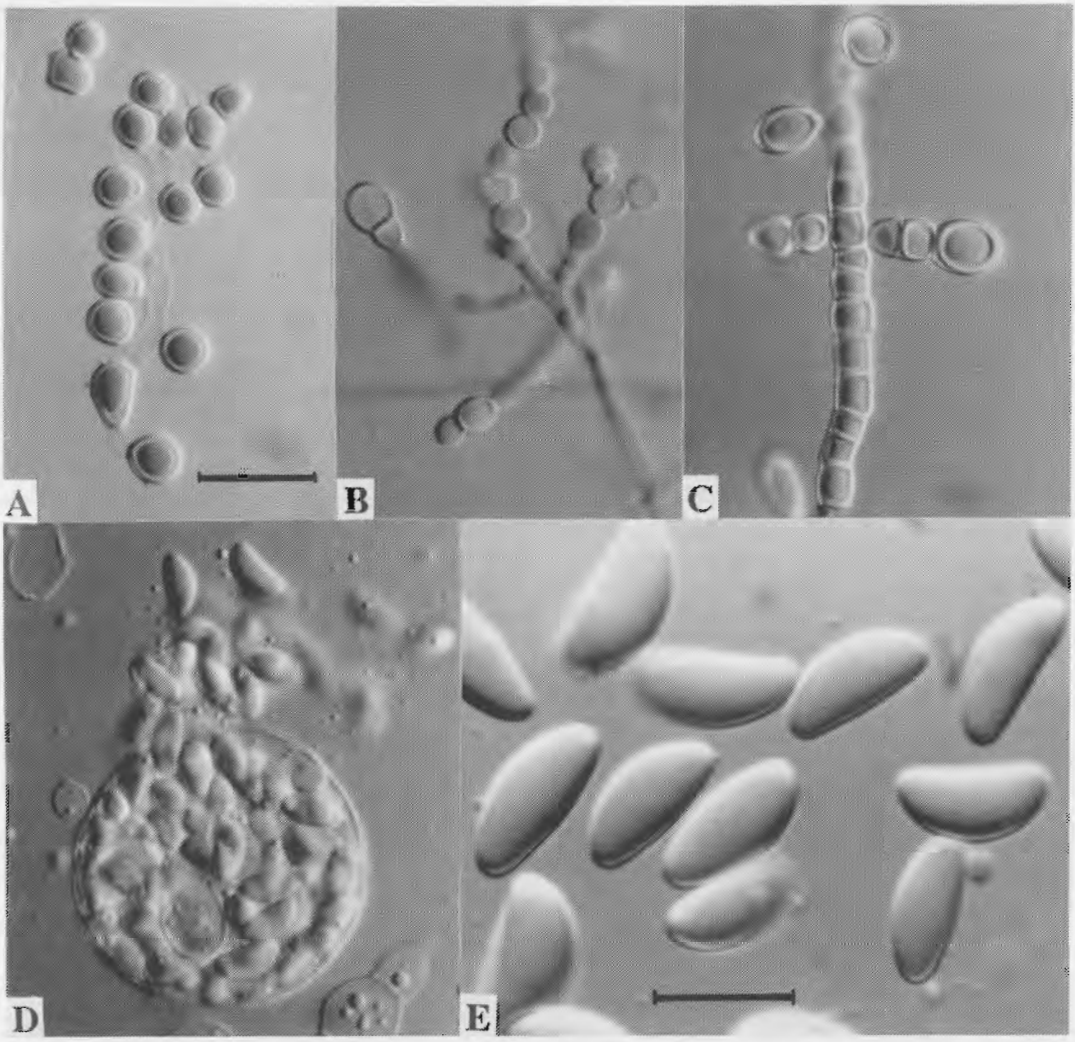

Fig. 4. Xerophilic fungi found in high sugar foods. (a) *Chrysosporium fastidium*; (b) *C. inops*; (c) *C. xerophilum*; (d) *Xeromyces bisporus*, showing a mature cleistothecium releasing ascospores; (e) *X. bisporus* ascospores, showing the characteristic 'D' shape. A–D, Bar = 20 µm; E, Bar = 10 µm.

wall or hyphae, each ascus containing 8 smooth-walled subglobose ascospores. The species are differentiated by slight differences in their ascus initials.

Xeromyces bisporus (Fig. 4d,e)

X. bisporus is the only member of this genus and is the most xerophilic of all the fungi. On MY50G, *X. bisporus* forms low, sparse, translucent colonies which are either colourless or pale pinkish brown. After 4–6 weeks, 'D'-shaped ascopores are formed in thin-walled, colourless cleistothecia (Figs 4d,e). At very low a_w values (below 0.70), thick-walled aleurioconidia may also be formed.

Wallemia sebi, *Basipetospora halophila*, and *Polypaecilum pisce* (Fig. 5)

These three fungi are grouped together because they are associated with salty foods. *W. sebi* differs from the other two in that it is often found in many other low moisture environments as well, and it has a lower optimum temperature for growth than the two white halophilic fungi (Wheeler et al. 1988a, 1988b). *W. sebi* is the only member of its genus. It is an easily recognised fungus because it forms characteristic small, chocolate brown colonies, 2-5 mm in diameter, after one week on most media. Although it is a xerophile, it can also grow on some high a_w media. It forms small, brown, rough-

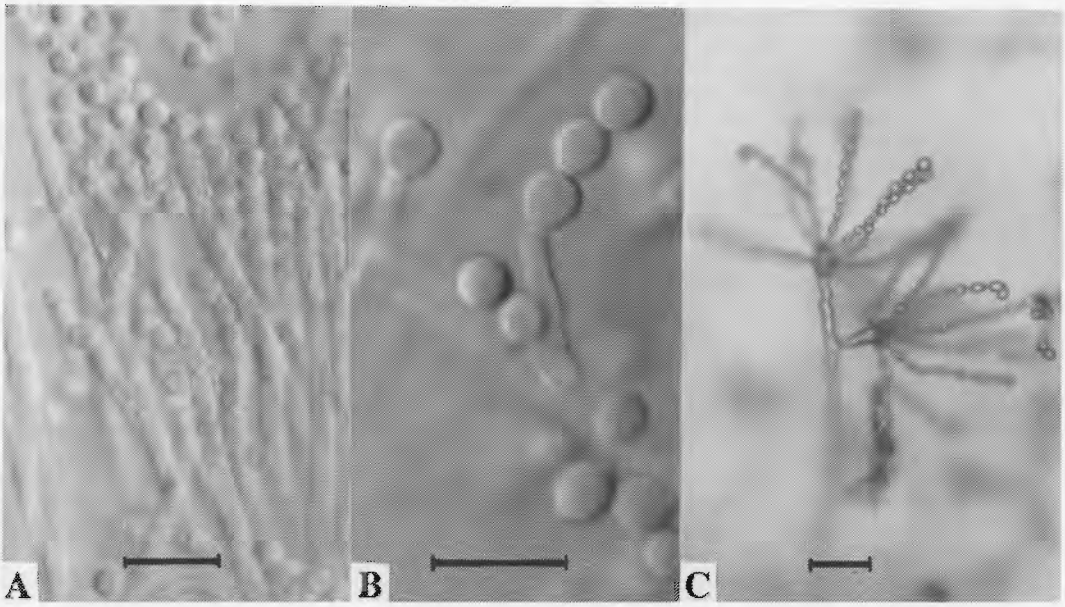

Fig. 5. Xerophilic fungi often associated with salty foods. (a) *Wallemia sebi*; (b) *Basipetospora halophila*; (c) *Polypaecilum pisce*. A, B, Bar = 10 µm; C, Bar = 25 µm.

walled conidia from the ends of short, fertile hyphae (Fig. 5a).

B. halophila and *P. pisce* both form white colonies, and grow better on media which contain some salt (Pitt and Hocking 1985b; Andrews and Pitt 1987; Wheeler et al. 1988b). *B. halophila* forms aleurioconidia in short chains from simple conidiophores which get shorter as more conidia form (Fig 5b). Chlamydoconidia and arthroconidia are never produced.

P. pisce is a recently described species (Pitt and Hocking 1985b) which is common on dried salted seafoods, particularly those from tropical regions. It is a Hyphomycete which has phialides with many openings (polyphialides) producing delicate chains of lemon-shaped conidia (Figs 5c). The conidiophores may be quite complex, with a number of branches. *P. pisce* has an optimum temperature for growth near 30°C (Wheeler et al. 1988b).

Conclusion

It is important to remember that detection and enumeration of specialised fungi requires specially adapted media and techniques. Many xerophilic fungi will not grow on normal, high a_w enumeration media. In choosing an isolation medium, the characteristics of the food being analysed should be taken into consideration. For high sugar foods, a high sugar medium such as MY50G (Pitt and Hocking 1985a) may be most appropriate for isolating the spoilage fungi. For salted, dried fish, MY5-12 (Pitt and Hocking 1985a) would better reflect the composition of the food, and thus provide more suitable growth conditions for halophilic xerophiles.

For routine use in enumerating the flora of stored commodities (grains, nuts, spices), a reduced a_w enumeration medium such as DG18 (Pitt and Hocking 1985a; Samson et al. 1991) is recommended. This medium is good for detecting common xerophiles like *Eurotium* species, *Aspergillus restrictus*, *A. penicillioides*, *Wallemia sebi*, and most *Aspergillus* and *Penicillium* species. However, it will not detect *X. bisporus* and some of the other more fastidious sugar-loving xerophiles. If in doubt about which medium to use, use more than one.

Intermediate and low moisture foods provide some extremely specialised ecological niches, inhabited by highly evolved species of fungi. The physiological adaptations that enable microorganisms to grow at reduced a_w are complex and varied. The most successful group of microorganisms colonising these low a_w niches, both in number of species, and ability to grow at low a_w, has been the xerophilic fungi (Hocking 1988).

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Culture Collections: Their Role and Importance

R. A. Samson and J.A. Stalpers*

Abstract

Culture collections are important germ plasm resources. They conserve representatives of all kind of microorganisms which can be made available to others for propagation and use. This paper reviews the various types of culture collections, research on and methods used for preservation, dispersal of information, and their value for fundamental and applied research. For research on fungi and mycotoxins in stored products, culture collections not only provide provide isolates for biochemical research but also reference cultures for identification and educational purposes. The International Commission on *Penicillium* and *Aspergillus* (ICPA) is, for example, currently developing a set of *Penicillium* and *Aspergillus* isolates which can be used for the latter purpose.

Culture collections maintain isolates reported to produce mycotoxins. However, there are many misidentifications of either the fungus or the mycotoxin. In several cases, isolates producing specific mycotoxins or originating from interesting cases were not deposited in major culture collections. This paper reviews some examples and makes recommendations regarding deposition of important isolates.

Most major culture collections are located in the developed countries and their existence is secured by continuous support by governmental or industrial bodies or by income gained from services to scientific, technological, and commercial customers. In developing countries, the need for culture collections is increasing but many constraints make their establishment difficult. Suggestions are given for operating a small culture collection responsive to local requirements.

CULTURE collections are germ plasm resources similar to botanical and zoological gardens or seed banks. There are many collections maintaining strains of micro-organisms. Takishima et al. (1989) list more than 200 collections maintaining fungi; the major ones are listed in Table 1. Demand for cultures, for isolates with particular properties, and for various related services, has greatly increased in the last decade mostly but not solely as a result of the rapid advances in biotechnology (Batra and Ijima 1984; Hawksworth 1985).

For research on fungi occurring in food, feed, and stored products, culture collections play a key role. Several investigators dealing with taxonomic, biochemical, and especially mycotoxicological aspects have stressed the importance of depositing strains in well recognised collections in which isolates can be kept under optimal conditions. This paper briefly reviews the current status of culture collections. For a more detailed account the reader is referred to Hawksworth and Kirsop (1988).

Types of Culture Collections

There are various types of culture collection, depending on size and aims (Hawksworth 1988). Cultures can be preserved for pure or applied research, or for commercial purposes. Many microbiological laboratories maintain small-scale culture collections for research use. Facilities for optimal preservation are sometimes lacking and important isolates may be lost during or after research projects. It is therefore recommended that important isolates be deposited in major culture collections. These collections are professionally managed and generally employ more than one preservation technique. Moreover, data on isolates and their properties are stored in databases available to users through catalogues, by requests to the collection, or even through on-line access if the database is computerised. In some cases, where a strain has potential commercial value or has an industrial application, services are provided for safety-deposit. Where patents are involved, the strain must be deposited in a collection with IDA (international depository authority) status.

Methods and Research

Basically, two types of storage methods can be recognised in culture collections: those in which metabolism is maintained (although often reduced) and those in which metabolism no longer occurs (Heckly 1978; Kirsnop and Snell 1984). With the exception of liquid drying and

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Table 1. Major culture collection maintaining fungal isolates

Australasia
- Australian National Reference Laboratory in Medical Mycology (AMMRL), St Leonards, NSW, Australia
- Victoria University of Wellington (DBVU), Wellington, New Zealand
- CSIRO Division of Food Research (FRR), North Ryde, NSW, Australia
- Materials Research Laboratories (MRL), Ascot Vale, Vic., Australia
- International Collection of Micro-organisms from Plants (PDDCC), Auckland, New Zealand
- Plant Pathology Branch Culture Collection (QA), Department Of Primary Industries, Indooroopilly, Qld., Australia
- Plant Research Division Culture Collection(WA), Department of Agriculture, South Perth, WA, Australia

Europe
- Aquatic Phycomycete Culture Collection (AP), Reading, UK
- Centraalbureau voor Schimmelcultures (CBS), Baarn, The Netherlands
- Culture Collection of Basidiomycetes (CCBAS), Prague 4, Czechoslovakia
- Culture Collection of Fungi (CCF), Prague 2, Czechoslovakia
- Czechoslovak Collection of Microorganisms (CCM)
- Collection de Champignons et d'Actinomycètes Pathogènes de la CNCM, Paris, France
- Deutsche Sammlung von Microorganismen [German Collection of Microorganisms] (DSM), Braunschweig, Germany
- CAB International Mycological Institute (IMI), Kew, UK
- IPO-Collection of Fungal Pathotypes (IPO), Wageningen, The Netherlands
- Laboratoire de Souches de Cryptogamie du Museum National d'Histoire Naturelle (LCP), Paris, France
- Mycothèque de l'Université Catholique de Louvain (MUCL), Louvain-la-Neuve, Belgium
- Friedrich-Schiller-Universität Jena, (MW) Weimar, Germany
- National Collection of Pathogenic Fungi (NCPF), London, UK
- National Collection of Wood Rotting Fungi (NCWRF-FPRL), Garston, Watford, UK
- Portsmouth Polytechnic Culture Collection (PPCC), Portsmouth, UK
- All-union Collection of Microorganisms (VKM), Pushchino, USSR

North America
- Atlantic Regional Laboratory (ARL), Halifax, Nova Scotia, Canada
- American Type Culture Collection (ATCC), Rockville, Maryland, USA
- Canadian Collection of Fungus Cultures (CCFC), Ottawa, Canada
- Mycological Culture Collection (DLR), Albany, USA
- Forintek Collection of Wood-inhabiting Fungi (EFPL), Ottawa, Canada
- Fungal Genetics Stock Center (FGSC), Kansas, USA
- Agricultural Research Service Culture Collection (NRRL), Peoria, USA
- Ontario Ministry of Health Fungal Collection (TPHL), Toronto, Ontario, Canada
- University of Alberta Microfungus Collection and Herbaria (UAMH), Alberta, Canada
- Upjohn Culture Collection (UCR), Michigan, USA

South America
- Departamento de Micologia, Fundacao Instituto Osvaldo Cruz OCF, Rio de Janeiro, (RJ), Brazil

freezing at ultra-low temperatures (<–135°C), Smith and Onions (1983) list methods currently used for the preservation of fungi (Table 2), with comments on costs (material and labour), longevity, and stability.

Methods with metabolic activity maintained

1. Storage on agar slants. This is the most general method, requiring periodic transfer at intervals varying from 6 weeks to 6 months at room temperature or up to a year at lower (e.g. 4°C) temperatures. The method is laborious, but does not require expensive equipment. A disadvantage is that the chances of degeneration (irreversible changes in properties) and contamination are relatively high.

2. Storage on agar slants under mineral oil (Onions 1977; Ellis 1979). In this method metabolism is reduced to about 10% or less, which is considered to be useful for storage periods up to about 10 years, though Smith and Onions (1983) report good results for some strains after 32 years. In general, the chances of irreversible changes are worse than for normal subculturing, except for a number of richly sporulating strains.

3. Storage at –20°to –40°C is acceptable only for a short period (< 2 years) (Kramer and Mix 1957; Alexander et al. 1980), but can be useful for maintaining cultures during a research project. Freezing of cultures at –70° or –80°C is generally considered as reliable for up to 5 years. However, this is an average and many fungi such as thermophilic isolates may not survive or may be viable for shorter periods.

Table 2. A comparison of methods of preservation

Method of preservation	Cost		Longevity	Genetic stability	General comments
	Material	Labour			
Regular subculturing on agar					
Storage at room temperature	Low	High	1–6 months	Variable, depends on curation	Keep stock culture in case of contamination of working culture
Storage in refrigerator	[a]Medium	High	6–12 months	Variable	
under oil	Low	Low/medium	1–32 years	Poor	
in water	Low	Low/medium	2–5 years	Moderate	
deep freeze	[a]Medium	Low/medium	4–5 years	Moderate	Must not allow thawing of material whilst sub-culturing
Drying					
in soil	Low	Medium	5–20 years	Moderate to low	
silica gel	Low	[b]Medium	5–11 years	Good	Recent work reports
freeze drying	High	Initially medium	4–40 years	Good	that overdrying can cause DNA damage
Freezing					
liquid nitrogen storage	High	Low	Infinite: 14 years to date at CMI	Good	

[a]Refrigerator and deep freeze costs included
[b]Initial processing costly depending on the method although maintenance is negligible
Source: Smith and Onions (1983)

4. Drying in soil or with silica gel (Onions 1977, 1983). These methods are not generally applicable, but may give good results for 5–10 years. They are relatively cheap and are extremely useful for maintaining *Penicillium* and *Aspergillus* cultures when other preservation methods are not available. They are not suitable for long-term storage of *Fusarium*.

Methods with metabolically inactive organisms

1. Freeze-drying (Raper and Alexander 1945; Ellis and Roberson 1968). This method consists of preserving the organisms by reducing the residual moisture content to less than 3% (and more than 1.5% to avoid permanent damage) by freezing and subsequently sublimating the ice, followed by a period of secondary drying. It is useful for many sporulating fungi, but for more sensitive species (e.g. with large, septate, or coloured spores or conidia), the parameters (cryoprotectant, freezing rate, preculturing conditions) must be optimal. Cultures are generally viable for periods of at least 10 years and often much longer, especially when the ampoules are stored at temperatures between 1° and 4°C. Differences in longevity of more than a factor of 10 are often observed in cold storage. The stability of the cultures is generally good. For mycotoxigenic species, freeze drying is usually appropriate.
2. Liquid drying. This method, much used for storage of bacteria, can also be successfully applied to storage of yeasts and is often used for preservation of bulk samples. Not enough data are available to estimate the stability of samples in time.
3. Ultra deep freezing (freezers operating at –135°C or lower, or in liquid nitrogen) (Hwang and Howells 1968; Smith 1983; Stalpers et al. 1987). Fungi are stored in a cryo-protectant after prefreezing at a specified rate. The method has generally applicability, but some groups of fungi require special procedures. For the resuscitation of the fungus, thawing must be rapid. It is also often advisable either to pregrow in liquid medium or to wash the cryoprotectant from the inoculum to avoid metabolic production of toxic substances.

Recent years have seen much research directed in particular at more permanent methods of preservation. In an EEC project involving culture collections from Belgium, France, The Netherlands, and the UK, the optimum cooling rate of many organisms was established and the influence assessed of various preservation methods on the fatty acid composition, metabolite production, protein production, and membrane stability. Using these results, Tan et al. (1991) developed a method to

freeze-dry hyphae that is especially successful for ascomycetous strains, but also strongly improves the results of the freeze-drying of species with large, dark, or complex conidia. Cultures are grown on cellophane, and then transferred to a blender containing a culture medium with the addition of 4% trehalose. After three days the colonies are harvested, transferred to ampoules containing skim milk, cooled at a rate of 1°C/minute to –45°C, and freeze-dried to a residual moisture content of about 3%. For revival, pellets are suspended in malt-peptone for 16 hours and then transferred to solid media.

Importance of Culture Collections for Research on Fungi and Mycotoxins in Stored Products

Due to identification problems many reports in the literature are based on doubtful or incorrect names. Brayford (1990) discusses the problem in *Fusarium* where the taxonomy of the genus is hampered by inaccurate identification of strains, either through misapplication of names or erroneus determinations. Particularly in physiological and molecular studies which can provide important criteria for the systematics, researchers are often taxonomically unskilled and serious mistakes can be made, rendering the data unreliable. Another problem relates to the fact that many mycotoxins have been named after isolates which were misidentified or carried incorrect names. Frisvad (1989) provides examples of incorrect naming of mycotoxins (Table 3). To correct these mistakes, the relevant isolates need to be available for examination, but often they were never deposited in a culture collection. Nevertheless, Frisvad (1989), Marasas et al. (1984), Wei and Jong, (1980) and van Reenen-Hoekstra et al. (1990) have investigated misidentified isolates of *Penicillium, Aspergillus,* and *Fusarium*. The International Commission on *Penicillium* and *Aspergillus* (ICPA) is also considering this problem and has plans to investigate, in a collaborative study, important toxigenic isolates of *Penicillium* and *Aspergillus* in major service collections. Moreover, ICPA is currently preparing a set of reference cultures of *Penicillium* and *Aspergillus* with morphological and biochemical descriptions for use by applied researchers who need to be able to identify members of these important groups of fungi.

Culture Collection and Developing Countries

Whereas most culture collections are in the developed countries, the need for such resources in developing countries is increasing. In many Third World countries small research collections exist but too often their sustainability is limited because of financial constraints. Important isolates obtained from research projects are often lost or not kept for long term preservation.

The World Federation of Culture Collections has recognised these problems, establishing, in 1975, a network of microbiological resources centres (MIRCENs) to organise conferences, training courses, and fellowship programs. These centres are designed: (1) to provide the infrastructure for building a world network incorporating regional and interregional functional units geared to the management, distribution, and utilisation of the microbial gene pool; (2) to strengthen efforts relating to the conservation of microorganisms with emphasis on *Rhizobium* gene pools in developing countries with an agrarian base; (3) to foster the development of new, inexpensive technologies that are appropriate to the particular regions; (4) to promote the application of microbiology to the strengthening of rural economies; and (5) to serve as focal points for the training of manpower and the dissemination of microbiological knowledge.

Recently, the Netherlands and Indonesia initiated a joint program on agricultural biotechnology, establishing a central facility for a culture collection at the University of Bogor. This cooperative program will emphasise not only training of culture collection staff, but also the incorporation of research program curricula for PhD students, to build expertise in taxonomy and applied microbiology. Several research topics are focusing on the application of biotechnology of indigenous food and the Indonesian culture collection may thus play an important role in research on fungal contaminants and mycotoxins in tropical foodstuffs.

Table 3. Examples of misidentified mycotoxins and isolates reported in the literature

Mycotoxin	Incorrect identification	Correct taxon
Cyclopiazonic acid	*Penilillium cyclopium*	*P. commune/P. camemberti*
	Aspergillus versicolor	*A. oryzae*
Verruculogen	*P. verruculosum*	*P. brasilianum*
Viridicatum toxin	*P. viridicatum*	*P. aethiopicum*
Ochratoxin	*P. cyclopium*	*P. verrucosum*
Neosolaniol	*Fusarium solani*	*F. sporotrichioides*
Deoxynivalenol	*F. nivale*	*F. sporotrichioides*

Source: Frisvard (1989)

For the preservation of fungal isolates in collections in developing countries, the various methods should be assessed in the light of the personnel, facilities, and financial support available. Methods requiring continuous and expensive consumables, such as ultra deep freezing, are for the most part not suitable. In general, cultures in collections in tropical countries should be freeze dried or kept in soil or silica gel. It is often useful for researchers from existing service collections to help in setting up the new collections in developing countries and to propose adaptations depending on local needs and resources. Much attention should be given to the training of the technical staff who carry out the routine work. The skill of these persons is important and often has a significant effect on the quality of the collection. In addition, the scientific staff who will work on or supervise the collection must be properly trained in the taxonomy of the organisms kept in the collection. A good taxonomic knowledge not only secures the quality of collection, because the isolates can be checked regularly, but can also be used to offer an identification service to research or industrial institutions. Together with the income gained by selling cultures, the service can become a substantial source of funds permitting the facility to become partly or completely self-supporting.

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The Fungi
Session Summary

Chairman: Professor Dr Hj. Sitti Soetarmi Tjitrosoma, BIOTROP, Bogor, Indonesia
Rapporteur: Professor U. Samarajeewa, University of Peradinya, Sri Lanka

FIVE papers were presented in this session covering isolation, identification, culture collection, taxonomy, and biology of mycotoxin-producing fungi.

In the first paper, Dr John Pitt noted that the taxonomy of fungi, including those producing mycotoxins, has traditionally been based on morphological characters, such as colony diameters of isolates grown at standardised temperatures on special media.

More recently, however, there have been new approaches to fungal taxonomy, most importantly, studies on the pattern of production of mycotoxins and other secondary metabolites. Other, more direct approaches are to study patterns of electrophoretically separated enzymes, and DNA restriction enzyme fragment polymorphism.

As further detailed by Dr Samson in the second paper, the taxonomy of *Penicillium* and *Aspergillus*, the most important genera of food spoilage fungi, is now more firmly established as a result of these new approaches. While this is not yet the case for *Fusarium* species, practical identification manuals are available for of the genus.

In his paper on the taxonomy and biology of dematiaceous hyphomycetes and their mycotoxins, Dr Sivanesan described some 32 species that are reported to produce mycotoxins and other metabolites. The majority, if not all, of these are more widely known as plant parasites or plant pathogenic fungi. A more detailed discussion was presented on the genera *Alternaria*, *Bipolaris*, *Cercospora*, *Curvularia*, *Drechslera*, and *Exerohilum*.

Dr Ailsa Hocking then discussed methods of isolation of xerophilic fungi. She stressed that modification of the media normally used for detecting fungi in food is essential. For general purpose enumeration of fungi in stored products DG18 (a_w 0.95) is recommended, whereas for extreme xerophiles and halophilic fungi, MY 50G (a_w 0.89) and MY 5-12, respectively, are recommended.

In the final paper in this session, Dr Rob Samson outlined the role and importance of culture collections in mycological and mycotoxicological studies. Dr Samson stressed that after isolation and identification of any food-borne fungus, establishment and maintenance of a culture of that fungus for future reference should always be considered. He outlined the research and educational functions of the main collections of fungal cultures in developed countries, and reviewed methods for preservation, dispersal of information, and the value of culture collections for fundamental and applied research.

In developing countries, the need for culture collections is increasing, but there are many constraints to their establishment. Specific local requirements should be considered before setting up even a small culture collection in a university laboratory or a research institute.

The establishment of culture collections, particularly in developing countries, was the main topic of discussion at the end of the session. The following recommendations emerged from this discussion.

- Techniques for isolation of food-borne fungi should be standardised, as should the media used for culturing specific groups of mycotoxin-producing fungi.
- Training courses should be developed for technicians and young scientists, either in-country or in overseas institutes.
- Government and non-government agencies should be alerted to the need for, and practical benefits of culture collection and maintenance.

The Mycotoxins — Part 1

Aflatoxins: Successes and Failures of Three Decades of Research

Ramesh V. Bhat*

Abstract

The discovery of aflatoxins in the early 1960s was followed by research on chemical aspects, biological effects in animals and plant systems, natural occurrence, sampling methodology, ecological conditions needed for their formation, analytical methods, metabolism, biosynthetic pathways, and disease outbreaks in humans and animals, prevention methods (including decontamination and detoxification), economic losses and impact on international trade, and bases for regulatory standards.

Aflatoxins have been reported to occur in over 50 countries of the world in a variety of agricultural commodities. Although sophisticated methods of analysis are available, simple thin-layer chromatography is still the most widely used technique. The importance of validation of methods and collaborative programs is increasingly being recognised.

Acute diseases in humans and animals, as well as chronic diseases such as primary liver cancer, have been linked to aflatoxin consumption. However, with many diseases, such as Reyes Syndrome, a cause-and-effect relationship has yet to be fully established.

Realisation is growing of the extent of economic losses due to mycotoxins. They are substantial for certain countries, particularly those which depend on agricultural exports for earning foreign exchange. Various prevention measures have been suggested to minimise the harmful effects. Recent efforts in this direction have called on genetic engineering techniques. Since eradication of aflatoxins from foods may not be achievable in the near future, management strategies which minimise their occurrence and harmful effects must be devised.

AFLATOXINS are secondary metabolites of the moulds *Aspergillus flavus* and *A.parasiticus* produced in agricultural commodities before or after harvest. Although the aflatoxins were formally discovered only during the early 1960s, the harmful effects of ingestion of moulds contaminated commodities in humans and animals had been recognised much earlier (Bhat et al. 1976).

Aflatoxins are basically difuranocoumarin compounds. The group of compounds includes aflatoxin B_1 B_2, B_{2a}, B_3, G_1, GM_1, G_2, G_{2a}, M_1, M_2, M_{2a}, GM_2, P_1, Q_1, R_0, RB_1, RB_2, AFL, AFLH, AFLM, and methoxy, ethoxy, and acetoxy derivatives. However, only a few of them, most importantly aflatoxin B_1, have been reported as naturally occurring compounds; the rest are metabolites or derivatives. Next in importance to B_1, found mainly in agricultural commodities, is aflatoxin M_1 secreted in milk by dairy cattle consuming aflatoxin-contaminated commodities. The common names of various aflatoxins are primarily descriptors of their fluorescence, mobility on thin layer chromatography plates during analysis, or the nature of derivatives occurring in milk/urine.

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Occurrence

Aflatoxins have been reported from most of the continents. The Afro–Asian countries from which aflatoxins have been reported include Bangladesh, Bhutan, Burma, the People's Republic of China, Côte d'Ivoire, Egypt, Ethiopia, Gambia, Ghana, Hong Kong, India, Indonesia, Iran, Iraq, Israel, Japan, Kenya, Republic of Korea, Malawi, Malaysia, Mozambique, Nepal, Nigeria, Pakistan, the Philippines, Senegal, Sierra Leone, Singapore, Sri Lanka, Sudan, Swaziland, Taiwan, Tanzania, Thailand, Tunisia, Turkey, Uganda, Vietnam, Zaire, Zambia, and Zimbabwe (FAO 1990 a,b; ICRISAT 1989). In the tropical, subtropical, and Mediterranean climate regions of Latin America, North America, Australia, and Europe, the aflatoxins are formed under natural conditions. The reported occurrence of aflatoxins in agricultural commodities and their by-products in countries with more temperate climates could be due to importation of these commodities. Over the last decade or so, the FAO/WHO/UNEP Global Environmental Monitoring System (GEMS) has been attempting to standardise the reporting system of occurrence of aflatoxins in high-risk commodities such as maize, peanuts, and various other nut species. (Jelinek et al. 1989).

The various commodities in which aflatoxins have been reported are listed in Table 1. Among these, high-risk commodities for aflatoxins are maize, peanuts, parboiled rice, cottonseed, copra (dried coconut), and pistachio nuts. In some commodities, such as cassava and coffee, naturally occurring fluorescent substances which have been mistaken for aflatoxin have been reported (Nagarajan et al. 1973).

Table 1. Occurrence of aflatoxins in food and feeds

Cereal grains	maize, rice, wheat, sorghum
Oilseeds	groundnut, cottonseed, coconut, soyabean, sunflower
Vegetable oils	Groundnut oil, coconut oil, cottonseed oil, olive oil
Pulses	various beans (Africa), peas (Asia/Africa)
Root crops	cassava, sweet potato
Tree nuts	pistachio nuts, almonds, walnuts, peanuts, arecanuts
Vegetable products	coffee, cocoa, figs, peaches, spices
Animal feeds	extractions of cottonseed, groundnut, coconut
Animal products	milk, cheese, fish, shrimp
Fermented products	alcohol, beer, sauces, wines

In most instances, aflatoxins are formed after harvest, particularly when harvesting takes place during floods, cyclones or unseasonal rains, or when there is improper storage of insufficiently dried agricultural commodities. Nevertheless, preharvest occurrence of aflatoxins in maize and peanuts in the field has received some attention, particularly in the USA.

There are several factors which affect aflatoxin production. The major factors include the strain of the fungus, substrate potential, moisture content, relative humidity, and temperature. Minor factors are the inoculum potential, crop variety, maturity, the extent of damage, insect/microbial interaction, and storage conditions.

Biological Effects

The history of aflatoxin investigations began with the discovery of their harmful effects in turkey poults. Ducklings have been found to be highly susceptible to aflatoxin occurrence. Besides these, a variety of animals such as quail, chickens, rainbow trout, rats, mice, guinea pig, hamsters, ferrets, rabbits, dogs, cattle, sheep, swine, horses, monkeys, and mink are known to be affected by aflatoxins. Species that have been studied for aflatoxin toxicity under laboratory conditions include the honey bee, salmon, carp, brine shrimp, and Japanese medaka. The major target organ of aflatoxins is the liver. Malnourished animals have been reported to be more susceptible than well nourished animals.

The carcinogenicity of aflatoxins in laboratory animals including primates has been unequivocally proven (Gopalan et al. 1972). In addition to liver tumours, AFB_1 can induce tumours in the kidneys, colon, stomach, and nervous system. Recent immunocytochemical studies have helped to accurately quantify DNA adduct level in various tissues (Wild et al. 1990). Other recent studies have focused attention on the effect of AFB_1 in the induction and development of liver cancer in Pekin ducks congenitally infected with duck hepatitis B virus (Cova et al. 1990).

Analytical Methodology

During the last three decades several methods for detecting aflatoxins have evolved. These include various screening techniques, as well as quantitative determination methods such as thin layer chromatography (TLC), minicolumns, fluorodensitometry, liquid chromatography, radio immunoassay, and enzyme linked immunosorbent assay (ELISA). Among these, TLC has been the most widely accepted, specially in developing countries of the world. A survey by the UNEP/FAO/WHO Global Environmental Monitoring System of the methods used by various laboratories in different parts of the world during the last decade has indicated that, among the participating laboratories, 35% use high-pressure liquid chromatography methods (HLPC), 29% use various other methods, 20% use the CB method, 8% BF method, and 4% each the EEC method and immunoassay method (GEMS 1991).

Several ready-to-use kits for aflatoxin detection — based mainly on ELISA methods — are being marketed aggressively . The type of tests include affinity column, microtitre well, cup, probe, card, and selective absorptions and are either visual–semiquantitative or instrumental–quantitative in nature. While the methods are rapid and easy, their application in tropical countries is limited because of their heat sensitivity, short life, and prohibitive cost.

Various method validation programs, such as those of the Association of Official Analytical Chemists, the American Oil Chemists Society, the American Association of Cereal Chemists and the International Union of Pure and Applied Chemists, are available for aflatoxins. The check sample program facility of the International Agency of Research on Cancer has been functioning for over a decade, providing participating laboratories with opportunities to compare the results of analyses of the same sample batch. At the regional level, check sample programs are encouraged by organisations such as Food and Agriculture Organisation.

Health Hazards of Aflatoxins

Several attempts have been made in various parts of the world to correlate the consumption of various foods

contaminated with aflatoxins with acute or chronic diseases in humans. During the second world war, Korean prisoners of war who were forced to consume mould damaged maize were reported to have suffered from toxic hepatitis. Sporadic cases of acute liver disease due to consumption of aflatoxin-contaminated cassava in Uganda and rice in Taiwan were reported in 1967. Outbreaks of aflatoxic hepatitis in humans, with supporting epidemiological, mycological, mycotoxic, and pathological evidence were reported from India in 1975 and Kenya in 1982 (Krishnamachari et al. 1975; Ngindu et al. 1982). A recent unconfirmed report indicated a similar episode in Malaysia in 1988.

Aflatoxins have also been linked with the occurrence of various chronic diseases in humans (Table 2). In most of these instances the linkage is based on detection of aflatoxin in biological fluids, and exact cause-and-effect relationships have not been established. Another important feature is that all these disease conditions, except primary liver cancer, have not been reported in other parts of the world where widespread occurrence of aflatoxins has nevertheless been reported (Bhat 1989).

Primary liver cancer (PLC) is one of the leading causes of cancer mortality in Asia and Africa. Studies carried out in the People's Republic of China, Thailand, the Philippines, Kenya, Swaziland, Mozambique, and Uganda, provide evidence for involvement of aflatoxin consumption in the causation of PLC. A major confounding variable in many of these studies has been the role of chronic hepatitis B virus infection which may inherently predispose individuals to the cancer-initiating effects of aflatoxin (Groupman et al. 1988). The role of aflatoxins in the aetiology of PLC is elucidated by measuring the aflatoxin exposure through retrospective and prospective epidemiological studies. For this purpose, exposure assessment used to be carried out by relatively crude methods such as extrapolation of data from food sample contamination. However, recent studies have indicated that aflatoxin–albumin adduct analysis is the most promising approach. Such adducts are found in the peripheral blood after exposure to aflatoxin B_1 (Wild et al. 1990).

Despite intensive research, whether or not aflatoxin is a human carcinogen remains uncertain. A World Health Organisation task force concluded in 1979 that aflatoxin ingestion may increase the risk of liver cancer and, according to the International Agency for Research on Cancer (IARC) in 1987, 'sufficient evidence exists to indicate that aflatoxin is a probable human carcinogen'. However, critics of this view hold that the published evidence is sufficient to indicate that aflatoxin is not a probable human carcinogen (Stoloff 1989) and that primary liver cancer mortality in China is unrelated to aflatoxin intake (Campbell et al. 1990). Perhaps follow up studies on survivors of the 1975 aflatoxic hepatitis outbreak in India for the possible development of liver cancer will help to resolve the problem.

Economic Losses and Impact on International Trade

The problem of aflatoxin contamination has posed serious problems in commerce and international trade. The magnitude of economic losses due to aflatoxins is not

Table 2. Association of aflatoxins with chronic diseases

Disease	Evidence	Countries
Primary liver cancer	Experimental in animals Molecular epidemiology Detection in biological samples	Kenya, The Gambia, Mozambique, Swaziland, Uganda, Zimbabwe, the Philippines, People's Republic of China, Thailand, Transkei (S. Africa)
Indian childhood cirrhosis	Experimental animals Accidental exposure Detection in biological samples	India
Chronic gastritis	Epidemiological Detection in biological samples	Kenya
Kwashiorkor	Detection in biological samples	Sudan, Ghana, Nigeria, S. Africa
Reyes Syndrome	Detection in biological samples	Thailand, USA
Respiratory diseases	Occupational Epidemiological	Czechoslovakia, Netherlands, USA

known. However, they hold serious economic implications in terms of visible and invisible costs at national and international levels. The volume of international trade in aflatoxin-susceptible commodities such as maize, peanuts, copra, pistachio nuts, spices and oilseed extracts are considerable and the stringent quality parameters on aflatoxins imposed by importing countries affect trade (Bhat 1988).

Economic losses due to aflatoxins occur at various levels. They may be to the national exchequer in terms of lowered foreign exchange earnings from reduced exports, costs involved in potential loss of overseas outlets, and increased costs of control, surveillance, preventative, and training measures, as well as costs of detoxification. In addition, in a few countries there is a demand for subsidies for the contaminated produce. Losses to primary producers (farmers) are in terms of outright food or feed losses, reduced selling prices for contaminated commodities, and reduced productivity of livestock leading to lowered income. The traders who are middlemen in the agricultural produce marketing chain experience losses due to product refusal, lowered prices, loss of reputation, and losses due to litigation and compensation to be paid. The consumers, who are always at the receiving end, incur costs due to impaired health and productive capacity, and possibly medical and veterinary costs (FAO 1977).

Since 1973, aflatoxins in finished products of commercial feed manufacturers have been controlled by legislation in the European Community (EC), with maximum permissible levels of aflatoxins prescribed. Community regulations state that the maximum levels of aflatoxin B_1 in unfinished feed may not exceed 0.05, 0.03 and 0.01 mg/kg, the actual level depending on the animal to be fed and the nature of intended produce i.e. milk, eggs, or meat. To increase the level of control, in 1988 the regulations were extended to raw materials such as maize, cottonseed, copra, Babassu, peanuts, and palm kernel, and their derivatives. If the materials are found to have a level above 0.2 mg/kg of aflatoxin, the importer would be obliged to have the goods returned to their origin, destroyed, or sold outside the EC. As a result of these stringent regulations the importation of peanut meal to the EC declined from 0.91 Mt in 1979–80 to 0.43 Mt in 1989–90. Imports of copra have declined from 0.26 Mt to 0.08 Mt (Cappuccio 1989). The economic implications of such fall in exports are considerable, especially for the countries whose main sources of foreign exchange earning are agricultural commodities and their by-products. The export of peanut extractions from India has declined from 550 t valued at US$42.5 million in 1977–78 to 265 t valued at US$32.5 million in 1985–86, mainly because of aflatoxins (Bhat and Rao 1990).

The African Groundnut Council and its member states (The Gambia, Mali, Niger, Nigeria, Senegal, and Sudan), in conjunction with UN agencies (FAO and UNDP) and EC countries, have developed and implemented a program to reduce aflatoxins in peanut and its products by strengthening monitoring capacity, and introducing measures for prevention, quality assurance and standards, control, detoxification, and warranty. The total economic cost to international agencies, donor countries, and countries of the region for these programs in terms of both cash and kind has been estimated to be US$ 7.5 million (Coulibaly 1989).

The financial losses incurred by the poultry sector are considerable. Losses to the US broiler industry are estimated to be US$ 143 million (Hesseltine 1986). A UK feed manufacturer is reported to have paid 0.5 million pounds in compensation to a single client because of aflatoxin (Austwick 1978). A recent case study in India estimated that a poultry farmer lost 10% of his investment over a 21 day period because of 0.7 ppm of aflatoxin contamination in feed (R.B. Sashidhar, R.V. Sudershan, R.V. Bhat, and H.V.V. Murthy, unpublished data). The benefit–cost and cost-effectiveness of lowering aflatoxin tolerance levels in terms of reduced cancer mortality rates in humans have also been studied (Dichter and Weinstein 1987).

Prevention and Control

Despite intense work over three decades in several countries, a satisfactory method of preventing aflatoxin contamination in agricultural commodities has not yet been found. Various agronomic approaches, such as proper crop water management, use of pesticide/soil amendments, crop rotation practices, control of insect pests, appropriate harvesting practices, sowing in the correct season, and so forth have been advocated. Use of resistant cultivars of peanuts and maize, although showing initial promise, has yet to be widely accepted as a safe means of preventing aflatoxin contamination. Peanut cultivars showing resistance to seed invasion and colonisation by *Aspergillus* species include 'Basse' (Gambia), RMP-12 (Burkina Faso), AL 7223 (Nigeria and Egypt), Lampang 55-437 (Malawi) and 72-30 (Senegal), U4-47-7 (Uganda), and J 11 (India) (ICRISAT 1989).

Sophisticated and precise innovative solutions based on genetic engineering for aflatoxin prevention have been suggested. One of them is to alter the fungus to make it non-toxic either by producing genetically engineered *A. parasiticus* or by selecting aggressive non-toxigenic biocompetitive strains. In this approach, the gene in the strain which makes the enzyme that produces the toxin is altered or removed. The enzyme methyl transferase responsible for conversion of aflatoxin precursors found in the late stages of the pathway to aflatoxin B_1 has been isolated. If a genetically engineered fungus that will not make aflatoxin is produced in the laboratory it can be

multiplied and made to compete with the potent toxin-producing strain for a niche in the environment of the maize/cottonseed or peanut field. Another approach is to alter the plants to make them more fungus resistant by locating genes responsible for production of phytoalexins or introducing genes in cotton plants that code for resistant strains. A large molecular weight substance, probably a glycoprotein, is present in the seed coats of cottonseed at a particular stage in seed development. While the material does not stop fungal growth, it does inhibit synthesis of aflatoxin. A gene in the cotton plant which codes for this protein has to be found (Lee 1989). However, these exciting discoveries are still in the preliminary stages and it is too early to expect their practical application.

Development of improved postharvest practices, such as drying the produce to safe moisture levels, improving storage structures and practices, and storing under modified atmospheres, appear to be more practical solutions. Similarly, a variety of natural plant extracts (such as those from spices), and industrial chemicals (such as volatile fatty acids), found to be effective in minimising aflatoxin contamination, also appear to have practical potential. However, widespread use of these substances at the field level needs to be collaboratively studied and their efficacy proven unequivocally if they are to be adopted by farmers. Education and extension activities on prevention of mycotoxin contamination have gained momentum in recent years (Bhat 1987).

Following aflatoxin contamination of commodities, segregation and detoxification appear to be the ideal approaches. In Australia, Malawi, and the USA, segregation of aflatoxin-contaminated peanuts by visual or mechanical means has been attempted. In Senegal, Sudan, and India, industrial detoxification of aflatoxin contaminated peanut oilseed cake by processes such as ammonia and formaldehyde treatment is considered to be practical and effective. Plants with capacities ranging from 0.5 t to 600 t are in operation (Bhat, 1990). However, such cakes are useful only as animal feeds. Regulatory measures permitting the marketing of such detoxified products have yet to be formulated even in European countries.

Hybrid chickens evolved during the last decades are becoming increasingly susceptible to harmful effects of dietary aflatoxins. Efforts are currently under way to develop aflatoxin resistant chickens. Chicks selected for five generations for resistance to acute aflatoxicosis when fed with dietary aflatoxins did not show any changes even in sensitive parameter of aflatoxicosis such as gamma-glutamyl transferase activity (Manning et al.1990). Another way of minimising aflatoxin toxicity is to use dietary supplements such as hydrated sodium calcium aluminosilicate or various vitamins in the diet of chickens/quail to ameliorate the harmful affects of aflatoxins (Kubena et al. 1990; Johri et al. 1990).

Among the measures that are aimed at minimising aflatoxin contamination, more practical approaches that are successfully adopted in developing countries are the following (i) segregation of contaminated peanuts in Malawi; (ii) detoxification of peanut meal for export in Senegal; (iii) regulatory aspects of animal feed ingredients in Zimbabwe; (iv) extension services in Tanzania; (v) varietal differences in peanut varieties approach in Burkina Faso; (vi) produce handling practices in Nigeria; and (vii) field aflatoxin detection kit in India. These approaches are worth considering for other developing countries.

Current regulatory limits on aflatoxins adopted by most countries are based on analytical capability and economic considerations. The emphasis has to be on appropriate risk assessment studies. To arrive at an estimated safe dose, a no observed effect level (NOEL) divided by a safety factor approach and a mathematical extrapolation to a virtual safe dose has recently been suggested (Kuiper-Goodman 1990).

Conclusion

Three decades of research on aflatoxins has yielded voluminous information on various aspects of the problem. However, these efforts have yet to result in concrete action programs which could eradicate the problem of aflatoxins in food and feeds. The major lesson that has to be learnt from experience to date is that it would be more prudent to accept the existence of the problem and adopt management strategies to cope up with it rather than attempt to eradicate aflatoxins.

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Fusarium and *Alternaria* Toxins

B.J. Blaney*

Abstract

Fusarium and *Alternaria* species are adapted to high moisture contents, as occur in living plants. Grain crops will continue to support growth if they are not dried, such as is the case with maize stored as the cob, or as may occur during intermittent sun drying. Fruit and vegetables can support growth of these fungi during growth and storage.

This paper reviews the known prevalence of *Fusarium* and *Alternaria* species and their mycotoxins in stored food products. Contamination of potatoes, tomatoes, bananas, and citrus fruit by *Fusarium* and/or *Alternaria* mycotoxins during storage has been reported. The toxic effects of these mycotoxins is briefly examined. Recent research has focused on links between production of carcinogenic fumonisins by *F. moniliforme* and occurrence of oesophageal cancer. The mutagenic properties of several metabolites of *A. alternata*, including the altertoxins, are also of particular concern. These two species are among the fungi most prevalent on food crops throughout the world, including the tropics. In extrapolating from growing crops to storage, it is to be expected that production of mycotoxins and other secondary metabolites by the same fungus might differ greatly, because of environmental factors and the needs of the fungus. Future research needs to take a more dynamic look at secondary metabolic changes stimulated by the storage environment and the nature of microbiological competition in it.

MEMBERS of the genera *Fusarium* and *Alternaria* are among the most widespread and important plant pathogens in the world, and few crops are not attacked by one or more species. Members of both groups of fungi are known for producing mycotoxins in crops (grain, fruit, vegetables) in the field, and clearly this can give rise to mycotoxin contamination of products that are subsequently stored. But do these same fungi produce these same mycotoxins in the storage environment, or not? In any case, what is storage? Grain held in sealed bins and silos is certainly stored, ears of maize held in bins open to the weather is also stored, but what about fresh fruit and vegetables in an Asian market place, or even mature crops left in the field until markets are found? It seems that the dividing line between production and storage becomes a little blurred, and a generic definition that I will adopt is that storage is 'any stage in the process between maturation of our food crops on their parent plants, and their final consumption by people or domestic animals'. Having said this, it is clear that different storage situations can hold quite unique environments which influence the nature of the microbiological competition for potential food sources.

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Fusarium and *Alternaria* species as Storage Fungi

As plant parasites, *Fusarium* and *Alternaria* species are adapted primarily to growth at the high water activities found in living plants. They are far less limited by temperature than by the reduced water activity often found in stored products. In growing crops, some members of each genus have a limited host range, and some of these relationships show a complex balance between fungal phytopathotoxins or phytotoxins and plant phytoalexins. Other members of each genus are less host-specific, surviving by an ability to invade a range of previously injured or stressed hosts and to defend the substrate against competitors by physical or chemical means. Members of these genera that survive by their ability to circumvent the microbial opposition by host-specific mechanisms, are probably less able to compete with the non-specialists when it comes to invasion of damaged tissue. It is thus common to find several species on a single plant.

If a plant has been invaded by a *Fusarium* or *Alternaria* species during growth, the fungus can continue to grow within the invaded part while the moisture content remains high. Crops such as fruit and vegetables that are not dried before storage will continue to be capable of supporting growth of these species. Grain crops will

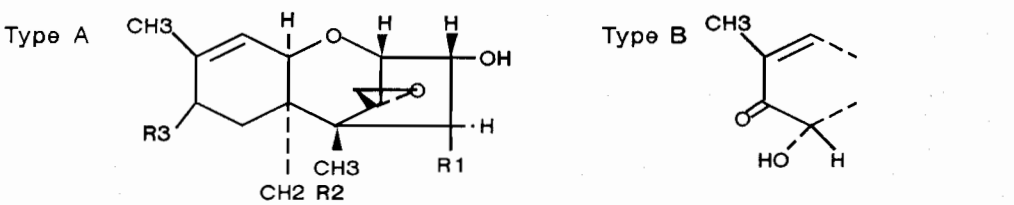
continue to support growth by these species in storage only if they are not dried, e.g. with maize stored on the cob in cribs (Mirocha and Christensen 1974) or in high-moisture maize inadequately treated with acid preservatives (Coppock et al. 1990). Small grains such as wheat and barley also will support continued growth of *Fusarium* species if drying is not attempted, or is hampered by husk and other trash in the grain (Moore et al. 1985). However, the same fungus may produce quite different mycotoxins and other secondary metabolites in a field and storage environment, as is further discussed below.

Mycotoxins produced by *Fusarium* and *Alternaria*

In beginning this section, I must note that our present knowledge of mycotoxins has largely resulted from investigations into the cause of acute intoxications in humans or their livestock. Much less is known of the effect of long term ingestion of fungal metabolites that have not been associated with a specific disease syndrome. For example, in reviewing phytopathogenic toxins and phytotoxins of *Alternaria, Fusarium,* and other fungi, Luke and Biggs (1976) warned that some of these occur in higher concentration in infected plants than do mycotoxins and that their animal toxicity had not been investigated at that time.

The fact that certain fungal chemical agents are acutely toxic to mammals when enough is ingested should not necessarily give us major cause for alarm. After all, we are an omnivorous species that has evolved a wide range of very effective defences against low concentrations of toxins in our food. What has become of more concern in recent years in the developed world, as a direct result of affluence and of increasing longevity, is the long term effect of ingestion of these toxins: and also the indirect effect that this fear can have on the orderly marketing of food between countries, often to the disadvantage of less developed nations.

With some exceptions, mycotoxins of *Fusarium* and *Alternaria* fall into several common biosynthetic categories of secondary metabolites: the polyketides (e.g. zearalenone, alternariol); the tetramic acids (e.g. tenuazonic acid); and the sesquiterpenes (e.g. trichothecenes). Structures of the mycotoxins that have attracted most interest are depicted in Figures 1, 2, and 3.



		R1	R2	R3
Type A	T-2 toxin	OCOCH3	OCOCH3	OCOCH2CH(CH3)2
	HT-2 toxin	OH	OCOCH3	OCOCH2CH(CH3)2
	Neosolaniol	OCOCH3	OCOCH3	OH
	Scirpenetriol	OH	OH	H
Type B	Deoxynivalenol	H	OH	
	Nivalenol	OH	OH	

* Acetylation or methylation of hydroxyl groups produces many related trichothecenes

Fig.1. Trichothecene mycotoxins from *Fusarium* species

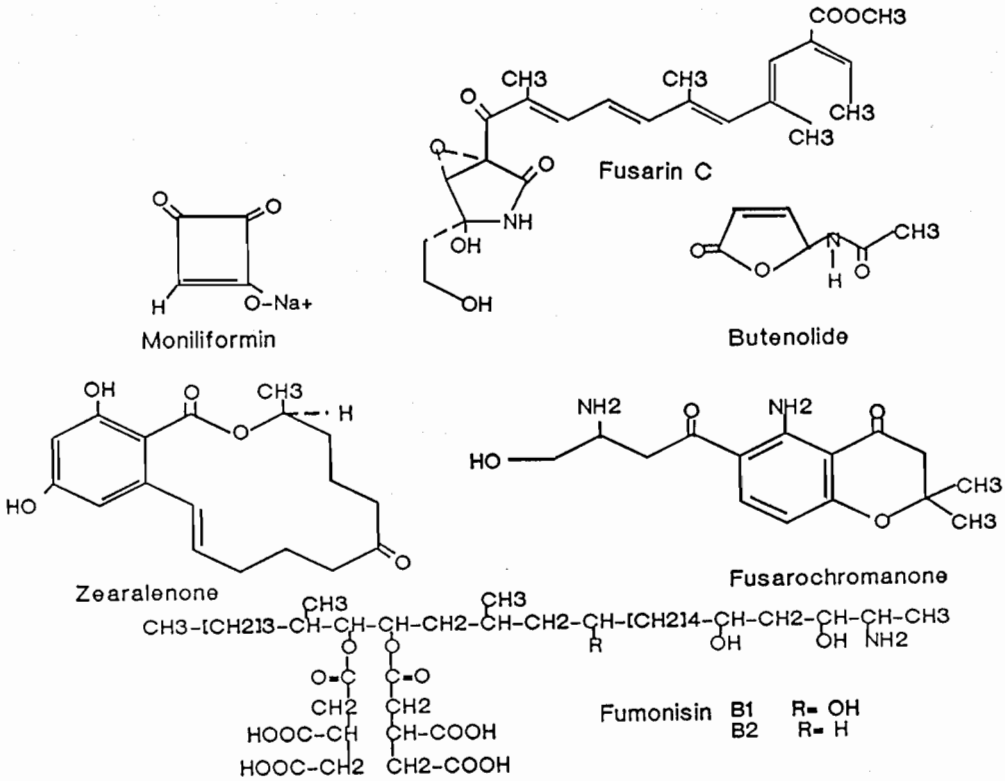


Fig.2. Non-trichothecene *Fusarium* mycotoxins

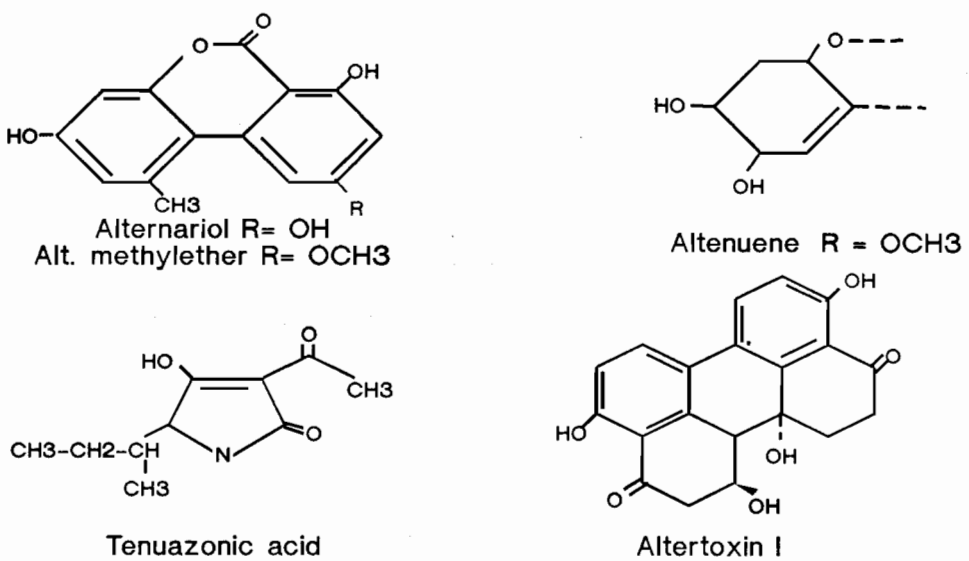


Fig. 3. Mycotoxins from *Alternaria* species

Fusarium species

Fusarium species are widely distributed in soil and organic substrates and have been isolated from the full range of climatic environments. They have a capacity for rapid change, both morphologically and physiologically, to a new environment. The classification system used here is that of Booth (1971), but with the separation of *F. graminearum* into groups 1 and 2 (Francis and Burgess 1977) and the addition of *F. crookwellense* (Burgess et al. 1982).

Most, if not all, *Fusarium* species are capable of producing some mycotoxins. However, some are more important in terms of human and animal disease because of their prevalence on the edible portions of crops, and capacity for production of high concentrations of highly toxic mycotoxins on those substrates. Marasas et al. (1985) examined 200 toxigenic *Fusarium* strains in the international toxic *Fusarium* reference collection and found them to belong to 20 species. These are given in Table 1, together with the major mycotoxins known to be produced by them. This is not intended as an exhaustive list — producers of zearalenone may also produce lower concentrations of zearalenols, while trichothecene producers each tend to produce a range of trichothecenes, with one or two dominant.

On the basis of their involvement in diseases, the most important toxigenic *Fusarium* species were regarded by Marasas et al. (1985) as: *F. graminearum* (Grp2), producing oestrogenic, emetic, and feed-refusal syndromes, and akakabi-byo — mainly due to zearalenone and type B trichothecenes; *F. moniliforme*, producing human oesophageal cancer and equine leucoencephalomalacia — probably due to fumonisins; *F. equiseti*, producing degnala disease and fescue foot — probably due to butenolide and type A trichothecenes, and tibial dyschondroplasia — due to fusarochromanone; *F. poae*, alimentary toxic aleukia — probably due to type A trichothecenes, haemorrhagic syndrome, Kasin Beck disease; *F. sporotrichiodes*, alimentary toxic aleukia, haemorrhagic syndrome, fescue foot, bean hulls poisoning, akakabi-byo — possibly due to type A trichothecenes.

Table 1. Toxigenic *Fusarium* species

Toxigenic species[a]	Mycotoxins produced
F. graminearum Grp 1	Zearalenone, deoxynivalenol
F. graminearum Grp 2	Zearalenone, deoxynivalenol, acetyldeoxynivalenol, nivalenol, acetylnivalenol, other trichothecenes, fusarin C, moniliformin
F. culmorum	Zearalenone, deoxynivalenol, acetyldeoxynivalenol, other trichothecenes
F. sambucinum	Scirpenols, other trichothecenes
F. sulphureum	T-2 toxin, scirpenols
F. crookwellense	Zearalenone, acetylnivalenol, butenolide
F. moniliforme	Fumonisins, moniliformin, fusarins
F. subglutinans	Moniliformin
F. proliferatum	Fumonisins
F. anthophilum	Uncertain
F. oxysporum	Zearalenone, neosolaniol, scirpenols, other trichothecenes, moniliformin, wortmannin
F. solani var *coerulum*	Scirpenols
F. equiseti	Zearalenone, scirpenols, neosolaniol T-2 toxin, acetyl-nivalenol, other trichothecenes, butenolide, fusarochromanone
F. acumimatum	T-2 toxin, HT-2 toxin, scirpenols, neosolaniol, other trichothecenes
F. semitectum	T-2 toxin, acetyl-nivalenol, other trichothecenes
F. avenaceum	Uncertain
F. sporotrichiodes	Zearalenone, T-2 toxin, HT-2 toxin, neosolaniol, scirpenols, deoxynivalenol, nivalenol, other trichothecenes, butenolide
F. poae	T-2 toxin, scirpenols, other trichothecenes, butenolide
F. chlamydosporum	Trichothecenes
F. tricintum	Uncertain
F. nivale	Nivalenol, acetyl-nivalenol, other trichothecenes
F. larvarum	Uncertain
F. merismoide	Uncertain

[a] According to Marasas et al. (1985): except for *F. sulphureum*, placed with *F. sambucinum*; *F. crookwellense*, recently described; and *F. graminearum* into groups 1 and 2.

Part of the infamous argument that trichothecenes (Fig. 1) were used as an agent of war as 'yellow rain' (Mirocha et al. 1983) was based upon the proposition that trichothecenes were not produced naturally in warm climates, since previous data showed them being produced more profusely at 0–8°C by certain *Fusarium* isolates. This was despite a report by Rukmini and Bhat (1978) that T-2 toxin had been detected in Indian sorghum. Ramakrishna et al. (1989) have since reported deoxynivalenol production by Indian fungal isolates from the subtropical Kashmir Valley. My colleagues and I have demonstrated the presence of nivalenol, acetyl-nivalenol and zearalenone in maize grown in high altitudes of the tropics in Queensland (Blaney and Dodman 1988). A *Fusarium* species from Queensland sorghum produced copious quantities of T-2 and HT-2 Toxins (2500 mg/kg) in sorghum and maize culture at 28°C (unpublished data). We have also confirmed by gas chromatography–mass spectrometry the presence of nivalenol and deoxynivalenol in maize extracts from the highlands of central Java (Widiastuti, personal communication). It is thus proven that all of the components of yellow rain can occur naturally in southeast Asia, but is not yet shown that they are either common, or that they naturally occur in the precise mixtures found in 'yellow rain'. It is interesting to speculate upon the possible role of bees feeding on infected pollen as delivery agents for such a mixture, since 'yellow rain' was heavily contaminated with bee faeces.

Occurrence of Toxigenic *Fusarium* Species and Mycotoxins in Stored Products

In this section, the species known to be toxigenic and listed in Table 1 are reviewed in line with their possible occurrence in stored products.

F. graminearum Grp1.

Although this fungus produces high concentrations of zearalenone and deoxynivalenol in wheat and barley straw, (Blaney et al. 1987), it rarely invades grain, and is not reported on other crops.

F. graminearum Grp2.

Although predominantly attacking cereals and other graminaceous hosts, it can be transmitted from maize to tomato and from tomato to maize (Booth 1971). It commonly infects seed of maize, wheat, barley, oats, rye, and rice. Spread is favoured in crop rotations combining wheat or barley with maize or rice (Cook 1981). Two chemotypes occur in *F. graminearum* Grp2 in respect to trichothecenes, those which produce predominantly deoxynivalenol and its acetyl derivatives, and those which produce predominantly nivalenol and its acetyl derivatives (Ichinoe et al. 1983). There is a very clear geographical separation of these two chemotypes in Australia, with only nivalenol-chemotypes occurring in north Queensland where maize is grown, while deoxynivalenol-chemotypes predominate in southern Queensland where wheat is the main crop (Blaney and Dodman 1988). Faifer et al. (1990) report that only deoxynivalenol chemotypes occur in Argentinian wheat crops. Among the more likely explanations that occur to us are that either deoxynivalenol-chemotypes have an affinity for wheat, or that the specific maize-breeding program in northern Queensland has also placed a selection pressure on secondary metabolism of the maize pathogen (*F. graminearum*) in the region. Naturally, either explanation strongly supports the role of these trichothecenes in the host invasion process. Various phytoalexins are believed to be produced in resistant maize hybrids, and 6-methoxybenz-oxazolinone is most frequently isolated —breakdown of this by the host is thought to produce resistance (Booth 1971).

F. graminearum Grp2 is the most important producer of zearalenone and type B trichothecenes such as deoxynivalenol and nivalenol. Production of acetyl-deoxynivalenol tends to parallel or even lead mycelial growth in culture, and concentrations are lower in mature culture (see below). For this reason and others advanced below, I tentatively suggest that concentrations of these particular trichothecenes are likely to reduce rather than increase during grain storage, even if conditions are otherwise suitable for fungal growth. On the other hand, concentrations of zearalenone are known to increase in certain storage circumstances, and infected maize with high moisture contents stored in open cribs under fluctuating temperatures can develop high zearalenone concentrations (Mirocha and Christensen 1974). However, different isolates may be well adapted to different climatic zones, and Australian isolates from the subtropics do not appear to increase zearalenone production in response to lower storage temperatures (Blaney and Dodman, unpublished data).

F. culmorum

This fungus is a soil inhabitant possessing highly competitive saprophytic ability and unusual tolerance of antibiotics. Infection of wheat straw with *F. culmorum* prior to burial in the soil prevents colonisation by other fungi (Booth 1971). It is a pathogen of wheat, rye, barley, oats and maize, co-occurring with *F. graminearum* and more abundant in cool climate regions such as northern Europe and southern Australia. *F. culmorum* is regarded as an important producer of zearalenone and deoxynivalenol in growing crops. Production of zearalenone in barley stored at 34% moisture has been reported (Gross and Robb 1975), but no information is available on the production of deoxynivalenol in storage conditions (Mills 1989). *F. culmorum* is sometimes associated with dry storage rot of potatoes, but is not as important as *F. solani* and *F. sambucinum* in this regard (Booth 1971).

F. crookwellense

Probably closely related genetically to *F. graminearum* and *F. culmorum*, this species produces acetyl-nivalenol and nivalenol, zearalenone and zearalenols (Bottalico et al. 1988). Isolates from dry rotted potatoes in central Poland produced these toxins plus the mutagen fusarin C (Golinski et al. 1988).

F. moniliforme

This fungus is widespread in humid and subhumid temperate zones, extending into the subtropics and tropics but uncommon in cooler temperate zones. The most common fungus on maize in most parts of the world, and a major parasite of rice, sugar-cane, and sorghum, it also attacks stored bananas, causing collar or crown rot, and stored pineapple and tomato.

Consumption of *F. moniliforme*-infected maize is associated with a high incidence of human oesophageal cancer in the Transki, in southern Africa. The fungus is extremely toxic to a variety of experimental animals and hepatocarcinogenic in rats. It is a poor producer of zearalenone and moniliformin. Gelderblom et al. (1988) have isolated fusarin C (a potent mutagen), and the fumonisins (which have cancer-promoting activity) from isolates of *F. moniliforme* from maize intended for human consumption in theTranski. Significantly higher levels of fumonisins were recently reported in mouldy maize from high-incidence cancer regions in the Transki than in low incidence areas (Sydenham et al. 1990). It is not yet clear to what extent these toxins are produced in storage rather than preharvest, but slow, sun drying of maize on the cob is normal practice, and Alberts et al. (1990) found fumonisin B1 production in maize cultures to increase during the stationary growth phase, and to be quite heat stable. Marasas et al.(1989) have also shown that fumonisins may be the cause of leucoencephalomalacia in horses.

Reports persist that *F. moniliforme* may produce trichothecenes, and Chakrabarti et al. (1986) found trichothecolone, diacetoxyscirpenol, T-2 toxin, and zearalenone in banana fruit infected in the field and in storage with *F. moniliforme*. Ramakrishna et al. (1989) also reported production of low concentrations of deoxynivalenol by *F. moniliforme*. However, Mirocha et al. (1990) remain adamant that this fungus does not produce trichothecenes—they cultured 34 isolates from all parts of the world on various substrates but produced none of 14 trichothecenes, or moniliformin, fusarochromanone, fusarin C, or wortmannin. While it certainly seems likely that *F. moniliforme* is often misidentified, it cannot be ruled out that certain strains produce trichothecenes only in the wild.

In regard to the mycotoxins produced by *F. moniliforme*, particularly those with demonstrated carcinogenic potential like fumonisins, it seems essential that surveys be performed into their possible occurrence in essential foodstuffs such as maize, and bananas and other tropical fruits. This is made even more urgent when it is realised that very little is known about the potential for growth of *F. moniliforme* in tropical storage conditions, and the production of mycotoxins in that environment.

F. subglutinans

This fungus has been extensively grouped and confused with *F. moniliforme*. It appears to predominate in cooler, more temperate areas than *F. moniliforme*, and is the major producer of moniliformin. No reports have been found of trichothecene production or otherwise. It may be involved in storage rots, but confusion with *F. moniliforme* makes this uncertain.

F. oxysporum

The most economically important member of the genus *Fusarium*, this fungus has a worldwide distribution and causes many serious diseases such as Panama disease of bananas. It was recently shown to produce the haemorrhagic mycotoxin wortmannin (Mirocha and Abbas 1989) which also has immunosuppressant effects in rodents (Gunther et al. 1989). Mustard seed infected with this fungus contained T-2 toxin and diaceto-xyscirpenol (Chakrabarti and Ghosal 1987).

F. solani

This species is found wherever plants are grown, but its chief economic significance is as a storage rot of potatoes. Apparently present in all potato growing regions of the world, *F. solani* var *coeruleum* causes a powdery, dry or white rot of stored potatoes, and superficial infection can occur in the soil. Most invasion occurs via wounds from other fungi or physical damage during lifting. Disease develops most rapidly at 20°C and high humidity. Firstly, small brown patches appear on the skin, and these enlarge as the skin wrinkles and the tuber shrinks. Pustules of the fungus may then burst through the skin, forming a stroma from which spores are produced. Control is by resistant varieties. El-Banna et al. (1984) reported that *F. solani* var. *coeruleum* can form deoxynivalenol in potatoes, while acetyl-deoxynivalenol and HT-2 were also detected in a few tubers.

F. equiseti

This fungus is common in warm temperate and tropical regions, and frequently recorded on cereals, including maize. It has also been isolated from avocado fruit, beans, cabbages, onions, potatoes, tomatoes, and soybean (Booth 1971). While it is regarded as a weak pathogen, its wide range suggests an ability to colonise and sporulate on a variety of substrates. Recorded as producing butenolide, zearalenone, and several trichothecenes, particularly the scirpenols, nivalenol, and T-2 toxin, this fungus is likely to invade grains harvested and stored with high moisture contents.

F. semitectum

Extremely common, particularly in the tropics and subtropics, this fungus is a secondary invader of plant tissue, often causing a serious storage rot of groundnut, banana, citrus, tomato, melons, and cucumber, and often being associated with disease complexes. It has been reported to produce diacetoxyscirpenol (Cole and Cox 1981), but reports of it producing toxins in stored products are lacking.

F. avenaceum

This species has a very broad host range and a worldwide distribution, though it is not quite as common as the other cereal-attacking *Fusarium* species. While chiefly a fungus of temperate zones and often a severe parasite of overwintering cereals, such as wheat, barley, and oats, *F. avenaceum* has been also reported in the humid tropics. It is a serious pathogen of broad bean in the USSR. Isolates from seed potatoes in Italy produced moniliformin (Logrieco et al. 1987).

F. poae

This species has a wide geographical and host range and is regarded as a weak parasite or saprophyte. It is common on graminaceous hosts in temperate regions, but also reported in heart-rot of sugar cane in South Africa, and on rice in Australia. *F. poae* is reported as causing head blight of maize in Europe and North America, and on decayed citrus fruit in Georgia, USSR (Booth 1971). It can produce type A trichothecenes and butenolide.

F. sporotrichioides

F. sporotrichioides has been described as 'widespread but sparse' on a wide variety of plants and soils in temperate regions (Booth 1971). A weak parasite, it causes wilting of seedlings, pea plants and squash. Many isolates of this fungus have been misidentified, so its prevalence may be greater than is presently known. It has a high potential for type A trichothecene production, but also produces type B trichothecenes, butenolide, and zearalenone (Marasas et al. 1985).

F. sambucinum

This species causes 'common rot' of stored potatoes in eastern Canada, Europe, and parts of southern Australia, but avoidance of physical damage will prevent storage rot. In France, an isolate from potatoes with fusarial dry rot produced diacetoxyscirpenol, which Lafont et al. (1983) considered a possible public health hazard. In the USA, Desjardins and Plattner (1989) isolated 15 strains from dry-rotted potato, which when grown on culture on potato slices, produced 15 mono- and 4,15 diacetoxyscirpenol, traces of T-2 toxin, HT-2 toxin, and neosolaniol. In Poland, isolates from dry rotted potato produced 20–330 mg/kg of trichothecenes in wheat grain (Perkowski et al. 1989). In the USA, Richardson et al. (1989) confirmed production of eight members of the scirpene group of trichothecenes in *F. sambucinum* cultures.

F. sulphureum

Economically important as a storage rot of potatoes, this fungus may compete or be associated with *F. sambucinum*. Marasas et al. (1985) include this with *F. sambucinum*, and their toxigenic potential should be considered together.

Alternaria species

Alternaria species are widely distributed in soil and plants. Those recorded on food crops are given in Table 2. Other species infect plants such as tobacco, cotton and flower crops (Ellis 1971). *Alternaria* species require a high moisture activity for growth. As a consequence, infection usually occurs in grains and seeds before harvest. Post-harvest infection is more likely to occur in fruit and vegetables that remain high in moisture during storage. Growth also can continue in refrigerated storage of fruit and vegetables since *Alternaria* species can grow at low temperatures.

Alternaria isolates grown in laboratory culture are toxic to chickens and rats (Sauer et al. 1978), chicken embryos and human cell cultures (Harvan and Pero 1976; Griffin and Chu 1983). Cultures are also teratogenic and foetotoxic in mice. Crude culture extracts and some purified *Alternaria* mycotoxins are mutagenic in the Ames test (Scott and Stoltz 1980), and the mutagenic activity of altertoxins was confirmed by Stack et al. (1986). The possibility that *A. alternata* may be a factor in the aetiology of oesophageal cancer in Linxian, China has been suggested by Dong et al. (1987).

The toxigenic potential of some *Alternaria* species has not yet been studied in detail. Altertoxins are highly mutagenic, alternariol mono-methyl ether is slightly mutagenic and the possibility of synergism is high. Tenuazonic acid is possibly the most acutely toxic of these mycotoxins and has been associated with onyalai, a haematologic disease among African blacks (Steyn and Rabie 1976), but in this case the tenuazonic acid was produced by *Phoma sorgina*. Alternariols have been found in 'weather-damaged' sorghum around the world (Seitz 1984), and up to 15 mg/kg was detected in very mouldy Queensland sorghum in 1983, as a result of prolonged wet weather and flood-submersion of crops (Williams et al. 1986). Traces of altenuene and altertoxin-I also have been found in sorghum. Alternariols have also been found in discoloured pecans. Tomatoes and apples naturally infected with *Alternaria* spp. and oranges and lemons naturally infected with *A. citri* have been found to contain most, if not all, of the *Alternaria* mycotoxins, albeit at low concentrations (Stinson et al. 1981). Tenuazonic acid at concentrations up to 7.2 mg/kg has been reported in black-moulded Italian tomatoes infected with *A. alternata* and *A. tenuissima* by Visconti et al.(1987). Mislivec et al. (1987) reported tenuazonic acid in fresh but mouldy tomatoes in the USA at concentrations ranging up to 70

mg/kg (average 4.9 mg/kg). *Alternaria* mycotoxins have also been detected in olives (Visconti et al. 1986) and sunflower seeds (Palmisano et al. 1989) in Italy.

Table 2. *Alternaria* species found on food crops

Species[a]	Toxins produced
A. alternata	Alternariols, altenuenes, altertoxins, tenuazonic acid
A. tenuissima	Tenuazonic acid
Alternaria state of *Pleospora infectoria*	Alternariols
A. brassicicola [b]	Unknown
A. brassicae [b]	Unknown
A. citri	Alternariols, altenuene, altertoxins, tenuazonic acid
A. solani [b]	Unknown
A. mali [b]	Unknown
A. cucumerina[b]	Unknown

[a] From Ellis (1971)
[b] See Sivanesan (this volume)

A. alternata

An extremely common saprophyte, this fungus is found on many plants and foodstuffs, and in soil and textiles (Ellis 1971). The fungus infects developing grain in most parts of the world via airborne spores at any time after anthesis. Persistent wet weather during maturation favours growth of *Alternaria* species until the stage where a darkish discoloration ('black point') becomes visible on the ends of some grains (Southwell et al. 1980). *A. alternata* (Fr.) Keissler is the most common fungus on ripening ears of wheat in all parts of Australia (Rees et al. 1984; Klein 1987). In 1983, wheat crops in south-eastern Queensland suffered severe weather-damage. In the course of a survey for *Fusarium* mycotoxins, alternariols were tentatively identified in half of the wheat samples assayed, but not quantified (Blaney et al. 1987). In sorghum grain, the situation is more variable. In some seasons, *Alternaria* species (probably *A. alternata*) are most common, but in other seasons, *Alternaria* are far less common than *Curvularia* and other fungi in the head mould complex.

Southwell et al. (1980) found that infection in wheat occurs 10–20 days after anthesis, when the water content is around 70% and water activity = 1.00. Magan and Lacey (1985) found that changes to water activity in *A. alternata* cultures affected the relative production of alternariols and altertoxins and that production of tenuazonic acid might be strain dependent. This means that the relative toxicity of *Alternaria*-infected grains could be poorly correlated with degree of fungal invasion. Magan and Lacey (1985) also found that growth and production of mycotoxins in culture was best at water activities above 0.95. Consequently, persistent wet weather during grain maturation may be needed for contamination, and continued growth in grain storage seems unlikely. However, factors not known are the *dynamics* of mycotoxin production in crops as the moisture content gradually falls during grain maturation, and the influence of competing fungi.

Alternaria state of *Pleospora infectoria*

This fungus is common on many plants, especially wheat, barley, oats, and rye (Ellis 1971). *A. alternata* and the *Alternaria* state of *Pleospora infectoria* also occur in soybeans in New South Wales (Stovold and Francis 1987). Unpublished work in Australia (Andrews and Lucas, personal communication) found it to be a weak producer of alternariols.

A. tenuissima

Plurivorous. Isolates from cottonseed produced tenuazonic acid (Young et al. 1980).

A. citri

Black rot of orange starts at the blossom end of fruit and causes premature ripening, followed by gradual progression through the tissues. Stem end rot of lemon starts at the stem end, with peel last to be affected. Oranges and lemons naturally infected with *A. citri* have been found to contain most, if not all, of the *Alternaria* mycotoxins, albeit at low concentrations (Stinson et al. 1981).

A. brassicicola

This species can cause considerable damage to cabbages and cauliflowers in transit. The potential for mycotoxin contamination during storage is unknown.

A. radicina

This fungus causes black rot of carrots, common in storage, a progressive softening and blackening of the tissue, most frequently starting at the crown. It also attacks celery, dill, and parsnips. The potential for mycotoxin contamination during storage is unknown.

A. longissima

A. longissima is found on fallen pollen grains of maize, and on husks and grains of rice (Ellis 1971).

A. brassicae

This species is found almost worldwide, producing dark brown spots on leaves of various Cruciferae, including broccoli, cabbage, cauliflower (black spots on heads), radish, turnip, and kohlrabi. The potential for mycotoxin contamination during storage is unknown.

A. solani

A. solani attacks the above-ground parts of plants of potatoes, egg plant, and tomatoes.

A. passiflorae

This species occurs on all aerial parts of the passionfruit plant, including the fruit, causing brown spot disease. The potential for mycotoxin contamination during storage is unknown.

A. cucumerina

A. cucumerina attacks melons and cucumbers.

Secondary Metabolism in *Fusarium* and *Alternaria* in Storage

It can be seen from the lists of fungi above, that many species are known to be toxigenic in culture, and some species are known to produce mycotoxins following infection of crops in the field. However, the influence of the slow maturation, harvest, and storage processes on mycotoxin production are much less well known. To gain some insight into the problem of *Fusarium* and *Alternaria* mycotoxins in stored products, I attempted to look at the problem of survival from the perspective of the fungi. Fungi generally are rich sources of chemicals that can be toxic to other fungi, bacteria, viruses, and insects. Bu'Lock (1980) has summarised opinion about the function of secondary metabolites, rejecting the hypothesis that the process of secondary metabolism is not useful to fungi. He refers to Muller (1974) who proposed that the general function of secondary metabolism is to provide a reservoir of non-functional diversity out of which new functional processes can emerge at some future time. Bu'Lock (1980) also accepts that it is likely that particular organisms may derive an additional selection advantage from having evolved certain uses for certain secondary metabolites.

I consider it useful to look at the whole problem of mycotoxins as one minor facet in a prolonged cycle of chemical warfare between microorganisms that commences when crops are planted, and ends only when the crop is finally consumed, and the microorganisms have provided for their next campaign in the war. It could be argued that some storage environments are an artifice of man, and that fungi have not evolved to compete in that environment. However, such a view ignores the incredible adaptability of fungi, their genetic diversity, and the plasticity of secondary metabolic processes.

We should not be surprised to find that the chemical agents used for attack or defence by a fungus are strongly adapted to a given environment and the competitors found in it. If a certain organism does not produce the appropriate chemical agents, it will not survive, unless it finds some other means of circumventing the competition (which includes the strategies used by many host-specific pathogens of *Alternaria* and *Fusarium* species). However, these chemical agents need not be toxic to a competitor. For example, it would be beneficial to a microorganism facing the end of a growth cycle because a certain nutrient is depleted *or the environment is slowly changing (e.g. cooling down or drying out)*, to convert as much substrate as possible into a form useful to itself if the environment again becomes favourable, *but is difficult for a competitor to use because it lacks those unique enzymatic biosynthetic pathways*. It will be even more advantageous, *if the agents are toxic, or repellent, or interfere in another organism's growth or reproduction.*

Having restated the case that secondary metabolites help fungi to survive, I wish to take this further, and ask whether the factors determining onset of their production are related to the needs of the fungus at different stages of its life cycle. Bu'Lock (1980) has stated that under optimal growth conditions, a fungus will display little or none of its potential for secondary metabolism, whereas under suboptimal growth conditions, the precise pattern of secondary metabolism depends on the history of the culture and current environmental conditions. This should be seen as a reflection of the high cost to the fungus of secondary metabolism, and emphasises the situation that 'wild' fungal cultures (on first isolation) bear little resemblance to those derived from subsequent subcultures. *This is particularly so for those pathogenic members of the* Alternaria *and* Fusarium *whose normal mode of survival is in living plants, rather than in stored products. Fusarium* species are quite notorious for rapid loss of ability to produce trichothecenes in subcultures.

Even in the 'wild', certain types of secondary metabolite may be more useful at certain times than others. For example, *Alternaria* and *Fusarium* species firstly need chemical agents that can breach the defences of a host plant. These include phytopathogenic toxins that initiate disease and phytotoxins that do not initiate disease but may be involved in the latter part of the disease syndrome (Luke and Biggs 1976). Secondly, they need agents to deter competitors from consuming this same substrate [and negative associations are often observed between different fungi on crops, as for example between *F. graminearum* and *F. moniliforme* on maize (Blaney et al. 1986)]. Thirdly, once it has exhausted growth-limiting nutrients, it needs to convert remaining substrate into a form that remains potentially available to itself, preferably with toxic or repellent properties against predators such as other microorganisms and insects during its period of dormancy.

Despite limited knowledge of their life-cycles, I can speculate that *Alternaria* and *Fusarium* species may produce phytotoxins in the early stage of growth, and antibiotics in the latter stages, since they specialise in high moisture environments in which the main competitors are bacteria. I will speculate further that those species which are more successful in a high moisture postharvest environment, may produce less phytotoxins and more antibiotics, and xerophilic fungi may spend less energy on producing phytotoxins and antibiotics than they do on producing anti-fungal agents and insecticides.

I also make the point that a substance that kills the host may not be in the long term survival interests of a true pathogen. Is it only chance that *Fusarium* pathogens of growing plants produce mainly type B trichothecenes, while those saprophytes that have to compete for damaged

or dormant plant material produce the more phytotoxic type A trichothecenes ?

It can be argued that most fungi produce antibiotics and insecticides, and that there is no apparent correlation between this and their mode of survival. I think that we have to be a little careful as to how we interpret the properties of these chemical agents. It seems quite likely to me that most 'wild' fungi have the genetic potential to produce a very wide range of these compounds. Clearly, some chemical agents are much more effective than others against bacteria. However, the effectiveness of a chemical agent to a fungus will be determined by a combination of factors, including toxicity to specific bacteria, broad spectrum activity against different bacteria, the relative cost of production, mode of delivery to target, and whether one agent can perform several additional functions.

It may be that a single mycotoxin can fulfil different functions in different fungi. In Figure 4 I have presented graphs of the production of zearalenone by isolates of *F. graminearum* Grps 1 and 2 in maize culture (Blaney and Dodman, unpublished data). Zearalenone regulates sexual reproduction in Grp2, inhibiting production of perithecia at high and low concentrations. Grp2 members attack aerial plant parts, causing head scab of wheat and barley, and ear rot of maize. On the other hand, Grp1 members rarely form perithecia or cause crown rot of wheat and barley.

It can be seen from Figure 4 that there is a different pattern of production of zearalenone by the Grp1 and Grp2 isolates. After 7 days incubation, the Grp1 isolate had produced 94 mg/kg, 5% of the maximum zearalenone concentration attained in that culture. With the Grp2 isolates, zearalenone production by 7 days was only 0.5 mg/kg, less than 1% of the maximum attained. This is consistent with regulation of sexual reproduction in Grp2, and probably a role other than sexual reproduction for zearalenone in Grp1 isolates. We also found a tendency for Grp1 isolates to produce more zearalenone and less trichothecene (Blaney and Dodman 1988). Perhaps zearalenone has no function at all in Grp1, but I strongly doubt it. To illustrate the point that fungal isolates conserve energy in secondary metabolism, I found a highly significant negative correlation between the production of zearalenone and trichothecenes in single spore cultures derived from the isolates of *F. graminearum* Grps1 and 2.

Initiation of production of different mycotoxins is triggered by different stimuli. In liquid culture of a *F. culmorum* isolate, Greenhalgh et al. (1985) found that butenolide was produced in the first few hours but then disappeared. Production of 3-acetyl deoxynivalenol was triggered when the free nitrogen was reduced to zero, and reached a maximum after 7 days: liquid culture favours production of acetylated trichothecenes, which are deacetylated on solid maize media (Figure 5, Blaney and Dodman, unpublished data).

The production of acetyl-deoxynivalenol by the Grp2 isolate reached a maximum concentration within 7 days. Deoxynivalenol continues to accumulate because acetyl-deoxynivalenol is its biogenetic precursor, and a constant rate of acetyl-deoxynivalenol production is probably balanced by a similar rate of de-acetylation. This is different from the pattern of production associated with

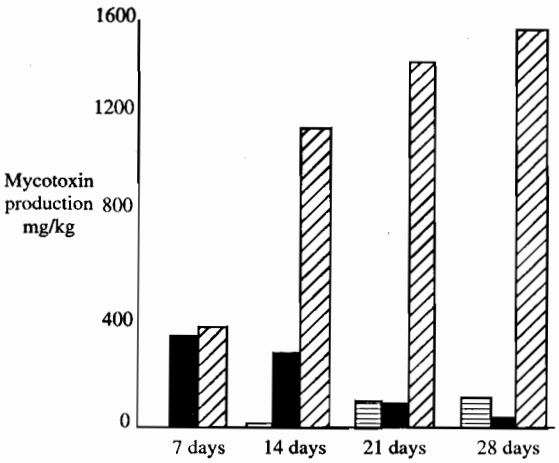


Fig. 4. Mycotoxin production by a southern Queensland isolate of *F. graminearum* Group 2 in maize meal culture at 28°C: zearalenone; acetyl-deoxynivalenol ; and deoxynivalenol

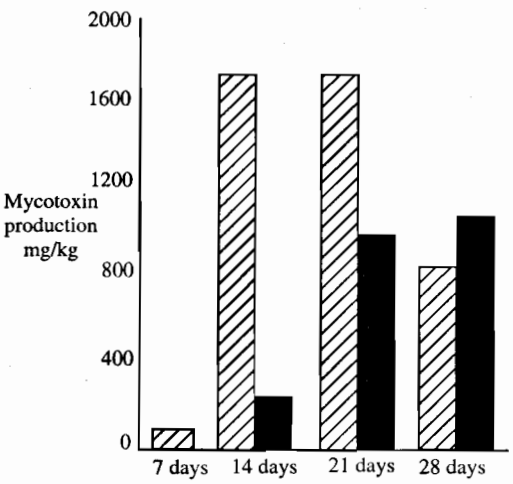


Fig. 5. Zearalenone production by southern Queensland isolates of *F. graminearum* Groups 1 and 2 in maize meal culture at 28°C: Group 1 ; Group 2 (×10)

many other secondary metabolites like alternariols, where production by *A. alternata* lags well behind culture growth (Magan and Lacey 1985). These different rates of production are consistent with a role in plant invasion for acetyl-deoxynivalenol in *F. graminearum* and a role of antibiosis by alternariols during survival of *A. alternata.* Of course, a sharp distinction between these roles should not be made, since multiplicity of function and synergistic interaction between these compounds should be expected.

If one of these chemical agents should be undesirable from the human viewpoint, our aim should be to influence the outcome of microbial competition so that the winners are those species of benefit to us. If we look at storage methods through the ages, it becomes clear that this has often been our approach — e.g. by using (in fermentation) our fungal allies to limit the growth of harmful organisms by producing chemical agents like alcohol, organic acids, carbon dioxide, or perhaps even specific antibiotics like kojic acid as in food cultured with *A. oryzae.*

Conclusions

What further research is needed in this area? Clearly, we need to continue to explore the toxigenic potential of those *Alternaria* and *Fusarium* species occurring in products stored in different environments, particularly fruit and vegetables, and the extent of mycotoxin contamination resulting from storage rots. We need to determine the extent to which *Fusarium* and *Alternaria* can continue to produce mycotoxins during slow sun drying of crops, particularly entire-ear maize, and unthreshed small grains. Finally, and most importantly, we should take a more dynamic look at the production of mycotoxins, and how their interaction with plant phytoalexins within common storage systems display gradually changing moisture contents, temperatures, atmospheres, and fungal or insect competition.

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Penicillium Toxins

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Abstract

Penicillium species produce an extraordinary range of toxic metabolites: at least 27 compounds with widely varied structures are regarded as genuinely toxic to animals or man. It is fortunate that most are produced only in wet conditions, are only acutely toxic, or are formed by species commonly found only in soils or other nonfood sources. The most toxic species grow best at low temperatures, and hence are most commonly found in temperate rather than tropical zones.

The major *Penicillium* toxins are described in this paper. The most important is ochratoxin A, which causes kidney damage in domestic animals and man. It is produced by *P. verrucosum*, which is common in European barley.

Penitrem A, produced by *P. crustosum*, is a tremorgenic toxin which causes sustained trembling in domestic animals, and may be rapidly lethal. Effects in man remain unknown. About 20 other tremorgenic compounds are made by *Penicillium* species, but these rarely occur in foods.

In past times, citreoviridin was of major importance, as the cause of acute cardiac beriberi in Japan. It is produced by *P. citreonigrum*, the cause of 'yellow rice': after yellow rice was banned from sale in Japan in 1910, acute cardiac beriberi ceased to be a problem. Other toxins found in yellow rice include luteoskyrin, erythroskyrin, cyclochlorotine, and islanditoxin. Produced by *P. islandicum*, each compound is quite toxic, but their significance in human and animal health remains unclear.

Other commonly occurring *Penicillium* toxins are patulin and citrinin, small molecules with definite acute toxicity, but no known long term effects. Patulin is produced by *P. expansum* when it causes apple and pear rot. Its occurrence can be controlled by careful culling of apples used for juice production. Although patulin has a relatively low toxicity, its presence is being monitored with increasing care around the world as an indicator of poor standards in apple and pear juice manufacture. Citrinin is produced by the ubiquitous species *P. citrinum*. Domestic birds are very sensitive to citrinin, so it is of importance in animal feeds. Human toxicity of citrinin and patulin appears to be quite low.

EXPERIMENTAL evidence that common microfungi can produce toxins is popularly believed to date only from about 1960. However, the study of mycotoxicology began 100 years ago. In 1891, in Japan, Sakaki demonstrated that an ethanol extract from mouldy, unpolished 'yellow' rice was fatal to dogs, rabbits, and guinea pigs, with symptoms indicating paralysis of the central nervous system (Ueno and Ueno 1972). The sale of yellow rice was subsequently banned in Japan in 1910.

Alsberg and Black (1913) reported isolation of *Penicillium puberulum* from mouldy maize in Nebraska. An extract of the fungus was toxic to animals when injected at 200–300 mg/kg body weight. They called the toxin penicillic acid. This was the first reliable account of toxin production by a microfungus in pure culture. Their study, carefully carried out, aimed to resolve the question of whether common fungi or fungal products could have an injurious effect on animals, and was far ahead of its time. Such direct evidence that common fungi could be toxic was largely ignored.

Miyake et al. (1940) published a study on *Penicillium toxicarium*, which they isolated and described from yellow rice. It produced a highly toxic metabolite, subsequently named citreoviridin. Perhaps because it was published in wartime, this study also failed to alert the world to the potential or actual danger of the toxicity of common fungi.

The discovery of penicillin in 1929 gave impetus to a search for other *Penicillium* metabolites with antibiotic

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properties, and ultimately to the recognition of citrinin, patulin, and griseofulvin as 'toxic antibiotics' or, as they later became called, mycotoxins.

The literature on toxigenic Penicillia is now quite vast. Of about 120 metabolites of common fungi with demonstrated toxicity to higher animals (Cole and Cox 1981), 42 were reported to be produced by one or more *Penicillium* species. No fewer than 85 *Penicillium* species were listed as toxigenic. The impression gained from the literature is that toxin production by *Penicillium* lacks species specificity, that is most toxins are produced by a variety of species. This viewpoint is commonly accepted. For example, citrinin has been reported to be produced by at least 22 species (Leistner and Pitt 1977; Cole and Cox 1981; Frisvad 1987). This picture has, however, been profoundly changed as a result of recent improvements in *Penicillium* taxonomy (Samson and Pitt 1990). Species concepts have been refined, and careful studies have shown that most *Penicillium* mycotoxins are produced by a small, well defined range of species.

Pitt and Leistner (1991) listed 27 mycotoxins, produced by 32 species, which possessed demonstrated toxicity to man or domestic animals. Molecular composition is diverse in the extreme. Patulin is an unsaturated lactone with a molecular weight of 150, while penitrem A has 9 adjacent rings with 4 to 8 atoms in each and a molecular weight of more than 650 (Cole and Cox 1981; de Jesus et al. 1981). While the toxicity of the *Penicillium* toxins is also very diverse, most toxins can be placed in one or other of two broad groups: those that affect liver and kidney function, and those that are neurotoxins.

Brief descriptions follow of the most important mycotoxins produced by *Penicillium* species.

Ochratoxin A

For half a century or more, nephropathy has been an important disease in Danish pigs. Etiological studies first showed it to be associated with mouldy grain, and then with a fungus identified as *P. viridicatum* (Krogh and Hasselager 1968). A representative isolate was first shown to produce oxalic acid and citrinin, and then ochratoxin A (Krogh et al. 1973). The major source of fungus and toxin was barley (Krogh 1978). Pitt (1987) showed that the species producing ochratoxin A in temperate zones was correctly named *P. verrucosum*, not *P. viridicatum*.

Ochratoxin A is fat soluble and not readily excreted, so it accumulates in fatty tissues. In northern Europe, where barley may be heavily contaminated with *P. verrucosum* and forms a major part of the diet of pigs, pork and bacon may contain high levels of ochratoxin. In consequence, ochratoxin poses a serious health risk to humans, especially in rural areas where pigs are not subject to rigorous inspection.

Toxicity

Ochratoxin A is an acute nephrotoxin, with oral LD_{50} values of 20 mg/kg in young rats and 3.6 mg/kg in day-old chicks. It is also lethal to mice, trout, dogs, and pigs (Scott 1977). Necroses of the renal tubules and periportal liver cells were the main pathological changes observed after fatal doses.

In humans, ochratoxin A appears to be responsible for kidney degeneration, which in extreme cases can lead to death. Kidney failure rates in rural Scandinavian populations are high, and the probable cause is the ingestion of pig meat containing excessive levels of ochratoxin A (Krogh et al. 1974). While Balkan endemic nephropathy, a devastating disease in certain areas of Yugoslavia, Roumania, and Bulgaria, may be caused by ochratoxin A (Krogh et al. 1977; Austwick 1981), direct or experimental evidence is of this lacking.

Distribution

P. verrucosum has been reported almost exclusively from temperate zones. It is associated with Scandinavian barley: in one survey of farms where pigs were suffering from nephritis, 67 of 70 barley samples contained high levels of *P. verrucosum*, and 66 contained ochratoxin A (Frisvad and Viuf 1986). This species has also been isolated quite frequently from meat products in Germany and other European countries. It appears to be uncommon elsewhere (Pitt and Hocking 1985).

Yellow Rice Toxins: Citreoviridin

The Oriental disease known as 'beriberi' has traditionally been regarded as a nutritional disease, an avitaminosis. However, beriberi is more than a single disease, and one form of it, known in Japan as acute cardiac beriberi, has been recognised for the past three centuries (Ueno and Ueno 1972). The disease frequently occurred in young healthy adults, and death could occur within a few days. The work of Sakaki in the 1890s (Ueno and Ueno 1972) implicated mouldy 'yellow rice' as a probable cause, and led to a ban on the sale of yellow rice in Japan in 1910. The disease subsequently disappeared.

Uraguchi (1969) and Ueno and Ueno (1972) showed that acute cardiac beriberi was a mycotoxicosis resulting from the growth in rice of *P. citreonigrum* (synonyms *P. citreoviride, P. toxicarium*), and that the mycotoxin responsible was citreoviridin.

Acute cardiac beriberi in Japan is now of only historical interest. However, *P. citreonigrum* and citreoviridin may still occur in other parts of Asia.

Citreoviridin is also produced by *P. ochrosalmoneum* (ascomycete state, *Eupenicillium ochrosalmoneum*). It is not closely related to *P. citreonigrum*.

Toxicity

Citreoviridin is a neurotoxin, acutely toxic to mice, with intraperitoneal and oral LD_{50}s of 7.5 mg/kg and 20 mg/kg, respectively (Ueno and Ueno 1972).

In several animal species, citreoviridin causes vomiting, convulsions, ascending paralysis, and respiratory arrest. Less frequent symptoms includes ataxia, enforced movements or stiffness in the extremities, and, later, cardiovascular disturbance, flaccid paralysis, and hypothermia (Uraguchi 1969). In higher mammals, neurological symptoms or depressed sensory responses are also evident. In many respects, the acute symptoms in man paralleled those observed in animals (Uraguchi 1969).

Distribution

P. citreonigrum is not a commonly isolated species, but is widely distributed (Pitt and Hocking 1985). According to Miyake et al. (1940), as reported by Uraguchi (1969), *P. citreonigrum* grows in rice after harvest, when the moisture content reaches 14.6%. At 1% higher moisture, other fungi will overgrow it, so the moisture band for invasion is narrow. The fungus is reported to be favoured by the lower temperatures and shorter hours of daylight occurring in the more temperate rice growing areas.

P. ochrosalmoneum is also an uncommon species in most environments. However, it has been found colonising unharvested maize in the USA, where it may produce citreoviridin under natural conditions (Wicklow and Cole 1984).

Yellow Rice Toxins: Toxins from *P. islandicum*

Because of the toxic 'yellow rice' syndrome, Japanese scientists have taken a particular interest in *P. islandicum*, which also can cause yellowing of rice (Saito et al. 1971). When tested against experimental animals, *P. islandicum* has been shown to produce several highly toxic compounds. However, the significance of these toxins, and of *P. islandicum* as a toxigenic fungus, remains in doubt. The species is included here as a potential problem, rather than because of known outbreaks of disease.

Toxicity

P. islandicum produces at least four mycotoxins that are unique to the species. Cyclochlorotine and islanditoxin are chlorine containing cyclic peptides which have the same toxic moiety, a pyrrolidine ring with two attached chlorine atoms, and share a number of other physical and chemical properties (Scott 1977). Both compounds are very toxic: cyclochlorotine has an oral LD_{50} in mice of 6.5 mg/kg, while that of islanditoxin by subcutaneous injection is 3 mg/kg. Fed to mice at the rate of 40 µg per day, cyclochlorotine caused liver cirrhosis, fibrosis and tumours (Uraguchi et al. 1972).

Luteoskyrin is a dimeric anthroquinone and erythroskyrin a heterocyclic red pigment. Both are liver and kidney toxins, though less acutely toxic than cyclochlorotine. Luteoskyrin is also carcinogenic.

Distribution

Reports of *P. islandicum* in nature have been infrequent (Pitt and Hocking 1985). Considering the striking appearance of colonies and the ease with which this species can be identified, the indications are that it is uncommon, at least in the temperate zones where most studies of *Penicillium* in foods have been undertaken.

Penitrem A, a Major Neurotoxin

The occurrence of serious outbreaks of tremorgenic and other neurotoxicity in domestic animals has been linked to several *Penicillium* species, notably *P. cyclopium* (now *P. aurantiogriseum*; Pitt 1979a), *P. palitans* (now *P. commune*; Pitt 1986) and *P. viridicatum*. The toxin responsible is now known as penitrem A, and its major source has been shown to be *P. crustosum* (Pitt 1979b). *P. crustosum* is now recognised as a very common species in foods and feeds (Pitt and Hocking 1985).

Toxicity

Naturally occurring compounds which can cause sustained trembling are rare, and most of those known are produced by fungi. Penitrem A is of the most potent. *P. crustosum* is by far the most important source, as it commonly occurs in foods, and virtually all known isolates are producers.

Penitrem A is a potent neurotoxin, with an intraperitoneal LD_{50} of 1 mg/kg in mice. Oral LD_{50} data do not appear to be available, but death or severe brain damage has been reported in field outbreaks involving sheep, cows, horses, and dogs (Wilson et al. 1968; Ciegler 1969; Richard and Arp 1979; Hocking et al. 1988).

In laboratory animals, the main symptom of poisoning by penitrem A is the onset of sustained trembling, which may continue for long periods without appearing to interfere with the normal functions of the animal. Trembling has been sustained in experiments for as long as 18 days without apparent ill effects or residual symptoms (Jortner et al. 1986). However, relatively small increases in dose (5 to 20 fold) can be rapidly lethal (Hou et al. 1971a). Post mortem diagnosis of tremorgenic toxins such as penitrem A is virtually impossible, as no pathological effects are evident.

The symptoms of penitrem A are essentially the same as those of a range of other fungal tremorgens, including those from *Claviceps paspali* growing in *Paspalum* grass, *Acremonium lolii* growing in *Lolium perenne* (ryegrass), or any of several *Penicillium* and *Aspergillus* species growing in foods or feeds (Pitt and Leistner 1991).

The potential hazard of penitrem A to man remains unknown, and puzzling. Its known toxicity to large domestic animals and dogs is such that it is unlikely to be nontoxic to humans. However, the only symptoms in man that can be attributed to *P. crustosum* have been unlikely, though quite well documented, instances of dizziness and vomiting after consuming beverages which contained mould growth. Recovery of patients was complete in all cases. Available evidence is fragmentary, and direct experimentation impossible. The role of penitrem A and perhaps other fungal neurotoxins in human illness or neurological disorders awaits elucidation.

Distribution

P. crustosum is a ubiquitous spoilage fungus. Pitt and Hocking (1985) reported isolating it from the majority of cereal and animal feed samples examined by them over more than a decade. *P. crustosum* causes spoilage of maize, processed meats, nuts, cheese, and fruit juices, as well as being a weak pathogen on pomaceous fruits and cucurbits (Pitt and Hocking 1985). The occurrence of penitrem A in animal feeds is well documented (Wilson et al. 1968; Ciegler 1969; Hou et al. 1971b; Richard and Arp 1979). Its occurrence in human foods appears equally certain.

Citrinin

Citrinin was discovered during the 1940s, and considered then to be a potentially valuable antibiotic. Like several other *Penicillium* metabolites, it proved to be too toxic for therapeutic use, and became known in time as a potentially hazardous mycotoxin.

Citrinin is produced by *P. citrinum, P. expansum,* and *P. verrucosum.* Literature citations (Leistner and Pitt 1977; Cole and Cox 1981; Frisvad 1987) indicate that at least 22 *Penicillium* species produce citrinin, but the great majority of these are either regarded as synonyms, or require confirmation (Pitt and Leistner 1991).

Toxicity

Citrinin is a significant renal toxin to monogastric domestic animals, including pigs (Friis et al. 1969) and dogs (Carlton et al. 1974). Domestic birds are also susceptible: citrinin causes watery diarrhoea, increased food consumption, and reduced weight gain due to kidney degeneration in chickens (Mehdi et al. 1981), ducklings, and turkeys (Mehdi et al. 1984). LD_{50} figures have been reported to be imprecise due to delayed deaths. The oral LD_{50} in mice is about 110 mg/kg (Scott 1977).

Chronic kidney degeneration in Danish pigs was at first considered to be due to citrinin (Friis et al. 1969), but later ochratoxin A was shown to be more significant (Krogh et al. 1973).

The effect of citrinin on humans remains undocumented. However, kidney damage appears to be a likely result of prolonged ingestion.

Distribution

P. citrinum is a ubiquitous fungus, and has been isolated from nearly every kind of food surveyed mycologically. The most common sources are milled grains and flour, and whole cereals, especially rice, wheat, and maize (Pitt and Hocking 1985). Toxin production is also likely to be a common occurrence.

Patulin

Like citrinin, patulin was discovered in the 1940s. For a time was considered to have potential as a cure for the common cold. Like citrinin, it was found to be too toxic for therapeutic use.

The main source of patulin in foods is *P. expansum*, the common apple rotting fungus. It is also produced by some isolates of *P. roquefortii*, the cheese mould, but patulin is apparently not formed in cheese during ripening.

Toxicity

Although patulin was at one time considered to be a carcinogen, and therefore a dangerous toxin, this is now considered to be unlikely. Its acute toxicity by injection has been reported to be 10–25 mg/kg in mice and rats (Ciegler 1977). However, toxicity by the oral route is relatively low, so patulin appears to pose little threat to humans.

Distribution

Because it commonly occurs in rotting apples, patulin occurs from time to time in apple juices, sometimes at very high levels. As it occurs only in tissue in which *P. expansum* has grown, it is an effective indicator of the use of substandard apples in juice manufacture.

Other Toxins

As has already been noted, *Penicillium* species produce a wide variety of other compounds shown to be toxic to man or animals. None can be regarded as a definite threat to human or animal health, however. An up-to-date and detailed account of *Penicillium* mycotoxins is given by Pitt and Leistner (1991).

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The Mycotoxins — Part 1
Session Summary

Chairman: Mr J.A. Conway, BULOG–NRI Development Project, Jakarta, Indonesia
Rapporteur: Dr B.D. Shukla, Central Institute for Agricultural Engineering, Bhopal, India

THREE papers were presented in this session.

Dr Ramesh Bhat gave an overview of aflatoxins research, beginning with some historical background to the discovery of aflatoxins in the early 1960s. Their discovery was followed by research on chemical aspects, biological effects in animal plant systems, natural occurrence, sampling methodology, ecological conditions needed for their formation, analytical methods, metabolism, biosynthetic pathways, disease outbreaks in man and animals, prevention methods including decontamination and detoxification, economic losses and impact on international trade, and basis for regulation.

Dr Bhat pointed out that the voluminous data generated in over 50 countries were mostly on fundamental aspects. Now, however, more emphasis is being placed on applied research to overcome the aflatoxins problem. Research in developing countries is concentrated on basic aspects, occurrence, and biological effects. In certain countries of Africa, the work is confined to practical aspects of minimising the extent of the problem.

Dr Bhat summarised research work on aflatoxins carried out in developing countries such as Senegal, Malawi, Nigeria, Tanzania, Zimbabwe, Kenya, Malaysia, and India. He said that limited success had been obtained, especially in Afro-Asian countries, in elucidating health hazards to humans and animals.

Dr Bhat noted that simple, rapid, and sensitive methods of analysis had been developed and were being used, though in some developing countries, because of lack of facilities, no work has been done.

Mr B.J. Blaney spoke on *Fusarium* and *Alternaria* toxins. Fungi in these genera are, he pointed out, adapted to high moisture conditions, as occur in living plants or in grain crops that are improperly dried. He described the toxic effects of their mycotoxins, noting that recent research had focused on links between oesophageal cancer and the ingestion of carcinogenic fumonisins produced by *F. moniliforme*. The mutagenic properties of several metabolites of *A. alternata* were also described.

In the final paper in this session Dr J.I. Pitt spoke on the toxins produced by *Penicillium* species. An extraordinary range of toxic metabolites is produced by these fungi, at least 27 compounds with widely varied structures, being regarded as genuinely toxic to animals and humans. Dr Pitt pointed out that it is fortunate that most are produced over a narrow range of water activities, are of acute rather than chronic toxicity, or are formed by species commonly found in soils and other non-food sources, and hence are rarely a direct threat to food supplies. Many of the toxic species also grow at relatively low temperatures and are more commonly found in temperate rather than tropical zones.

Ochratoxin A and penitrem A were described in some detail. The former causes kidney damage in domestic animals and humans, and is a potentially dangerous toxin principally produced by *P. verrucosum.*. Penitrem A, produced by *P. crustosum,* causes trembling in domestic animals, and may be rapidly lethal. Dr Pitt said that its effects on humans remain unknown.

The effects of the presence of *Penicillium* spp. in various commodities were described, as were measures needed to prevent infestation.

The discussion session following presentation of these papers was wide ranging, several speakers seeking the views of the panel on the most pressing subjects for study in relation to potential human health problems.

Dr Pitt's opinion was that aflatoxins remained of major concern but questioned whether we were actually facing a storage or a pre-harvest invasion issue. This was by no means clear on the basis of current knowledge.

Dr Bhat considered that attention should now be focused on the *Fusarium* toxins and was supported in this view by other speakers. Mr Blaney found the high levels of cyclopiazonic acid sometimes associated with significant aflatoxin levels in maize to be an area deserving of attention.

In the light of certain comments made by Dr Bhat during his presentation, the Chairman sought the views of the meeting on the efficacy of commercial testing kits. Responses were generally negative, with speakers citing high cost, frequent model changes, and narrow specificity.

Dr Pitt noted that standardisation of guidelines for the culturing of the toxigenic fungi and production of their metabolites to ensure consistency between research laboratories was sadly lacking at present. He made a plea for rationalisation of the current situation, citing it as a high priority problem.

Dr Bhat put forward a list of recommendations to guide research needs in the coming decade. These were welcomed by the Chairman who said they would be presented to a mycotoxins working group scheduled to meet later in the conference.

The discussion session concluded with a reminder from Dr J.D. Miller that the mycotoxins research arena was encumbered with a great deal of outdated and erroneous dogma which needs to be discarded in the move into the more complex area of *Fusarium* toxins. Subjects of fundamental research importance should be clearly defined, and a rational, coordinated, approach to research planning should be adopted.

The Mycotoxins — Part 2

Table 1. AOAC official methods of analysis (AOAC 1990)

Mycotoxin	Commodity	Method type	Date	Limit (μg/kg)
Aflatoxins	Foods/feeds	Romer minicolumn*	1975	5
Aflatoxins	Peanuts	CB method TLC	1968	1–5
Aflatoxins	Peanuts	BF method TLC	1970	–
Aflatoxins	Maize	AACC–AOAC TLC	1972	–
Aflatoxins	Cottonseed	TLC/HPLC	1980	15
Aflatoxin B_1	Cottonseed	ELISA[a]	1989	15
Aflatoxin M_1	Milk	TLC	1974	
Aflatoxin M_1	Milk	HPLC(fluorescence)	1986	0.005
Deoxynivalenol	Wheat	TLC	1986	300
Deoxynivalenol	Wheat	GC(HFBderiv)	1986	120
OchratoxinA	Barley	TLC	1973	–
Patulin	Applejuice	TLC	1974	–
Zearalenone	Maize	TLC	1976	–
Zearalenone	Maize	HPLC(fluorescence)	1985	200

appropriate experience to perform competently with the proposed method must be identified, and their agreement obtained to allocate time needed to undertake the study.

After 6–9 months work in undertaking the trial and evaluating the data, assuming that the results are satisfactory in terms of acceptable repeatability (r) and reproducibility (R) values, then the report of the trial has to undergo extensive peer review before final adoption as an official method. The length and overall cost of this evaluation process, coupled with the fact that not all collaborative trials necessarily yield the desired results, goes some way to explaining the lag between methodological innovation and adoption as official methods.

There are further problems with immunological developments in that there is such a large number of commercial ELISA-type products for mycotoxin screening and quantification, each of which may have different performance characteristics, that evaluation by the traditional approach of inter-laboratory comparison is difficult to undertake. Changes in format of these kits aimed at improved performance can be an added difficulty if costly collaborative testing is to be undertaken. In future, a different approach to that of inter-laboratory collaborative trial may be required for objective validation of these analytical approaches.

Sampling Procedures for Mycotoxins

A number of papers (Dickens and Whitaker 1986; Park and Pohland 1989) review the particular problems associated with sampling of commodities for mycotoxin analysis and the sampling schemes being used by various bodies. Unlike analytical methods, sampling schemes cannot be collaboratively tested and it is more a matter of a particular sampling plan being proposed based on measured toxin distribution, and thereafter being adopted as an official procedure. It is important to appreciate that sampling plans have different objectives, and an acceptable sampling plan for quality control purposes may be very different from a plan intended for use for enforcement purposes. In choosing to adopt a sampling plan, in addition to ensuring that it is based on sound statistical principles, practical considerations must be taken into account as there is little point in adopting a procedure so labour intensive that it is too costly to implement. General guidance is available from international bodies such as Codex (Codex Alimentarius 1987) on the factors that should be taken into account in considering sampling schemes. In enforcement situations there is some merit in specifying both the sampling scheme and the regulatory limit, so as to avoid dispute between parties on the level of mycotoxin contamination in a particular commodity when the individual samples have been taken in different ways.

The US Food and Drug Administration has well defined sampling procedures for aflatoxins (Park and Pohland 1989). These take account of the commodity type and whether samples are to be taken from retail or bulk commodities and, in the latter case, the lot size. For each circumstance the minimum number of subsamples to be taken is specified, as is the minimum unit size of each subsample. In addition, the equipment used for grinding and mixing of the bulk sample is specified, as is the subsample weight to be taken for analysis and the manner of its collection. For the analysis of aflatoxins in peanut butter, for example, the sampling requirement is for a minimum of 24 jars of 240 g size the contents of which are mixed before homogenisation of a portion (1100 g) in a blender with extraction solvent and, finally, collection of a 55 g subsample for analysis. For pistachio nuts in shell as a bulk consignment, 20% of the units have to be sampled to make up a total weight of 45 kg. The whole sample,

including shells, is then ground in a vertical cutter mill for 3 minutes and a final subsample of 200 g taken for analysis. Other sampling plans are in use in European countries, differing in detail but all requiring the collection of a large total weight of sample made up of as many subsamples as can reasonably be taken.

Nearly all work on sampling has addressed aflatoxins, where there is clear evidence of a highly skewed toxin distribution. For other mycotoxins that may be present in nuts, dried fruit, and cereals, although there are fewer data available on toxin distribution, as a rule much the same general principles of sampling tend to used as for aflatoxins.

Screening Methods for Aflatoxins

The Holaday-Velasco and Romer minicolumns are rapid screening methods for aflatoxins in foods and feeds that have been AOAC tested. These methods are inexpensive and rapid, but have the potential disadvantage of being non-specific, involving minimal clean-up, and relying to a large extent simply on the characteristic fluorescence of aflatoxins. Decisions as to whether or not fluorescence is present can therefore be rather subjective when using these columns, particularly where contamination is close to the operating limit. Equally rapid, but more specific screening methods for aflatoxins requiring minimal skill, to replace the minicolumn and for application in new field situations, were seen as a sizeable commercial market.

Antibodies initially produced for aflatoxin enzyme-linked immunoassays (ELISA) were utilised in the development of products designed specifically for rapid screening. Various commercial products have recently become available using absorbed antibodies in a sandwich format (Afla-20-cup, EZ-screen, and Cite-probe), antibodies bound onto a protein matrix in the form of affinity columns (Aflatest and Oxoid), or more directly on conventional ELISA principles (Agri-screen). The endpoint can be 'yes' at a predetermined limit depending on the absence of colour developing, or 'no' when a blue coloration occurs. Alternatively, the test may offer the possibility of a semi-quantitative estimation of aflatoxin concentrations by direct reading of either colour intensity, or fluorescence measurement.

When these kits were evaluated (Koeltzow and Tanner 1990) they were found to take, on average, between 4 and 15 minutes to carry out, the affinity column-based kits being the slower, presumably because of the time required to pass the extract through the column. For maize samples both naturally contaminated and spiked with total aflatoxins in the range 8.2–70.7 μg/kg there was no statistical difference in performance of Afla-20-cup, Aflatest, EZ-screen, or Oxoid compared with the minicolumn; performance being assessed on the basis of numbers of false positives and negatives (Koeltzow and Tanner 1990). The EZ-screen card test and the Afla-10-cup test were also evaluated for screening aflatoxins in raw peanuts, by comparing results for the same extract with those obtained by HPLC (Dorner and Cole 1989). Both tests correctly identified 95% of samples containing no detectable aflatoxin as negative, and more than 97% of samples containing over 10 μg/kg aflatoxin as positive (Dorner and Cole 1989). The ImmunoDot Screen (IDS) Cup from International Diagnostic Systems was collaboratively tested (Trucksess et al. 1989) and adopted as a first action AOAC method for screening of aflatoxins at ≥ 20 μg/kg for cottonseed and peanut butter, and at ≥ 30 μg/kg in maize and raw peanuts. It is recommended that positive samples detected by this screen be re-analysed by an official quantitative method.

ELISA methods for aflatoxins typically employ an initial aqueous extraction followed by a simple dilution of the extract in buffer, with aliquots of this solution being pipetted directly into the wells of a microtitre plate. Commercial kits employ single antibody, or more commonly, double antibody format which is less expensive but does require extra stages in the determination. Typically, for a 96 well ELISA plate, 16–24 wells would be used for running standards and, assuming samples are run in triplicate, the plate has the capacity for running around 25 sample extracts. Current commercial ELISA kits have limits of detection of about 2 μg/kg, and separate assays would need to be run to measure total aflatoxins as well as aflatoxin B_1 alone. A critical comparison has been carried out of three commercial ELISA kits (Patey et al. 1989). Although each kit was found to have its own merits, the overall assessment was that these ELISA methods were useful as screening procedures but were not sufficiently reliable to be used as quantitative tools. AOAC collaborative studies (Park et al. 1989a,b) were carried out of the Neogen Agri-screen kit, which is an ELISA method with visual or spectrophotometric estimation of colour intensity. The test was accepted as a first action screening method at > 15 μg/kg for cottonseed and mixed feed, and at > 20 μg/kg for maize and roasted peanuts.

ELISA methods are more expensive and elaborate to carry out than the rapid screening tests, but could suit the situation where very large numbers of samples are to be monitored. The choice between individual kits is to a large extent a matter of personal preference, and although the simple format of the card and cup tests seems particularly appropriate to field situations one must remember that any one of these kits will give a result that is only as good as the extract being analysed. Thus, although the kit may in itself be simple, other more elaborate equipment such as an homogeniser or wrist-action shaker is required for the extraction stage.

Affinity Column Clean-up for Aflatoxin Analysis

Affinity columns consist of an anti-aflatoxin antibody bound to a gel material (about 50–100 mg of gel on a dry weight basis) contained in a small plastic cartridge. The principle of the extraction is that the crude extract is forced through the column and the aflatoxins are left bound to the recognition site of the immunoglobulin. Extraneous material can be washed off the column with water or aqueous buffer, and the aflatoxins are finally obtained in a purified form by releasing them from the protein gel with an elution solvent such as methanol or acetonitrile. Columns are commercially available from different manufacturers, each performing differently in terms of recovery of aflatoxins depending on the specificity of the antibodies employed.

One of the attractions of affinity column clean-up for aflatoxin analysis is that, irrespective of the matrix to be analysed, the approach is much the same. Additionally, some of the mystique previously associated with the analysis of mycotoxins has been eliminated and anyone with basic skills in analysis should be able to get good results using affinity columns. Typically, using these columns, extraction of the commodity with either methanol/water or acetonitrile/water would be carried out as with the conventional approach. After filtration or centrifugation the extract is diluted with phosphate-buffered saline, and loaded onto the affinity column at a slow and steady rate of 5–10 mL/min. This is followed by washing the column with water and elution of the aflatoxins with either acetonitrile or methanol. Analysis for aflatoxins can then be by any method, although HPLC is the current preference.

Coefficients of variation (CVs) of 3–7% have been routinely obtained for the analysis of aflatoxins in naturally contaminated peanut butters. These results were obtained for 38 replicate analyses of a specially prepared homogenised smooth peanut butter that was being evaluated for certification as a reference material (Gilbert et al. 1991). This precision was at an aflatoxin B_1 level of 7 µg/kg and a total aflatoxin level of 10 µg/kg. Affinity columns can be applied to a wide range of different matrices such as nuts (peanuts, pistachios, Brazil nuts, and almonds), nut confectionery products, dried fruit, animal products, and animal feedstuffs.

An inter-laboratory evaluation of affinity columns for peanut butter analysis (Patey et al. 1990), where participants were allowed free choice of the end-determination, produced results giving CVs of the order of 30% at total aflatoxin levels ranging from 14–38 µg/kg. When a collaborative trial was conducted to AOAC guidelines (Patey et al. 1991), although a number of laboratories produced consistent results with quantitative recoveries, some laboratories clearly had problems with the use of the affinity columns achieving only low and highly variable recoveries. Relative standard deviations for repeatability [RSD(r)] and reproducibility [RSD(R)] of the method for three naturally contaminated peanut butter samples were 15–26% and 33–45% respectively. In view of the recovery problems and the less than satisfactory performance values the method was not considered for adoption as an official procedure. However, an essentially similar procedure using a different commercial immunoaffinity column and methanol/water extraction was collaboratively tested (Trucksess et al. 1991) and the recoveries were significantly better, averaging 80%. The method was recommended for adoption as an official AOAC, AOAC-IUPAC procedure on the basis of RSD(r) values ranging from 5–23% and RSD(R) values from 4–57% for aflatoxins B_1 and G_1. This immunoaffinity method could also be used without HPLC to estimate total aflatoxin concentrations using solution fluorimetry on the brominated column eluent.

Affinity column clean-up is particularly effective for the analysis of milks for aflatoxin M_1 as the liquid sample may be passed directly down the column and the aflatoxin eluted in an extremely clean extract for HPLC analysis (Mortimer et al. 1987). A mean recovery of 86% with a CV of 6.4% was obtained for replicate analyses of naturally contaminated milk containing 0.1 µg/kg aflatoxin M_1, and good agreement was obtained with certified values for European Community Bureau of Reference Materials (BCR) powdered milk certified reference materials. The chromatograms were considerably cleaner than using conventional silica gel columns, and the analysis was significantly faster with an average time including chromatography of 2.5 hours for the analysis of a batch of six samples. The International Dairy Federation (IDF) is currently organising a collaborative trial of an immunoaffinity column method for determining aflatoxin M_1 in milk.

HPLC Procedures forAflatoxins

HPLC analysis with fluorescence detection is now the preferred means of monitoring aflatoxins in the majority of modern laboratories. Fluorescence enhancement in reverse-phase HPLC solvents is generally achieved by pre-column formation of the hemiacetal derivatives by reaction with trifluoracetic acid (TFA) or by post-column iodination or bromination. TFA derivatisation has the disadvantage that the derivatives are sensitive to traces of methanol, and are of questionable long-term stability. Nevertheless an HPLC method using silica gel column clean-up and TFA derivatisation has been collaboratively tested and adopted as a first action AOAC method for determining aflatoxins in peanut butter and maize at concentrations of >13 mg/kg total aflatoxins (Park et al. 1990).

Iodination (Tuinstra and Haasnoot 1983; Shepherd and

Gilbert 1984) involves use of a post-column reaction coil heated at 75°C into which the column eluent is mixed with iodine-saturated water. The method can detect from 5 – 30 picograms of aflatoxin B_1 in a single HPLC peak, and is particularly attractive for use in automated HPLC systems. Post-column bromination has been proposed as an alternative (Kok et al. 1986), where bromide ion is included in the mobile phase and is converted to bromine post-column using an electrochemical cell.

A European Community (EC) collaborative study was conducted of a method for aflatoxin B_1 in animal feedstuffs using a two-stage clean-up of Sep-Pak florisil and reverse-phase cartridge columns and end-determination by HPLC with post-column iodination or 2-dimensional TLC (van Egmond et al. 1991). The method gave *r* and *R* values of 11% and 18%, respectively, at aflatoxin B_1 levels of 8 and 14 μg/kg and was recommended for adoption as an official EC method.

Analytical Methods for Trichothecenes

Of the trichothecenes most attention has been directed towards developing methods for monitoring the presence of deoxynivalenol (DON) and nivalenol (NIV) in foods and animal feeds. These compounds are particularly interesting in that, despite a number of advances in analytical methods, no one technique can yet be shown to be unequivocally superior and thus TLC, HPLC, and GC are all used alongside one another, each offering its own advantages as well as shortcomings.

Early methods for DON analysis involved a lengthy sample clean-up using a silica gel column with a GC end-determination. A more rapid clean-up was proposed (Trucksess et al. 1984) involving forcing the grain extract under vacuum through an alumina-charcoal-Celite column before TLC determination. This procedure was collaboratively tested, and found to give average recoveries of 78–96% with repeatabilities of 30–64% and reproducibilities of 33–87%, and was subsequently adopted as an official AOAC method (Eppley et al. 1986). The same approach was subsequently used for processed grain products — such as high sugar breakfast cereals, corn syrup, and beer — by inclusion of an additional C_{18} reverse phase cartridge clean-up stage (Trucksess et al. 1986). This use of charcoal as part of a clean-up system has found considerable favour for use in conjunction with TLC, HPLC, or GC as end-determinations.

GC and HPLC have found equal favour for instrumental analysis of trichothecenes. GC analysis requires derivatisation to form either trimethylsilyl (TMS) or heptafluorbutyrl (HFB) derivatives before capillary column determination with electron capture or flame ionisation detection (Ware et al. 1984; Scott et al. 1986). GC analysis is more sensitive and specific than HPLC, and samples can be readily confirmed by GC/MS, but the sample preparation is extensive and, for quantification, internal standardisation is to be preferred. A GC procedure was collaboratively tested (Ware et al. 1986) and shown to give an average recovery of 92% with mean repeatability and reproducibility of 32% and 41%, respectively, for spiked samples, and 31% and 47% for naturally contaminated samples. The greater separating capacity of GC compared with HPLC means that at least in theory it should be possible to monitor a number of different trichothecenes in the same extract. For HPLC analysis samples are run underivatised and using low wavelength UV for detection (Lauren and Greenhalgh 1987; Visconti and Bottalico 1983). Sensitivity is limited to 0.05–0.10 mg/kg which is adequate for DON and NIV, but interferences in HPLC can be a source of difficulty depending on the clean-up that has been chosen. To date there have been no collaborative trials of HPLC procedures for trichothecenes.

Mycotoxin Reference Materials

Although official methods must be fully tested and validated and their performance established by inter-laboratory collaborative trial, this is expensive, time-consuming, and inevitably means that their adoption lags some way behind developments in analytical methodology. An additional problem with some of the newer immunologically based procedures is the proliferation of various commercial products all of which it would be impossible to collaboratively test. The changes in design of these products as they are progressively improved and refined, also runs counter to the requirement of a stable product range after adoption of an official method based on a particular commercial product.

Although not a substitute for inter-laboratory comparisons, biological reference materials do offer the possibility of demonstrating both the accuracy and precision of a new method using naturally contaminated materials (Gilbert 1988). Two powdered milk reference materials are available from the European Community Bureau of Reference Materials (BCR) which have certified aflatoxin M_1 values of 0.307 ± 0.056 and 0.755 ± 0.052 μg/kg respectively (van Egmond and Wagstaffe 1987). These materials have proved useful both for validating immunoaffinity column procedures (Mortimer et al. 1987) and for demonstrating the possibilities of automating aflatoxin procedures (Gifford et al. 1990). Peanut butters containing certified levels of individual aflatoxins are now available from BCR (Gilbert et al. 1991), and work is nearing completion on wheat and maize samples naturally contaminated with deoxynivalenol. Current BCR projects include the certification of animal feedstuffs containing aflatoxins as well as barley and pigs kidneys containing ochratoxin A. This increasing range of certified materials should in future offer greater possibilities for rapid assessment of new methodology, both for different toxins and different matrices.

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Recent Developments in Methods for Sampling and Analysis of Mycotoxins

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Abstract

Efficient sampling, sample preparation, and analysis methods are required in order to identify the nature and extent of the mycotoxin problem, and also to form the basis of quality control procedures. The validity of the final analysis result (and any action based upon it) will be determined by the integrity of the initial sample. It is imperative that this sample and, consequently, the final result, can be identified with a specific population or batch.

The distribution of mycotoxins in stored products can be highly skewed. Consequently, the collection of truly *representative* samples requires carefully designed sampling protocols. This paper describes recent work performed on the distribution of aflatoxin in cereals and oilseeds, the use of distribution data in the identification of mathematical models, and the subsequent design of sampling protocols. Sample preparation methods and equipment are also described. Methods used for the analysis of mycotoxins include high performance liquid chromatography (HPLC), high performance thin layer chromatography (HPTLC), and enzyme linked immunosorbent assays (ELISA). Immunochemical methods are seeing increasing use for both the clean-up of samples and as rapid quality control tests. Solid-phase extraction cartridges have also proved to be valuable clean-up aids. A variety of analysis methods is described and compared, and their role in the control of the mycotoxin problem briefly reviewed.

An increasing awareness of the deleterious effects of mycotoxins on the health and productivity of man and animals has persuaded many countries to implement regulations to control the occurrence of these compounds in foods and feeds (van Egmond 1989).

The imposition of regulations by importing countries has placed severe pressure upon the exporting countries (frequently of Third World origin) who not only have to meet the stringent requirements of their customers but also have to accommodate, consequentially, the relatively higher levels of mycotoxins in their domestically utilised commodities.

If the interests of the Third World exporting countries are to be protected, it is vital that methods be available that are capable of effectively monitoring the mycotoxin content of a range of commodities within both the exporting and importing countries.

The components of these quality control methods will include sampling, sample preparation, and analysis.

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Sampling

It is imperative that the sampling step is performed accurately so that the sample collected is truly representative of the batch of food or feed under investigation. Needless to say, inaccurate sampling will completely invalidate the final result of the analysis.

Most of the current regulations are concerned specifically with aflatoxins and many workers have reported upon the heterogeneous distribution of these toxins in oilseeds and cereals (Jewers et al. 1989). Consequently, much effort is required to design efficient sampling protocols which can accommodate the highly skewed distribution of aflatoxins (and, presumably, other mycotoxins) in stored products.

Recent work at the Natural Resources Institute (NRI) and collaborating organisations has focused upon the design of sampling protocols for a variety of commodities including peanuts, cotton seed, maize and oil-palm (and their derivatives). The distribution of aflatoxins (total) in systematically collected, small incremental samples of foods and feeds is shown in Tables 1 and 2.

It is evident from Table 1 that 100 g incremental

Table 1. Distribution of aflatoxin in processed peanut kernels

Country	Batch size/samples	Contaminated samples	Aflatoxin content (ppb)	Batch average (ppb)
China	20 t 200 × 100 g	0/200(0%)	0	0.0
Sudan	20 t 200 × 100 g	1/200 (0.5%)	10	0.05
China	10 t 200 × 100 g	1/200 (0.5%)	60	0.3
China	20 t 200 ×100 g	6/200 (3.0%)	8–81	1.0
Sudan	15 t 200 ×100 g	3/200 (1.5%)	31,92,109	1.0
Argentina	20 t 150 × 100 g	3/150 (2%)	30,40,107	1.0
USA	15 t 200 × 100 g	2/200 (1%)	7,609	3.0
India	20 t 200 × 100 g	7/200 (3.5%)	8–1938	10.0

Table 2. Aflatoxin distribution within a batch

Commodity	No. of samples	Approx. sample size (g)	Range (μg/kg)	Mean (μg/kg)
Peanut kernels	154	100	0–3233	192.0
Peanut kernels	84	100	0–4235	209.1
Peanut kernels	200	100	0–13748	859.0
Peanut cake	204	100	87–249	163.0
Peanut meal	130	100	40–245	105.5
Maize	48	500	21–385	123.2
Cottonseed kernels	88	100	0–473	47.7
Cottonseed cake	93	100	12–128	57.2

Source: O. Roch, R.D. Coker, A. Sharkey, and G. Blunden, unpublished data.

samples of hand-picked selected (HPS) peanut kernels can contain as much as 200 times the mean aflatoxin content of the batch. A similar distribution pattern was exhibited by retail packs of peanuts (and peanut butter) which were systematically collected from the production line (Coker 1989).

The distribution of aflatoxin in fair average quality (FAQ) peanut kernels, peanut cake, peanut meal, cotton seed kernels, cotton seed cake and maize is shown in Table 2 (O. Roch et al., unpublished data). It can be seen that 100 g incremental samples of FAQ peanut kernels may contain approximately 16 times the mean aflatoxin content of the batch. Comparison with the data for HPS kernels (Table 1) indicates that the distribution of aflatoxins becomes relatively less skewed as the batch mean increases. It is also evident that a substantially more uniform distribution exists within peanut cake and meal. The distributions of aflatoxins in peanut kernels and the derived cake are compared in Figure 1.

The simplest approach towards the estimation of the batch mean is the collection of a single, representative sample followed by an analysis of the aflatoxins content. Sampling plans of this type are currently being developed at the NRI for peanut kernels, peanut/cotton seed/oil-palm meals, and maize. Composite, representative samples are composed of 100 g increments obtained by the systematic collection of spear samples from throughout the batch. It is anticipated that the required sizes of the composite samples will be, at least, equivalent to those shown in Table 3.

The nature of some commodities precludes the collection and/or comminution of large samples. Oil-palm kernels, for example, are reasonably simple to sample but notoriously difficult to comminute. On the other hand, peanut/cotton seed/oil-palm cakes are readily comminuted but extremely difficult to sample. The sampling of cotton seed kernels is also problematic.

Consequently, these problematic commodities require sampling protocols which involve the collection and comminution of small samples. Such sampling protocols are currently being developed at the NRI, and collaborating institutions, which require the collection

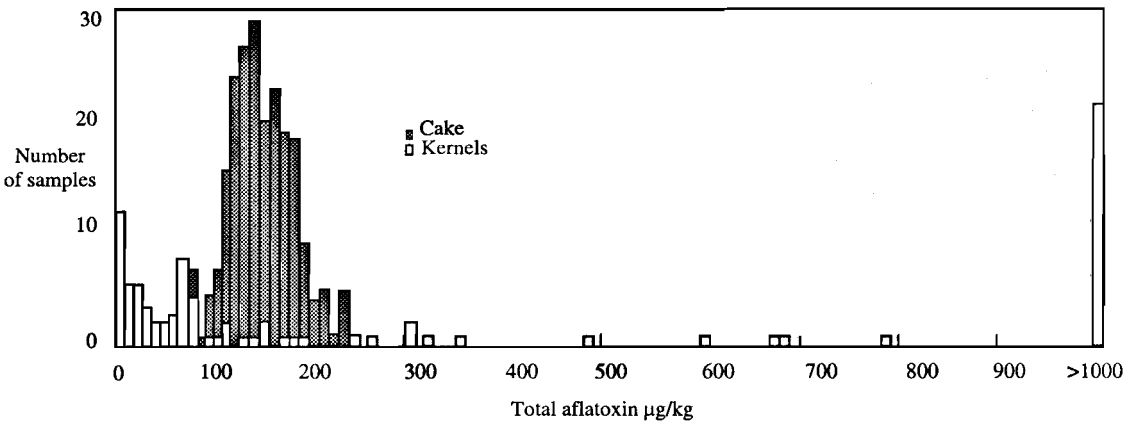

Fig.1. Aflatoxin distribution in peanut kernels and cake.

Table 3. Minimum sample size to obtain a single representative sample

Type of commodity	[1]Minimum sample
Very small particle (droplet) size e.g. milk, milk-products, and vegetable oils.	500 g
Particles of intermediate size, e.g. finely ground meals and flours, lime-treated maize, peanut-butter.	3 kg
a) Small grains, e.g. wheat, barley, oats, rice, sorghum	5 kg
b) Intermediate grains, e.g. maize, cotton-seed, [2]cottonseed-cake	10 kg
c) Large grains, e.g. peanuts, [2]peanut-cake, tree-nuts.	20 kg

[1]i) For a batch not exceeding 50 tonnes
ii) Composed of at least 100 subsamples, per 20 t, taken throughout the batch
[2]Portions of cake should be collected (by breaking larger slabs of cake, if necessary) that are not heavier than the appropriate subsample weight

and comminution of 1 kg samples (O. Roch et al. unpublished data; S. Nawaz et al. unpublished data). Each 1 kg sample is composed of ten 100 g aliquots. A brief description of the development of this approach to the sampling of problematic commodities follows.

An examination of the distribution of aflatoxins in a variety of oil seeds and maize has demonstrated that Weibull distributions can be fitted to the data summarised in Table 2 (Jewers et al. 1989). The three-parameter Weibull distribution has the distribution function:

$$F(x) = 1 - \exp(-(x-g)/n)^{b},$$

where g = minimum aflatoxin level, b = shape parameter, and n = scale parameter

The shape and scale parameters were estimated from '*n*' ordered observations according to the method described by Dubey (1967). The application of the Weibull model to the distribution of aflatoxin in peanut cake is illustrated in Figure 2.

The identification of a suitable Weibull distribution facilitated the computer generation of additional

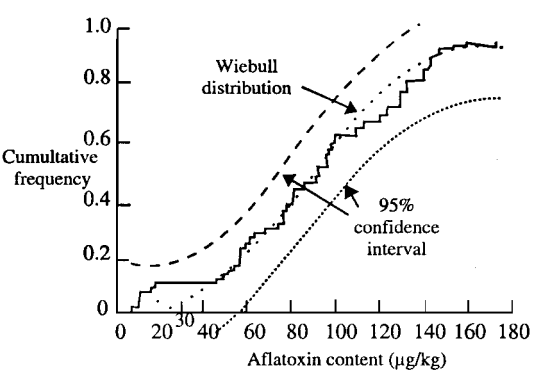

Fig. 2. A single Weibull distribution fitted to aflatoxin B_1 data from groundnut cake: g = 6.64; b = 2.48; and n = 94.01.

distribution data (typically 10 000 additional 'incremental samples') which were then utilised in the simulation of 1000 1 kg samples. Finally, the simulated 1 kg data were interpreted by utilising the z statistic (Jewers et al. 1989):

$$z = \frac{\bar{x} - u}{s / \sqrt{n}}$$

where x = sample mean, u = target mean, s = standard deviation, and n = number of samples

The utilisation of the z statistic may be summarised thus:

1. Define the maximum level of aflatoxin permitted in the batch (u, the target mean).
2. Define the other test parameters, for example:
   a) n, the number of 1 kg samples analysed should be at least three but no more than six.
   b) the batch is rejected if z is $\geq +2$;
   c) the batch is accepted if z is ≤ -2;
   d) further 1 kg samples are analysed if $-2 < z < +2$;
   e) the batch is rejected if any 1 kg sample exceeds, w, a predetermined aflatoxin level; and
   f) if six 1 kg samples are analysed the mean aflatoxin content of the samples is considered to be equivalent to the batch mean.

A simple computer program has been written which facilitates the application of the test parameters to successive 1 kg samples (O. Roch et al. unpublished data).

The application of the z statistic test to simulated 1 kg samples of peanut cake, cotton seed cake (O. Roch et al. unpublished data) and oil-palm kernels (S. Nawaz et al. unpublished data) is demonstrated in Table 4. It can be seen that a batch of peanut cake, with a mean aflatoxin content of 163 µg/kg, was correctly accepted by each of 276 tests when the target mean (the maximum accepted level) was set at 200 µg/kg. Each test, on average, required the analysis of between three and four 1 kg samples. Similarly, a batch of oil-palm kernels (at 214 µg/kg aflatoxin) was correctly rejected by 90% of 393 tests, each test requiring the analysis of between two and three 1 kg samples.

Work is currently in progress at NRI to finalise the design of sampling protocols for both problematic and non-problematic commodities.

Sample Preparation

One kilogram samples of problematic commodities (e.g. oil-palm kernels, cotton seed kernels, and oilseed cakes) may either be directly converted into an aqueous slurry (Coker 1984) (by blending with water) or slurried after comminution. Aliquots of slurry may then be analysed for aflatoxins (mycotoxins) by a method of choice.

For non-problematic samples (e.g. peanut kernels, maize, and oilseed meals) it is likely that the single, representative sample will be more than 10 kg in weight (Table 3) (Coker et al. 1984). Consequently it is necessary to reduce the initial sample to a representative 1 kg sub-sample.

If a subsampling mill is available, peanut kernels and maize can be simultaneously comminuted and subdivided to afford a representative 1 kg sub-sample. A prototype subsampling mill has been developed by the NRI and commercial partners (Fig. 3); pre-production models will be field-tested in India and Bangladesh in the latter part of 1991.

For oilseed mills, comminution and sample division must be performed as consequent steps. Rotary cascade sample dividers are routinely used at the NRI for the subdivision of free-flowing, comminuted samples. A cascade divider with a capacity of approximately 1 kg is shown in Fig. 4 . (A larger version with a capacity of approximately 15 kg is used, of course, for the subdivision of large samples.)

Analysis

The chosen analysis method will be determined by the nature of the job in-hand.

Table 4. Acceptance sampling protocol[a]

Commodity	Mean (µg/kg)	No. values	No. tests	No. accepted	No. rejected	% accepted
Peanut cake	163	1000	276	276	0	100
Cottonseed cake	57	1000	333	333	0	100
Palm kernels	214	1000	393	41	352	10

[a]Application of z value to simulated 1 kg samples
Minimum number of samples = 3
Target mean (max. accepted) 200 µg/kg
Max. accepted target value 250 µg/kg

Fig. 3. A prototype subsampling mill

Fig. 4. A rotary cascade sample divider

If a large number of samples is to be analysed, an automated procedure such as high performance liquid chromatography (HPLC), high performance thin-layer chromatography (HPTLC), or enzyme-linked immunosorbent assay (ELISA) will be advantageous. However, automated methods which offer the advantage of high sample throughput, accuracy, and precision require the purchase of expensive equipment. Such an investment will be justified for a busy quality control or research laboratory staffed by skilled analysts.

However, rapid methods are also required which are appropriate for the analysis of intermittent samples, requiring a quick decision, by relatively unskilled operators. Such methods must be simple to perform and require relatively unsophisticated equipment.

Sample Clean-up Methods

The efficiency of chromatographic analysis methods will be largely determined by the effectiveness of the sample clean-up step (i.e. the removal of interfering components from the initial, crude sample extract).

Two recent developments have resulted in an improvement in the precision of the clean-up procedure as well as facilitating the automation of this step.

The first development, known as solid phase extraction (SPE), involves the use of liquid phases bonded to an inert support, contained within a plastic cartridge (Coker and Jones 1988). Fig. 5 illustrates the use of SPE cartridges in combination with a vacuum manifold. SPE utilising a phenyl bonded-phase is used routinely at the NRI for the clean-up of sample extracts of peanut, cotton seed, maize, copra, and oil-palm prior to the quantification of aflatoxins. A small volume of the crude sample extract (normally in aqueous acetone) is applied to the SPE cartridge which is then washed with water. After drying by the passage of air, the aflatoxins are eluted with chloroform and the chloroform eluate dried by passing through a sodium sulphate cartridge (Fig. 6). Liquid handling equipment is now available which facilitates the automation of the SPE clean-up procedure (Fig. 7).

After SPE clean-up, quantification may be performed by HPTLC, HPLC, or other methods.

A combination of SPE (phenyl) clean-up and HPTLC quantification has been successfully applied at the NRI

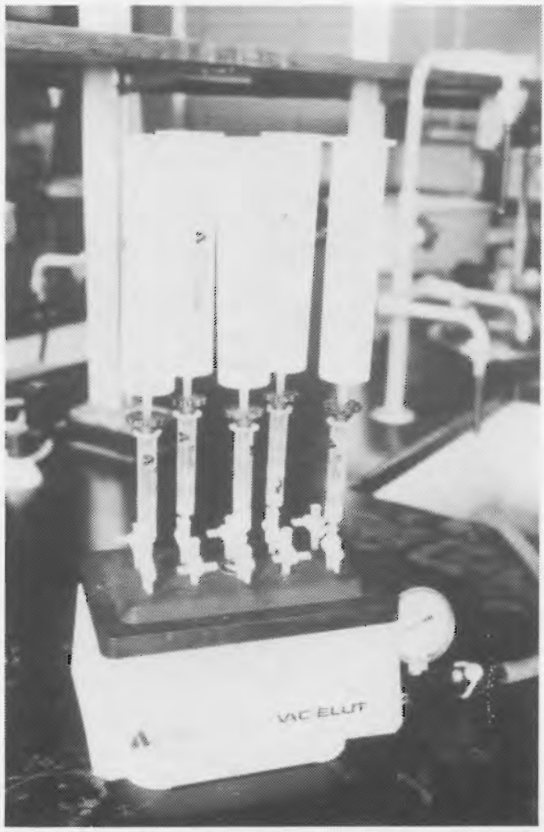

Fig. 5. Solid Phase Extraction cartridges and vacuum manifold

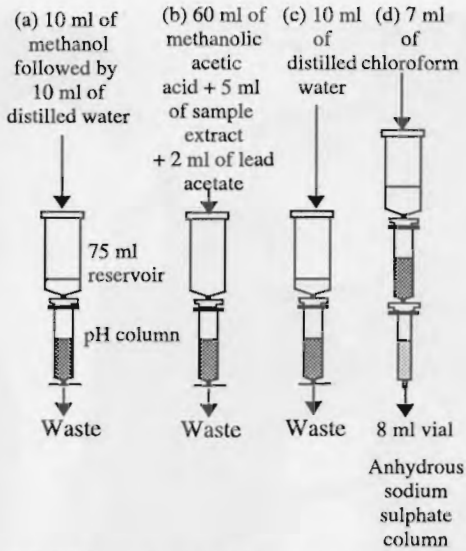

Fig. 6. Solid phase extraction. Aflatoxin sample clean-up using pre-packed disposable phenyl bonded columns. (a) Wetting the column; (b) isolation of aflatoxins; (c) rinsing the column; and (d) elution of aflatoxins.

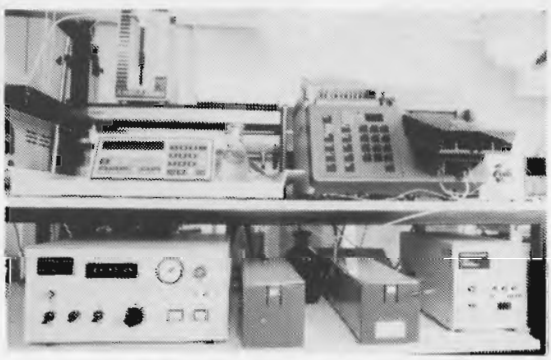

Fig. 7. Automated solid phase extraction and HPLC

to the analysis of aflatoxins in a variety of commodities including maize (Tomlins et al. 1989; Bradburn et al. 1990), cotton seed (Bradburn et al. 1989), oil-palm (S. Nawaz et al. unpublished data), copra (H.J. Nagler and J.Gibbs, unpublished data) and peanut butter (Dell et al. 1990). A typical chromatogram, after bidirectional HPTLC, is illustrated in Figure 8.

SPE clean-up methods have also been used in conjunction with HPLC quantification (Coker and Jones 1988) for the analysis of aflatoxin in peanuts (Hurst et al. 1987), cotton seed (McKinney 1981), and maize (Hutchins and Hagler 1983).

The second development in improved clean-up methodology is the immunoaffinity cartridge. In this approach, the crude extract is passed through a cartridge containing monoclonal mycotoxin antibodies adsorbed onto an inert support. The retained mycotoxins are eluted from the affinity column with methanol before quantification using a method of choice. Affinity cartridges are commercially available for the analysis of the aflatoxins B_1, B_2, G_1, G_2, and M_1.

After immunoaffinity clean-up, quantification may be performed by, for example, conventional HPLC methods or by using a simple fluorometer.

Immunoaffinity/HPLC methods have been applied, for example, to the analysis of aflatoxin in rodent feed using post-column derivitisation with iodine and fluorescence detection (Holcomb and Thompson Jr. 1991).

Immunoaffinity clean-up has also been used in conjunction with a simple fluorometer for the analysis of total aflatoxins in maize (Anon. 1990). In this procedure, the methanolic eluate from the affinity cartridge is treated with bromine solution in order to enhance the fluorescence of aflatoxins B_1 and G_1. This method has been adopted by the USDA Federal Grain Inspection Service (FGIS).

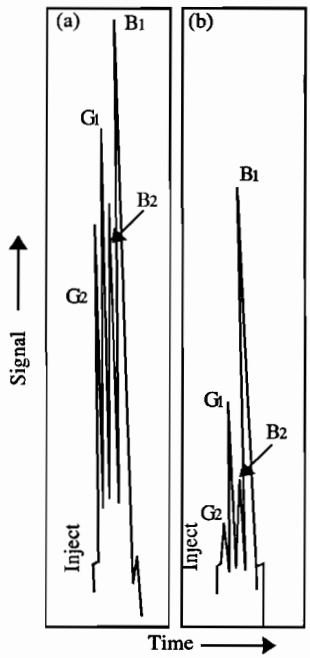

Fig. 8. HPTLC traces of (a) an aflatoxin standard and (b) a peanut butter sample. Densitometer light source; high pressure mercury lamp, Wavelength 306 nm, Scan speed 0.5 mm s^{-1}. Bi-directional plate development: the first solvent, anhydrous diethyl ether was used to remove interfering compounds. The plate was rotated through 180° and developed twice with chloroform-xylene-acetone (6+3+1) for 20 minutes.

Rapid Analyses Methods

The remainder of this paper will focus upon the development and utilisation of simple, rapid clean-up and quantification methods.

The utilisation of sophisticated HPLC, HPTLC, GLC, ELISA, and mass spectrometric procedures has been reviewed elsewhere (Coker 1984; Shepherd 1986; Coker and Jones 1988; Beaver 1989; Morgan 1989).

Effective quality control protocols require simple, rapid, efficient analysis methods which can be handled by relatively unskilled operators. Recently developed rapid methods include those that utilise immunochemical technology or selective adsorption agents.

The utilisation of immunoaffinity cartridges for the fluorometric analysis of total aflatoxins has already been discussed.

Antibody technology has also been applied to the development of rapid ELISA methods for a variety of mycotoxins including the aflatoxins, zearalenone, T-2 toxin, and deoxynivalenol in feeds and feed ingredients (Anon. 1989).

A rapid ELISA method for estimating aflatoxin in peanuts, cotton seed, maize, rice, and mixed feeds has been subjected to a collaborative study and recommended for First Action Approval by the Association of Official Analytical Chemists (AOAC) (Anon, 1989).

Solid phase ELISA kits have been developed for estimating aflatoxins, zearalenone, ochratoxin A, and T-2 toxin in a variety of commodities. An 'immunodot' cup test, where the antibody is immobilised on a disk in the centre of a small plastic cup, has been approved by the AOAC as an Official First Action screen for aflatoxin in peanuts, maize and cotton seeds (Trucksess et al. 1990).

Card tests have also been developed where the antibody is immobilised within a small indentation on a card similar in size to a credit card. Such tests have been developed for estimating aflatoxin, zearalenone, ochratoxin A, and T-2 toxin in maize.

The reported analysis (extraction, filtration, and estimation) time for solid phase ELISA kits is of the order of 5-10 minutes. Dorner and Cole (1989) have compared rapid ELISA and solid-phase ELISA methods with a conventional HPLC procedure for aflatoxins in peanuts. Good agreement occurred between the methods when the aflatoxin concentration of the sample was greater than 10 µg/kg.

Mini-columns containing selective adsorption agents have been developed for aflatoxin/zearalenone (single test) and the deoxynivalenol (Gordon and Gordon 1990).

In the aflatoxin/zearalenone test, for example, the extracted mycotoxins are partitioned into toluene which is added to the mini-column. Selective adsorption agents remove interfering compounds and then retain the mycotoxins as two discreet bands at the lower tip of the column. Designated levels of the mycotoxins can be observed under longwave UV light.

The FGIS has evaluated 8 commercially available, rapid tests for aflatoxin in maize. FGIS-approved kits include rapid ELISA, immunoaffinity cartridge, solid phase ELISA, and selective adsorbent mini-column procedures (Emnett 1989).

Conclusions

Any attempt to control the occurrence of aflatoxins, and other mycotoxins in stored products requires the implementation of efficient quality control protocols. The efficiency of these procedures will be determined, in turn, by the efficiency of their sampling, sample preparation, and analysis of components. Inefficient sampling methods will invalidate all subsequent activities.

It is therefore imperative that the development of efficient cost-effective sampling and analysis methods is pursued with considerable urgency.

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The Mycotoxins — Part 2
Session Summary

Chairman: Dr Mulyo Sidik, National Logistics Agency (BULOG), Jakarta, Indonesia
Rapporteur: Dr D.J. Webley, Australian Wheat Board, Melbourne, Australia

IN the first paper in this session Dr J. Gilbert discussed official methods for aflatoxins and trichothecenes analyses. He outlined the essential components of a collaborative test program, leading to an official reference method. These included the need for analytical standards, naturally occurring contaminated samples, blind duplicate test samples, determination of recovery, repeatibility, and reproducibility in a minimum number of laboratories. For aflatoxins, high performance liquid chromatography (HPLC) was now the method of choice, giving better separation and specificity than thin-layer chromatography (TLC), though during the discussion period it was agreed that TLC is extremely useful for experienced analysts. There is not yet an official ELISA method, although the Neogen kit had reached this stage for cotton seed analysis.

Sampling must be statistically based on distribution and be practical. It would be difficult to do a collaborative test of sampling methods. Various screening methods were described based on either ELISA or affinity columns. It was noted that the best extraction solvents were sometimes not compatible with kits, and that kits tend to change frequently. It was also noted that affinity columns could be used 10 or more times for clean-up, greatly reducing cost.

A number of official methods was described, including that of Tucksess (1991) for aflatoxin, the EC method for aflatoxin in feedstuffs, and methods for DON by TLC and GLC. The availability of certified reference materials, e.g. aflatoxin in milk powder, to allow assessment of recovery, accuracy, and repeatibility was described.

Dr R.D. Coker then covered recent developments in methods for sampling and analysis of mycotoxins. He began by describing different sampling situations, stressing that the validity of the analytical result depended on the integrity of the sample. It was shown that, because of the non-uniform distribution of aflatoxin in peanuts, only a few percent of samples may be positive from a contaminated batch. For example, 100 g samples could contain 200 times the batch mean, i.e. 2000 ppb in a mean of 10 ppb.

In the U.K., the Natural Resources Institute (NRI) was developing sampling plans for peanuts, maize, oilseeds, and meals. One hundred gram individual samples are combined into single composite samples per batch, depending on grain size and ease of grinding and mixing. The difficulty of sampling and a new NRI-developed sampling mill were described. The Weibull distribution can be fitted to the data of distribution of aflatoxin in a variety of commodities. A simple computer program has shown that the sampling plan described would rightly accept or reject consignments on nearly 100% of occasions.

Dr Coker then turned to the analysis of aflatoxin by clean-up on pre-packed disposable phenyl bonded columns, followed by bidirectional TLC on high performance aluminium backed commercial plates using an automated applicator and scanning densitometer read-out, describing, basically, the development and utilisation of simple, rapid clean-up and quantification methods.

Discussion ranged over a variety of topics including the relative merits of two-dimensional TLC, safety in laboratories, protection of samples from UV light, the value of recovery data from spiked samples, and further discussion on batch and sample sizes. It was concluded that any attempt to control aflatoxin and other mycotoxins in stored products requires the implementation of efficient quality control protocols, which are largely dependent on the efficiency of the sampling, sample preparation, and analysis components.

Professor U. Samarajeewa noted that analytical methods designed in the west are often not applicable to conditions in the Asian region for a range of reasons, some as basic as the fact that solvents evaporate more rapidly at higher temperatures. Other factors requiring attention are solvent mixture and substrate–solvent ratios, availability of solvents, particle size reduction for extraction, recoveries during extraction and analysis, check sample programs, and operator safety.

Dr Webley indicated that from his experience of the AOAC chloroform–water method, extracts of copra yielded gels and hence the method was unsuitable. A modified aqueous acetone procedure was shown in 1975 to be as good as any other methods and this method needs to be studied and popularised.

Dr Coker said that the most urgent need was for quantitative surveys of mycotoxin incidence, to supplement the qualitative data available. Associated quantitative sampling protocols would be required.

Management of the Microflora and Their Toxins — Part 1

Significance of Grain Mycotoxins for Health and Nutrition

J.D. Miller*

Abstract

Various metabolites of fungi are among the most potent natural toxins. These compounds are produced in the field during growth of crop plants or on the stored product. The mycotoxins of the 24 toxigenic *Fusarium* species are of most concern in the former category, along with ergot and sometimes *Aspergillus flavus*. Several species of *Aspergillus* and *Penicillium* are the most important producers of mycotoxins in storage. Most of the known *Fusarium* toxins were discovered only in the last 5–10 years. Although *Fusarium* toxins are most known from north-temperate climates, the species involved are widespread and the toxins can occur wherever cereals are grown. The toxicity of cereals and other crops infected by the toxigenic *Fusarium* species such as *F. graminearum* (deoxynivalenol, nivalenol, zearalenone, fusarin), *F. moniliforme* (fumonisin, fusarin), and *F. sporotrichioides* (T–2) is a result of the interaction between the major metabolites and numerous 'minor' metabolites. Crop plants can contain mixtures of toxins produced by the above species.

When crops containing mycotoxins from plant pathogens are put into storage, the edible material can become further contaminated with mycotoxins from *Aspergillus* and *Penicillium* species. There is evidence that grain contaminated with trichothecenes (produced by *Fusarium* spp.) may cause increased aflatoxin formation with the growth of *A. flavus/parasiticus*. In the final analysis, stored grain can contain a wide variety of toxins from several genera and species.

Acute trichothecene toxicosis involving tens of thousands of people has been reported in China and India. Liver and oesophageal cancers are high in areas with chronic aflatoxin and fumonisin/fusarin exposures in Southeast Asia, China, and southern Africa. Chronic exposures to trichothecenes and ochratoxin cause impaired immune function and probably indirectly cause increased bacterial and viral disease in a large part of the developing world. The serious effects on animal productivity caused by feeding the poorest quality grain containing mycotoxins are also a cause of malnutrition. These combined effects need to be taken seriously with respect to the many approaches for reducing mycotoxin contamination.

In 1989, A.L. Demain wrote 'It has always amazed me that the importance of secondary metabolites in ecological interactions between plant versus herbivore, insect versus insect, and plant versus plant interactions have been universally accepted but their importance in microbial interactions has been almost universally denied' (Demain 1989). The importance of secondary metabolites of moulds in their population ecology is becoming widely accepted. Unfortunately, this ecology involves limiting mammalian competitors. Pigs will refuse to eat maize contaminated by as little as 2 mg/kg deoxynivalenol (DON) if given a choice. The ancestral pig presumably was as turned off by DON in ancestral maize as domestic pigs. In such case, a colony of *Fusarium* had more food yielding more spores resulting in increased survival (Demain 1989; Wicklow 1981).

H.L. Trenholm and other colleagues at Agriculture Canada have spent a large amount of time and resources to elucidate the effects of *Fusarium graminearum* toxins on domestic animals, especially pigs. Some of the details of this work is covered in a review elsewhere in these proceedings (Trenholm et al. 1991a). However, we have learned that there is a series of effects depending on the amount of *Fusarium*-contaminated grain eaten. At absolute doses of perhaps a few mg trichothecene per kg body weight, a number of acute reactions occur, including death. At the other extreme of toxin exposure, subtle losses of animal productivity, reduced fertility, and increased bacterial and viral disease can be measured. In Ontario, there is little or no human dietary exposure to trichothecenes. However, the effects of exposure to *Fusarium*

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toxins on farm animal production cause considerable economic harm in some years. On an individual basis, the effects are not dramatic. However, the most modest impact with respect to feed conversion, lowered fertility, and disease incidence, multiplied by millions of pigs and other domestic animals is impressive (Trenholm and Prelusky 1988; Vesonder and Hesseltine 1981).

There are many data that indicate that contamination of grain with various mycotoxins has a major effect on public health, particularly in developing countries. This does not seem to be a widely held view. This is surprising given the effort in North America to keep food free of mycotoxins. Mycotoxin contamination of staple foods is very widespread, particularly with aflatoxins and *Fusarium* toxins (Jelinek et al. 1989; Scott 1989; Tanaka et al. 1988a). The significance of many *Fusarium* toxins is just beginning to be understood. Most were only discovered in the last 5–8 years. This includes the *F. moniliforme* toxin fumonisin which appears to be widespread.

A major problem in evaluating the significance of mycotoxins concerns the limited availability of expertise in this area and the high costs associated with such work.

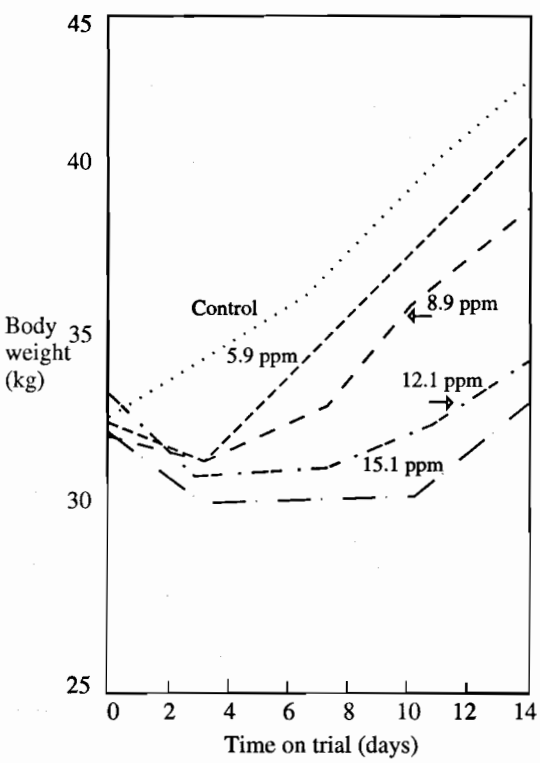

Fig. 1. Effect of pure deoxynivalenol on growth of swine (adapted from Trenholm et al. 1991b)

Most effort has been placed on aflatoxins, at least in part because this is a problem toxin in the USA. As a consequence, mycotoxin researchers polled in 1985 thought aflatoxins were the most important mycotoxins (41%), followed by *Fusarium* toxins (27%), and ochratoxin (12%) (Hesseltine 1986). If the poll is taken at the turn of the century, I think the results will be different. The widespread occurrence of *Fusarium* toxins such as trichothecenes and fumonisin noted above, plus the problem of co-occurring mycotoxins, will become more widely recognised.

A good example of the fragility of the mycotoxin data base for various parts of the planet can be illustrated with the question of *Fusarium* toxins in Southeast Asia. At the time of concerns over the possible use of trichothecenes in chemical warfare, an assertion was made that *Fusarium* toxins (trichothecenes) did not occur in this region (Mirocha et al. 1983; Robinson et al. 1987). This prompted considerable investigation and in fact toxigenic *Fusarium* species were found to be widespread on commodities in Southeast Asia. Trichothecene, fumonisin, and fusarin-producing species have been recovered from several countries. Trichothecenes were found in food and blood of people from some parts of this region (Miller 1991). Elucidating these facts involved laboratories from all over the western world over the last decade.

This paper will explore three important lines of evidence for an increased effort at improving the quality of staple grain foods in developing countries:

1. The common co-occurrence of mycotoxins with differing biological activities from different fungal genera and species, and the toxicological consequences of this fact.
2. Evidence of acute human mycotoxicosis in historical time and recent years.
3. Evidence of the direct and indirect effects of chronic exposure of mycotoxins on human health and nutrition.

Mycotoxin Mixtures

Toxins from *Fusarium graminearum*

The discovery of zearalenone and DON in grain infected by *Fusarium graminearum* in the mid 1970s led to considerable research on the toxicology of these two compounds. Pure DON has a considerable effect on feed intake and weight gain in pigs. Under experimental conditions, pigs fed 12–15 mg/kg DON in diets will gain little or no weight. Animals fed 2–9 mg/kg pure DON in diets will gain weight but less than animals fed clean grain. Animals fed < 2 mg/kg pure DON in diets will gain weight equal to controls (Fig. 1; Trenholm et al. 1991b). There is no effect of feeding pigs <10 mg/kg pure zearalenone on feed intake and weight gain (Young and King 1986).

Table 1. Frequency of *Fusarium* toxin contamination in cereal samples

	Nivalenol	Deoxynivalenol	Zearalenone
Europe			
Austria[a,b]	3/4	6/10	3/6
U.K.[c,d]	17/31	219/230	4/31
Poland[a]	14/48	40/48	
France[a]	1/2	2/2	
Hungary[a]	2/2	2/2	
Netherlands[e]	33/51		
Norway[f]	102/102	16/102	2/102
India[g]	24/65		
Africa			
Egypt[h]	3/64	36/64	
Zambia[i]	34/152	64/262	
South Africa[j]	34/43	35/43	43/43
Australia–Asia			
Australia[k]	2/12	11/12	
Korea[l]	74/89	87/89	64/89
China[m,n]	37/51	47/51	22/47
Taiwan[m]	6/12	9/12	9/12
Japan[o]	7/18	18/18	18/18
America			
Canada[p]	5/11	10/11	10/11
USA[q,r]	311/562	43/43	
Argentina[s]	123/123	54/209	

a. Ueno et al. 1985
b. Bottalico et al. 1981
c. Osborne and Willis 1984
d. Tanaka et al. 1986
e. Snijders 1990
f. Sundheim et al. 1985
g. Ramakrishna et al. 1990
h. Abdelhaimid 1990
i. Siame & Lovelace 1989
j. Thiel et al. 1982
k. Tobin 1988
l. Lee et al. 1985; 1986
m. Ueno et al. 1986a,b
n. Luo et al. 1990
o. Tanaka et al. 1985
p. Tanaka et al. 1988b
q. Scott 1990
r. Hagler et al. 1984
s. Resnick 1988

In female animals, the effects of zearalenone on fertility are significant above 0.25 mg/kg. From this information, it could be concluded that diets containing <2 and <0.5 mg/kg DON and zearalenone, respectively, should yield satisfactory pig performance. However, a series of discoveries has made this conclusion untenable.

The co-occurrence of zearalenone and DON is probably common in grain worldwide (Table 1; more detail can be found in Scott 1989). The effects of these two compounds together in pig diets were recently examined. The effect of pure zearalenone is antagonised by the presence of pure DON at low concentrations. In contrast, the effects of DON are slightly enhanced in combination over a range of zearalenone concentrations (Trenholm et al. 1988).

The situation with naturally-contaminated grain is more complicated. *F. graminearum*-infected grain was prepared into nutritionally equivalent pig diets containing about 5 ppm DON. The performance of the animals on a few of these diets was similar to that expected, based on the DON analysis. However, the response of the pigs to the

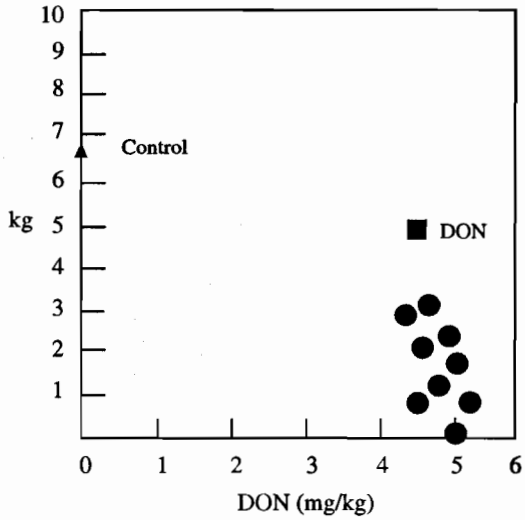

Fig. 2. Effect of grain naturally contaminated with deoxynivalenol on growth of pigs (adapted from Foster et al. 1986b)

majority of diets from naturally contaminated grain was very much inferior to the expected value. This indicated that there were other compounds present (Fig. 2; Foster et al. 1986b). Another complication is that animals fed 2 mg/kg DON will suffer immunological effects, as discussed later in this paper.

Fusarium graminearum has been shown to produce several dozen metabolites other than DON and zearalenone. (ApSimon et al. 1990; Greenhalgh et al. 1984, 1986). Many of these occur in quantity in naturally contaminated grain, including culmorin, hydroxycalonectrin, sambucinol, and acetyldeoxynivalenol (Foster et al. 1986a; Miller, unpublished data). The production of these 'minor' compounds—culmorin was found in corn in equal concentration to DON (Foster et al. 1986a)— is a strain-specific characteristic (Miller et al. 1991). Thus, each sector in a cereal plot could be contaminated by a slightly different mixture of metabolites additional to DON and zearalenone. The effect of mixing several of these metabolites with DON was tested with caterpillar species that are pests of maize. The metabolites culmorin, dihydroxycalonectrin, and sambucinol were not toxic to the insects tested at naturally occurring concentrations. When mixed with DON at natural ratios, these compounds were potently synergistic compared with DON alone (Dowd et al. 1989).

Monoacetates of DON occur as the 3- or 15-acetate. Almost all strains of *F. graminearum* in North America produce 15-acetyl deoxynivalenol (Abbas et al. 1986; Miller et al. 1983; Miller et al. 1991; Mirocha et al. 1989). In contrast, most strains from Japan produce 3-acetyl deoxynivalenol. Chinese strains tested to date have been found to be mostly 3-acetate producers although the

15-acetate has been found in Chinese grain (Luo et al. 1990; Miller et al. 1991). The toxicity of the two acetates to mice is different (Forsell et al. 1987; Yoshizawa and Morooka 1977). Which monoacetate of DON is formed, as well as the mixture of toxins produced by individual strains, is therefore a matter of considerable practical significance.

Toxins from more than two species of *Fusarium*

Table 1 also illustrates co-occurrence of two trichothecenes: DON and nivalenol. Nivalenol is produced by some strains of *Fusarium graminearum* and *F. crookwellense* (Miller et al. 1991). Naturally contaminated grain can contain DON, nivalenol, and toxins from *F. sporotrichioides* such as T-2, DAS, and HT-2 (Foster et al. 1986a; Scott 1990). The toxicology of co-occurring trichothecenes is also complicated. The natural combinations T-2/DAS, DAS/DON, and DAS/fusarenone have been reported to be synergistic in laboratory animals (Schiefer et al. 1986; Bhavanishankar et al. 1988). Experiments with yeasts have demonstrated that the toxicity of pairs of trichothecenes including the natural combination of T-2/HT-2 are synergistic in some ratios and antagonistic in others. An 'optimum' ratio was found which yielded the most potent combination of toxins at all doses (H.A. Koshinski and G.G. Khachatourians, unpublished data). The co-occurrence of the *F. graminearum* toxins DON and zearalenone with the *F. moniliforme* toxins fumonisin B_1 and B_2 has been reported from southern Africa (Sydenham et al. 1990). Fumonisins are potent carcinogens associated with high oesophageal cancer areas, as discussed later.

Toxins from more than one toxigenic genus

Co-occurrence of aflatoxin and trichothecenes has been reported in wheat in the USA and maize in Argentina (Hagler et al. 1984; Resnik 1988). Co-occurrence of zearalenone and aflatoxins has been reported in Brazil and Indonesia (Widiasuti et al. 1988a,b). Co-occurrence of aflatoxins and ochratoxin, and of aflatoxins, cyclopiazonic acid, and zearalenone was reported in Indonesia (Widiastuti et al. 1988b). It is most likely that DON was also present in these samples. The trichothecene T-2 toxin has been shown to synergise the toxicity of aflatoxin in several respects (Lindenfelser et al. 1974).

Co-occurrence of *Fusarium* toxins, aflatoxin, and cyclopiazonic acid has been reported from Indonesian samples (Widiastuti et al. 1988a,b). Co-occurrence of fumonisin and aflatoxin has been found in maize from the USA (Campbell, personal comm.). This is probably common and illustrates the contamination of grain by both field and storage toxin-producing fungi. The toxicology of such mixtures can only be imagined.

Synergism of the toxicity of metabolites from species of *Aspergillus* and *Penicillium* has been reported (Dowd 1988; 1989). Preliminary data suggest that metabolites of species of *Penicillium* that occur together in storage act synergistically (Wicklow, personal comm.). The presence of trichothecenes in grain put into storage may increase the amount of aflatoxin produced (Fabbri et al. 1984). There appears to be a very sophisticated ecology related to the production of mycotoxins by storage fungi (Wicklow 1984).

Acute Mycotoxicosis

In modern times, the effects of aflatoxins on humans have been considered important enough to require a sweeping and expensive regulatory system (Van Egmond 1989). However, trichothecenes (produced by *Fusarium* species) have been responsible for a number of large-scale human toxicoses in modern times. Alimentary toxic aleukia (ATA) is, according to Mirocha (1984), amongst mycotoxin researchers 'almost a household word'. This disease was described prior to 1900 and was associated with the ingestion of overwintered grain. Particularly during World War II, Russians were forced to eat grain left in the field. Thousands of people were affected, resulting in the elimination of entire villages. This syndrome has been shown to have been caused by T-2/neosolaniol/T-2 tetraol toxicosis (Mirocha et al. 1983). These are toxins of *F. sporotrichioides* which grows on wet grain left in the field.

So-called 'red mold poisoning' was reported in many parts of rural Japan in the 1950s (Udagawa 1988). This was found to be due to DON contamination. In fact, DON was first described in food that had caused human toxicosis (Morooka et al. 1972). Thousands of people were affected by DON toxicosis in an incident in the Kashmir Valley in India in 1987. Flour that in some cases had approximately 10 mg/kg trichothecenes was used by people as food. As with ATA, the symptoms were similar to those of other monogastric animals (Bhat et al. 1989). Luo (1988) reported ca. 10000 cases of acute trichothecene toxicosis in China over a 20-year period. Given the difficulties of acquiring and evaluating such information from rural China and India (Bhat et al. 1987), the Chinese data must be regarded as indicative of a larger problem.

These incidences point out the additional problem faced by people who eat grain as a staple in their diet. Campbell (1990) recorded cases of a syndrome that he regarded as associated with mycotoxin exposure (possibly *Fusarium*) in southern Africa. The number of cases rose dramatically in July and started to decline in December (Fig. 3). This rise was associated with the consumption of home-grown and stored grain. The low period was associated with the use of purchased grain subject to government control (Campbell 1990).

Acute human aflatoxicosis has been reported in Kenya and Mozambique. Cases of acute aflatoxicosis have probably also occurred in South Africa but been misdiagnosed (Marasas 1988).

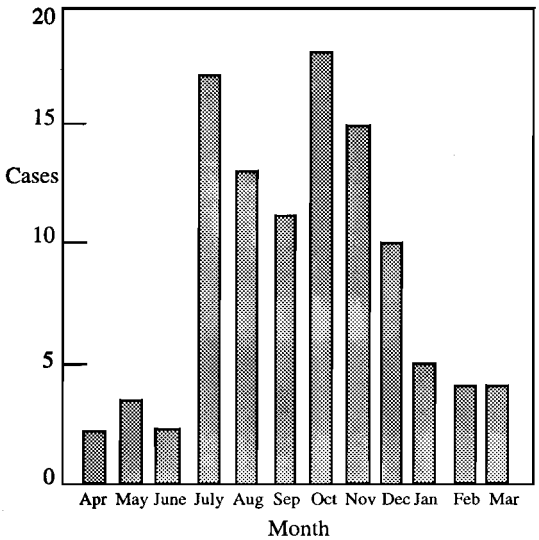

Fig. 3. Cases of suspected mycotoxicosis from an area in southern Africa (adapted from Campbell 1990)

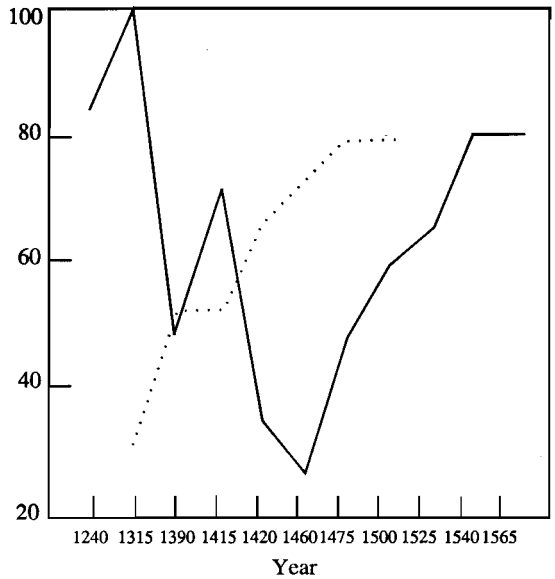

Fig. 4. Population index and rise of wheat consumption in eastern France from 1240-1570 (data from Matossian 1989): population index 1314 = 100, ————; wheat consumption .....

There are references to mycotoxin poisoning in the Bible (Hesseltine 1986). Ergotism has been blamed for several traumatic incidents in western history from the French revolution to the Salem witch trials in the USA (Matossian 1989). Analysis of information from the middle ages in Europe has also provided evidence of the dramatic effects of dietary exposure to trichothecenes. From the period from 1300–1500, much of western Europe suffered a severe depopulation. Data for part of France are shown in Fig. 4. During the period of greatest decline (1400–1500), there was a rise in the amount of wheat used as opposed to rye. The weather during this period (data for 1436–1487) was much colder than usual. In addition, the period between 1350–1479 had above average precipitation. Records exist of incidents of the demise of entire villages in this area. A good correlation between the wet, cold summers and these incidents ($r = 0.67$) was found (Matossian 1989). Rye is more resistant to fusarium head blight than wheat and wet summers favour this disease and the accumulation of DON.

Chronic mycotoxicosis

The strongest impression I have gained by observing the studies of *Fusarium* mycotoxins fed to pigs is that the effects of chronic exposure are by far the most important. Unfortunately, they have been much more difficult and costly to elucidate than the more dramatic acute responses. These studies have been done on animals with short lifespans hence the impact on humans of any outcome can only be conjectured.

Matossian's analysis of fertility patterns in relation to ergot alkaloid exposure in Russia through to the late 19th century suggest that chronic exposure to these mycotoxins disrupted human populations. Similarly, her assertion that chronic trichothecene exposure resulted in immune suppression promoting true plague epidemics in 14th century Europe indicates a massive impact on human populations (Matossian 1989).

Carcinogenicity

The most studied mycotoxin is aflatoxin. It is discussed in greater detail by R.V. Bhat elsewhere in these proceedings (Bhat 1991). This compound is a potent in vitro carcinogen. In warm climates (excepting the USA), there is appreciable human exposure to this compound. Dietary exposure and the presence of aflatoxin–albumin adducts from human sera have been quantified in the affected countries (e.g. Groopman et al. 1988; Wild et al. 1990). These exposures have been linked to the appreciable rate of liver cancer in these areas. On this evidence, regulations exist on the contamination of food with aflatoxins. These are vigorously policed in all western countries (Van Egmond 1989). It is well-known that there are a number of confounding variables with respect to the link between liver cancer and aflatoxin exposure. Nonetheless, the epidemiological data continue to show that chronic dietary exposure to aflatoxins causes liver cancer. This includes recent examination of the

relationship with hepatitis B virus (Kuiper-Goodman 1990a). *Inhalation* exposure to aflatoxin has also been demonstrated to promote liver cancer in humans. The dose-effect response is strikingly similar to that derived for dietary exposure (Olsen et al. 1988). Regardless, the International Agency for Research on Cancer classifies aflatoxin as a human carcinogen (Kuiper-Goodman 1990a).

A major problem that remains in unravelling the aflatoxin question is the implication of the probable co-occurrence of *Fusarium* toxins, particularly fumonisin and fusarin, in cereals. These toxins are produced, for example, by Southeast Asian strains of *Fusarium moniliforme* (M.E. Savard and J.D. Miller, unpublished data; J.D. Miller et al., unpublished data).

Fusarium moniliforme was associated with maize causing human toxicosis in 1881. This is rather earlier than the case of the notorious aflatoxin-producing aspergilli which were first thought to cause toxicosis in animals in 1940 and in humans perhaps 30 years later. Exposure to *Fusarium moniliforme*-contaminated maize has been linked to oesophageal cancer for more than 10 years in both southern Africa and parts of China (Marasas et al. 1988; Yang 1980). While it was known that this fungus produced moniliformin and some other relatively non-toxic compounds, a possible link eluded investigators until very recently. Two new classes of toxins have been now described from *F. moniliforme* fusarins and fumonisins.

Fusarins were described in 1984. Fusarin C is a mutagen of similar potency to sterigmatocystin and aflatoxin B_1. In addition, fusarin C is a macrophage inhibiter and causes a reduction in toxicity of the macrophages (Farber and Scott 1989). There are two less-toxic/ non-mutagenic forms, fusarin A and D. Another fusarin, E, has just been described, with unknown biological activity. However based on its chemical structure, it could have comparable activity to fusarin C (M.E. Savard and J.D. Miller, unpublished data). Fusarins occur naturally in maize in high oesophageal cancer areas of South Africa and China (Gelderblom et al. 1989). Fumonisins have been demonstrated to exhibit cancer-promoting activity in diethylnitrosamine-initiated rats and are toxic (Gelderblom et al. 1988). More recently, fumonisin has been demonstrated to be hepatotoxic and hepatocarcinogenic in rats fed 50 mg/kg (90%) fumonisin B_1 for 26 months (W.C.A. Gelderblom et al., unpublished data).

Significantly, the toxic effects reported from feeding purified fumonisin B_1 were more modest than expected, based on the feeding of culture material containing a similar amount of fumonisin B_1. Fumonisin concentrations have been shown to be higher in the high oesophageal cancer areas than the low rate areas (Sydenham et al. 1990). Interaction between fusarins and fumonisins and perhaps other toxins was indicated (W.C.A. Gelderblom et al., unpublished data). Previous work from South Africa demonstrated the co-occurrence of trichothecenes. Trichothecene contamination is common in the high oesophageal cancer areas of China (Marasas et al. 1979; Luo et al. 1990).

The effects of ochratoxin are sufficient to cause it to be regulated in a number of countries (Van Egmond 1989). This compound is carcinogenic and teratogenic (Kuiper-Goodman and Scott 1989). Exposure to this compound has been linked to an elevated occurrence of urothelial cancers in rural eastern Europe. This situation has not been sufficiently studied to affirm or deny the link (Castegnaro et al. 1990).

Immune Suppression

The immune-suppressing effects of mycotoxins may prove to be very important in terms of human health in developing countries. Regardless of their hepato-carcinogenicity, aflatoxins have been reported to cause reduced T-cell function, diminished antibody response, and suppressed phagocyte activity among other things in animals. Similarly, ochratoxin modulates various aspects of the immune response at both the cellular and humoral levels. Ochratoxin exposure induces natural killer cell activity and suppresses antibody response (Pestka and Bondy 1990). Fusarins inhibit the production and functioning of macrophages.

As noted above, the trichothecenes are widely distributed in staple foods all over the world. Animals exposed to trichothecenes show increased susceptibility to various fungal diseases (*Cryptococcus*, *Candida*) as well as to food-borne bacteria including *Listeria* and *Salmonella*. Trichothecenes affect B- and T-cell mitogen responses and macrophage function. Additionally, trichothecene exposure results in disregulation of IgA (for a review see Pestka and Bondy 1990). Naturally occurring concentrations of T-2, DON, and diacetoxyscirpenol (DAS) in food were found to result in serum trichothecene concentrations sufficient to cause immune-suppression (Mekhancha-Dahel et al. 1990).

Simultaneous exposure of T-2 and *Salmonella typhimurium* reduces the oral LD_{50} of the latter from 5×10^6 to 5 cells per mouse (Tai and Pestka 1988). A low oral dose of DON (2 mg/kg) causes a reduction in the time-to-death after exposure to *Listeria*. Grain containing this order concentration of DON and nivalenol is common in many parts of the developing world. The World Health Organization has identified the importance of these and other food-borne bacterial diseases in developing-country mortality (Kaferstein 1988).

Pestka and Bondy (1990) have noted the prevalence of IgA nephropathy and a possible link with trichothecene exposure. Up to 40% of patients with glomerulonephritis and some patients undergoing routine dialysis (10%) suffer from IgA nephropathy. This syndrome has been associated with diet and mucosal infection but the cause is not really known (D'Amico 1987).

Conclusions

This review has concentrated on the evidence for widespread dietary mycotoxin exposure in developing countries. There are periodic incidents of acute mycotoxicosis that have dramatic effects on the inhabitants of a given region. There are at least one and probably two families of mycotoxins (aflatoxins, fumonisins) that cause significant cancer mortalities in developing countries. Although the baseline concentrations of mycotoxins in staple foods are often modest, the interactions of these toxins have important toxicological significance. That is, the effects of a mixture of compounds at subacute concentrations are significant in monogastric animals and likely also in people. There is good reason to suspect that the immune-suppressing effects of many common toxins, particularly the trichothecenes, contribute to disease.

In developing countries, the worst quality grain is fed to animals. Grain containing mycotoxins will result in low animal productivity thus indirectly contributing to poor human nutrition. In extreme cases, there is some concern about mycotoxin residues in milk, meat, or eggs (Kuiper-Goodman 1990b). The economic effects of mycotoxin contamination in terms of precluding export of commodities to the developed world cannot be underestimated. This too would cause a degraded quality of life for many.

A critical beginning to dealing with this issue would be an effort to accurately assess mycotoxins exposure in developing countries. Scarce resources could then be effectively allocated towards a solution to this problem in the decades ahead.

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Safety of Mycotoxins in Animal Feeds and Approaches to Detoxification

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Abstract

Mycotoxins have been implicated in growth and reproductive problems, and disease outbreaks in farm animals. Mycotoxins of most importance to grain and livestock producers of northern USA and Canada include zearalenone, trichothecenes (deoxynivalenol, T-2 toxin, HT-2 toxin, diacetoxyscirpenol), ochratoxin A, ergot alkaloids and, recently, fumonisin B_1. Aflatoxins are the most prevalent mycotoxins found in the southern USA and throughout Southeast Asia. Cyclopiazonic acid is produced under similar conditions to aflatoxins. Each of these mycotoxins, when present in sufficient concentration in the diet, produces a wide variety of toxic signs dependent on the mycotoxin(s) involved and the animal species affected. Zearalenone is an oestrogen that causes reproductive problems. Trichothecenes reduce feed intake and weight gain and, at higher concentrations, cause emesis and complete feed refusal. Ochratoxin A is a nephrotoxin. Ergot alkaloids are associated with nervous disorders (tremors, convulsions), agalactia, and gangrene. Fumonisin B_1 is a carcinogen that is associated with equine leucoencephalomalacia, porcine pulmonary oedema, and spiking mortality in poultry. Aflatoxins cause liver damage and impaired immune function. Signs of cyclopiazonic acid intoxication include weight loss, weakness, diarrhoea, and convulsions. Grain and feed may be contaminated with several known, as well as unknown mycotoxins. Toxicosis may be associated with additive or synergistic combinations of mycotoxins. Regulatory officials worldwide are very concerned about the presence of mycotoxins in food and animal feed. Prevention of mould and mycotoxin contamination must continue to be a high priority area of research, though under certain environmental conditions the natural contamination of grain cannot be avoided. There is thus a body of postharvest research aimed at developing methods for detoxifying contaminated grain and feed. Approaches to detoxification include chemical, physical, and biological treatment of contaminated grain, and manipulation of the diet to reduce the effects of such mycotoxins on the animal itself. Until a commercially applicable detoxification method is developed, economic losses due to mycotoxin contamination of cereal crops worldwide will remain high.

MYCOTOXINS are naturally occurring secondary metabolites produced by several fungal genera (see Bhat 1991; Blaney 1991; Pitt 1991) on a variety of crops, especially cereal grains. They can be produced in the growing crop and during storage. Animals may die following ingestion of mycotoxins or suffer growth and reproductive problems. Mycotoxins have also been implicated in non-specific illnesses in farm animals. Considerable economic losses are attributed to reduced crop yields and grain quality following fungal contamination, to downgrading of cereals from human food grade to animal feed, and to decreased animal performance and increased incidence of disease in livestock consuming mycotoxin contaminated grain. Since the discovery of mycotoxins 30 years ago, attention has focused on the occurrence and biological effects of these compounds. However, during the last decade much research has concentrated on delineating the taxonomy and toxicology of the genus *Fusarium* as the mycotoxigenic potential of these moulds has become apparent, especially in North America and southern Africa.

This report will focus on the relative toxicity of mycotoxins found in northern areas of North America, methods of decontamination, and the economic impact of mycotoxins.

Mycology

Most varieties of maize and small grain cereals are susceptible to fungal infection and mycotoxin contamination.

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The most serious toxigenic fungi in eastern Canada and northeastern USA are those of the *Fusarium* genus. *F. graminearum* is the fungus associated with ear rot in corn and *Fusarium* head blight in wheat. The principle inoculum source of *F. graminearum* is host debris, e.g. old maize stalks, ears, and stubble, and debris of small grain cereals left on the soil surface. Weeds also serve as a source of mould inoculum.

For diseases to occur, environmental/weather factors favourable for production and dispersion of spores, growth on the host surface, and infection must coincide with the time that the grain is susceptible to mould infestation. Infestation is favoured by warmth and prolonged surface wetness. The concentration and profile of toxins produced by *Fusarium* species also depend on these environmental conditions. In general, moist, cool conditions favour mycotoxin production by *Fusaria*. Moreover, after harvesting, mould growth and mycotoxin production may continue if the grain is not dried sufficiently or properly stored.

Toxicology

Some of the most important mycotoxins found in the northeastern United States and eastern Canada are: zearalenone; the trichothecenes — deoxynivalenol (DON), T-2 toxin, HT-2 toxin, and diacetoxyscirpenol (DAS); ochratoxin A; ergot alkaloids; and fumonisin B_1 (FB_1). Although some of these mycotoxins have been associated with animal diseases in North America, aflatoxin is still of major concern in the southern areas of this region, as it is in Southeast Asia. A description of the toxic effects of certain mycotoxins is presented in Table 1.

1) Zearalenone

Zearalenone (ZEN) may be present in cereal crops in cooler, moist regions of the northeastern USA and eastern Canada. This toxin has strong oestrogenic influences in pigs and is often associated with swelling and reddening of the vulva, uterine enlargement, and vaginal and rectal prolapse (Chang et al. 1979; Trenholm et al. 1988; Young et al. 1987). Levels above 1 to 2 mg ZEN/kg diet have been associated with prolonged oestrus, infertility, stillbirths, and reduced litter size in pigs (Long and Diekman 1986). Friend et al. (1990) have observed that ZEN at very low levels (< 1 mg/kg) is oestrogenic to young female pigs (gilts).

Effects in other species are much less pronounced. High concentrations of ZEN in cattle diets have been associated with infertility and development of atypical secondary sexual characteristics in heifers (Bloomquist et al. 1982). Avian species are relatively resistant to the effects of this toxin and dietary concentrations up to 800 mg/kg are without effect on reproductive performance of mature chickens (Allen et al. 1981).

2) Deoxynivalenol

The high incidence of DON (also called vomitoxin) in grain makes this mycotoxin the most important trichothecene, although it is the least toxic of the more

Table 1. Toxic effects of mycotoxins

Mycotoxin	Clinical signs
Zearalenone	Swollen, reddened vulva, vaginal prolapse and sometimes rectal prolapse in pigs; suckling piglets may show enlargement of vulvae; fertility problems.
Deoxynivalenol	Decreased feed intake and weight gain in pigs with DON at >2 mg/kg feed; vomiting and feed refusal at very high concentrations of DON (>20 mg/kg diet).
Other trichothecenes T-2 toxin HT-2 toxin Diacetoxyscirpenol	More toxic than DON; reduced feed intake; emesis; skin and gastrointestinal irritation; neurotoxicity; abnormal offspring; increased sensitivity to disease; haemorrhaging.
Ochratoxin A	Mainly affects proximal tubules of the kidneys in pigs and poultry; kidneys are grossly enlarged and pale; fatty livers in poultry.
Ergot alkaloids	Nervous system disorders; tremors; convulsions; diarrhea; necrosis of the extremities (gangrene); reduced feed intake; abortion; stillbirth and agalactia (cessation of milk production); blackening of the comb, toes and beak in poultry.
Fumonisin B1	Equine leucoencephalomalacia (ELEM); porcine pulmonary oedema; 'spiking mortality' and 'toxic feed' syndrome in poultry; hepatocarcinogenic in rats; and possibly oesophageal cancer in humans.
Aflatoxins	Liver damage; reduced productivity; inferior egg shell and carcass quality; increased susceptibility to disease.
Cyclopiazonic acid	Liver, kidney, and gastrointestinal tract damage; weight loss, weakness; inappetance, diarrhoea, dehydration, depression, opisthotonos, and convulsions.

commonly occurring trichothecenes. Pigs are particularly susceptible to the adverse effects of DON. This mycotoxin causes reduced feed intake and weight gain in pigs when fed at concentrations greater than 2 mg/kg feed, and feed refusal and vomiting in pigs may occur when fed at concentrations greater than 20 mg/kg diet (Trenholm et al. 1988).

Other livestock species are more tolerant of the presence of DON in their feed. Poultry can tolerate much larger concentrations of DON in their diet than pigs (Bryden et al. 1987; Hamilton et al. 1985; Manley et al. 1988; Trenholm et al. 1981). At concentrations up to 5 mg DON/kg diet no major adverse effects have been observed other than decreases in egg weight and shell quality (Trenholm et al. 1984). There is a paucity of research on the effects of DON in ruminants. Limited studies indicate that cattle are more tolerant of DON than pigs. This may be attributed to the extensive degradation of DON to secondary metabolites in the rumen (King et al. 1984). However, there is evidence of reduced feed intake and milk production in dairy cows consuming diets containing DON (Whitlow and Hagler 1987).

3) T-2 toxin and DAS

These trichothecenes are less widely distributed than DON and they appear to be more toxic than DON to pigs, poultry, and ruminants.

Pigs: The toxicity of T-2 toxin has been studied using purified toxin. Following intravenous injection of 1.21 mg/kg body weight, clinical signs in pigs include emesis, posterior paresis, lethargy, and frequent defaecation (Weaver et al. 1978a). Death follows in a proportion of animals.

At natural levels of contamination in the diet, obvious effects are usually limited to reduced feed intake and animal performance. At low dietary exposure levels, T-2 toxin has been implicated in decreased immune function in pigs. The effect is primarily on the cellular aspect of the immune process, although some studies have shown an effect on certain humoral factors (Pang et al. 1988). This increases susceptibility to other diseases and the effects of poor management practices. At high concentrations in the diet, however, T-2 toxin is unlikely to produce clinical symptoms, other than diarrhoea. Due to its emetic and feed refusal actions, pigs will refuse to eat diets containing more than 16 mg T-2 toxin/kg (Mirocha 1983).

In pigs, DAS is more toxic than T-2 toxin (Weaver et al. 1978a). The no-effect dietary level is less than 2 mg DAS/kg diet. In pigs (8–40 kg body weight) fed 2 to 10 mg DAS/kg diet, in its purified form, reduced feed intake and weight gain were observed (Weaver et al. 1981). Complete feed refusal occurred at the 10 mg/kg level.

Following intravenous injection of 0.3–0.5 mg DAS/kg body weight (BW) to young pigs (9–23 kg BW), emesis occurred within 20 minutes, defaecation was frequent, and feed was consumed readily. Lethargy, paresis, and prostration occurred, and in some cases death within 18 hours (Weaver et al. 1978b).

Poultry: Birds are severely affected by T-2 toxin. Natural outbreaks of mycotoxicosis attributed to this toxin have been recorded (Wyatt et al. 1972). Signs include oral lesions, reduced feed consumption and growth rate in broilers, and reduced egg production in laying hens. Using the purified toxin, the LD_{50} dose has been estimated to be approximately 5 mg/kg BW for broiler chicks and 6 mg/kg BW for laying hens (Chi et al. 1977). Other researchers have shown that body weight gains decrease proportionally as the dietary T-2 toxin level is increased above 4 mg/kg diet (Wyatt et al. 1972). Below approximately 2 mg/kg diet, effects are minimal. Above this level, the impact on production is important.

As with pigs, an immunosuppressive effect of this toxin has been observed in poultry. There is evidence to suggest that DAS is slightly more toxic to broiler chicks than T-2 toxin (Chi and Mirocha 1978).

Ruminants: T-2 toxicosis in ruminants results in a wide range of responses, especially if doses exceed 0.6 mg T-2 toxin/kg BW. Toxic signs can be summarised as feed refusal, leucopenia, depression, diarrhoea, coagulopathy, enteritis, and posterior ataxia (Osweiler 1986). At lower concentrations, T-2 toxin reduced humoral immunity. Other experimental studies have reported that T-2 toxin produced radiomimetic effects in ruminants, including ulceration of the gastrointestinal tract (Ribelin 1978).

4) Ochratoxin A

Ochratoxin A is a nephrotoxic mycotoxin produced by several *Aspergillus* and *Penicillium* species. Adverse effects have been observed in pigs and poultry given naturally occurring levels of this mycotoxin (<2 mg/kg grain). In pigs, signs include impairment of proximal tubular function, impairment of urine concentration, and increased excretion of glucose in the urine (Elling et al. 1985; Carlton and Tuite 1986). In poultry, effects on growth and feed conversion have been recorded (Rotter et al. 1990). Mortality rate is also increased by feeding ochratoxin A contaminated feed (Schaeffer and Hamilton 1986). Ochratoxin A also elicits immune suppression in poultry. The effects are similar to those seen with aflatoxins.

5) Ergot alkaloids

These are produced by *Claviceps* species and are associated mainly with rye and triticale grown in northern latitudes. Consumption of these alkaloids can result in gangrenous symptoms leading to necrosis of extremities, central nervous system effects including ataxia, convulsions and paralysis, and gastro-intestinal disorders. All livestock species appear susceptible. Effects on animal

production include reduced weight gains, reproductive efficiency, and milk production, and agalactia (Lorenz 1979). It has recently been demonstrated that the endophyte (an *Acremonium* species) that infects many varieties of tall fescue in North America produces ergot alkaloids (Bacon et al. 1986). Ruminants grazing infected tall fescue during cold winters develop signs including lameness (fescue foot), loss of appetite, weight loss, arched back, swelling of hind limbs, sloughing of hooves, and foot deformities (Hemken et al. 1984). In contrast, during the summer, cattle experience summer syndrome or summer slump, which is characterised by lowered feed intake, reduced weight gain, higher respiration rates, higher body temperatures, rough hair coats, more time in shade or water, reduced blood prolactin concentrations, and reduced reproductive performance (Bacon et al. 1986). Interestingly, a condition identical to summer syndrome, in which hyperthermia is the prominent feature, was recently described in cattle in Australia (Ross et al. 1989). The cattle had been consuming a diet contaminated with the ergots of *Claviceps purpurea.*

6) Fumonisin B_1

Fumonisin B_1 (FB_1) is a recently identified mycotoxin produced by *Fusarium moniliforme* and related species, and is found to contaminate cereals, particularly maize, in many parts of the world. It has been chemically characterised as the causal agent of equine leucoencephalomalacia (ELEM). This toxicosis in horses is characterised by brain lesions, liver damage, blindness, abnormal behaviour, and death (Marasas et al. 1988). The lowest concentration of FB_1 found to cause ELEM was 10 mg/kg feed, given for 40–50 days. Porcine pulmonary oedema has also been associated with FB_1 contaminated feed. When sows were fed FB_1, clinical signs of mild erythema of ears, skin and nose, hydrothorax, cyanosis, dyspnea, weakness and recumbency were observed within 4–10 days (Ross et al. 1990). Mortality or morbidity of up to 100% was reported. A condition referred to as 'spiking mortality' or 'toxic feed syndrome' in poultry has also been linked to feed contaminated with FB_1 at 10–25 mg FB_1/kg diet. Clinical signs included extended legs and neck, ataxia, paralysis, wobbly gait, dyspnea, gasping, and poor growth.

7) Aflatoxins

Aflatoxins are the most studied group of mycotoxins, in particular aflatoxin B_1 which is carcinogenic and the most toxic member of this group. Numerous reviews have been published on the effects of aflatoxins in animals (Bryden 1982; Norred 1986) and the most notable effects relate to liver damage and impaired productivity, including reduced growth rate and milk production, inferior egg shell and carcass quality, and increased susceptibility to disease. The last effect is due to impairment in cell-mediated immunity, reduced levels of circulating antibodies, and factors of native resistance, including phagocytosis, complement, and other nonspecific humoral factors (Pier 1986).

Another aspect of aflatoxin that has serious human health implications is the transfer of an aflatoxin B_1 metabolite aflatoxin M_1 into the milk of lactating animals.

8) Cyclopiazonic acid

Cyclopiazonic acid (CPA) is a mycotoxin that may be produced under similar circumstances to the aflatoxins. Natural contamination of peanuts, maize, and cheese has been reported; there is a recent report from Indonesia of a suspected CPA toxicosis in quail following consumption of maize infected with *Aspergillus flavus* (Stoltz et al. 1988).

Clinical signs of CPA intoxication include weight loss, weakness, inappetance, diarrhoea, dehydration, depression, opisthotonos, and convulsions. Sheep are susceptible to CPA. The toxin also decreases egg shell quality (Cole et al. 1988). CPA passes into milk and eggs (Dorner et al. 1990).

Other Toxicological Aspects

It is now recognised that many mycotoxins can coexist in contaminated grain although the exact mycotoxin profile, concentration or toxicity characteristics are difficult to characterise (Foster et al. 1986a, b). Unknown mycotoxins may be acting alone, additively or synergistically with the known mycotoxins.

Food regulatory officials are very concerned about the presence of mycotoxins in food of both plant and animal origin for human consumption. Mycotoxins such as DON, and possibly FB_1, can be as high as 1000–2000 mg/kg in heavily contaminated maize kernels and it does not take many such infected kernels to contaminate feed and food products at 1.0–5.0 mg/kg. In addition, if mould contaminated grain is downgraded to animal feed, mycotoxins fed to farm animals may subsequently be transmitted to animal food products.

Approaches to Decontamination

Although desirable, the prevention of mycotoxin contamination of grain in the field is currently impossible. Under certain environmental conditions of temperature and humidity, *Fusarium* infestation and mycotoxin production are unavoidable. The development of inexpensive, effective, and commercially applicable methods for decontamination of mycotoxin contaminated grain is therefore very important. At the present time, no single, well established, safe and economical method for the decontamination of *Fusarium* mycotoxin contaminated grain or feed has been developed, although research in this area is increasing.

Approaches to detoxification of mycotoxin contaminated grain and feed have included physical, chemical, and biological treatment of such grain, as well as dietary manipulations to minimise the effects of contaminating mycotoxins on the animal. Cole (1989) has reviewed the methods of decontaminating feedstuffs containing aflatoxin. Ammoniation has been shown to be an effective method of detoxifying animal feedstuffs.

In cases of light to moderate *Fusarium* infestation and mycotoxin contamination, physical or chemical methods for cleaning the kernel surface, and hence removing the more heavily contaminated particulate matter, have proven effective in reducing mycotoxin concentrations. Such methods have included dehulling (Trenholm et al. 1990), washing (Trenholm et al., unpublished data), and roasting processes. Other methods, including density segregation of contaminated from the non-contaminated kernels (Huff and Hagler 1985; Babadoost et al. 1987), food processing practices (El-Banna et al. 1983, Scott et al. 1983,1984; Young et al. 1984; Seitz et al. 1985, 1986; Tanaka et al. 1986; Lee et al. 1987; Abbas et al. 1988), and treatment with chemicals including sodium bisulfite (Swanson et al. 1984; Young et al. 1986, 1987), ozone (Swanson et al. 1984), ammonia (Bennett et al. 1980; Young 1986), ammonium carbonate (Friend et al. 1984; Young 1986), sodium carbonate (Trenholm et al. 1990), and calcium hydroxide (Abbas et al. 1988) have been found to vary in their effectiveness at reducing mycotoxin concentrations in contaminated grain or feed.

An alternative approach to detoxification, which seeks to minimise the impact of such toxins on the animal itself by treating or modifying the contaminated diet prior to its consumption, has also been attempted with varying degrees of success. Such dietary manipulations have included dilution of mycotoxin-contaminated feed with uncontaminated grain, improvement of the nutritional content of the diet to compensate for mycotoxin-induced reductions in intake, and addition of potential mycotoxin-binding agents to the diet to inhibit the absorption and stimulate the metabolism of mycotoxins by the animal.

Dilution of mycotoxin-contaminated grain with uncontaminated grain is one of the simplest and most widely utilised methods for improving feed intake and weight gains in pigs. However, the success of this approach depends on the degree of contamination, the dilution achieved, and the availability of a source of uncontaminated grain. Increasing the energy, crude protein, mineral, and vitamin content of a contaminated diet by 20% also can improve weight gain in pigs, but only if feed intake has been reduced by 20% or less (Chavez and Rheaume 1986).

A diverse variety of substances has been investigated as potential mycotoxin-binding agents, including alfalfa (Smith 1980a, b; James and Smith 1982; Carson and Smith 1983b; Stangroom and Smith 1984), synthetic cation or anion exchange zeolite (Smith 1980a, b; Carson and Smith 1983a), bentonite (Carson and Smith 1983a; Smith 1984), spent canola oil bleaching clays (Smith 1984), Antitox Vana™ (Friend et al. 1984) and hydrated sodium calcium aluminosilicate (HSCAS) (Davidson et al. 1987; Orr 1987; Wyatt 1987; Trenholm et al. 1989). In some studies, alfalfa was shown to be beneficial in diets contaminated with ZEN. A dietary concentration of 15–25% alfalfa overcame the adverse effects of 250 mg ZEN/kg diet on feed consumption and body weight gain in rats (Smith 1980a; James and Smith 1982; Stangroom and Smith 1984), but had no effect on the uterine enlargement induced by 50 mg ZEN/kg diet in young gilts (Smith 1980a). However, 15% alfalfa did partially overcome the uterine enlargement induced by 10 mg ZEN/kg diet in similar young gilts (James and Smith 1982). James and Smith (1982) suggested that alfalfa, at a dietary concentration of 15%, could alleviate some of the adverse effects of ZEN on pigs if its dietary concentration was 10 mg/kg or less.

Alfalfa also has been found to be beneficial in diets contaminated with T-2 toxin. At a dietary concentration of 20%, alfalfa completely overcame the adverse effects of 3 mg T-2 toxin/kg diet on feed intake and body weight gain in rats (Carson and Smith 1983b). The results suggested that alfalfa exerted its effect by binding the toxin in the gastrointestinal lumen and thus inhibiting its absorption.

Synthetic anion exchange zeolite was shown to alleviate the adverse effects of ZEN in rats. Rats fed 250 mg ZEN/kg diet with this polymer showed increased faecal excretion of ZEN due to a reduction in intestinal absorption and an inhibition of the enterohepatic circulation of this mycotoxin (Smith 1980b).

The ability of other non-nutritive polymers, including cation and anion exchange resins and bentonite, to alleviate the adverse effects of T-2 toxin in rats, also has been investigated (Carson and Smith 1983b). Of these polymers, bentonite was found to be the most effective. At a dietary concentration of only 10%, bentonite completely overcame the adverse effects of 3 mg T-2 toxin/kg diet on feed consumption and body weight gain in rats. In another study, spent canola oil bleaching clays, also at a dietary concentration of 10%, overcame the adverse effects of 3 mg T-2 toxin/kg feed in rats (Smith 1984). Both bentonite and spent canola oil bleaching clays appear to exert their beneficial effects by adsorbing T-2 toxin and hence inhibiting its absorption within the gastrointestinal tract.

In another study, Antitox Vana™, the chemical binding agent polyvinyl-pyrrolidone, had no effect on reductions in feed intake and body weight gain observed in Yorkshire pigs fed diets containing 4 to 14 mg DON/kg diet (Friend et al. 1984).

Several studies have examined the efficacy of a hydrated sodium calcium aluminosilicate anticaking agent (HSCAS, Novasil™, Engelhard Corp., Cleveland, Ohio) in reducing the toxicity of a variety of mycotoxins in several animal species. Phillips et al. (1988) concluded that HSCAS was a high affinity sorbent for aflatoxin, capable of forming a very stable complex with the toxin and hence reducing its bioavailability. HSCAS at a dietary concentration of 0.5% significantly diminished the adverse effects of 7.5 mg aflatoxin B1/kg diet in broiler and leghorn chicks. In addition, HSCAS improved feed efficiency and growth rates (Harvey et al. 1988) as well as reduced liver, kidney, and muscle tissue concentrations of aflatoxin B_1, B_2, and M_1 (Beaver et al. 1990) in pigs fed aflatoxin-contaminated diets.

HSCAS at a dietary concentration of 1% was also found to cause significant improvements in body weight gain in young chicks fed mouldy maize-based rations containing low concentrations of *Fusarium* mycotoxins (T-2 toxin, DON, and ZEN) (Wyatt 1987). Davidson et al. (1987) suggested that HSCAS was binding irreversibly to the toxins present in the diet in a manner similar to that in which HSCAS binds aflatoxin in vitro. Orr (1987) investigated the effect of 0.5% HSCAS on feed intake, body weight gain, and feed efficiency in pigs fed variable concentrations of DON (0.5–2.25 mg/kg) and ZEN (0.2–1.5 mg/kg) from naturally contaminated feed. Although improvements in average daily gain of 6.1–19.6% and feed efficiency of 3.6–19.6% were reported for pigs fed HSCAS compared with pigs fed the same contaminated diets without HSCAS, these differences were not significant ($P < 0.05$).

Many approaches have been used to reduce the toxicity of mycotoxin contaminated feed. However, most methods have been tested on a limited number of specific toxins. Since contaminated grain may contain a broad range of toxins of differing chemical characteristics (Foster et al. 1986a,b), including heat stability, solubility, and adsorbent affinity, a detoxification procedure that works well for individual toxins may not be effective for the diverse mycotoxin combinations that occur naturally.

Economic Impact of Mycotoxins

The Food and Agriculture Organization estimates that 25% of the world's food crops are affected by mycotoxins every year. Though the mycotoxins discussed in this paper account for only a portion of the problem, their impact is still substantial. Aflatoxins are a serious problem in many countries of the world. However, in colder climates such as in Canada, aflatoxins are not found on indigenous crops. Economically, the most important mycotoxins in Canada are DON and ZEN.

Economic losses can be categorised as direct and indirect. Direct losses are related to reduced crop yields for growers, and reduced animal performance and increased losses due to disease for livestock producers. Reduced yields and decreased animal performance may be so widespread as to pass unnoticed in many instances. This is especially true when the level of infection and/or contamination is low. Disease symptoms are more readily apparent and losses can be catastrophic in individual cases.

Indirect economic effects of mycotoxins are probably as important as the direct effects, although more difficult to quantify. For the grower, mycotoxin contamination will restrict markets, reduce the marketable value of the crop, and may render crops unmarketable. Also, increased costs associated with fungicide or pest control have to be borne.

For the livestock producer, increased costs are associated with both searching for uncontaminated feed supply as well as secondary costs associated with feeding contaminated feed. The latter include veterinary bills, decontamination or dilution of contaminated feedstuffs, reproductive failures, and loss of markets.

There is also a considerable cost to the industry as a whole, in terms of research, monitoring, and extension, extra handling and distribution costs, increased processing costs, legal suits, and loss of consumer confidence in the safety of food products.

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Effects of Fumigation and Modified Atmosphere Storage on Growth of Fungi and Production of Mycotoxins in Stored Grains

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Abstract

The use of fumigants and modified atmospheres for insect control in the bulk storage of grains can have the added benefit of controlling mould growth and mycotoxin production. Mould deterioration accounts for significant losses in stored grains, particularly in tropical countries where the temperature and relative humidity are high. The problem is exacerbated where grains are inadequately dried before entering storage.

Phosphine (hydrogen phosphide) is being used with increasing frequency for insect control, because of its ease of application, and apparent lack of problems with residues. Phosphine, even at low levels (0.1 g/m^3) can slow the development of storage fungi in grains where the moisture content is above the levels normally accepted for safe storage. Fumigation with phosphine can also reduce mycotoxin production. However, phosphine appears only to affect growing fungi, and has little effect on dormant conidia and mycelium.

Atmospheres high in CO_2 are more effective in controlling fungal growth than those which exclude O_2 by replacement with nitrogen. Although most fungi require some oxygen for growth, many spoilage species are efficient scavengers and are capable of near normal growth in O_2 concentrations of < 1%. Atmospheres containing about 20% CO_2 generally inhibit mould growth, but > 80% CO_2 may be required to prevent fungal deterioration of high moisture commodities. Some *Fusarium*, *Aspergillus*, and *Mucor* species are particularly tolerant of high levels of CO_2.

Mycotoxin production is more sensitive than fungal growth to low O_2 and high CO_2 atmospheres. Concentrations of CO_2 between 20 and 60% have been shown to prevent or significantly reduce mycotoxin production by some *Fusarium*, *Aspergillus*, and *Penicillium* species. Reduction of O_2 content is less effective in preventing mycotoxin formation.

FUNGAL growth and mycotoxin production can be important causes of loss of quality in stored grain. Medium to long term storage of grains in tropical regions presents greater problems than in temperate regions, as grain is frequently stored at a higher moisture content than is desirable, and invasion pressure from insects is often high. Methods of mould control in current use, such as drying, aeration, cooling, and propionic acid treatment, are all either capital intensive and expensive, or unfit for use on human foodstuffs.

The introduction of modified atmosphere storage of commodities for insect control also offers considerable scope for control of fungal deterioration. However, many storage fungi are capable of growth in low partial pressures of oxygen (O_2), and reduction of available oxygen is often not sufficient to prevent moulding, particularly of high moisture grains. Elevated levels of carbon dioxide (CO_2) are more inhibitory to mould growth, but other factors, such as temperature and moisture content, will affect the degree of inhibition exerted by modified atmospheres.

Maintenance of a modified atmosphere, particularly one high in CO_2, requires a well sealed storage system. As such systems are often not available in tropical countries, fumigation is often used as an alternative means of insect control. Phosphine fumigation is practised widely in the tropics, as it is relatively cheap, easy to apply, and effective in controlling insect pests in grain (Bond 1984). There is evidence that phosphine fumigation may also assist in prevention of mould growth in stored commodities.

The effects of both fumigants and modified atmospheres on fungal growth and mycotoxin production in stored commodities will be discussed in this paper.

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Fumigants

Fumigants in common use for insect control include methyl bromide, phosphine, and carbon dioxide. Carbon dioxide will be dealt with in the section on modified atmospheres.

Methyl Bromide

Although methyl bromide has been used widely for insect control in stored products, there has been relatively little information in the literature on its effects on fungi in these commodities. More attention has been paid to the fungicidal effects of methyl bromide on soil fungi, particularly plant pathogens and mycorrhizal fungi.

The few published studies on effects of methyl bromide on fungi in stored commodities report mixed results. Raghunathan et al. (1969) reported 100% control of the internal fungi of sorghum (*Fusarium*, *Alternaria*, and *Helminosporium* spp.) after exposure to 64 mg/L methyl bromide for 48 hours. Harry et al. (1972) reported greater than 99% reduction of viability in dry conidia of *Aspergillus fumigatus* after fumigation with 40 mg/L methyl bromide for 20 hours at 25°C. A wide range of fungal species was shown to be susceptible to methyl bromide fumigation by Lee et al. (1973), when they exposed naturally contaminated peppercorns to 0, 120, and 240 mg/kg methyl bromide for 30 days at 26° and 30°C. After fumigation with 240 mg/kg methyl bromide, fungi were not detected in 18 of 20 samples, and the counts in the remaining two samples were reduced to 2–6% of the untreated controls. Fumigation with methyl bromide under these conditions killed a wide range of *Aspergillus*, *Penicillium*, *Chaetomium*, and *Cladosporium* species, and *Paecilomyces variotii*. *Eurotium* species and *P. variotii* were the most resistant, surviving in 11 of 20 samples treated with 120 mg/kg methyl bromide.

Phosphine

Effects of phosphine on growth of fungi in stored commodities

Phosphine fumigation is used throughout the tropics because it is relatively cheap and effective, and easy to apply. In normal fumigation practice, concentrations of phosphine up to about 3 g/m^3 are used against insects. Where long exposure times are possible, target maximum phosphine concentrations may be 0.5 g/m^3 or less. The lowest level that is effective against insects has not been determined precisely, but appears to be in the region 0.02–0.1 g/m^3 (Winks 1986). Phosphine is an inhibitor of respiratory enzymes (Nakakita et al. 1971), and could therefore be expected to have some effect on fungi also.

Although phosphine has been in widespread use for insect control for some decades, there has been relatively little work done on the effects of phosphine fumigation on the development of storage fungi in commodities, particularly over extended periods. Most of the few published studies on the effect of phosphine on growth of moulds, either on natural substrates or in culture media, have been carried out over relatively short periods. It appears that, at best, phosphine has only a minor influence on non–growing moulds (see, for example, Raghunathan et al. 1969; Sinha et al. 1967; Hocking and Banks 1991a) but there are indications of some useful effects under storage conditions where moulds are active.

Natarajan and Bagyaraj (1984) noted some reduction in fungi on pulses exposed to very high phosphine levels (100 g/m^3), particularly at 15% moisture content (m.c.), approximately 0.8 water activity (a_w), the highest value they tested. Inhibition of growth of a number of fungal species on culture media at high a_w in the presence of phosphine was noted by Bailly et al. 1985 and Leitao et al. 1987.

Hocking and Banks (1991a) inoculated wheat of 0.80 and 0.86 a_w with a common storage fungus, *Eurotium chevalieri*, and a mycotoxigenic species, either *Aspergillus flavus* or *A. parasiticus*. The wheat was then exposed to a stream of phosphine (0.1 g/m^3) for 2 weeks at 28°C, with control samples exposed to air under the same conditions. Storage of the moist grain in phosphine rather than air led to a less rapid development of most storage fungi, but did not completely prevent growth (Fig. 1). The level of inhibition observed suggested that phosphine may be useful in retarding fungal spoilage during short–term storage of high moisture grain (15–19% m.c. for wheat). Phosphine at 0.1 g/m^3 caused only a slight decrease in populations of fungi that were unable to grow at the a_w of the stored grain. There was no elaboration of aflatoxins in the grains, since the a_w was just marginal for growth of the aflatoxigenic fungi, and below the level at which toxin can be formed (Wheeler et al. 1988; Northolt et al. 1976).

The effect of phosphine on aflatoxigenic fungi and aflatoxin production was studied by Hocking and Banks (1991b) when freshly harvested paddy rice, equilibrated to 0.92 a_w (equivalent to 20.8% moisture content, wet basis), was inoculated with a mixture of *Aspergillus parasiticus* and *Eurotium chevalieri*. The inoculated samples, and uninoculated controls, were exposed to 0.1 g/m^3 (100 ppm) phosphine for 14 and 28 days at 28°C. There was extensive development of storage fungi, particularly *Penicillium* species, in both the inoculated and uninoculated samples held in air (Fig. 2). The population of *A. parasiticus* rose substantially in the inoculated samples, but development was less rapid in the phosphine treated samples. Storage of moist paddy rice in phosphine slowed the growth rate of most storage fungi, but did not prevent growth altogether. *Penicillium* species appeared to be particularly resistant to phosphine. Because of the

relatively high a_w, *Eurotium* species were rapidly overgrown by *A. parasiticus* in both the phosphine–treated samples and the air controls. Use of phosphine to inhibit mould growth and mycotoxin formation appears promising as a method of short term preservation of undried paddy. However, phosphine levels higher than 0.1 g/m^3 may be necessary if moist rice is to be held for longer than a few days.

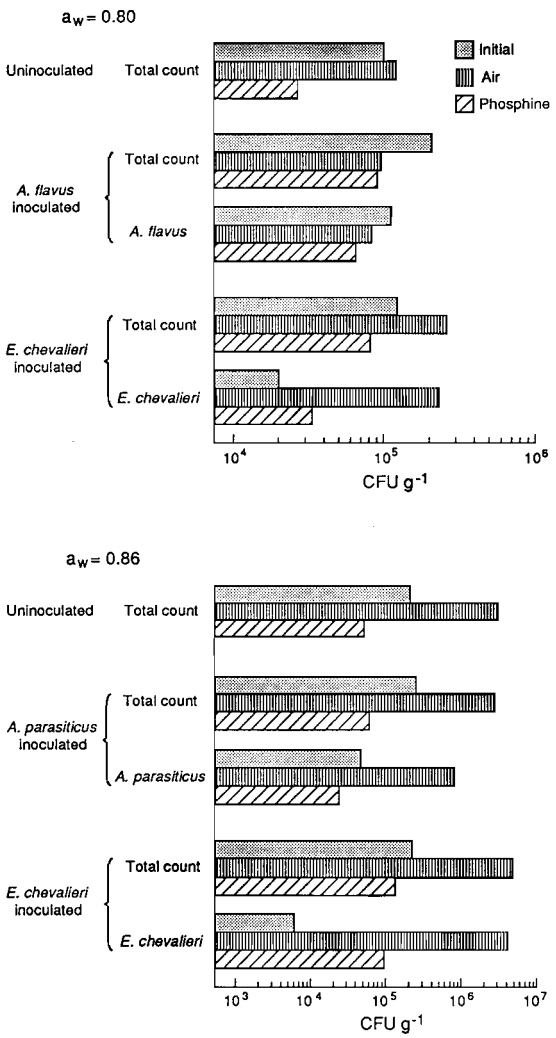

Fig. 1. Fungal counts (CFU/g) in uninoculated wheat and wheat inoculated with *Eurotium chevalieri*, *Aspergillus flavus*, or *A. parasiticus*, held for 14 days at 28°C in air or 0.1 g/m^3 phosphine, compared with counts before incubation. The upper chart shows data for wheat held at 0.80 a_w, the lower chart shows data for wheat held at 0.86 a_w.

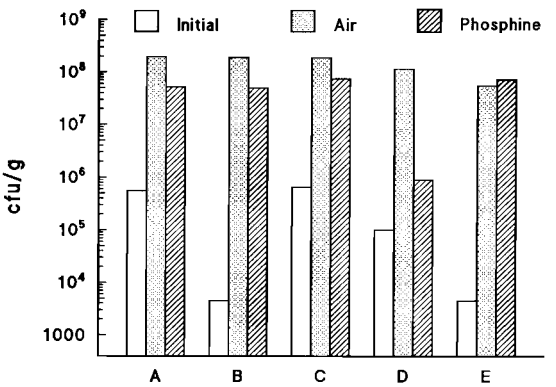

Fig. 2. Fungal counts on DRBC agar from rice, 0.92 a_w, exposed to air or 0.1 g/m^3 phosphine for 4 weeks. (A) total fungi in uninoculated rice; (B) *Penicillium* spp. in uninoculated rice; (C) total fungi in inoculated rice; (D) *Aspergillus parasiticus* in inoculated rice; (E) *Penicillium* in inoculated rice.

Effects of phosphine on fungi in pure culture

Studies on the effects of phosphine on fungi growing in pure culture have yielded varying results. Phosphine at a concentration of 0.3 g/m^3 reduced growth (measured as dry weight of mycelium) of pure cultures of various *Aspergillus* species grown on liquid medium (Leitao et al. 1987), but the reduction varied from as little as 3.5% for one strain of *Eurotium rubrum* up to 86.5% for one strain of *A. flavus*. There was considerable strain to strain variation in the level of inhibition. Ren et al. (1983), using phosphine concentrations between 0.31 and 3.08 g/m^3 for 3, 5, and 10 days at 30°C, demonstrated that *Eurotium amstelodami* was more sensitive to phosphine than *A. flavus*. Germination of *E. amstelodami* was inhibited by the lowest concentration of phosphine applied, but *A. flavus* germinated after 10 days in 0.97 g phosphine/m^3. Once growth was established, the fungi were considerably more resistant to phosphine. Ren et al. (1983) also found that phosphine was more effective in preventing fungal growth if it was applied in low (< 1.0%) oxygen atmospheres.

Bailly et al. (1985) demonstrated inhibition of a number of fungal species held for 21 days at ambient temperature in an atmosphere of 1.6 to 2.0 g phosphine/m^3. After phosphine treatment, cultures were incubated at 28°C for 15 days. Of the 100 strains tested, 41 were killed by the phosphine treatment, and 41 were inhibited, but subsequently grew when removed from the phosphine fumigation chamber. They found that *Fusarium* species were the most resistant, and *Penicillium* strains the most sensitive to the treatment. Of the 46 strains of *Aspergillus* tested, 9 were killed, and 31 inhibited by the phosphine. Similar results were reported by Bailly et al. (1987) using

higher concentrations of phosphine 4.3 – 4.9 g/m^3) over a longer time period (up to 90 days). However, four *Fusarium* isolates and the single isolate of *Byssochlamys* tested survived exposure for 90 days.

Effects of phosphine on mycotoxin production

Phosphine was shown to have no effect on preformed aflatoxin in naturally contaminated wheat, even when applied at 25 times the normal insecticidal dose (Brekke and Stringfellow 1978). Vandegraft et al. (1973) obtained mixed results with phosphine–treated wheat inoculated with aflatoxigenic strains of *A. flavus* and *A. parasiticus*, and ochratoxigenic strains of *A. ochraceus* and *P. verrucosum* (reported as *P. viridicatum*). The wheat (25 kg) was treated with 300 mg phosphine for 48 hours at 21°–27°C, then aerated for 5 minutes before being inoculated. Some strains produced more toxin on the treated wheat, and some produced less. Toxin production was higher in wheat that had been autoclaved after the phosphine treatment. However, phosphine at a concentration of 0.3 g/m^3 was shown to reduce aflatoxin production by a factor of 10 to 100 in pure cultures grown on liquid medium (Leitao et al. 1987).

When freshly harvested paddy rice (a_w 0.92) was inoculated with *Aspergillus parasiticus* and exposed at 28°C to 0.1 g/m^3 phosphine, Hocking and Banks (1991b) reported that considerable amounts of aflatoxins were formed in the inoculated samples, both in air and in phosphine. However, after 2 weeks exposure, aflatoxin levels in the phosphine–treated rice were less than half those in the air controls, and after 4 weeks, the phosphine–treated rice contained just over half the amount of aflatoxins that were detected in the air controls (Fig. 3).

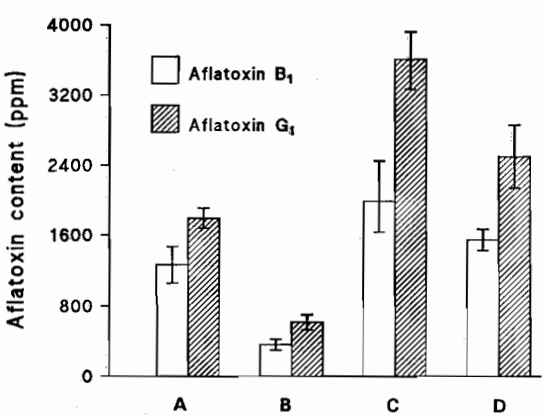

Fig 3. Aflatoxin content of rice inoculated with *Aspergillus parasiticus* and exposed to air or 0.1g/m^3 phosphine for 2 and 4 weeks. Bars show range of aflatoxin in duplicate samples. (A) 2 weeks in air; (B) 2 weeks in phosphine; (C) 4 weeks in air; (D) 4 weeks in phosphine.

Modified Atmospheres

Effect of modified atmospheres on mycoflora of stored commodities

Investigations into the effects of reduced O_2 and increased CO_2 on moulds in stored commodities date back at least to the early 1950s, when hermetic storage of grains was proposed as a new technology (Vayssière 1948). The earliest studies were undertaken with maize (Bottomley et al. 1950) and wheat (Petersen et al. 1956) and dealt mainly with spoilage by storage fungi. Studies undertaken after the mid 1960s were more concerned with the proliferation of mycotoxigenic fungi, and the effects of modified atmospheres on mycotoxin production.

Bottomley et al. (1950) investigated the effects of reduced oxygen on maize stored at relative humidities between 75 and 100%, and temperatures from 25° to 45°C, but their storage period was only 12 days. They found that mould growth was significantly reduced but not prevented by storage in an atmosphere of 0.1% O_2 and 21% CO_2. Different moulds predominated depending on the storage conditions. At 80% relative humidity, *Penicillium* species were dominant at 25°C, *Aspergillus flavus* at 30°C, and *Eurotium* and *Penicillium* species at 35°C (Table 1). Mould growth was less at 40° and 45°C, but *Mucor* was predominant at 45°C, especially when the oxygen concentration was 5% or less. In maize at 90% equilibrium relative humidity (ERH) or higher, *Candida* species proliferated in the 0.1% O_2 and 21% CO_2 atmosphere at 25°C, but not at higher temperatures.

Wilson et al. (1975) investigated the effects of modified atmospheres on the survival of the toxigenic moulds *A. flavus* and *Fusarium moniliforme* in freshly harvested high moisture maize (29.4% m.c.) and maize rewetted to 19.6% moisture. The maize was inoculated with *A. flavus*, and exposed to atmospheres of air, N_2 (99.7%, balance O_2), CO_2 (61.7%) and low O_2 (8.7%), and a modified atmosphere (MA) mixture of 13.5% CO_2, 0.5% O_2, and 84.8% N_2. In the freshly harvested maize, *A. flavus* levels increased in the air control to 90% kernel infection after 2 weeks, but with the other treatments kernel infection rate was only 5–18% after 4 weeks (Fig. 4). *F. moniliforme* was initially recovered from 21% of the kernels , but in subsamples exposed to modified atmospheres for 4 weeks, then held for 1 week in air, was present in 100% of kernels from all three treatments.

In a longer term experiment, Wilson et al. (1977) used maize with a moisture content of 18.8% for a storage trial in an atmosphere of 14–15% CO_2 and 0.5–1.0% O_2. Maize stored for 35 and 109 days in this atmosphere was tested for aflatoxins and the presence of *A. flavus* and *F. moniliforme*. No aflatoxin was detected after 35 or 109 days, whereas a control sample stored in air contained 472 µg/kg total aflatoxins. A significant proportion of the kernels contained *A. flavus* (30–47%) and *F. moniliforme* (35–47%), after both storage periods.

Table 1. Predominant mycoflora in corn stored for 12 days at 80% ERH in 20% CO_2 and 0.1% O_2 (data of Bottomley et al. 1950)

Temperature (°C)	Species (%)
25	*Penicillium* (55); *A. flavus* (45)
30	*A. flavus* (90)
35	*Eurotium* (50); *Penicillium* (50)
40	*Eurotium* (70); *A. flavus* (25)
45	*Mucor* (25); *Penicillium* (50); *A. flavus* (15)

The effects of CO_2 (in the presence of 20% O_2) on growth and sporulation of *A. flavus* on high moisture peanuts were reported by Landers et al. (1967) and Sanders et al. (1968). Concentrations of CO_2 in excess of 20% were required before there was any inhibition of growth of *A. flavus* in high moisture peanuts. Growth and sporulation were reduced with each 20% increase in CO_2 from 20% to 80%, with no growth occurring in 100% CO_2. Growth was much reduced in atmospheres containing less than 5% O_2, and almost completely inhibited at less than 1% O_2. However, Jackson and Press (1967) reported that the incidence of *A. flavus* at 27°C on shelled peanuts of 5.0% m.c. or unshelled peanuts at 7.5% m.c. was not reduced by storage in atmospheres containing 3% O_2 or 82% CO_2 in air compared with air storage over 12 months.

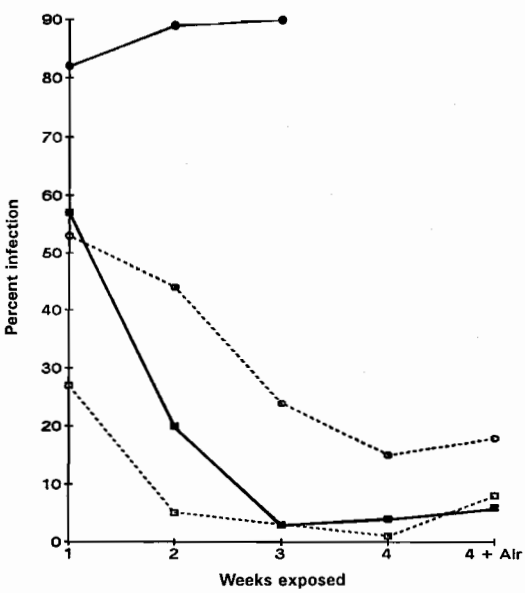

Fig. 4. Effects of modified atmospheres on Aspergillus flavus infection of inoculated high miosture maize. (●) air; (○) CO_2 + low O_2 (61.7% CO_2, 8.7% O_2, 29.6% N_2); (■) N_2 (99.7% N_2, 0.3% O_2); (□) CA (13.5% CO_2, 0.5% O_2, 84.8% N_2). Data of Wilson et al. 1975.

Wilson et al. (1985) used pilot scale experiments to determine if long term storage of peanuts was practical in modified atmospheres with minimal deterioration due to mould spoilage, aflatoxin contamination, and insect infestation, without use of refrigeration or pesticides. Two large bins of peanuts (1996 kg and 6451 kg) were stored in an atmosphere of approximately 60% CO_2 (balance air) at a moisture content of 6–7% for one year. The smaller (metal) bin experienced moisture migration due to condensation of water on or near the surface at night, and the peanuts were visibly mouldy after 16 weeks, the most common species being *A. flavus, Eurotium* species, and an unidentified white yeast, possibly a *Candida* species. Other *Aspergillus* species (*A. candidus, A. ochraceus* and *A. niger*) were also recorded, but *Rhizopus* and *Penicillium* were isolated less frequently. Despite the high incidence of *A. flavus*, no aflatoxins were detected. In the second trial with the larger (fibreglass) bin of peanuts, the atmosphere of 55–60% CO_2 was recirculated, and there was no moisture migration. The only major change observed in the mycoflora was a decrease in superficial *Penicillium* contamination from 64 to 16%. Aflatoxins were not detected during the 54 week trial.

Petersen et al. (1956) stored wheat of 18% m.c. at 30°C for 16 days under atmospheres with varying concentrations of oxygen and carbon dioxide (Table 2). In 4.3% O_2, the mycoflora was dominated by *Eurotium* species (80%), with *Penicillium* species and *A. flavus* also present (10% each). When O_2 was reduced to 2.3%, only *Eurotium* (67%) and *Penicillium* (33%) were present. In 0.2% O_2, only *Eurotium* species were detected, and their numbers were much reduced. With gas mixtures containing 21% O_2 and varying concentrations of CO_2, there was no significant change in numbers of fungi present in CO_2

Table 2. Effect of oxygen concentration on mould population and distribution in wheat stored for 16 days at 18% m.c. and 30°C. Data of Peterson et al. 1956.

Oxygen (%)	Moulds/g	Species
0.2	7.0×10^3	*Eurotium*
2.3	1.9×10^5	*Penicillium*
	2.9×10^5	*Eurotium*
4.3	1.0×10^5	*Penicillium*
	8.0×10^5	*Eurotium*
	1.0×10^5	*A. flavus*
8.0	1.0×10^5	*Penicillium*
	5.6×10^5	*Eurotium*
20.6	6.8×10^5	*Penicillium*
	3.9×10^5	*Eurotium*
	5.6×10^4	*A. flavus*

concentrations up to 18.6%. However, growth was almost completely inhibited by 50% and 79% CO_2 (Fig. 5). *Eurotium* species were the most tolerant of elevated levels of CO_2.

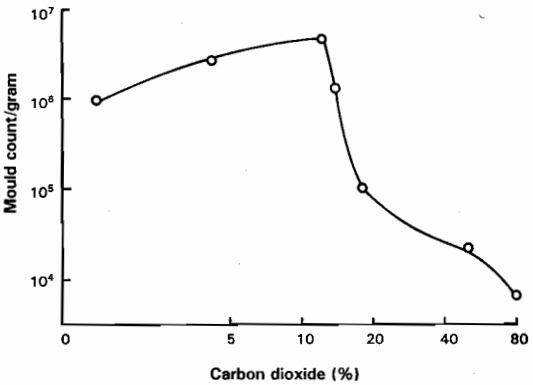

Fig. 5. Effect of CO_2 tension on mould count in wheat incubated for 20 days at 30°C and 18% moisture. All gas mixtures contained 21% O_2. Data of Peterson et al. 1956.

Shejbal and Di Maggio (1976) and Di Maggio et al. (1976) stored wheat of 18% m.c. in pure nitrogen, and found that mould growth was inhibited, and fungi gradually decreased with time. After 30 and 54 weeks, there was an increase in *Aspergillus candidus*. Under 0.2% O_2, mould growth at 18°–26°C on wheat of 17.4% m.c. was substantially inhibited in comparison with the air control. However, with both treatments, *A. candidus* eventually proliferated, reaching counts of 6×10^5/g after 3 weeks in air and about 20 weeks in 0.2% O_2.

In a study on naturally contaminated rice, Richard–Molard et al. (1986) investigated the effects of oxygen deficiency on microflora of grain rewetted to 0.87 and 0.94 a_w and stored for 2–4 months. They found that in the samples where the moisture content was low enough to prevent bacterial growth (0.87 a_w), most storage fungi, including *Penicillium* and *Aspergillus*, were inhibited by atmospheres of less than 1% O_2. However, yeasts (*Candida* spp.) and the yeast–like fungus *Aureobasidium pullulans* were able to develop, even with less than 0.5% O_2, and the higher the a_w, the more rapid the growth. In the complete absence of O_2 (under 100% CO_2 or N_2), there was no fungal growth. At a_w values higher than 0.90, lactic acid bacteria proliferated, and were not inhibited by any of the atmospheres studied.

Effect of gas mixtures on growth of fungi in pure culture

The two factors that need to be considered in preventing fungal growth in modified atmospheres are: (1) the minimum amount of oxygen required for fungal growth; and (2) the inhibitory effects of high levels of CO_2. Atmospheres high in nitrogen are effective only because of their low O_2 content, as nitrogen itself has no inhibitory effects.

Oxygen requirements

Many fungi are able to grow in the presence of very small amounts of oxygen (Miller and Golding 1949; Follstad 1966; Wells and Uota 1970; Walsh 1972; Yanai et al. 1980; Gibb and Walsh 1980; Magan and Lacy 1984). Anaerobic growth has also been reported for several fungi, for example, *Fusarium oxysporum* (Gunner and Alexander 1964) and some species of *Mucorales* that are used as starter cultures for food fermentations in Asia (Hesseltine et al. 1985). Tabak and Cook (1968) reported 'good to very good' growth of a range of species under 100% nitrogen. The strongest growth was exhibited by *Geotrichum candidum*, a yeast–like fungus, *Mucor heimalis*, *Fusarium oxysporum*, and *F. solani*. However, 'good' growth was observed in *Aspergillus niger*, *A. fumigatus*, *Penicillium aurantiogriseum*, and *P. brevicompactum*, and the black yeast–like fungus *Aureobasidium pullulans*. Such anaerobic growth can take place only if a number of growth factors (vitamins, oxygen donors in the form of higher oxidation states of certain elements) are supplied.

What is perhaps more relevant to MA storage of commodities, is the ability of many common field and storage fungi to grow in atmospheres containing less than 1% O_2 (Fig. 6). Of the field fungi present on grains at harvest, e.g. *Fusarium* species, *Alternaria*, other dematiaceous hyphomycetes, *Rhizopus*, yeasts etc., some grow very well in low levels of oxygen. *Fusarium moniliforme*, *F. oxysporum*, *F. culmorum*, and *F. solani* all grow

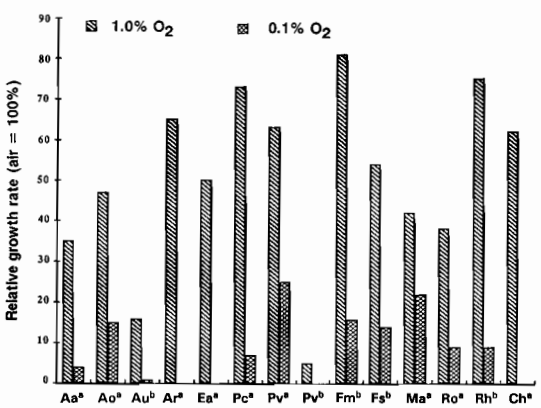

Fig. 6. Effects of reduced O_2 tensions on growth of some field and storage fungi. [a]Data of Yanai et al. 1980; [b]Gibb and Walsh, 1980. Aa, *Aspergillus awamori*; Ao, *A. oryzae*; Au, *A. ustus*; Ar, *A. restrictus*; Ea, *Eurotium amstelodami*; Pc, *Penicillium cyclopium*; Pv, *Paecilomyces variotii*; Fm, *Fusarium moniliforme*; Fs, *F. solani*; Ma, *Mucor ambiguus*; Ro, *Rhizopus oryzae*; Rh, *Rhizopus* sp.; Ch, *Cladosporium herbarum*.

strongly in atmospheres containing 1.0% to 0.1% O_2 or even less (Gunner and Alexander 1964; Tabak and Cook 1968; Walsh 1972; Gibb and Walsh 1980; Magan and Lacey 1984), provided that other growth conditions such as temperature and water activity are favourable. Some *Rhizopus* and *Mucor* species can also grow at low oxygen tensions (Wells and Uota 1970; Gibb and Walsh 1980; Yanai et al. 1980) or even anaerobically (Hesseltine et al. 1985), and can proliferate in high moisture commodities stored under low oxygen atmospheres (Bottomley et al. 1950; Wilson et al. 1975). Other field fungi such as *Alternaria* and *Cladosporium herbarum* are more sensitive to reduced oxygen tensions (Magan and Lacey 1984) and gradually die out during storage.

Storage fungi such as *Penicillium* and *Aspergillus* species are generally more sensitive to low levels of O_2 than the more tolerant field fungi. With the exception of *P. roquefortii*, the growth rates of most *Penicillium* species are reduced by more than half in atmospheres of 1% O_2 or less (Yanai et al. 1980; Magan and Lacey 1984). Of the Aspergilli, *A. candidus* and some *Eurotium* species are the most tolerant of reduced O_2 conditions (Magan and Lacey 1984; Yanai et al. 1980).

In our laboratory, studies on a number of spoilage fungi isolated from low O_2 environments have shown that most are inhibited only slightly when grown in nitrogen atmospheres with 0–1.0% O_2 (Fig. 7). Isolates of *Penicillium corylophilum* and *P. glabrum* from vacuum packed jams were able to grow at 66–90% of their control rate (air) when sealed in barrier film with an atmosphere of nitrogen. *Fusarium equiseti* and *F. oxysporum*, which caused fermentative spoilage of UHT fruit juices, grew at 88–97% of their normal rate. A *Cladosporium* species isolated from inside a UHT pack of apple juice was little affected by lack of oxygen, growing at 95–100% when sealed in an atmosphere of nitrogen. *Mucor plumbeus* and *Absidia corymbifera* also grew strongly in nitrogen. *Eurotium repens* grew at 60–90% of the control rate, depending on the growth medium, and the extreme xerophile *Xeromyces bisporus* grew at the same rate in air and in nitrogen (Fig. 7).

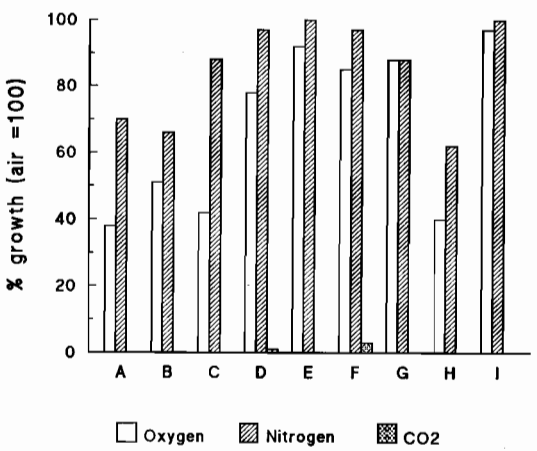

Fig. 7. Growth rates (relative to air) of nine fungi in O_2, N_2, and CO_2. Fungi were grown in pure culture on Petrislides (Millipore Corp.), sealed inside barrier film containing the appropriate atmosphere, and incubated at 25°C. Growth was measured by radial growth rate of colonies. A, *Penicillium corylophilum* ; B, *P. glabrum*; C, *Fusarium equiseti*, D, *F. oxysporum*; E, *Cladosporium* sp.; F, *Mucor plumbeus*; G, *Absidia corymbifera*; H, *Eurotium repens*; I, *Xeromyces bisporus*.

Effects of increased carbon dioxide levels

Levels of CO_2 from 4–20% can be stimulatory to growth of many fungi in atmospheres containing low levels of O_2 (Wells and Uota 1970; Gibb and Walsh 1980), conditions that may well arise during sealed storage of commodities, particularly during the early stages. However, elevated CO_2 concentrations are generally much more effective in controlling fungal growth than oxygen depletion. Thus, atmospheres rich in CO_2 are more likely to prevent mould deterioration of MA–stored high moisture commodities than atmospheres of nitrogen with traces of O_2. Typical insecticial atmospheres used for grain storage are 1% O_2 in nitrogen and 60% CO_2 40% air (Banks 1981), and while both may be equally effective in controlling insect populations in stored grain, the CO_2–enriched atmosphere would be more effective in controlling fungal growth in high–moisture commodities.

Atmospheres containing more than 50% CO_2 will substantially inhibit growth of most spoilage fungi (Petersen et al. 1956; Wells and Uota 1970) but there is little information in the literature on their actual CO_2 tolerances. Stotzky and Goos (1965) recorded slight growth of *Rhizopus stolonifer*, *Mucor hiemalis*, and a *Trichoderma* species in 100% CO_2. The same three species grew well in an atmosphere of 50% CO_2, 45% N_2, 5% O_2. *Fusarium oxysporum* grew in 95% CO_2, 5% N_2, but not in 95% CO_2, 5% O_2. *Paecilomyces lilacinus* grew reasonably well in 50% CO_2, 45% N_2, 5% O_2.

Wells and Uota (1970) showed that growth of *Alternaria alternata*, *Botrytis cinerea*, *Rhizopus stolonifer*, and *Cladosporium herbarum* in atmospheres of 10, 20, 30, and 45% CO_2 plus 21% O_2 decreased linearly with increasing CO_2 concentrations and was inhibited about 50% in an atmosphere of 20% CO_2 (Fig. 8). Growth of a *Fusarium* species, cited as *F. roseum*, was stimulated at 10% CO_2, and inhibited 50% at 45% CO_2.

Magan and Lacey (1984) reported that a CO_2 concentration greater than 15% was required to halve the linear growth rate of most of the 14 species of field and storage fungi tested at 0.98–0.90 a_w and 23°C. The species most sensitive to elevated CO_2 concentrations were *Penicillium brevicompactum*, *Aspergillus fumigatus*, *A. nidulans*, and *A. versicolor* (Table 3). However, no upper

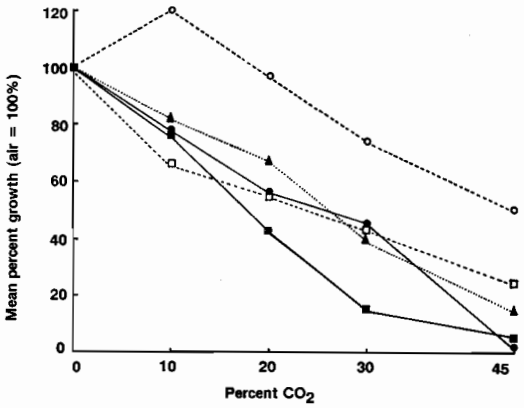

Fig. 8. Growth of five fungi in 21% O_2 with different levels of CO_2, cultured on liquid media at 19°C. Growth was measured by dry weight of mycelia. Data of Wells and Uota, 1970. (○) *Fusarium 'roseum'*; (▲) *Rhizopus stolonifer*; (□) *Alternaria alternata*; (●) *Botrytis cinerea*; (■) *Cladosporium herbarum*.

limits of CO_2 tolerance were determined, as the maximum concentration of CO_2 tested was 15%.

Nine species were tested in our laboratory for their ability to grow in an atmosphere of 97–99% CO_2 with trace amounts of O_2 and N_2. Only *Fusarium oxysporum* and *Mucor plumbeus* grew, and their growth rates were only 0.5–4% of those in air (Fig. 7).

Table 3. Concentrations of CO_2 required to halve the linear growth rate of field and storage fungi at 23°C (data of Magan and Lacey 1984)

	Water activity		
	0.98	0.95	0.90
Field fungi			
A. alternata	>15.0	>15.0	>15.0
C. cladosporioides	>15.0	>15.0	>15.0
C. herbarum	13.0	>15.0	>15.0
E. nigrum	>15.0	>15.0	>15.0
F. culmorum	14.0	13.5	>15.0
Storage fungi			
P. brevicompactum	11.5	8.5	15.0
P. aurantiogriseum	4.5[a]	4.0[a]	>15.0
P. hordei	>15.0	8.5	9.5
P. piceum	>15.0	>15.0	14.5
P. roquefortii	>15.0	>15.0	4.5
A. candidus	>15.0	>15.0	>15.0
A. fumigatus	>15.0	5.2	12.5
A. nidulans	>15.0	6.5	13.5
A. versicolor	12.0	>15.0	14.5
E. repens	>15.0	>15.0	>15.0

[a] Stimulation of growth occurred at higher CO_2 concentrations

Reports of the effects of CO_2 concentrations on fungi growing in stored commodities seem to vary. Landers et al. (1967) and Sanders et al. (1968) reported that growth of *A. flavus* on high moisture peanuts was inhibited by 80% CO_2/20% O_2, but Jackson and Press (1967) found no reduction in *A. flavus* on peanuts stored at 5% m.c. (approximately 0.7 a_w) in 82% CO_2 in air for 12 months. This perhaps indicates that although 80% CO_2 will inhibit growth of *A. flavus*, conidia of this species are not killed by exposure to high levels of CO_2 at low a_w. Peterson et al. (1956) reported that *Eurotium* species survived and grew in wheat stored in 50% CO_2/21% O_2 and 79% CO_2/21% O_2.

The exact mechanisms of CO_2 inhibition of microbial growth are unknown. It is obvious that it is not simply an oxygen displacement effect. Most studies have been carried out on bacteria, and little is known of the effects on fungi. Research on mechanisms of inhibition of bacterial growth have been summarised by Daniels et al. (1985) as follows: (a) the exclusion of oxygen by replacement with CO_2 may contribute slightly to the overall effect; (b) the ease with which CO_2 penetrates cells may facilitate its chemical effects on the internal metabolism; (c) carbon dioxide is able to produce a rapid acidification of the internal pH of cells with possible ramifications relating to metabolic processes; and (d) carbon dioxide appears to exert an effect on certain enzyme systems, though these effects differ for different species and with differing growth conditions.

Effects of gas mixtures on mycotoxin production

Aflatoxins

There has been a number of studies of the effects of various atmospheres and other environmental conditions on aflatoxin production, both in stored commodities and in pure culture. Landers et al. (1967), investigating aflatoxin production in stored peanuts, reported that aflatoxin production decreased with increasing concentrations of CO_2 from 0.03% (air) to 100%, and that, in general, reducing the O_2 concentration also reduced aflatoxin production, particularly from 5% to 1% O_2 (Fig. 9). The inhibitory effect of CO_2 was greater at 15°C than at 30°C. Sanders et al. (1968) reported similar results in storage experiments with peanuts at reduced a_w and temperature. They found that aflatoxin levels decreased as a_w decreased from 0.99 to 0.86. At a constant temperature, an increase in CO_2 concentration caused a decrease in aflatoxin formation, and lowering the temperature also decreased the amount of toxin formed.

Epstein et al. (1970) studied the effects of a modified atmosphere (10% CO_2, 1.8% O_2, 88.2% N_2) on aflatoxin production in liquid medium and in inoculated maize at ambient temperature (25°–35°C) and at temperatures from 29°C to 1°C. At ambient temperature, *A. flavus* grew well and produced toxin in both air and MA. At 15°C, aflatoxin

production, but not growth, was inhibited in the MA. Aflatoxin was not produced at 12°C, and there was little growth at this temperature in air and none in MA. The minimum temperature for aflatoxin production varies with strains, but is generally around 12°–13°C (Northolt et al. 1976).

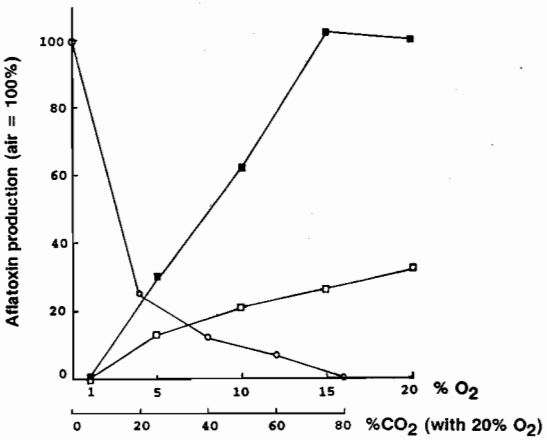

Fig. 9. Influence of various concentrations of O_2 and CO_2 on aflatoxin production in peanuts with kernel moisture content of 27-30% held at 30°C for 2 weeks (data of Landers et al. 1967): (○) CO_2 with 20% O_2; (■) O_2 with no CO_2; (❑) O_2 with 20% CO_2.

Wilson and Jay (1975) found that maize inoculated with *A. flavus* and stored at 27°C for four weeks in three different modified atmospheres accumulated less than 20 µg/kg total aflatoxin, compared with up to 1021 µg/kg for the air control. Remoistened maize was more susceptible to aflatoxin production than freshly harvested high moisture maize. Aflatoxin production in moistened (18.5% m.c.) wheat incubated at 32°C for up to 21 days was minimal (<1 µg/kg) in an atmosphere of N_2, compared with 123 µg/kg in air (Fabbri et al. 1980). Clevström et al. (1983) also found that small quantities of aflatoxins were produced when *A. flavus* was cultured under an atmosphere of nitrogen, and that production increased approximately 15–fold with the addition of B vitamins and a supply of traces of air. Carbon dioxide enrichment hindered aflatoxin formation on a defined medium even in the presence of B vitamins, but small quantities (5 to 15 µg/litre) were formed when formic acid was added.

Carbon monoxide (CO) can also suppress growth of *A. flavus* and aflatoxin formation. Buchanan et al. (1985) reported that, after growth of *A. flavus* for 32 days in cooked rice medium or raw pistachio nuts in an atmosphere containing 2% O_2 and 10% CO, aflatoxin production was less than 2% of the production in an atmosphere containing 2% O_2 or air without CO.

Ochratoxin

Paster et al. (1983) grew *A. ochraceus* on solid synthetic medium at 16° ± 1°C for 14 days in atmospheres containing various concentrations of O_2 and CO_2 (Fig. 10). In atmospheres of 1% and 5% O_2 without CO_2, ochratoxin production was similar to the air control. Increasing the O_2 level up to 40% reduced ochratoxin production by 75%, whereas at 60% O_2 ochratoxin production was enhanced. In atmospheres of 10% and 20% CO_2, ochratoxin production decreased when O_2 concentrations were below 20%, and was enhanced when they were 40% or 60%. Ochratoxin production was completely inhibited by 30% or more CO_2, regardless of the oxygen concentration. Colony growth was partially inhibited at 60% CO_2, and there was no growth in 80% CO_2.

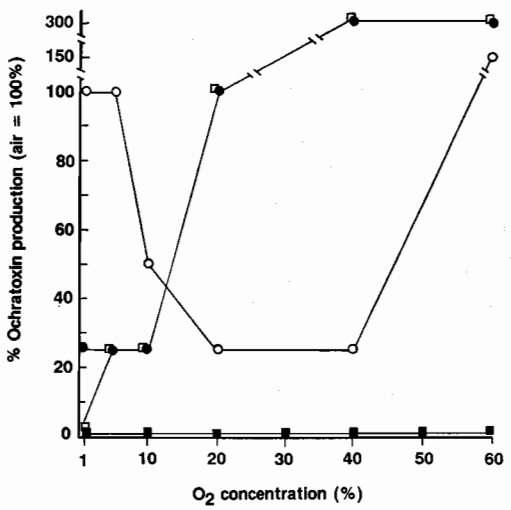

Fig. 10. Ochratoxin production by *Aspergillus ochraceus* grown under modified atmospheres on solid synthetic medium at 16°C ± 1°C for 14 days (data of Paster et al. 1983): (○) 0% CO_2; (●) 10% CO_2; (❑) 20% CO_2; (■) 30% CO_2.

Penicillium *toxins*

Paster and Lisker (1985) reported that *Penicillium patulum* (now *P. griseofulvum*) grown for 7 days in 1% or 5% O_2, but no CO_2, produced less patulin than the control (Fig. 11). In 10% O_2 without CO_2, patulin production and mycelial dry weight were similar to the controls. Increasing the O_2 content to 60% or 70% decreased patulin production to 20 and 1.3 mg/40 mL respectively. Toxin production was also inhibited when CO_2 concentration was raised to 20% or more in the presence of 20% O_2. Spores incubated in 100% CO_2 or N_2 did not germinate , but when subsequently exposed to air grew normally and produced patulin in amounts comparable to the controls .

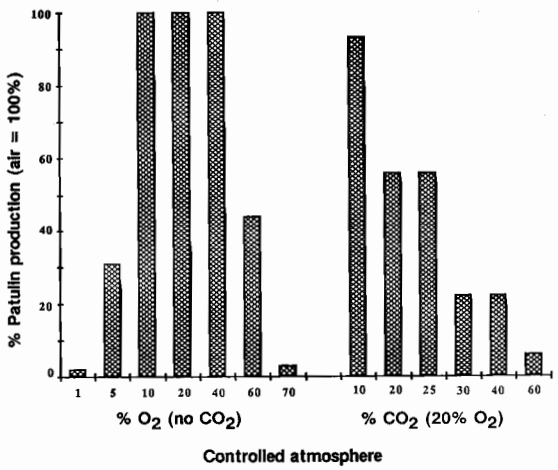

Fig. 11. Effects of controlled atmospheres on patulin production by *Penicillium aurantiogriseum* grown for 7 days in Czapek agar at 26°C (data of Paster and Lisker 1985).

Penicillic acid production by *Penicillium martensii* (now *P. aurantiogriseum*) was studied in inoculated maize over a temperature range of 5°–20°C in air and in atmospheres containing 20%, 40%, or 60% CO_2, with 20% O_2 (Lillehoj et al. 1972). Penicillic acid production decreased with increasing CO_2 concentration. Toxin production was greatest in air at 5°C, but was completely blocked at this temperature by 20% CO_2, and by 40% CO_2 at 10°C over a 4 week incubation period.

Fusarium toxins

As with *Penicillium* species, little work has been done on the effects of modified atmospheres on toxin production by *Fusarium* species, although it is known that many Fusaria are tolerant of low O_2 tensions and high CO_2 concentrations.

The effects of MA on production of T–2 toxin by *F. sporotrichioides* have been investigated both in synthetic media (Paster et al. 1986) and in remoistened irradiated maize (Paster and Menasherov 1988). In the synthetic medium, T–2 production after 7 days at 27°C in an atmosphere of 50% CO_2/20% O_2 was reduced to about 20% of the air control (Fig. 12). At 60% and 80% CO_2 with 20% O_2, there was a significant reduction in fungal growth. Toxin production in 80% CO_2 was only 1.1 µg/45 ml. When the same strain of *F. sporotrichioides* was grown for 14 days at 26° ± 1°C on irradiated maize remoistened to 22% m.c., the production of T–2 toxin was totally inhibited under 60% CO_2/20% O_2, and only trace amounts were detected when the gas combination was 40% CO_2/5% O_2. Fungal growth was not inhibited by any of the gas mixtures examined, and the growth rate was identical to that for grains kept under air.

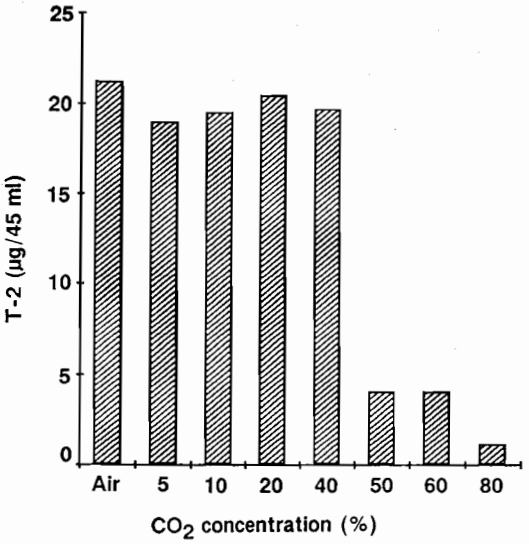

Fig. 12. T-2 toxin production by *Fusarium sporotrichioides* under controlled atmospheres containing various concentrations of CO_2 in 20% O_2. Cultures were grown on potato dextrose agar for 7 days at 27°C (data of Paster et al. 1986).

Conclusions

Storage of commodities in modified atmospheres containing high (> 60%) levels of CO_2 to prevent insect infestation can also inhibit mould growth and mycotoxin production, while atmospheres of nitrogen need to contain less than 1% O_2 to retard fungal growth. Mycotoxin production is more sensitive to fumigation and modified atmosphere conditions than is fungal growth, but may still occur if other conditions (temperature and a_w) are favourable.

Fungal deterioration cannot be completely prevented in high moisture commodities (a_w between about 0.90 and 0.80) either by fumigation or by MA storage, as some fungi, particularly some *Fusarium*, *Mucor*, and *Aspergillus* species, are tolerant of levels of 60–80% CO_2 and some are also resistant to phosphine. Yeasts and yeast–like fungi can also develop in CA stored high moisture commodities, causing rancidity and off odours.

However, these storage strategies may be very useful in extending (by days or weeks) the periods at which grains can be safely held before proper drying facilities become available.

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Effective Use of Mould-Damaged Commodities

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Abstract

Feeding mouldy food to domestic animals is a time-honoured method of salvaging such material, is often economically desirable, and should not be prohibited. Mouldy feed can be safely fed to animals depending upon the extent of changes to nutritional quality and palatability, and whether mycotoxins are in sufficient concentrations to affect animal performance. Mould growth reduces starch and lipid content of commodities, with consequent increases in fibre. Decreased palatability affects pig and poultry industries since growth rates and profitability are functions of feed intakes. For example, at 1990 prices for the Queensland pig industry, a 15% decrease in feed intake by young pigs produced a 7% decrease in profit margin, but this could be compensated for by a 22% decrease in the price of grain. A common effect of mycotoxins or poor nutrient balance is reduced feed conversion efficiency, which also affects profits. In 1990 in Queensland, a 15% decrease in feed conversion efficiency by pigs would reduce profits by 10%, but this could be avoided if the cost of the grain were reduced by 24%. Similar relationships hold for chickens. Mycotoxins are probably common in feeds but have only slight effects on animal productivity, either because the concentration is too low or the animal species is tolerant of the toxin. Even with the more important mycotoxins, prediction of types of contamination occurring in given situations — through knowledge of crops and associated fungi — together with accurate mycotoxin assay, will allow mouldy feed to be effectively used by processes of dilution and diversion between animal species and classes of livestock.

INCREASING knowledge of the long term effects of ingestion of mycotoxins suggests that mouldy commodities should be avoided as human food wherever possible. However, feeding mouldy food to domestic animals is a time-honoured method of salvaging such material, is often economically desirable, and should not be proscribed. Just as with people who eat mould-cultured products, domestic animals often voluntarily consume mouldy feed without ill effects. Similarly, farmers often seek to use mouldy feed, either because this is all that is available or because it is cheaper than sound feed. Is this a dangerous practice to be avoided at all cost — or perhaps only if the cost outweighs the benefit? After all, one of the most important roles of the mycotoxicologist should be to advise the community on decisions regarding use of mouldy stored commodities.

We believe that there are four factors that should be considered when faced with a question on the use of damaged feed: whether the nutrient content has been altered for better or worse; whether the palatability has been affected; whether mycotoxins are present in concentrations sufficient to affect animal performance; and whether the lower price of the feed, or its total value to a particular farmer in a particular situation, compensates for these effects and the risk involved.

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Effects of Moulds on Nutrients

Mould infection of grain slightly affects its feed value for monogastrics through the removal of storage starch and the hydrolysis of protein (Castor and Frederiksen 1981). Losses in lipid content due to fungal lipases also occur (Bartov et al. 1982). Consequently, the relative amount of fibre in the grain will increase in proportion to the decline in starch, protein components, and lipid, sometimes leading to reductions in digestible energy content (Taverner et al. 1975). However, we found digestible energy to be only slightly affected in several batches of severely weather-damaged sorghum infected with *Alternaria* and *Fusarium* species (Williams et al. 1986). Where mould invasion is slight, some improvement in nutritive value may occur as a result of starch hydrolysis similar to that seen in early germination. Deteriorated grain will be lighter in weight, and discoloured and darkened if mould invasion is extensive and the endosperm is likely to have a chalky appearance due to the partial hydrolysis of the nutrient stores. Gross

energy on a weight basis may be unaffected, but fibre values are invariably higher and protein levels may also be increased.

With pigs and poultry, the nutritional deficiencies of damaged grains can be compensated for during dietary formulation by increasing the digestible energy with lipids. Naturally, these changes are of much less significance in ruminant nutrition than with pigs and poultry. In ruminants, the digestibility of fibre from grain cell wall is about 50%, much greater than in monogastrics, while moderate reductions in lipids and amino acids are unimportant compared with total nitrogen. Consequently, very badly damaged grain can be used as ruminant feed provided that palatability is satisfactory and high concentrations of toxins are not present. The nutritional effects of moulds on hays and other forms of stored fodder are similar in kind to those on grains.

Effects of Moulds on Palatability

Damaged grain that has undergone fungal invasion may exhibit 'off' aromas and flavours, and be unpalatable to pigs, which exhibit strong taste preferences when food is freely available (Baldwin 1976). However, when there is no choice, the pig will eat unpalatable food out of hunger and may even become accustomed to the taste if fed over a long period. With chickens, the appearance of the food seems to be more important in deciding what is eaten (Adret-Hausberger and Cumming 1985).

Horses are also susceptible to 'off aromas'. Cattle, sheep, and goats are less sensitive to aroma but may reject some mouldy feeds, particularly if dusty. Methods used to overcome poor palatability in intensive animal industries include use of sugar, molasses, flavour enhancers, and ingredients such as peanut and coconut meals. Addition of oil also reduces dust, another contributor to reduced feed intakes. In some situations, reduced palatability can even be desirable since it tends to even-out intakes over a period of time.

It is important to distinguish between palatability as such, and other effects causing reduced feed intakes. In intensively fed animals, decreased feed intake is usually the first sign of chronic intoxication, e.g. by aflatoxins (Allcroft 1969). Other mycotoxins such as trichothecenes have specific systemic effects causing nausea and emesis, which may not be overcome by flavour additives (Forsyth et al. 1977).

General Effects of Mycotoxins

Mycotoxins occur frequently in animal feeds. In most cases, no adverse effects are noticed, either because the mycotoxins are not in sufficiently high concentration, the particular animal species is tolerant to the mycotoxin, the mycotoxin is not fed for a long enough period of time, or a good balance of nutrients allows the animal to withstand the toxicity.

In a few cases, fungi are sufficiently prevalent in a given pasture, grain crop, or storage situation to produce mycotoxins in sufficient concentrations to cause significant animal production losses through deaths or impaired growth. To date in Australia, mycotoxins in this category include aflatoxins, zearalenone, and deoxynivalenol occurring mainly in grains, and phomopsins, sporidesmin, and ergot alkaloids occurring mainly in forages. Elsewhere, ochratoxin is clearly a problem for pig producers in the colder climates of Europe and North America: T-2 toxin and related trichothecenes appear to be a sporadic problem in stored fodders and grains in cold climates.

In most cases, reduced growth rates or feed conversions caused directly by mycotoxins are hard to differentiate from those caused by reduced nutritional value. For example, *Alternaria* spp. are very common in grains and forage in Australia, and their mycotoxins are occasionally suspected of contributing to poor animal performance (usually in the absence of evidence for any other possible diagnosis), but no cases have ever been confirmed. Certain mycotoxins appear to have no noticeable effect on animals in the short term until a certain threshold dose is ingested; others cause a gradual reduction in growth rate as the dose increases.

As we consider the effects of specific mycotoxins, it is important to remember that animals have evolved with the ability to cope with the presence of small amounts of toxins in feeds, and it is only when the detoxification mechanisms are overloaded that sickness occurs. Clearly, the carcinogenic and mutagenic effects of mycotoxins are extremely important for humans, but much less so for our domestic animals, which are usually kept for only a short proportion of their potential lifetime. We must also be aware that a farmer's decision to use mouldy feed will be largely determined by economic considerations.

Economic Factors

To satisfactorily advise a farmer, we must always take account of the social and economic systems that will guide decision making. For example, does the farmer have an alternative source of feed, and what value is placed on the livestock? Is the mouldy feed only a dietary supplement for foraging animals? Is it possible to dilute the mouldy feed with better feed, or to restrict access by younger animals?

In Australia, intensive pig and poultry production is characterised by its high capital investment and its vulnerability to feed costs, as feed represents from 60 to 80% of operating costs. Annual farm profitability is very sensitive to the price received for the product sold, the price paid for the inputs (predominantly that of the feed) and the volume of product per annum. Since the volume of product produced per annum is closely correlated with total feed intake and the conversion of feed into product, any changes affecting feed intake or feed conversion due

to nutrient balance, palatability, or toxins can seriously influence profit.

Result of reduced feed intakes

We examined the consequences of reduced feed intake or impaired feed conversion on costs of production using 'what-if' cost-of-production computer models developed by the Queensland Department of Primary Industries. Calculations were based on ruling prices in southeastern Queensland for the first quarter, 1990. The standard models used were as follows: for pigs, a 100-sow piggery turning off 17 pigs/sow/year with a gross income of $230 000/year and a margin-over-feed-cost (MOFC) of $873/sow/year; for meat chickens, a 60 000-bird farm raising 5 batches/year at an average turn-off weight of 1.8 kg, feed usage of 3.6 kg/bird and a gross rearing fee of $125 000/year; and for layers, an 8000-hen farm producing 21 dozen eggs/hen/year with a gross income of $225 000/year and a MOFC of $0.78/dozen eggs.

The effects of reduced feed (energy) intake and worsening feed conversion on pig performance and carcass grading were based on predicted responses as described by Black (1987) and Campbell and Dunkin (1990). It was assumed that the deterioration in feed conversion was compensated for by increased feed allowance, such that growth rate remained unaffected until feed consumption was limited by the pig's maximum voluntary feed intake. In the economic model, turnoff age was held constant at 23.9 weeks on the basis that pen accommodation in commercial piggeries is usually fully utilised so that reductions in growth rate cause pigs to be turned-off at lower liveweights.

The direct effect of reduced feed intake is reduced growth rate which directly relates to profitability. However, for a given reduction in growth rate, there is a correspondingly lower purchase price of the feed ingredients that would restore profitability (but greatly reduced feed intakes over a long period of time may lead to additional adverse effects that are not readily compensated for). These relationships are shown in Table 1.

A reduced feed intake by pigs during growth from 20 to 50 kg caused the overall herd profitability to decrease by 0.5 to 1% for each 1% feed intake depression. During growth from 50 to 90 kg the effects of reduced feed intake were much less because pigs in Australia are normally fed restrictively (80% of *ad libitum*) in this period as this leads to a leaner carcass, attracting a premium price. Therefore, intakes must be retarded by more than 20% before any effect will be seen. At slightly reduced intakes, there is also a compensating benefit of improved feed conversion. Clearly, it may be advantageous to have slightly unpalatable feed for older pigs.

Reduced feed intakes will also affect the chicken-meat and egg industries causing decreased growth rates and egg production but these effects are difficult to quantify. Meat chickens are far more tolerant than pigs to 'off' flavours and odours and extremely mouldy and rotten wheat has been fed to meat chickens without detriment to performance after incorporation into a nutritionally balanced diet (Mannion et al. 1987). The economic consequences of diet unpalatability in egg production are complex. Pullets are usually controlled-fed during rearing

Table 1. The effects of reduced feed intakes by pigs during growth from either 20 to 50 kg or 50 to 90 kg, on margin-over-feed-cost (MOFC) of a 100-sow piggery

% of Voluntary intake period	MOFC ($ sow^{-1} year^{-1})	Purchase price to restore MOFC ($ tonne^{-1})	
		Total diet[a]	Grain alone[a]
20 to 50 kg period			
100	873	272	150
95	849	264	139
90	829	256	129
85	808	247	117
80	786	238	105
75	755	223	85
70	723	208	65
50 to 90 kg period			
100	873	272	150
80	873	272	150
75	864	269	146
70	855	265	141

[a]Based on the average prices of dietary ingredients in southern Queensland, first quarter 1990

to reduce obesity with the degree of restriction being 20 to 30% of the feed consumed by birds fed *ad libitum* (Karunajeewa 1987). During lay, restricting food to 85–94% of *ad libitum* intake improves feed to egg conversion but more severe feed restriction progressively reduces egg output (Connor et al. 1977; Robinson and Sheridan 1982).

Result of poor feed conversion

The efficiency of feed conversion to animal product is affected by nutritional balance, disturbance to metabolism, infectious disease, and the presence of toxins in the feed. In the simplest sense, the ratio of crude fibre and ash to fat, protein and carbohydrate will tend to vary inversely with feed conversion efficiency. In such a case, the animal may maintain growth rate by increasing intake up to certain limits. The presence of toxins may affect metabolism by increasing energy wastage or by causing irritation to the intestinal tract mucosa, leading to diarrhoea, and thereby decreasing the efficiency of absorption of nutrients. On the other hand, low concentrations of toxins or mouldy feed sometimes give a slight improvement in feed conversion (Mannion et al. 1987). The reasons are unknown, but perhaps they affect the intestinal microflora, as do antibiotics.

In any case, poorer feed conversion has a similar but more marked effect than reduced feed intakes on profitability and these relationships for pigs, meat-chickens and laying-hens are shown in Tables 2 and 3.

Intensively fed and stalled ruminants do not require such fine nutritional balance as pigs and poultry, and the rumen protects against certain mycotoxins. On the other hand, ruminants may be offered feed considered unsuitable for other species. This includes maize cobs, in which toxins can be concentrated. Growth rate is less closely monitored in ruminants than in pigs or poultry, although this also depends upon the circumstances; intensive cattle grain feedlots must achieve rapid weight gain to be economically viable, but the villager with a few sheep or goats may well be content with survival and moderate breeding or milking performance.

Specific Effects of Mycotoxins

There is little doubt that different countries have their own unique situations in respect to mycotoxins, and mycotoxins produced by certain fungi are apparently influenced by local cropping practices (Blaney and Dodman 1988). However, there are many similarities, since the prevalence of fungi is determined by environment and host, and most countries provide microenvironments in which most crops can be produced. As an example of how it is possible to cope with mycotoxins, we will provide a brief account of the occurrence of mycotoxins in Queensland grains, and our general recommendations on the subject. The documented occurrence of mycotoxins in Australia is given in Table 4.

Table 2. The effects of poor feed conversion by pigs during growth from either 20 to 50 kg or 50 to 90 kg on margin-over-feed-cost (MOFC) of a 100-sow piggery

Change in feed conversion (% worsening)	MOFC ($ sow^{-1} year^{-1})	Purchase price to restore MOFC ($ tonne^{-1})	
		Total diet[a]	Grain alone[a]
20 to 50 kg period			
0	873	272	150
5	851	265	141
10	828	258	131
15	789	245	114
20	750	232	97
25	691	210	67
30	631	189	39
50 to 90 kg period			
0	873	272	150
5	847	264	139
10	820	256	129
15	794	249	119
20	768	241	109
25	742	235	101
30	715	228	91

[a]Based on the average prices of dietary ingredients in southern Queensland, first quarter 1990

Table 3. The effects of poor feed conversion by either chickens or laying hens on feed costs

Change in feed conversion (% worsening)	Feed cost[a] ($)	Purchase price to maintain lowest feed cost ($ tonne^{-1})	
		Total diet[a]	Grain alone[a]
Meat chickens			
0	1.15	320	150
5	1.21	304	124
10	1.27	289	101
15	1.33	275	78
20	1.39	261	56
25	1.45	248	35
30	1.51	236	16
Laying hens			
0	0.43	255	150
5	0.46	240	129
10	0.48	226	109
15	0.51	213	90
20	0.53	200	71
25	0.56	187	53
30	0.59	177	39

[a]Feed costs are per bird for meat chickens and per dozen eggs for laying hens and are based on the average prices of feed ingredients in southern Queensland, first quarter 1990

Table 4. Mycotoxins known to occur in Australia, fungal sources and prevalence (from Blaney and Williams 1991)

Mycotoxin	Fungal source	Prevalence
Aflatoxins	*Aspergillus flavus* *A. parasiticus*	Common in peanuts; uncommon in sorghum and maize; occasionally in other grains, oilseeds and mixed feeds
Cyclopiazonic acid	*A. flavus*	Unknown; produced in culture
Ochratoxin A	*A. ochraceus*	Occasionally in maize and mixed feeds
Zearalenone	*Fusarium graminearum* Grps 1 and 2	Common in maize and sorghum in wetter regions; occasionally in wheat and triticale
Deoxynivalenol	*F. graminearum* Grps 1 and 2	Occasionally in wheat and triticale
Nivalenol and derivatives	*F. graminearum* Grp 2	Common in maize in northern Queensland
T-2 and HT-2 Toxins	*Fusarium* sp.	Unknown; produced in culture
Cytochalasins H and J	*Phomopsis longicolla*	Unknown; produced in culture
Phomopsins	*P. leptostromiformis*	Common in lupins
Sporidesmin	*Pithomyces chartarum*	Sporadic in pasture in southern Australia
Ergot alkaloids	*Claviceps* spp.	Mainly in pasture, uncommon
Alternariols	*Alternaria alternata*	Common in sorghum occasionally in wheat
Altertoxins, Altenuene Tenuazonic acid	*A. alternata*	Unknown; produced in culture
Penitrem A	*Penicillium crustosum*	Mouldy dog food
Patulin	*P. expansum*	Apple juice
Viriditoxin	*Paecilomyces variotii*	Unknown, produced in culture

Aflatoxicosis

In northern Australia, aflatoxins are far more common in summer crops — peanuts, maize and sorghum (Blaney 1985) — than in winter crops — wheat and barley — although aflatoxins can develop in any grain if it is stored moist at temperatures of 25–40°C. High summer temperatures combined with irregular rainfall and insect damage, are probably important factors in allowing *Aspergillus flavus* and *A. parasiticus* to invade summer crops in the field, and this is a vital factor in creating a large initial inoculum of aflatoxin-producing fungi. However, aflatoxin concentrations in maize and sorghum before harvest are usually too low in Australia to affect animal production.

By far the most important cause of aflatoxin problems is failure to dry grains adequately before storage. Inexperience in the use of dryers by farmers is a major factor. Cases of aflatoxicosis in pigs commonly occur from March to August, about 4 weeks after harvesting the grain, when there has been 2–3 weeks for the fungus to develop, and the pigs have been fed for 1–2 weeks. The first sign noticed by the farmer is often a decreased feed intake usually about 2–3 days after the grain is first fed. However, onset of toxicity will depend on the amount of toxin present, and Ketterer et al. (1982) described peracute and acute cases involving mouldy bread and peanut waste. Aflatoxins have caused problems in piggeries on the Darling Downs, the Burnett region, and Central Queensland, involving mouldy bread, sorghum, peanut by-products, and maize, and aflatoxins in stored barley caused problems in a piggery in northern New South Wales, Australia (Blaney and Williams, unpublished data). The case histories indicate serious economic losses to individual farmers, even though the total number of cases is not large (we have observed 2–3 cases of subacute aflatoxicosis yearly from 1980–90. The losses result from deaths, reduced growth rate, poor feed conversion, and carcass condemnation. We consider that there could be a greater number of cases in which aflatoxicosis was not suspected, particularly if the only effect was a worsening of feed conversion.

Farmers are occasionally faced with a situation in which stored grain has become mouldy, perhaps through unavoidable circumstances. To simply dump many tonnes of such grain would entail severe financial hardship — and what should be done with it anyway? If farmers wish to utilise mouldy grain, they should undertake a risk assessment, preferably based on accurate aflatoxin analyses. (Although other mycotoxins may occur in such grain, aflatoxin is clearly the major risk in stored summer crops, and we have not identified other types of mycotoxicosis in pigs in these particular circumstances.) Obviously mouldy pockets of grain should be discarded (having regard for human exposure) and the remainder dried, mixed, and several representative samples taken for assay. If aflatoxins are detected by a reputable laboratory, the concentrations need to be carefully evaluated. The adviser can then determine the best strategy for using the grain.

There are some problems in setting acceptable levels for aflatoxins in that tolerances are obtained experimentally by using relatively small numbers of animals fed on well balanced diets. In the field, there can be great variation in individual susceptibilities, confounded by stresses not present under experimental conditions. Subject to these limitations, we suggest feeding strategies that include prevention of aflatoxin exposure to young pigs of less than 20 kg liveweight, feeding moderate concentrations (< 0.1 mg/kg) to growing pigs and breeding animals, and the highest concentrations (< 0.5 mg/kg) to pigs in the finishing stage of production (50–100 kg). We also advise that every opportunity should be taken to further dilute the contaminated feed, and to restrict the period of feeding, so as to increase the margin for safety. The well known practice of test-feeding a few animals for several days before exposing the entire herd is also recommended. We accept that aflatoxin-containing feed might contain other toxins such as cyclopiazonic acid which is also produced by Queensland isolates of *A. flavus* (Blaney et al. 1989). However, we have not yet detected this compound in naturally mouldy feed, and have no indication than it is highly prevalent. Harvey et al. (1988) have explored the use of alumina-silicates such as bentonite in alleviating aflatoxicosis. Bentonite is often used as a cheap pellet-binding and non-specific anti-diarrhoeal agent in Australian pig diets. It might be a useful adjunct to other aflatoxin-counteracting strategies, but we have not yet tested its efficacy in Australian conditions.

Chickens are relatively tolerant of aflatoxins. In contrast, ducklings are highly susceptible, while turkeys and other game fowl are intermediate. In the original Turkey X disease caused by heavily contaminated peanut meal, ducks and turkeys were far worse affected than chickens (Austwick 1978). In chickens, the minimum known harmful feed concentration is about 0.2 mg/kg, although this concentration is quite toxic to ducklings. Maize and poultry feeds in Southeast Asia may attain this concentration, but not often exceed it (Ginting 1985; Widiastuti et al. 1988a). Aflatoxins might be limiting potential poultry production by interacting with other stress or disease factors, and cyclopiazonic acid co-occurs with aflatoxin in Indonesian maize (Widiastuti et al. 1988b). However, there appears to be only anecdotal evidence at present to support this. In our present state of knowledge, it would seem that chicken production is probably the most effective way to use the contaminated maize.

By comparison, aflatoxins are not common in Australian poultry feed. In 1971–88, 161 poultry feeds suspected of causing reduced production were assayed

at our institute. Aflatoxins were detected in 13 samples, mostly from peanut meal, and only 4 samples contained > 0.05 mg aflatoxin B_1 equivalent/kg (Blaney, unpublished data). However, our experience is that the fairly centralised Australian meat chicken industry is reluctant to use obviously mouldy grains because of fears of reduced palatability or toxicity — an attitude clearly supported by the availability of feed grade wheat and barley and their usage as blends (dilution) with maize and sorghum.

Once the rumen has fully developed, cattle (and other ruminants) are moderately resistant to aflatoxin, with the lowest concentration associated with slightly reduced growth in calves being 0.1 mg/kg while adult cattle may tolerate 1 mg/kg or more for short periods (Keyl 1978).

Ochratoxin A

Ochratoxin A, perhaps in combination with citrinin, causes kidney disease in pigs in Denmark and several other European countries and in North America (Krogh 1978). Residues of ochratoxin can persist in meat, and are of concern in marketing of pig meat. It can be produced by *Penicillium verrocusum* in grains such as barley stored with high moisture content and in cool conditions (10–20°C) or by *A. ochraceous* in sorghum and maize stored at higher temperatures. In Queensland, ochratoxin A has been assayed routinely in more than 100 suspect animal feeds since 1980 (Connole et al. 1981; Blaney, unpublished data). It was detected on six occasions in sorghum, maize, or mouldy bread, but acute intoxication has not been confirmed. Ochratoxin A has been found with aflatoxin in sorghum grain but at low concentrations of 0.001–0.1 mg/kg (Ketterer et al. 1982; Blaney, unpublished data). Concentrations of 2 mg/kg had only very mild effects on pigs in feeding trials in our department (Tapia and Seawright 1984, 1985). Traces of ochratoxin have also been detected in Indonesian maize (Widiastuti et al. 1988b). Cases of ochratoxicosis in chickens involving mouldy maize have been described in the USA, but the risk of this in Australia seems remote, and there is insufficient knowledge of its prevalence in other tropical parts of the world. Contaminated material can be fed to ruminants, which are quite resistant to ochratoxin (Ribelin 1978).

Zearalenone

Young female pigs are the animals most sensitive to the effects of zearalenone, with poultry quite resistant. Concentrations of 1–5 mg/kg will induce red, swollen vulvas in about 4–7 days (Young et al. 1981). Concentrations of 5–20 mg/kg may reduce fertility of sows and boars (Chang 1979). Up to 50 mg/kg has been fed to baconer-pigs in experiments in north America giving only slightly impaired growth and feed conversion (Smith 1980).

Zearalenone contamination of maize, wheat and barley is common in temperate climates, but it also occurs in maize grown at high altitudes in the tropics of Indonesia (Stoltz et al. 1986). The red–purple discoloration produced by *F. graminearum* is a reasonable warning indicator of the possible presence of zearalenone, and it can thus be diverted into poultry feed, or fed to pigs only in the finishing stage. Zearalenone is converted to the more oestrogenic zearalenol in the rumen and may cause infertility.

In Queensland, zearalenone is common in maize on the Atherton Tableland where it is associated with *F. graminearum* ear rot (Blaney et al. 1984b, 1986). It can also occur in wheat infected with *F. graminearum* (Blaney et al. 1987) and in sorghum which has sustained heavy rainfall during maturation and then been stored without drying (Blaney 1985). However, concentrations above 1 mg/kg in grains are generally infrequent and only a few cases of intoxication have been recorded (Blaney et al. 1984a). The impact on Australian intensive animal production of zearalenone thus appears to be slight.

Trichothecenes

Trichothecenes and other *Fusarium* toxins are likely to be present in grain containing zearalenone. In Queensland, deoxynivalenol occurs with zearalenone in wheat (Moore et al. 1985), nivalenol occurs with zearalenone in maize (Blaney and Dodman 1988, unpublished data), and other *Fusarium* toxins probably occur in mouldy sorghum (Williams et al. 1986). Deoxynivalenol, nivalenol, T-2 toxin, and related trichothecenes occur in all parts of the world, including the tropics (see B.J. Blaney, *Fusarium* and *Alternaria* toxins, these proceedings).

The effect of deoxynivalenol on pigs is to reduce feed intake without affecting feed conversion except at concentrations greater than 8 mg/kg. We have calculated that voluntary feed intake is depressed by 6% for each 1 mg/kg of dietary deoxynivalenol (Williams et al. 1163U,8. Poultry are far more resistant to deoxynivalenol than pigs although slight reductions in feed intake may still occur at high concentrations (Mannion and Blaney 1988). Farmers seeking to use such grain should feed it either to ruminants as a supplement, to chickens at moderate concentrations (< 6 mg/kg), or to pigs at low concentrations (< 2 mg/kg) and only in the finisher stage (50–90 kg). Deoxynivalenol appears to be of very minor importance in Australia, with its known occurrence mainly around elevated country in the New South Wales–Queensland border region (Blaney et al. 1987; Tobin 1988).

Maize from the northern Queensland tablelands can contain nivalenol and acetyl-nivalenol in addition to zearalenone. Some maize crops can contain up to 2 mg zearalenone and 5 mg nivalenol/kg, but the centralised drying and marketing system used in the region results in bulk maize with much less than this. We used maize screenings containing about 13 mg nivalenol and 3 mg zearalenone/kg in pig feeding trials (Blaney and Williams, unpublished data). In an acute study of 2 weeks duration,

40 kg pigs were offered maize-based diets containing up to 8.9 mg nivalenol and 1.5 mg zearalenone/kg. No vomiting occurred but voluntary feed intakes were only half of those of pigs on control feed. Except for failure to cause vomiting, these results were similar to those we had obtained with equivalent concentrations of deoxynivalenol in wheat (Williams et al. 1988). However, in comparison with our deoxynivalenol results, a worsened feed conversion contributed to a greater impact on growth rate. These effects were subsequently confirmed in a pair-feeding study. Lower concentrations of nivalenol (< 3.6 mg/kg), appeared to be tolerated by the pigs without observable effects on performance. Bentonite has been found useful in preventing T-2 toxicosis in rats (Carson and Smith 1983), but we detected no useful effect of bentonite from an Australian source in improving feed intake of pigs fed nivalenol in one experiment.

Any effects of mycotoxins on the breeding herd would have severe economic consequences. Hence, we fed the same maize to pregnant sows in two experiments to test the effects of nivalenol and zearalenone on fertility and reproductive performance, since nivalenol is an abortifacient in mice. In these studies, there was some feed refusal associated with feeding the mouldy maize, but at slaughter we found no differences between test and control groups in either the number or weight of embryos or ratio of corpora lutea to embryos.

These same screenings were fed to chickens and produced similar results to deoxynivalenol, with growth adversely affected only by about 12% at a dietary concentration of 6 mg nivalenol/kg (Kopinski, pers. comm.).

In summary, we consider that pigs and chickens are unlikely to be affected by the concentrations of nivalenol and zearalenone normally occurring in bulk maize in northern Queensland. Screenings, or materials in which the mouldy kernels may be concentrated, would be better used as ruminant feed, which is the usual practice. Cattle are also fairly resistant to deoxynivalenol. T-2 toxin has affected calves in cooler climates, but the prevalence of this toxin in the tropics is currently unknown. If trichothecenes are shown in future to be a major problem in Southeast Asia, then the best option would appear to be to feed such material to poultry or ruminants as a supplement. At the village level, hand-sorting of visually mouldy maize ears may be feasible, facilitating distribution between man and his livestock.

Fusarium moniliforme and *Diplodia maydis*

F. moniliforme is undoubtedly a very toxic fungus, producing the carcinogenic fumonisins, and we should be very concerned about its presence in human food. Nevertheless, it is also the most prevalent *Fusarium* species in maize and sorghum in northern Australia and, as yet, no cases of animal disease associated with it have been reported. *Diplodia maydis* is another toxic fungus common on maize, but apparently without real impact on animal production (Blaney et al. 1981).

We will illustrate the economic consequences that often apply with use of mouldy grain, with the results of a feeding trial that we performed using *F. moniliforme*- and *D. maydis*-infected maize in pig diets (Blaney and Williams 1991). Four batches of grain with 4–21% visually mouldy kernels were obtained from southern Queensland in 1985 and incorporated as the sole grain portion into pig feeds. The results are shown in Table 5.

Table 5. The effects on pigs of diets based on batches of *Fusarium moniliforme*- and *Diplodia maydis*- infected maize, purchased at different prices, and the margin-over-feed-cost (MOFC) of a piggery using the grain

Attribute	Maize batch 1	2	3	4[a]
Maize cost ($ tonne^{-1})[b]	160	135	110	110
Mouldy kernels (%)	4	17	21	15
Daily feed intake	1.94	1.88	1.84	1.94
Feed conversion ratio) (0–2 weeks)[c]	2.39	2.46	2.68	2.50
Daily feed intake	2.35	2.09	2.23	2.25
Feed conversion ratio (0–5 weeks)[c]	2.54	2.57	2.57	2.57
MOFC[d] ($ sow^{-1} year^{-1})	879	894	916	916

[a] Batch 4 was heavily infected with both *Diplodia maydis.* and *F.moniliforme*; other batches mainly with *F.moniliforme*

[b] Costs in April 1987

[c] Period of experimentation from a start live weight of 27.4kg; data from Williams and Blaney (unpublished)

[d] Profitability calculated on the basis that the experimental results applied for the period of growth from 20 to 50 kg live weight but thereafter no difference occurred in performance until sale at 90 kg live weight

In the first 2 weeks of experimentation from 27 kg, there was some reduction in feed intake and worsened feed conversion efficiency in the batches with most damage, indicating some toxicity. However, over the full 5 weeks of experimentation these effects diminished, indicating developing tolerance. As a consequence, profits increased by using the most damaged maize because of its lower purchase price.

Other Mycotoxins

Alternaria species can produce mycotoxins in crops such as sorghum maturing in the field, and perhaps in some storage circumstances in fruit or vegetables. This is naturally of concern for human food, but there is less reason to consider this a danger to domestic animals. Mouldy sorghum containing around 15 mg alternariols/kg (and probably other toxins) has been fed to pigs and chickens (Bryden et al. 1984;Williams et al. 1986), worsening feed conversion efficiency by up to 14% (see Table 2). However, this was very mouldy material, fed as the sole grain component of the diet, and dilution of the grain may well have avoided any ill effects.

Several other fungi growing in field crops have caused problems in grazing animals around the world. This includes *Claviceps* species (ergot), *Phomopsis* species (lupinosis), and *Pithomyces chartarum* (facial eczema), but these are not known to cause problems in stored commodities, unless the storage of previously contaminated hays is considered in this category.

So, to summarise this section, there are certainly a large number of mycotoxins occurring in animal feeds in Australia, but they rarely cause serious illness or death. A combination of reduced nutrient content, poorer palatability, and low concentrations of mycotoxins can reduce feed intakes and feed conversion, but it is an effect that can often be balanced against the lower price paid for the grain. We are confident that similar situations exist throughout the world.

Residues in Meat

Many people in affluent countries are becoming more and more concerned with undesirable residues in meat. The issue is often a weapon in international trade wars rather than a real human health issue, but we cannot neglect its great impact in marketing animal products. Mouldy feed has the potential to cause two different types of residue in meat. Firstly, specific residues of aflatoxin, ochratoxin and other mycotoxins may occur. Secondly, most moulds produce a range of antibiotics, and these might be detected in bacterial inhibitory tests used to screen meat for residues of therapeutic antibiotics. As a broad generalisation, most mycotoxins and other fungal antibiotics are not stored in fat depots, and tend to be eliminated from the body within 3–14 days, often via the urine. It seems wise not to give mouldy feed to animals within 2 weeks of slaughter, in order to reduce the risk. However, definite recommendations cannot be made, since accurate withholding periods have not been determined for many fungal metabolites.

Conclusions

Effective use of mould damaged commodities means getting the best return in terms of food suitable for human consumption. There are certain methods such as physical segregation and chemical or microbiological treatment of contaminated commodities to reduce their mycotoxin content. However, the cheapest and most time–honoured method, available to most farmers and villagers, is to convert it into animal protein by feeding it to domestic animals. Once the circumstances governing certain types of mycotoxin contamination are recognised, there are many options for distributing the material between animal species, and categories of animals within those species, that should enable most material to be effectively used. Most problems arise in circumstances where people are unable to quantify the degree of risk, and it is an appropriate role for a mycotoxicologist to assess this real risk in the framework of the farmer's social and economic situation.

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Strategies for Managing Spoilage Fungi and Mycotoxins: a Case Study in Thailand

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Abstract

Thai maize has been noted for its bright yellow colour and high protein content. However, samples have sometimes been found to contain unacceptably high levels of aflatoxin and have therefore been discounted and even rejected by foreign buyers.

In 1984 a 4-year program was initiated with the aim of identifying the causes of aflatoxin contamination and establishing the best approach for eradicating the problem. The project was coordinated by Rural Investment Overseas (UK) Ltd together with the Department of Agriculture (DOA) and Bank for Agriculture and Agricultural Cooperatives (BAAC) in Thailand, and the Natural Resources Institute (NRI) and Silsoe College of the United Kingdom.

The initial stages of the project established that maize collected and dried to 14% moisture content within 48 hours of harvest showed the lowest possible levels of aflatoxin. This combined system of grain management and drying is now referred to officially as the UTP system (UK–Thai Project System). The resulting maize, collected and dried using the UTP system was found to have aflatoxin levels at a mean value of 2.5 ppb. Control samples confirmed that maize at local merchants and regional silos had values of more than 50 and 100 ppb, respectively.

Ignorance of the real significance of aflatoxin is a major constraint to progress. Unless higher premiums can be offered for low toxin levels, the incentives necessary to change the situation will not exist. The remaining stages of the project therefore involved alerting those affected by the aflatoxin problem and investigating ways of introducing financial incentives that would increase awareness of the problem. Incentives would also ensure that merchants and others invested in the equipment (e.g. dryers) needed.

The project showed that, through the application of grain management and drying procedures in the commercial sector, together with the introduction of realistic financial incentives, aflatoxin contamination can be reduced to acceptable levels.

In the mid 1980s agriculture accounted for up to 25% of gross domestic product (GDP) in Thailand and about 55% of exports by value. Whilst rapid expansion over the past 5 years in non agricultural sectors has reduced agriculture's relative contribution to the economy, it remains very significant.

Much of the success of Thai agriculture can be attributed to the existence of a private sector responsive to market requirements and to government programs which emphasised the development of rural infrastructure. Private sector merchants were quick to identify export opportunities and provide supporting production, processing, and marketing services which encouraged farmers, by means of a relatively efficient market mechanism, to expand the output of such crops as maize, kenaf, cassava, and sugar.

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However, expanded production was still largely attained through increase in cropping area. This was recognised in the Thai Government's Fifth Development Plan (1982–86) which stated that agriculture's continued contribution to exports and employment must come from improved yields and cropping intensity rather than an increase in cropping area.

The World Bank's 1980s sector review also identified a number of major constraints requiring policy attention. These were:

- market constraints for some important Thai products, namely rice, tapioca products, and sugar, and quality control problems for others, namely maize and some livestock products;
- limits on resource availability, especially arising from the use of marginal land;
- pricing and regulatory policies such as export levies which reduce farmer incomes and incentives;

- insecurity of land tenure, reducing incentive to invest; and
- inadequate design or unavailability of appropriately improved technology packages to aid intensification.

Maize is a major crop in Thailand and in the 1970s and early 1980s commanded a premium price on world markets because of its high quality. However, maize cropping typified the problems highlighted in the Thai Government and World Bank reports. Given its importance in generating income and employment, maize production was rapidly expanded during this period, but little note was taken of falling quality and inadequate incomes to growers. The result was that Thai maize began to be discounted on world markets and at times shipments were rejected because of poor quality.

A major factor in quality deterioration was the increasing presence of aflatoxins. A request to assist in solving problems relating to aflatoxin contamination, particularly its effects on maize exports and a growing poultry industry reliant on maize for feed, was made by the Thai Government to the United Kingdom in 1984. It was estimated that, at that time, these problems were costing Thailand some $25 million per year through lost revenue and reduced feed conversion efficiency.

This paper gives an overview of the findings and recommendations of a resulting 4-year project. Copies of full reports covering the three phases of the project are available on request from the author.

UK–Thai Aflatoxin Project

Maize in Thailand is grown, stored, and marketed largely in the rainy season when susceptibility to fungal infection and aflatoxin contamination is greatest. Although the aflatoxin problem was not generally appreciated by many growers and middle merchants, a number of organisations and institutions, in both the public and private sectors, were implementing research and control measures. The UK-Thai project sought to focus attention on the aflatoxin problem, bring together the interested parties, and undertake practical field and laboratory work.

The project team comprised members from the Thai Department of Agriculture (DOA), the Bank for Agriculture and Agricultural Cooperatives (BAAC), the U.K. Tropical Development and Research Institute (TDRI); now the Natural Resources Institute (NRI); and Silsoe College, and was coordinated by Rural Investment Overseas (U.K.) Ltd.

A feature of the project was the linkages forged between the public and private sectors, such that the field trials were, in practice, undertaken both at government field stations and with private sector merchants and exporters. In addition, it was sought to undertake trials that were both scientifically valid and commercially relevant.

The overall objective of the project was to 'find a practical and economic solution to the incidence of aflatoxin and related postharvest problems in Thailand'.

Maize Harvesting and Marketing in Thailand

Maize harvesting involves hand picking and stripping of the cobs, and transportation to farm storage by manual, animal, or motorised means.

At the farm, the maize cobs are either bulked up in preparation for immediate shelling and sale, or are placed in storage because immediate sale is not possible due to inaccessibility during the rainy season or because the farmer anticipates a price rise.

On farm postharvest practices, particularly drying and storage, are heavily influenced by farmer circumstances, such as the need to settle debts and the availability of on farm storage facilities, and local market conditions.

Shelling is normally undertaken by the merchant and, since there is little on-farm drying, the common practice of shelling above 23% moisture content (m.c.) can result in broken and damaged grains, exacerbating the risk of aflatoxin contamination.

Payment for maize is usually made on the basis of weight after shelling, less a discount for moisture content, impurities, and broken and mouldy seeds. Moisture content, often measured by touch and smell, provides the main basis of pricing and, as found in a BAAC survey in 1984, many farmers mistrusted estimations of weight and moisture content made by traders.

With crop sales linked to maize shelling and with traders better placed to exploit market incentives for drier grain, maize drying has become primarily a trader function..

Few up-country merchants, however, have installed mechanical dryers and still rely heavily on sun drying. Much has been said and written about the high losses associated with sun drying, particularly in the rainy season, but what has not been fully realised up till recently is the degree to which inefficient sun drying can contribute to the incidence of aflatoxin contamination. In addition, because the Thai Maize Producers and Traders Scale (TMPTA) scale cuts out at 18% m.c., maize is usually only dried to this level before being shipped to exporters' silos, as there is no initiative to dry to 14% m.c.

The initial objectives of the project were therefore to show that, through fast mechanical drying of the maize, the aflatoxin levels could be reduced. It was also contended that drying should take place as close to the point of production as possible and the dryers chosen were therefore small and portable.

For practical and funding reasons the project was split

into three phases (I, II, and III) with the objectives of phases II and III largely developed from the findings of the preceding phase.

Phase I — Approach and Results

Initial research revealed little evidence of aflatoxin contamination in the field before harvesting. It was generally accepted that the problem largely manifested itself during the storage of damp grain before its sale and transport to the merchants and exporters. Nevertheless, preharvest contamination was encountered during Phase III.

Phase I aimed at developing and evaluating a method of mechanical drying which would dry the maize to 14% m.c. and could control aflatoxin production, especially during the high risk rainy season. Two drying systems incorporating mechanical dryers were evaluated, and their effectiveness and economics compared with normal handling practices.

Trial 1 — Operation and results

A two-stage drying process aiming to minimise damage at shelling was designed. Ten tonnes of maize cobs were dried in a bin to approximately 18% m.c., then shelled and further dried in a second bin to 14% m.c. by using a Lister Moisture Extraction Unit (LMEU).

As already noted, shelling grain at high moisture contents can result in excessive cracking and damage. This trial was therefore designed to test whether cobs could be successfully dried to an ideal moisture content for shelling — 18%, then shelled, and the resulting bulk grain dried to 14% m.c. without significant increase in the aflatoxin level.

Following the purchase of maize for this trial, it was found that the cobs had not been freshly harvested, as originally designed, but had a history of 1–4 weeks temporary farm storage. This was reflected in a mean total aflatoxin content of 71 ppb, significantly higher than the level (21 ppb) for freshly harvested cobs sampled on farm (Table 1). Nevertheless, in this trial the average aflatoxin content appeared to increase only marginally to 97 ppb during cob drying. There was a further apparent increase to 126 ppb during the second stage 'bulk' drying.

The trial indicated that control was possible using this method. However, although less kernel damage was found to result through shelling at 18%, the practical complexities of using this type of two-stage drying were too great.

Trial 2 — Operation and Results

In this trial, shelled maize in bulk was LMEU-dried over a central duct within a wall of bags to 14% m.c. The maize used was as received by regional traders but, as with Trial 1, was found to be already highly contaminated with aflatoxin, at an average 176 ppb. Using this system it took 2 days to reduce the moisture content to 14%, during which time the aflatoxin contamination did not increase significantly (176 to 181 ppb) (Table 1).

Samples from both Trials 1 and 2 were stored for a further 2 months, at the end of which aflatoxin levels had fallen, as they also did in sun dried control samples (Table 2).

Table 1. Mean total aflatoxin contents measured in Phase 1 of the UK–Thai Aflatoxin Project

Source	Mean total aflatoxin content (ppb)		
	On cob	After shelling	After drying
Farm	(13) 21	(10) 76	– –
Merchant			
i. All	(10) 45	(20) 239	(19) 187
ii. Matched	– –	(10) 223	(9) 246
Trial 1	(9) 71	(10) 97	(10) 126
Trial 2	–	(10) 176	(9) 181

( ) = number of samples

Table 2. Mean total aflatoxin content measured in storage trial during Phase 1 of the UK–Thai Aflatoxin Project

Source	Mean total aflatoxin content (ppb) during storage		
	Commencement	2 weeks	8 weeks
Trial 1	(10) 76	(10) 61	(10) 49
Trial 2	(10) 194	(10) 122	(10) 136
Merchant control	(10) 162	(10) 143	(10) 104

( ) = number of samples

Conclusions and recommendations from Phase I

The results of Phase I trials showed that, by drying wet maize to 14% m.c., in as short a time as possible, significant increases in aflatoxin production could be avoided. The trials also showed that, by initially drying the cobs to 18% m.c., then shelling and drying the grain to 14% m.c., shelling damage and aflatoxin contamination froze at existing levels. In practice, however, management and equipment needs make this two-stage method

impractical. Shelling after harvest and one-stage drying to 14% m.c. was found to be the most practical method.

The most significant finding was the high levels of aflatoxin contamination in the maize delivered to merchants. On-farm sampling indicated that aflatoxin content immediately after harvest was low, averaging 21 ppb, but rose very quickly during and after farm storage to, for example, 239 ppb, in the samples taken at merchants. This also indicated that by the time maize reached the large dryers at the exporters it is too late to control aflatoxin contamination. It was therefore recommended that Phase II concentrate on confirming the drying results from Phase I and institute a collection system which minimised the time between harvest and drying.

Phase II—Approach and Results

Specific objectives of Phase II were:

- production of high quality, low aflatoxin maize during the rainy season by drying to 14% m.c. within 48 hours of harvest, maize which had a history of 1-4 weeks field drying;
- assessment of the economics of producing maize with low levels of aflatoxin;
- evaluation of small scale drying systems; and
- confirmation of Phase I results.

The Phase II program included drying and storage trials in the provinces of Lopburi and Loei. In order to produce high quality, low aflatoxin maize, a grain management system was applied whereby drying of the maize to 14 or 16% m.c. commenced within 48 hours of harvest. As in Phase I, a number of drying trials were conducted to determine the most practical and economical method of minimising aflatoxin contamination.

Trial 1—Operation and results

Shelled grain with a history of 1–4 weeks field drying was dried to 14% m.c. within 48 hours of harvest and stored for 2 months.

As in Phase I, a Lister Moisture Extraction Unit was used to dry shelled grain loaded into a drying bin with a perforated floor. This method gave uniform drying and was simple to operate and sample. It was shown that single-stage drying of field dried, freshly harvested and shelled maize could produce maize with very low levels

Table 3. Summary of aflatoxin results for Phases I and II of the UK–Thai Aflatoxin Project

Drying trials	Mean total aflatoxin content (ppb)		
	1984 Phase I	1985 Phase II	
	Lopburi	Loei	Lopburi
Trial 1			
2-stage[a] (maize received as at local merchants)	71 --->126 (4–5 days)		
Trial 2	dry		
1-stage[b] (maize received as at regional merchants)	176 --->181 (1–2 days)		
		dry	dry Trial 1
1-stage (maize received < 48 hrs after harvest)		4 --->4 (1–2 days)	2 --->2 (1–2 days)
— stored 8 wks at 14% m.c.	–	4	1
		dry	dry Trial 2
1-stage (maize received < 48 hrs after harvest)	–	1 --->2 (1–2 days)	1 --->2 (1–2 days)
— stored 1 wk at 16% m.c.	–	5	4
		dry	– Trial 3
2-stage[a] (maize received < 48 hrs after harvest)	–	3--->30 (3 + days)	
a. stored 1 week at 9%	–	500+	
b. stored 8 wks at 14%	–	22	

[a] Two-stage drying = cob drying to 18% moisture content: shelling; further drying to 14% moisture content.
[b] One stage drying = drying maize, after shelling, to 14% moisture content.

of aflatoxin. In Loei, a mean total aflatoxin content of 4 ppb was found for 10 replicates dried to 14% m.c., while in Lopburi, 20 batches averaged only 2 ppb (Table 3).

Twenty 2.5-3 tonne batches of maize which had been dried to 14% m.c. were then stored for 2 months at an average relative humidity of 75%. During this period, there was no change in the moisture content of the stacks. The aflatoxin content of the maize remained constant at 4 ppb in Loei and fell to 1 ppb in Lopburi (Table 3).

This study therefore confirmed the Phase I finding that maize at a moisture content of 14% can be stored safely for at least 2 months, provided that moisture gradients within the batch are low and storage conditions are good.

It should be stressed that these results were achieved at a time when a mean total aflatoxins level of 89 ppb was found in maize held by local merchants in Loei, 57 ppb in Lopburi, and 100 ppb after mechanical drying at export silos.

Trial 2—Operation and results

The aim of this storage trial was to determine whether partially dried maize could be stored for up to 1 week without incurring any significant increase in aflatoxin levels. Should safe storage be possible under these conditions, then maize could be partially dried up-country and transported to a silo within 1 week for completion of drying to 14%.m.c.

Maize collected as in the first trial of Phase II was dried to 16% m.c. and stored for 1 week. There were 10 experimental replicates in Lopburi and 5 in Loei. The mean aflatoxin contents after drying were 1.9 ppb in Loei and 2.2 ppb in Lopburi and after 1 weeks storage the levels had increased marginally to 4.7 ppb and 4 ppb, respectively, neither increase being statistically significant (Table 3).

The results were therefore encouraging, indicating that maize could be stored safely for at least a week at 16% m.c. before drying to 14% for longer term storage.

Trial 3—Operation and Results

Since the two-stage drying strategy tested in Phase I was deemed to be too complex, further trials were undertaken in this trial using a simpler method. Using this method, although damage was reduced when the grain was shelled at 18% m.c., the extended time taken to dry it to 14% m.c. resulted in significant increases in aflatoxin content, i.e. from 3 to 30 ppb over the 3 day drying period (Table 3).

The results of Trial III showed that batches of maize dried to 14% m.c. incurred no significant increase in aflatoxin content during storage. However, one batch dried to only 17% over 3–4 days had a level of 68 ppb after drying, increasing to 260 ppb after only 1 week. Another sample at 19% m.c. had an initial aflatoxin level of 32 ppb which rose to 592 ppb after 1 week of storage!

These results thus yielded further evidence that fast drying to 14% m.c.was required if aflatoxin levels were to be 'frozen' during storage.

Economics of up-country drying

While the economics of up-country drying are hard to assess accurately, they are at the heart of the aflatoxin problem because on them hinges the implementation or otherwise of any technical solution proven. Phases I and II of the project indicated that the maize should be dried as quickly as possible after harvest and shelling. It follows that dryers must be located close to the production area and shelling point.

Given the small plot size of average Thai farmers it is doubtful whether they will have the means to purchase dryers. The up-country merchant, on the other hand, is ideally placed to offer a drying service. Nevertheless this costs money, which will have to be recovered in the final price of the maize. Similarly, the exporter (or feedmiller) will need to spend more on sampling, quality control etc. if he is to produce high quality maize.

During the term of the project in the mid to late 1980s, there were three main market incentives to supply of low aflatoxin maize:

(i) incentives for up-country drying;

(ii) incentives for better quality grades; and

(iii) emerging incentives for maize with low aflatoxiin levels.

Table 4 shows that maize at a wholesale price of Bt2250 when dried from 23–18% attracts a premium of Bt212/t or Bt42/t for each unit of percentage moisture reduction (Bt42/t/%).

Table 5 shows estimated drying costs for the major types of dryer, from which it can be seen that a premium of Bt41/t will cover the costs of only the larger, continuous-flow dryers. Additional benefits are therefore needed to provide incentives to all the parties in the production and marketing chain.

The incentives provided by the world market for meeting Grade A quality standards were around Bt130/t and the incentive for low aflatoxin maize < 20 ppb about Bt260/t in 1988.

The incentives applying can be summarised as follows:

	Bt/t
Drying 23–18%	212
Quality	130
Aflatoxin < 20 ppb	260
Total	602

Drying premiums should be paid to those who do the drying. The other premiums should be distributed between the three main agents: farmers, merchants, and exporters (feedmillers). A 2:2:1 share (where the up-country merchant does the drying) would provide the following incentives:

	Bt/t
Farmers	145
Merchants (212 + 145)	360 (includes drying cost)
Exporters	95
Total	600

The UK–Thai Project System (UTP System)

The Phase I and II studies were successful in delivering maize containing low levels of aflatoxins. From these investigations it was possible to specify a set of conditions, called the UTP system, for production of low-aflatoxin maize. These are as follows.

1. Maize should be left to dry on the plant in the field for 1–4 weeks before harvesting so as to reduce its moisture content to no more than 22%.
2. The field dried maize should be shelled within 24–48 hours of harvest.
3. Shelled maize should be loaded into a dryer within 12 hours of shelling.
4. Drying to average 14% m.c. should be completed within 48 hours, with no portion exceeding 15%. Alternatively, maize should be partially dried to a moisture content of 16% within 48 hours and further dried to 14% within 1 week.

Phase III—Objectives

Phases I and II of the project had demonstrated that it was possible to produce dry, low aflatoxin maize in the rainy season. However, although the dryers had been operated in conditions approximating those encountered in the open market, further work was needed to verify that the UTP system of fast maize collection after harvest could function successfully within the existing marketing chain.

The aim of Phase III was therefore to apply the Phase I and II findings within the existing maize marketing chain during the rainy season harvests of 1986–87 and 1987–88 in order to establish that commercially realistic improvements in the production of low aflatoxin maize could be obtained.

To achieve this objective the following work was undertaken:

1. Selected merchants and buyers with suitable grain dryers were provided an incentive to participate in the

Table 4. Price incentives for drying to 18% moisture content in Thailand

Maize price Bt/t	3000		2500		2250		1750	
Discounts	Bt/t	Bt/t/%	Bt/t	Bt/t/%	Bt/t	Bt/t/%	Bt/t	Bt/t/%
Moisture content								
17.5 – 18.0	0	0	0	0	0	0	0	0
18.1 – 18.5	21	42	18	35	16	32	13	26
18.6 – 19.0	39	39	33	33	30	30	23	23
19.1 – 19.5	78	52	65	43	59	39	46	30
19.6 – 20.0	114	57	95	48	86	43	67	33
20.1 – 20.5	168	67	140	56	126	50	98	40
20.6 – 21.0	222	74	185	62	167	56	130	43
21.1 – 22.0	252	63	210	52	189	47	147	37
22.1 – 23.0	282	56	235	46	212	41	165	33
(23.1 – 27.0)	444	46	370	38	333	34	259	26

Unofficial

Table 5. Summary of estimated costs for alternative systems for maize drying in Thailand

	Capacity	Capital cost (Bt)	Bt/t/%
Solar crib	2.3 t	2000–3000	6
On-floor sun drying	–	180/sq.m	11
Engine-powered Lister LT1	3.3 t/batch	150 000	56–75
ST3	6.0 t/batch	317 000	38–47
Medium continuous flow	5–10 t/hr	720 000	30
Large continuous flow	75–200/hr	10 million	25

project by an offer to buy BAAC farmer clients maize at a premium price.

2. Farmers and merchants were interviewed to establish what difficulties there might be in changing from current practices to the new system.
3. Studies were undertaken to develop a farm level sprayer for application of a chemical fungal inhibitor to maize cobs in locations which are inaccessible in the rainy season.
4. Small laboratories were set up at one export silo and one up-country merchant's godown to monitor aflatoxin levels.

Phase III Observations

Harvesting

In Phase II it had been recommended that the time from harvest to shelling not exceed 48 hours. However, in Phase III it was found that the labour input required to harvest sufficient maize to warrant shelling and transport was not available and farmers did not have the cash to pay for it. Thus, the UTP criteria were not rigidly adhered to and farmers were advised instead to harvest as quickly as circumstances permitted.

In addition, during the 1986–87 harvest it was found that farmers were reluctant to speed up harvesting as the premiums available for low aflatoxin maize were not flexible enough to accommodate maize slightly above 20 ppb.

In 1986–87 the average time elapsing between the beginning of harvest and shelling was 9 days and in 1987–88 16 days.

Shelling

In most cases, shelling and delivery to the drying silo were completed within 24 hours, with drying being completed within 3 hours. However, in Petchaboon, delivery took 1.5–3 days because maize has to be brought down from the mountain by tractor and then delivered by truck to the silo.

Tonnage collected and aflatoxin levels

In 1986–87 294 tonnes of project maize were collected from four sites. Of this 52% had aflatoxin levels of less than 20 ppb.

In 1987–88, 2098 tonnes of project maize were collected. None of the crop had less than 20 ppb aflatoxins, 9.25% was between 20–50 ppb, and the remainder was over 50 ppb.

Tables 6 and 7 show that aflatoxin levels in project maize in 1986-87 were greatly lower than those in control samples. Note, however, that the quantity involved is small, reflecting the unwillingness of farmers to produce UTP maize when prices were low and they wished to hold onto their crop in the hope that prices would rise.

In 1987–88, the price of maize was very high, largely because of reduced plantings and drought conditions.

Table 6. Aflatoxin levels determined during year 1 (1986) of Phase III of the UK-Thai Aflatoxin Project

Classification (Code)	No. of samples	Total mean	Aflatoxin range (ppb)
Project Maize			
BAAC collection prog.	8	22	0–94
Up-country drying	5	82	8–262
Combined	13	45	0–273
Control Maize 1			
Dry maize			
Silo 1	15	121	58–212
Silo 2	30	191	55–348
Combined	45	168	55–348
Control Maize 2			
Trucks arriving			
Silo 1	17	126	23–298
Silo 2	57	162	0–684
Combined	74	154	0-684
Control Maize 3			
Trucks arriving			
Silo 1	20	103	55–181
Silo 2	15	150	100–219

Again farmers did not want to release their maize early in case prices rose even further! In practice, it was found that the levels of aflatoxin in the project maize were extremely high and that there was no significant difference between project and control samples (see Table 8).

The UTP system (allowing for the increased allowable harvesting time) had clearly failed to produce low aflatoxin maize in 1987–88, and the control samples collected at the merchants' dryers had also shown much higher aflatoxin levels. It was concluded that the high levels of aflatoxin were almost certainly due to atypical preharvest contamination caused by serious drought during the late growing period, leading to stress fractures of the grain and subsequent infection during the rainy season harvest.

Evaluating the Role of Mould Inhibitors

In Petchaboon and other areas maize is often grown in hilly, inaccessible areas. In the rainy season, this means that the crop has to be stored on-farm for extended periods because of transportation difficulties.

An alternative to fast drying of freshly harvested maize is to apply a fungicidal chemical, NILSPOR PLUS, which has been shown to prevent fungal and bacterial deterioration in grain and fodder crops. A trial was therefore undertaken to determine if NILSPOR PLUS, when applied to fresh maize, could prevent aflatoxin development under conditions typical of Thai farms. This work was carried out by the Asian Institute of Technology, in Bangkok, with a custom-designed, continuous-flow spray applicator.

Table 7. Aflatoxin levels determined during year 2 (1987) of Phase III of the UK-Thai Aflatoxin Project

Classification (Code)	No. of samples	Total mean	Aflatoxin range (ppb)
Project maize 1			
Up-country drying			
Petchaboon	16	550	46–3518
Chantaburi	16	606	46–2898
Nan	4	1029	571–1734
Control maize 1			
Merchant			
Petchaboon	8	339	67–721
Chantburi	14	717	32–2669
Nan	5	545	264–972
Farm			
Petchaboon	10	21	0–80
Chantaburi	18	121	0–829
Project maize 2			
Siam grain project:			
Petchaboon	21	220	8–735
Kampaengpet	22	171	2–632
Loei	10	251	94-482
Control maize 2			
Siam grain project collected:			
Tha Rua	24	366	99–584
Combined controls			
From merchants	51	462	32–2669
From farm	28	85	0–829

The results of these trials indicated that while it was practical to use the cob sprayer at farm level, the economics of using chemicals was marginal.

Laboratory determination indicated control of fungal activity but, largely because of low levels of contamination in the area in which the maize was grown, aflatoxin levels of treated and control samples were similar. Further tests were recommended.

Recommendations Based on Phase I, II and III Results

The findings and recommendations discussed and agreed on by the various sections of the Thai maize industry, following the UK-Thai Aflatoxin project, are summarised in this section.

Harvest and postharvest practice

a. Farmers should be encouraged to allow maize to dry out for 1-2 weeks on the plant, after the crop has reached maturity.

b. There is clear evidence that temporary storage of undried maize cobs leads to a build up of aflatoxin contamination. Because farm storage is so widely practiced it is recommended that further research work be undertaken on field drying and temporary farm storage.

c. Merchants should be encouraged to:

- dry maize rapidly after harvest;
- shell maize within 5 days of harvest;
- start grain drying immediately or at latest within 12 hours of shelling; and
- use drying systems capable of drying maize to 14% m.c. within a 48-hour period.

d. The use of chemical mould inhibitors should be further investigated together with additional work on the cob sprayer developed by AIT.

Table 8. Levels of aflatoxin levels in project and control maize samples

	Mean total aflatoxin (ppb)		
	Chantaburi	Petchaboon	Nan
Project maize	606	551	1029
Control maize	717	339	545

Quality control

a. Simple aflatoxin quality control should be used by all merchants with drying facilities to identify truck loads of maize which are within 50 ppb and these loads should be kept separate and dried as per c) above.

b. The establishment of laboratories to pursue better methods of quality control in the maize producing areas should be encouraged. In particular, these laboratories would provide a service to farmers and merchants for moisture content assessment, aflatoxin analysis of samples, and monitoring and reporting on levels of pre- and postharvest contamination of aflatoxin. The laboratory staff could also carry out training programs in the area.

c. The government should establish standard methods for sampling consignments of maize for exporters and feed millers

Incentives for quick release of maize after harvest

a. Pledging and contract schemes should be developed to encourage farmers to release their harvested crops immediately after harvest e.g. BAAC pledging schemes.

b. Feed millers who have identified supplying merchants and who follow acceptable quality control procedures, should experiment with forward contracts and price incentives.

c. To provide incentives for farmers and merchants to dry maize mechanically, the TMPTA weight scale should be altered to provide an incentive of at least 20 kg/tonne per 1% of moisture removed down to 14%. Such a scale should replace the existing scale which does not provide an incentive to dry below 18% m.c.

d. Merchants operating in the maize-producing areas, who are prepared to mechanically dry maize that has been freshly harvested and will offer a premium to farmers, should be encouraged, recognised, and approved.

e. In order to encourage merchants to pursue the production of quality maize through mechanical drying and to encourage local farmer participation, the B.O.I. should consider awarding privileges to these merchants.

Training

a. Greater training, through extension work, should be given to merchants and farmers as to the effects of poor post harvest management, aflatoxin control, equipment suitability, incentives available etc.

Summary

The success of the UK–Thai Aflatoxin Project is probably best judged by the fact that the maize industry officially recognised the UTP system and recommended its adoption throughout the supply and marketing chain. However, from the findings in Phase III it is obvious that when methods of reducing the incidence of aflatoxin contamination can be considered proven it will take time and effort to ensure that they are fully implemented.

The major weapon in the fight against aflatoxins would appear to be incentives. Incentives must be available to all those people involved in the chain from farmers to exporters if they are to commit themselves to solving this problem.

If incentives are available to compensate for changes in farming practices, investment in dryers and the costs of sampling and quality control, then, together with training and extension work, there is a real chance that many of the problems associated with mycotoxins and other storage fungi will be solved.

Management of the Microflora and Their Toxins — Part 1 Session Summary

Chairman: Dr J. Gilbert, Food Science Laboratory, Ministry of Agriculture, Fisheries and Food, Norwich, U.K.
Rapporteur: Ms Sarah Phillips, Natural Resources Institute, Chatham, U.K.

PAPERS in this session concentrated on health and nutritional implications of mycotoxins, utilisation of contaminated commodities, and strategies for the control of mould growth and toxin production.

The impact of mycotoxins on health and nutrition was considered by Dr J.D. Miller. Emphasis was placed on ecology, microbial/mycotoxins/crop interactions, and effects on animal productivity. The occurrence of toxins, particularly those produced by *Fusarium*, and toxicology, was summarised. Grains or feeds may be contaminated with a mixture of toxins. Work on the combined effects of different toxin mixtures was described. Normally, contaminated grain with toxin mixtures may be more highly toxic with possible synergistic or antagonistic effects.

Although acute trichothecene toxicoses have been reported, chronic exposure to trichothecenes may affect the immune system.

The main point arising during discussion following this paper was need to increase knowledge of toxin occurrence, particularly in developing countries: future work should include screening of *Fusarium* isolates, prioritising of important toxins and their occurrence, and toxicology related to immune suppression.

Dr H.L. Trenholm discussed the safety of animal feeds, covering the toxicology of a range of animals, economic impact, and approaches to detoxification. Concern was expressed over contaminated grain, rejected or downgraded, which may be consumed by humans. The sensitivity of different animals to *Fusarium* toxins which have toxicological effects in horses, sheep, and poultry was discussed with particular reference to fumonisin. Although mycotoxins can cause mortality at low levels, toxic effects can include loss in productivity (grain yield and livestock production), weight loss, and immunosuppression. Mycotoxin prevention should always take priority, detoxification being considered only as a last resort. Detoxification methods include binding agars to absorb ingested toxin, chemicals, and removal of contaminated grain by flotation dehulling.

Discussion following Dr Trenholm's paper centred on the 'spiking' effect in poultry mortality, and the movement away from chemical detoxification.

The problem of salvaging mould- and mycotoxin-contaminated feed was then addressed by Mr B.J. Blaney, particularly by a degree of dilution dependent on a knowledge of the nutritional effects of mycotoxin contamination on animal performance and target livestock. In poultry and pigs, the relationship between reduced palatability of the feed is closely linked to growth rate, profitability margin, and cost of contaminated feed.

Mr Blaney's paper stimulated much discussion on the ethics of feeding contaminated grain and concern over mycotoxin residues in milk or meat. Since dilution level estimates are dependent on analytical methods used to determine levels of toxin present, it was suggested that diluted feed should first be administered to a few animals to observe effects before finalising a feeding strategy for contaminated grain for greater numbers of livestock. Feasibility of utilisation of contaminated feed for conversion to starch or alcohol was discussed.

Control of mould growth and mycotoxin formation was dealt with in two papers. A paper presented by Dr Ailsa Hocking examined the scientific basis for control of mould growth by the use of modified atmospheres and phosphine fumigation, procedures used primarily to control insects. Phosphine fumigation may retard

development of mycelial growth, in addition to mycotoxin formation, in grain of higher moisture content. The results of research in this area were reviewed.

The effects of carbon dioxide, oxygen, and nitrogen on fungi were discussed in relation to modified atmosphere storage. High CO_2 was more effective in controlling mould growth than a reduction in oxygen. Mycotoxin formation was also more sensitive than mould growth to low O_2 and high CO_2. The practical application of phosphine fumigation for 'holding' high moisture grains was discussed. Implications for grain storage systems should be considered.

An integrated approach to the problem of aflatoxin in maize by implementation of grain management procedures at all stages of the postharvest chain was described by Mr M. Cutler, who reported on a 4-year project in Thailand aimed to reduce aflatoxin levels in maize. The project initially involved assessment of drying procedures and survey of aflatoxin content of maize, and scientific research leading to recommendations for drying practice which could reduce levels of aflatoxin in the wet season. A further phase then sought to implement appropriate drying practices at farm and merchant level, coupled with a quality control system for monitoring aflatoxin and the introduction of a scheme of price incentives. The importance of price incentives, without which improvements would not occur, was stressed. The discussion centred on the issue of incentives and difficulties in ensuring that the benefits went to the right people. Training and extension were considered important.

The following general points were discussed during this session.

- The need for research on the ecology of fungi and toxins, including co-occurrence of toxins and the question of immunosuppression.
- The need for more information on the natural occurrence of mycotoxins in developing countries.
- Breeding for cultivar resistance, susceptibility of varieties to kernel cracking, insect attack, toxicity characteristics.
- Particularly in developing countries, further information on fumonisins is needed.
- The need for a regional working group on food and feed safety issues.

Management of the Microflora and Their Toxins — Part 2

Aflatoxin Studies in the Philippines

Raquel T. Quitco*

Abstract

Aflatoxin contamination of agricultural commodities is a problem in the Philippines. This is due to the high temperatures and humidities prevailing, which favour the growth of the toxin-producing fungi, *Aspergillus flavus* and *A. parasiticus*.

Under natural conditions, maize, peanuts, cassava, and copra were found to be susceptible to aflatoxin contamination, whereas rice, soybeans, and sorghum were poor substrates for aflatoxin production. Nevertheless, both rough and milled rice were found to support high levels of aflatoxin when artificially inoculated with toxigenic strains of *A. flavus* and *A. parasiticus*.

The moisture content and temperature necessary for maximum production of aflatoxin varied with the commodities. In addition, certain postproduction practices were observed to predispose agricultural commodities to *A. flavus* infection and consequent aflatoxin production.

Contamination of agricultural commodities was observed at harvest, and significant levels were observed in commercial storage. In cassava, however, aflatoxin contamination was observed only in processed cubes.

This paper discusses studies on the control of aflatoxin in agricultural commodities, including measures aimed at controlling the toxin-producing fungi and detoxification methods, investigations of the effects of aflatoxin in animals, and an epidemiological study involving primary liver cancer in humans and its correlation with consumption of maize contaminated with aflatoxin.

AFLATOXIN contamination of agricultural commodities is a problem in the Philippines. This is due to the prevailing high temperatures and humidities which favour the growth of the toxin-producing fungi, *Aspergillus flavus* and *parasiticus*. The problem is aggravated when harvesting coincides with the rainy season and immediate drying of grains is not possible.

Aflatoxins are a group of metabolites which are considered highly toxic and whose ingestion could lead to death in animals. It is a well studied mycotoxin, but somehow control remains elusive. This paper reviews the researches undertaken in the Philippines and presents the perceived gaps that may be relevant in controlling the toxin.

Commodities Susceptible to Aflatoxin

An initial study on aflatoxin was started in 1967 by the Food and Nutrition Research Institute (FNRI) of the Philippines, which undertook a survey of the aflatoxin content of various food items (Salamat 1978). It showed that peanuts, maize, cassava, and copra (dried coconut meat) contained high levels of aflatoxin under natural conditions (Table 1). In another survey, peanuts, maize, copra, and their by-products were again found to contain high levels of aflatoxin (Table 2) under natural conditions (Santamaria et al. 1972). In both surveys, rice and soybeans were found to be poor substrates for aflatoxin production under natural conditions. However, when artificially inoculated with toxigenic strains of *A. flavus* and *A. parasiticus*, brown rice and milled rice were found to support high levels of aflatoxin (Ilag and Juliano 1982). Soybean, on the other hand, was found to be a poor medium for aflatoxin production (Fandialan and Ilag 1973).

Factors Affecting the Production of Aflatoxin

Moisture content, relative humidity, and temperature are the important factors that affect the growth of *A. flavus* and *A. parasiticus* and the production of aflatoxin in natural substrates. Laboratory studies showed that the optimal temperature for aflatoxin formation by *A. flavus* isolates inoculated in their natural substrates was 30°C for rice and maize, 25°C for peanut and copra isolates, and 20°C for soybean isolates (Fandialan and Ilag 1973). Similarly, copra inoculated with *A. flavus* at an initial moisture content of 8% had maximum aflatoxin production at 30°C while no aflatoxin was obtained in

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Table 1. Aflatoxin levels of various Philippine foods

Food	Total no. of samples	% of negative samples	% of samples > 20 ppb	Range (ppb)	Mean positive levels (ppb)
Raw, fresh maize	9	100	0	neg.	0
Boiled maize	24	33	0	neg–9	9
Dried maize	660	5	65	neg-1152	76
Canned maize	4	25	0	neg-25	6
Maize starch	9	33	11	neg–25	12
Maize bran	2	0	100	37-71	54
Raw, fresh peanut	169	35	23	neg–885	58
Boiled peanuts	8	0	38	trace-103	24
Roasted peanuts	212	29	27	neg-818	68
Peanut butter	522	1	87	neg–6600	186
Milled rice	82	79	4	neg-43	12
Rough rice	10	70	0	neg–18	15
Rice bran	15	20	33	neg–38	16
Pop rice	6	83	0	neg–3	3
Sorghum	2	0	2	29	29
Cowpea (paayap)	16	37	19	neg-86	16
Kidney beans	20	10	45	neg-222	63
Lima beans (patani)	7	43	43	neg–118	58
Mungbean (munggo)	33	12	18	neg-46	13
Peas (guisantes)	8	63	12	neg–40	13
Pigeon pea (kadyos)	9	44	12	neg–23	7
Soybean (utaw)	25	52	16	neg–48	18
Grated fresh coconut	4	100	0		0
Coconut milk	1	100	0		0
Processed coconut	29	69	14	neg–26	11
Copra	182	29	31	neg–513	39
Crude coconut oil	3	0	0	trace-9	3
Cooking oil	25	40	0	neg–7	3
Refined oil	2	100	0		0
Cassava, raw	23	0	57	too high	too high
Ube	8	50	0	neg-14	4
Sweet potato	37	22	24	neg-780	39
Gabi	38	82	18	neg–86	13
Singkamas	16	88	6	neg-30	16
Cocoa	8	25	25	neg–28	18

copra samples stored at 20°C. Furthermore, at 30°C, copra had up to 247 ppb (µg/kg) aflatoxin in 4 days and 15 ppb in 5 days when stored at initial moisture contents (m.c.) of 12.3% and 7.8%, respectively (Tuason and Madamba 1980).

Under natural conditions, postproduction practices predispose crops to *A. flavus* invasion and aflatoxin production. It was shown that dried maize samples had higher infection by *A. flavus* and *A. parasiticus* when stored in shelled form than unshelled form. Furthermore, maize stored with husk had very minimal *A. flavus* infection. The degree of infection was directly related to the amount of *A. flavus* inoculum in the air, which was least in maize fields, higher in drying areas, and highest in storage (Ilag 1973). The formation of aflatoxin in maize while the crop is still standing in the field has been studied to some extent. It has been shown that the soft dough stage of maize was most susceptible to *A. flavus* infection followed by the milk stage, the hard dough stage, and the ripe stage, in decreasing order (Mojica 1986). *A. flavus* and *A. parasiticus* were found to infect maize in the field particularly when the ears were injured prior to inoculation (Ilag 1973).

While initial surveys reported the absence of aflatoxin at harvest in susceptible agricultural commodities, subsequent studies showed otherwise. In southern Mindanao where maize is largely grown, aflatoxin was observed in freshly harvested maize at 10 µg/kg. The climate in this area is characterised by an even distribution of rainfall throughout the year. Figure 1 illustrates an aflatoxin build-up pattern found to be typical in this area. After harvest, the aflatoxin level increased rapidly until drying commenced. This was the period when maize was held for some days at a high moisture content because of delays in drying (Paz et al. 1989). Temporary storage of wet maize takes place when drying is not possible. Guerrero and

Table 2. *Aspergillus flavus* infection and aflatoxin content of various agricultural commodities in the Philippines

Sample	Units yielding *A. flavus* (%)	Aflatoxin B_1 (ppb)
Whole maize, shelled, in drying yard	55	107
Whole maize on cobs, drying on cement, rural areas	70	1074
Whole maize on cob standing in field	70	929
Whole maize for animal feed, Cebu market	90	817
Whole maize for human consumption, shelled, ready for milling	30	503
Maize grits, taken from mill at bagging spout	25	86
Maize germ, for oil milling	47	233
Whole maize on cobs, wet, in sacks	35	948
Maize germ and bran from milling	100	367
Freshly dug peanuts	5	14
Farmer stocks of peanut for commerce, dry, in bulk storage	1	257
Shelled peanuts from farmers stock	46	964
Whole peanuts in shell	25	0
Large segregated peanuts separated from smaller discards, used for peanut products	90	114
Crude copra, mouldy, in pile	100	314
Sorghum, in pile	25	208
Sorghum, in storage, dry	0	0
Rice, unmilled, mouldy, from storage	0	0
Rice, 2 yrs old, in storage	2	5
Soybean, drying on mat	16	21
Gabi, mouldy, from public market	15	0
Coffee beans, in sacks	41	150

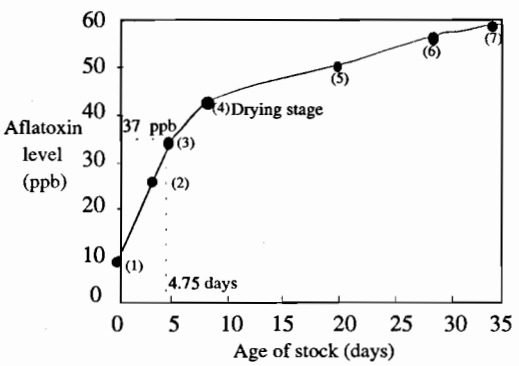

Fig. 1. Typical aflatoxin build-up pattern at various stages during the post harvest operation. (1) harvesting, (2) shelling, (3) drying, (4) trader's level, (5) wholesaler's level, (6) miller's level (pre-milling), and (7) miller's level (post-milling).

Bautista (1986) showed that storage of wet maize increased aflatoxin content to 20 µg/kg in 5 days for shelled maize and 8 days for unshelled maize. The aflatoxin level reached 50 ppb in 10 days during storage of wet shelled maize. Studies showed that aflatoxin continued to increase even after drying, because of the farmers' practice of drying maize to 15–17% m.c. then mixing it with maize of 12–14% m.c. This results in a non-uniformly dried grain (Paz et al. 1989). Furthermore, most stocks of the maize entering trade channels were found positive for aflatoxin (Tiongson and Gacilos 1990) and their aflatoxin contents were higher than those observed on farm (Paz et al. 1989).

Similarly, studies on peanuts showed that they contain aflatoxin at harvest. In addition, the practice of windrowing peanuts for 3–6 days on the field before threshing was found to increase aflatoxin content. This was attributed to the slow drying of nuts while on the field (Quitco et al. 1989). Recently, however, it was shown that aflatoxin did not increase in peanuts during windrowing, provided there is rapid decrease in moisture content during this period (Quitco and Prudente 1991). Because farmers do not thoroughly dry peanuts before storage, aflatoxin was observed to increase in peanuts during storage on the farm. In addition, significant levels were observed at the trader level (Fig. 2) (Quitco et. al. 1989).

In cassava, aflatoxin was not detected in 7–14 month old roots. However, during storage, trace amounts of

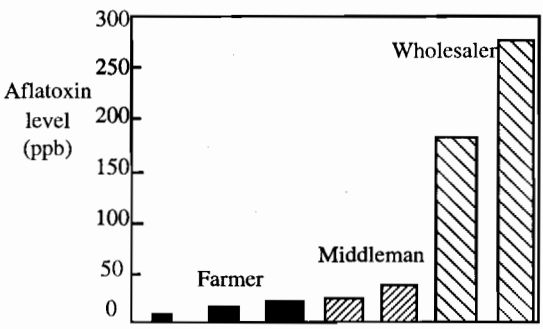

Fig. 2. Aflatoxin contents in peanuts at farmer and trader levels, Isabela and Quirino Province, Philippines 1985.

aflatoxin B_1 were observed in stored cubes but not in stored chips and unprocessed roots. The difference was attributed to the fact that cassava cubes required longer drying times than cassava chips and unprocessed roots (Sajise and Ilag 1987). Furthermore, the same authors observed that, in the absence of competing fungi, fresh cassava roots may be a good substrate for the growth of *A. flavus* and *A. parasiticus* but not for aflatoxin production.

Control of Aflatoxin

Studies on the control of aflatoxin have taken two approaches, namely : 1. the control of the toxin-producing fungi; and 2. detoxification of aflatoxin. The greatest amount of research has targeted the prevention of *A. flavus* growth.

Since it was observed that aflatoxin production commenced at farm level and that significant build-up could occur during on-farm operations, the reduction of moisture to a level unfavourable for the growth of *A. flavus* is considered a practical approach for minimising aflatoxin at the farmers level.

A study showed that maize should be dried uniformly to 14% m.c. within 2 days from harvest to prevent significant on farm build-up of aflatoxin (Paz et al. 1989). On the other hand, peanuts had to be dried uniformly to 8% m.c. in 5 days during the dry season and within 3 days during the rainy season to prevent significant build-up of aflatoxin at the farm level (Quitco and Prudente 1991). A drying scheme suited to the needs of farmers is being developed in the control of aflatoxin at farm level. The segregation of mouldy, damaged, and immature maize kernels, either by handpicking or using a mechanical grader, was found to reduce the level of aflatoxin. However, a very low correlation was observed between aflatoxin content and the percentage mouldy and damaged maize (Paz et al. 1989).

The application of fungicides to grains on a small scale to prevent *A. flavus* growth and aflatoxin production has been investigated. Maize treated with 0.1% thiabendazole at the time of inoculation with *A. flavus*, and grain treated 1–2 days before inoculation, were free of infection and aflatoxin contamination 2 weeks after incubation (Table 3). Appreciable levels of aflatoxin were produced when the fungicide was applied 1 or 2 days after inoculation (Ilag et al. 1974). Garcia (1989) showed that *A. flavus* isolates from rice and maize grains exhibited no apparent growth in Czapek's and Czapek yeast autolysate agar infused with 10 ppm benomyl (Table 4). The growth of *A. flavus* was progressively inhibited by increasing concentrations of benomyl. Captan also inhibited *A. flavus* growth, but required a higher concentration (Table 5). Benomyl applied at 5 g/kg of maize grains of 12.7 and 16.5% m.c. resulted in lower infection by storage fungi than the controls, even following 9 weeks of storage. Aflatoxin was not detected at this rate of benomyl application after 9 weeks of storage (Garcia 1987).

Heat treatment and the application of chemicals were found to reduce aflatoxin in food commodities. Boiling of mungbean seeds previously inoculated with toxin-

Table 4. Effects of different concentrations of benomyl on the growth of *A. flavus* isolates on agar media after 7 days at 25°c.

Aspergillus isolates	Benomyl concentration (ppm)	Average colony* diameter (mm)
A. flavus rice isolates	0	56.5
	1	37.7
	2	33.6
	5	21.3
	10	**
A. flavus maize isolates	0	52.8
	1	37.1
	2	34.5
	5	21.4
	10	**

* Average of 4 trials with 3 replicates per trial
** No apparent growth was observed

Table 3. *Aspergillus flavus* infection and aflatoxin content after two weeks' incubation of maize grains that were variously treated with 0.01 and 0.1 per cent thiabendazole.

Treatment	Per cent infection*		Aflatoxin content* ppb B_1	
	0.01% thiabendazole	0.1%	0.01% thiabendazole	0.1%
Fungicide applied one day after inoculation	80	0	2812	22
Fungicide applied two days after inoculation	70	10	5625	100
Fungicide applied at the time of inoculation	100	0	3625	0
Fungicide applied one day before inoculation	10	0	157	0
Fungicide applied two days before inoculation	20	0	739	0
No fungicide applied on inoculated grains	100	100	7500	7429
No fungicide applied; grains were not inoculated	0	0	0	0

*Average of two trials with 2 replicates per trial

Table 5. Effects of different concentrations of captan on the growth of *A. flavus* isolates on agar media after 7 days at 25°C

Aspergillus isolates	Captan concentration (ppm)	Average colony* diameter (mm)
A. flavus rice isolates	0	56.88
	50	42.08
	100	6.33
	150	**
	200	**
A. flavus maize isolates	0	52.43
	50	31.62
	100	6.96
	150	**
	200	**

* Average of 4 trials with 3 replicates per trial
** No apparent growth was observed

producing *A. flavus* at 100°C for 30 minutes reduced the aflatoxin content substantially. Indeed, when the boiling period was extended for 3 hours, total elimination of the toxin was observed (Table 6). A combination of boiling and autoclaving was found to be more effective in reducing the aflatoxin than boiling alone (Perez and Ilag 1977). Celino and Madamba (1985) showed that steam cooking of maize grits (naturally contaminated with aflatoxin B_1) caused a 9% reduction in aflatoxin content. They observed, however, that more aflatoxin was lost during washing than cooking (Table 7). They showed also that dry roasting or roasting with oil of artificially contaminated maize grains led to 94% and 86% reductions in aflatoxin B_1 content, respectively (Table 8).

Table 6. Effect of boiling only and autoclaving plus boiling on the aflatoxin B_1 content of inoculated mungbean

Length of heat treatment (hr)	Aflatoxin (B_1) content (ppb) Boiling only*	Autoclaving plus boiling**
0.5	36	14
1.0	21	11
2.0	13	0
3.0	0	0
Unheated (control)	129	

* Boiled at 100°C
** The samples were first autoclaved at 121 kPa (17.5 lbs/sq. in.) for 15 minutes and then boiled

Washing of maize grains with lime in preparation for 'binatog' (a maize food preparation) resulted in a 68% reduction in aflatoxin B_1 and G_1 (Table 9). Cooking the lime washed maize grains resulted in further reductions of 26% in aflatoxin B_1 and 22% in aflatoxin G_1 (Celino and Madamba 1985).

Chemical detoxification methods using ammonium hydroxide, calcium hydroxide, sodium hypochlorite, sodium hydroxide, hydrogen peroxide, and bisulfite were tested on copra artificially inoculated with an aflatoxin-producing isolate of *A. flavus*. Ammonium hydroxide, calcium hydroxide, and sodium hypochlorite were found to be more effective in reducing aflatoxin in copra at an initial moisture content of 24 %. Ammonium hydroxide was found to be most effective in reducing aflatoxin when the moisture content was reduced to 7%. Effective reduction at 9% m.c. was shown after 5 days of storage with 1.5% NH_3, 10 days storage with 1.0 and 1.5% NH_3, and 15 days storage with 0.5, 1.0, and 1.5% NH_3 (Mercado 1988).

A search for peanut varieties resistant to aflatoxin-producing fungi is currently under way at the Institute of Plant Breeding (IPB). Initial results have identified peanut varieties resistant to *A. flavus*. Further study is aiming to ascertain their levels of resistance. Other studies are investigating fungal infection and aflatoxin contamination of peanuts under preharvest and postharvest drying conditions (IPB 1990).

Studies On Mycotoxicoses

Case studies on the effects of aflatoxin-contaminated feed on certain animals have shown the potential risk to livestock.

Feeding day-old ducklings with feeds treated with aflatoxin B_1 resulted in depressed growth, pathological lesions in the liver, and even death after 7 days of feeding on higher doses (0.5 mg-1.0 mg/kg body weight) of aflatoxin B_1 (Ilag 1976). Similarly, incorporating increasing doses of aflatoxin (0.625, 1.25, 2.5 and 5.0 µg/g diet) into the feed resulted in decreased growth rate. The extent of depression of growth rate was dependent on the concentration of aflatoxin. Aflatoxin doses of 2.5 and 5.0 µg/g diet reduced protein and feed utilisation significantly (Gonzales and Salamat 1980).

A strong positive association has been established between consumption of aflatoxin-contaminated maize in the Philippines and the development of primary liver cancer. The incidence of primary liver cancer in the country was found to be higher in maize-consuming regions. This effect was found to be synergistically aggravated by alcohol consumption (Jayme at al. 1982). In contrast, however, a much lower incidence of liver cancer has been observed in Bicol province where there is a high intake of maize. This has been linked to an unusually high intake of coconut in the province and suggestions that a significantly high intake of coconut in the diet offers some protection from liver cancer (Jayme et al. 1976).

Table 7. Effects of washing and cooking on the aflatoxin B_1 content of naturally contaminated maize grits

Cooking method	Stage	Toxin content (μg/50g)	Toxin reduction (%)
Rice cooker	Initial sample	9.4 ± 0.2	–
	Washed and cooked	5.7 ± 0.4	39 ± 2
	Washings	3.2 ± 0.4	–
	Unwashed and cooked	8.5 ± 0.5	
Cooking pan	Initial sample	9.3 ± 0.2	–
	Washed and cooked	5.8 ± 1.4	37 ± 4
	Washings	2.9 ± 1.0	–
	Unwashed and cooked	8.4 ± 0.2	9 ± 1

Table 8. Effects of roasting on the aflatoxin content of artificially contaminated maize kernels

	Toxin content (μg/50g)	Toxin reduction %
Initial toxin content	38.0 ± 2.0	–
Dry roasted	2.0 ± 0.8	94 ± 8
Oily roasted	5.0 ± 1.0	86 ± 4
Oil	0.6 ± 0.4	–

Table 9. Effects of lime washing on the aflatoxin content of artificially contaminated maize kernels for 'binatog' preparation

	Toxin content (μg/50g)	Toxin reduction %
Initial toxin content	38.0 ± 2.0	–
Lime washed/ uncooked	12.0 ± 3.0	68 ± 6
Lime washings	21.0 ± 1.0	
Lime washed/ cooked	2.0 ± 0.7	94 ± 8

Summary and Recommendation

Aflatoxin contamination of agricultural commodities is a serious problem in the Philippines. This is due to the prevailing environmental condition and postharvest practices. Various control methods investigated have shown potential in minimising aflatoxin levels.

Despite the large amount of information generated, there is still a lack of awareness among farmers, processors, and consumers regarding aflatoxin and its potential risk to human and animal health. While an information campaign is needed, caution must be exercised in alerting consumers and producers of the gravity of the problem. Experience has shown that such campaigns may cause panic among consumers and anger among the traders and processors who bear the brunt of the aflatoxin problem. Nevertheless, it is imperative that farmers, traders, and processors be informed and trained on the proper handling of commodities.

Success in preventing aflatoxin contamination of agricultural commodities, however, must involve more than farmers and traders. Government participation is imperative if an environment conducive to the sustained implementation of an aflatoxin control program is to be created. This requires the formulation of policies that will regulate aflatoxin in agricultural commodities. In support of this, grades and standards must be established for each commodity and a pricing system must be developed so that incentives can be provided to the farmers and processors alike to encourage them to produce good quality grains.

Regulation of aflatoxin-contaminated food demands constant monitoring. For this purpose, an aflatoxin screening method suitable for monitoring must be developed. In addition, there is a need to develop sampling protocols for susceptible agricultural commodities. Furthermore, people who will be involved in monitoring need to be trained.

There is no doubt that research directed to the control of the aflatoxin-producing fungi will be pursued and researchers will discover effective and economical control measures. An integrated approach towards this end should be a priority.

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Aflatoxin in Maize in Thailand

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Abstract

Survey studies indicated that aflatoxin contamination in Thai maize was low at harvest and increased during storage. Maize ears were less contaminated by *Aspergillus flavus* and aflatoxin than the kernels. Most contamination started in kernels that were damaged by shelling and were not dried properly. There was no regional or seasonal difference in the *A. flavus* population in soil samples from maize cultivation areas. Several alternative approaches are currently being investigated to control aflatoxin, including effective drying, application of chemicals, ensiling of high moisture maize, breeding for resistant varieties, and cropping patterns. Field and mechanical drying are the most effective practical control measures currently available.

MAIZE is one of the most important crops in Thailand. It is second to rice in cereal grain production. Total production in 1988 was 4.6 Mt grown on 1.84 Mha, giving yields equivalent to 2.62 t/ha. Up to 1985, about 70% of the crop produced in Thailand was exported. This figure accounts for only 2.5% of world trade. The remaining 30% was domestically consumed, mainly as feed for livestock, and a small percentage as human food with daily consumption of 0.8 g/person. Recent trends show a dramatic increase of domestic consumption up to 75% of total production. This is due to the growth of poultry industries. Aflatoxin is a major problem in maize production worldwide, Thailand not excepted. Research on aflatoxin in Thailand began in 1978.

This paper aims to provide an understanding of when and how aflatoxin production occurs in Thai maize. In addition, the results of some research studies that have been conducted to deal with the problem will be presented.

Maize Production and Marketing

On the basis of the 1983 crop, about 50% of total production of maize in Thailand was in the lower part of the northern region. The northeast region accounted for about 26%, and the central region 24%. According to Boonma et al. (1980), maize farms are commonly between 4–12 ha, with an average size of 7.3 ha.

Maize varieties that are grown in Thailand are the open pollinated group, which comprise the composite and the synthetic, and the hybrid group. Maize growing relies solely on natural rainfall. It can be produced in two seasons. The first is from April–May to July–August–September, that is the beginning and middle part of the rainy season. The second crop is grown from July–August–September to October–November–December, and harvested at the end of the rainy season. Harteveld (1984) found that almost 90% of production occurs in the first season.

Maize marketing in Thailand can be categorised into three main levels: local markets in which farmers sell directly to merchants (also known as farm gate markets); assembly or central markets at district and provincial levels; and terminal markets for feed mills and export.

By providing credit in the form of seed, fertiliser, and tractor services for cultivation and shelling, merchants exert a great influence on the production and marketing of maize in Thailand.

Postharvest Practices

In order to pinpoint when, where, and how aflatoxin contamination of maize occurs, some understanding of postharvest practices is necessary.

Onfarm postharvest practices — harvesting and shelling

The main harvest time is during the rainy season, usually about 110 days after planting. However, the timing of harvesting sometimes depends on other factors, such as re-use of the land to plant other crops, avoidance of flood, or a need for cash. In such instances, the harvest may occur 80–90 days after planting. On the other hand, if farmers want to hold the crop for a better price, they will leave it standing in the fields for 120–130 days after planting in order to increase the storage quality.

Maize harvesting involves hand picking, dehusking,

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and packing first into bamboo baskets and then into jute bags which are transported by human, animal, and engine-powered vehicles. Virtually all farmers will complete their harvest within 30 days but, on average, the length of harvest time for most farmers is 10–14 days. Harvesting is often expedited by use of hired or communal workers.

At the farm, the maize ears are bulked up in preparation for shelling and sale. If immediate sale is not possible due to inaccessibility, or if the farmers anticipate a price rise, then the maize will be placed in storage. The harvested maize may sometimes accumulate on farms up to 3–5 days waiting for machine shelling and sale. This delay could be critical from an aflatoxin control viewpoint, if the maize is moist and stored in unsuitable conditions, a problem that many farmers are aware of.

According to Boonma et al. (1980), 79% of farmers sell their maize at the earliest opportunity, 52% immediately after harvest, and 27% as soon as the crop is transportable. This is perhaps due to the need for money, or lack of suitable storage facilities. Thus, only 21% of the farmers store their maize in anticipation of a price rise.

Storage

Rudimentary cleaning and grading of maize is made at farm level but little attempt is made to dry it. About 20% of farmers are reported to have undertaken some form of sun drying before storage (MOAC 1983; Wirrawat 1984). Field drying is a widely accepted practice but is hard to implement in areas where double cropping is practiced.

When drying, ears are often spread on a drying bed made of bamboo located about 50 cm above the ground. Tarpaulin, plastic, or galvanised sheets are used to cover the maize ears at night or during rain. Regional variation in storage practices is evident (MOAC 1983). Storage facilities sometimes involve simply piling the maize ears under the house, or in stores built for the purpose. Typically, these are constructed of a timber frame, have ventilated bamboo walls, an earth, wooden, grass, or concrete raised floor, and a corrugated pitched roof. Mostly, these stores do not provide conditions suitable for storing maize for long periods. The average storage period for maize held in anticipation of a price rise is, according to Boonma et al. (1980), 2–4 months. Wirrawat (1984) found that 63% of farmers who stored their maize did so for over 60 days.

Traders in assembly and central markets

After shelling, maize quickly enters the marketing system. The most critical factors are the length of time that shelled maize is kept in store or in transit, and its moisture content and the storage temperature, all of which may be conducive to aflatoxin contamination. The moisture content in maize will depend largely on the time of year, averaging 30% in July, 25% in August and September, about 21% in October, and about 18% at other times (Wirrawat 1984).

During the peak harvesting and selling period, maize moves quickly through the marketing system, reaching the feed millers' or exporters' silos within a matter of days. However, initial high moisture content and the inadequacy of existing drying methods mean that the grain is very susceptible to contamination as it passes through the marketing channel. Virtually all maize moves from the farms to the assemblers' depots in 100 kg bags. Small traders will accumulate bagged grain until quantities justify transhipment further up the marketing channel. They try not to hold moist grains in the early season for more than a few days.

Sun drying on concrete floors is widely practiced by merchants. Maize is normally delivered to the merchants' godowns, usually by trucks or farm tractors. On delivery, moisture content is assessed, either by touch or meter, increasingly the latter, and the need for further drying determined. If moisture content exceeds about 18–19%, and there is space on the drying floor, the grain is then debagged and spread out to dry, using either tractors (with front spreading blades and rear rakes), or manual labour. The grain is raked at intervals. A drying floor on a good sunny day can achieve a moisture reduction of 7–10%. Reducing the moisture content of early season crop to the level needed for safe storage could therefore take 2 days, even with favourable ambient conditions. Maize that can be sun dried in a day or two under sunny condition is often of high quality. However, the sun drying floor must be clean and the use of a tractor must be limited in order to avoid cracking the kernels.

Most traders are aware of the risk of deterioration if maize is bulked up at high moisture content for any length of time and they normally try not to hold undried maize for more than 2–3 days. For many traders, however, sun drying capacity during the peak trading period is insufficient to cope with the amount of incoming grain, and bulking up problems are therefore inevitable. During one rainy period, maize was observed to remain on the drying floor in heaps at over 23% moisture for over 3 days, while more incoming wet maize had to be redirected to silos. Some merchants appreciate the improvement in maize quality that can be obtained through mechanical rather than sun drying methods.

Transportation in the merchants' sector is mainly, but not exclusively in bags, on trucks carrying at least 13 t. Grain can be delivered from most of the up-country merchants to silos within at least 10 hours by road, and is not considered to present a bottleneck in the marketing channel. During long transit periods of wet grain, the maize may heat up markedly, creating conditions conducive to aflatoxin formation.

The price premiums for crop condition in the terminal markets usually influence traders' practices, particularly with respect to moisture content, and to a lesser extent, quality grading. Price differentials in the terminal markets are mostly based on moisture content. This is intended as an incentive for traders to undertake crop drying, as well as to grade maize deliveries. Maize delivered to the terminal buyers at 14.5% moisture content, which is considered the minimum acceptable moisture content for safe storage and shipment, commands the prevailing wholesale market price.

One important feature of the present schedule is that no price incentives are given for drying to less than 18%. This is mainly because silo operators themselves assume that the maize will need to pass through their own large dryers at least once. However, maize in temporary stores and in transit at 18% moisture content is still at risk of aflatoxin contamination. Pricing at farm level appears less sensitive to moisture content than at silo level.

Terminal markets: exports and feed millers' silos

Maize is lorry-delivered by traders to silos, in bulk or bagged form. In some cases, large silo operators may appoint commission agents to act as intermediaries between themselves and the traders. On delivery, most silo operators try to separate grains according to moisture content, physical attributes, and the need for subsequent treatment. With the increase in the maize trade and the greater emphasis on improved marketing, there has been a rapid growth in silo storage and drying capacity. Simultaneously, there has been an increase in drying capacity such that most silos operate high output continuous-flow dryers. Silo operations range from 111 000 t storage with two dryers of 225 t/hour output to about 2000 t storage with one 35 t/h dryer.

Charges for grain handling, drying, storage, bagging, loading, and stowing levied by silo operators for export maize are all set by the Maize and Produce Association. They vary according to time of year and whether the maize is in bulk or bags.

Incidence of Aflatoxin in Maize

A comprehensive survey of foodstuffs in Thailand (Shank et al. 1972) identified peanuts and maize as commodities particularly susceptible to aflatoxin contamination. The incidence of aflatoxin contamination of maize at various stages in the processing and marketing chains has been investigated (Anon. 1985a; Asanuma and Vayuparp 1985; Goto et al. 1986; Siriacha et al. 1983). Samples were taken from fields, farmers' and merchants' storages, and silos. These surveys indicated that the problem is a postharvest one. Aflatoxin contamination of Thai maize is generally believed to begin in the farmer's storage house and to become serious during storage by a middleman, on a drying floor, or in a storage house.

The population of *Aspergillus flavus* and the amount of aflatoxin in maize ears and kernels collected from four areas in Thailand in 1987 were studied (Siriacha et al. 1988b). The results showed more external and internal contamination of *A. flavus* in the kernels than in the maize ears. Moreover, it has been shown that the amount of aflatoxin in maize ears was much lower than in kernels (Siriacha et al. 1988a; Pratchaya 1986).

Postharvest Contamination

Postharvest contamination of Thai maize with *A. flavus* and aflatoxin was traced from farmers' fields through middlemen's storages (Kawashima et al. 1990; Siriacha et al. 1989). These studies sought to pinpoint when and where *A. flavus* infected maize and formed aflatoxin. The results indicated that the infection by *A. flavus* started in kernels that were damaged by shelling and insufficiently dried.

The undried and mechanically shelled kernels were infected quickly by *A. flavus* and aflatoxin if the initial moisture content was over 20%. The infection did not occur if moisture content was less than 17%. For the undried and hand-shelled kernels, infection proceeded quickly if moisture content was over 23%. If the moisture content was less than 20%, the infection appeared to be halted.

It was found that kernels that were sun dried immediately after mechanical shelling on a drying floor (moisture content less than or around 15%) could be stored without significant *A. flavus* infection for more than 8 weeks after sun drying, and aflatoxin content did not increase. Maize ears might remain free from heavy *A. flavus* infection even after long-term storage, provided that they were stored with the proper initial moisture content and in a well ventilated storage.

The Population of *A. flavus* in Soil

During the dry and wet seasons from 1986 to 1988, soil samples were collected from several maize fields for analysis of seasonal and geographical variations of *A. flavus* populations in the soil (Kawasugi et al. 1988a; Saito et al. 1986a; Tsuruta et al. 1986; Siriacha et al. 1988b). The results showed that *A. flavus* could be isolated from almost all (91%) soil samples. Although the *A. flavus* population in soil samples differed significantly from field to field, consistently higher levels were noted in some locations. There was, however, no consistent evidence of geographical variability. There was also no difference in the fungal population of soil samples obtained during dry and wet seasons.

Continuing this investigation, Saito et al. (1986b) studied the aflatoxin-producing ability of *A. flavus*, and isolated various strains of the fungus from Thai maize fields. Three atypical strains (B-1, B-2, and C strains) of

A. flavus were identified. The B-1 strain, which formed abundant microsclerotia and showed high aflatoxin B_1 producing ability, was detected from about half of the soil samples examined. The occurrence of *A. flavus* B-1 strain in the southern region was less than that in other regions of Thailand. While the B-2 and C strains produced B as well as G groups of aflatoxins, their distribution in soil seemed to be limited.

Control of Aflatoxin in Maize

Since 1981 many government and private organisations have investigated aflatoxin problems in Thailand. In March 1985, the National Committee to Solve Mycotoxin Problems in Agriculture Commodities was established in Thailand. This committee comprises three sub-groups, the Research and Development group, the Extension and Information group, and the Marketing group. These groups work in conjunction with both private and government sectors. Measures to control aflatoxin contamination involve field drying, mechanical drying, ensiling, breeding for resistant varieties, and manipulation of cropping pattern.

Field drying

Field drying is a process whereby maize is left standing unharvested in the field for a period of time after plant maturity, so as to allow the maize ears to dry as they attain equilibrium with the relative humidity of the air. Studies in Thailand (Kawasugi et al. 1988b; Kawashima et al. 1990; Nagler et al. 1989) strongly suggest that field drying from 2–4 weeks may have a significant role in controlling aflatoxin in maize, reducing the moisture content to 18–22%. This renders subsequent mechanical drying to achieve 14% moisture content more economical. No trend towards an increase in levels of aflatoxin contamination was found over a 6-week period of field drying. Physical quality of the grain was maintained during field drying and the percentage of kernels damaged during mechanical shelling was reduced relative to that of maize which was harvested at field maturity.

Disadvantages of field-drying are: 1. Planting of a second crop may be delayed and may not be possible in some instances; 2. There may be an increased risk of losses due to storms or other natural hazards in some areas; and 3. The farmers may have to bear a financial loss unless they are adequately compensated for weight loss due to the reduction of moisture content.

Mechanical drying

It has been established that mechanical drying to 14% moisture content could efficiently prevent further aflatoxin contamination (Anon. 1985a). Dry maize (14% moisture content) can be stored for at least 2 months without an increase in aflatoxin level.

The UK-Thai Aflatoxin Maize Project, 2nd Phase (Anon. 1985b, 1986; M. Cutler, these proceedings) has identified a set of criteria called the UK-Thai Project (UTP) System, which has been shown to reliably produce low aflatoxin content maize. With this system, maize is first field-dried on the stalk for 1–2 weeks before harvesting, in order to reduce the moisture content to 18–22%. It is next shelled within 24–48 hours of harvest, and loaded into a dryer within 12 hours of shelling. Thus, within 48 hours, it is dried to 14% moisture content with no part exceeding 15%. Maize dried to 14% moisture content by this system can be stored safely for at least 2 months with no increase in aflatoxin content. Using this system, 25 batches of maize, each of 3 t, were successfully processed with a mean total aflatoxin content ranging from 2.5 ppb to 16 ppb at drying sites.

An extension of these experiments on control of aflatoxin by mechanical drying attempted to apply the results obtained to the production and marketing systems in the maize industry in Thailand (Anon. 1988; M. Cutler, these proceedings). It was reported that farmers and merchants have both found it difficult in practice to harvest, shell, and deliver their maize to a dryer within 48 hours. About one-third of all maize in Thailand is cultivated in mountainous areas which increases transportation times especially during the rainy season. Thus, as far as is technically and economically feasible, mechanical dryers should be located near the point of harvest. A cost-benefit analysis indicated that mechanical drying is the method with the highest potential to control aflatoxin contamination (Arunotong 1987).

Chemical treatment

Various chemical agents have been studied as inhibitors of *A. flavus* and aflatoxin formation in Thai maize, including ethylene oxide, sulphur dioxide, theobromine, ethyl alcohol, methyl alcohol, acetic acid, propionic acid, sodium diacetate, sodium bisulphite, ammonia, and ammonium polypropionate (Chualprasit et al. 1985; Ilangantileke 1987; Ilangantileke and Noomkorn 1988; Kawashima 1990; Pupipat et al. 1986; Tanboon-Ek 1987; Tanboon-Ek 1989).

While chemical treatments were reported to be effective in inhibiting *A. flavus*, it should be noted that, except for one chemical treatment — a mixture of propionic acid and ammonium bis propionate, which was tested on a commercial scale (Tanboon-Ek et al. 1987) — the experiments were conducted mostly at laboratory level. Application of the mixture mentioned, at a rate of 6–7 L/t maize, could prevent the incidence of aflatoxin for more than 3 months. Discoloration of maize kernels, odour, and corrosive characteristics to users were reported, however.

The costs and benefits of alleviating aflatoxin problems by chemicals in Thai maize have been studied (Arunotong 1987). The results generally indicated that the possibility and feasibility of these chemicals being used in the

marketing system are still unclear. Some chemical treatments appeared to be effective only under some constraints, e.g. use of ammonia gas in high moisture content maize, and ammonium propionate in maize with an initially low aflatoxin content.

Ensiling of high moisture content maize

In Thailand, high moisture maize (moisture content over 20%) is widely distributed in the marketing system. It takes from several days to more than a week before maize can be dried. During this long distribution period, high moisture maize is readily infected by *A. flavus* and many other fungi, and aflatoxins can accumulate. If *A. flavus* and other fungi could be controlled for a short period until maize can be dried, say a week or two after shelling, the quality of the grain would markedly improve.

A preliminary laboratory study showed that holding maize in airtight plastic bags could control the growth of *A. flavus* for more than 2 weeks. In a subsequent study (Siriacha et al. 1990), maize at 29% moisture content was artificially inoculated with *A. flavus* and stored in packages made from a range of materials including jute bags, plastic bags, fabricated plastic bags, and double bags consisting of a high density polyethylene lining inside another fabricated polypropylene bag. These packages variably influenced the growth of *A. flavus* and degree of aflatoxin contamination. In traditional jute bags and fabricated polypropylene bags, maize that was initially free from aflatoxin was completely infected by *A. flavus* within four days after storage, with aflatoxin contents of 56 and 104 ppb respectively. However, the maize packed in polyethylene film bags and double polyethylene lined bags showed no growth of *A. flavus* or of aflatoxin contamination after 20 days of storage.

When high moisture content maize was packed in airtight plastic bags or containers, the atmosphere inside the bag was modified. Respiration of the kernels and associated microorganisms rapidly depleted the oxygen in a day or two. This can inhibit the growth of many aerobic microorganisms, including *A. flavus* (Hyde 1965; Peterson et al. 1956). Other anaerobic or facultative aerobic microorganisms can survive and produce some metabolites, leading to natural fermentation or ensiling. Wilson and Edward (1975) reported that in modified atmospheres with low oxygen, concentrations of aflatoxin were low.

Subsequent studies confirmed the effectiveness in controlling aflatoxin by packing freshly harvested maize in airtight plastic bags. Maize packed in traditional jute bags showed very high concentrations of aflatoxin and thus lost commercial value after 3–5 days. In comparison, maize packed in plastic bags was found to contain no *A. flavus* and aflatoxin contamination even after up to 2 months storage. However, a sweet–sour odour resulting from natural fermentation was characteristic of maize kept in plastic bags for more than 3 days. This maize was chemically analysed to compare it with high quality maize used as animal feed. No significant distinction was found between the two. Since this simple storage method controls aflatoxin contamination, its application on a larger scale for holding high moisture maize until it can be dried should be investigated.

Breeding for resistant varieties

In Thailand, both the Department of Agriculture and Kasetsart University are undertaking research on breeding varieties of maize resistant to aflatoxin contamination. Varieties such as the synthetics, the inbreds, the hybrid, and the early-maturing are being used in these studies.

The objectives of these studies are:

- to screen germplasm with aflatoxin resistance, crossed to well-adapted local varieties and selected for low fungus invasion and aflatoxin formation in progeny;
- to develop and evaluate varieties for lodging resistance, so as to keep the maize ears on stalk until harvest;
- to develop varieties for tight husk cover and enclosure beyond tip of the ear;
- to develop varieties for drooping ear, in which the ear shank bends and points towards the ground after physiological maturity, and selected for lower seed moisture content; and
- to evaluate maize varieties resistant to preharvest aflatoxin contamination.

However, little is yet known about possible genetic resistance to *A. flavus* and the formation of aflatoxin. 'Resistant' varieties have not been effective under field conditions conducive to preharvest contamination. Currently, there are no genotypes known to inhibit aflatoxin biosynthesis.

Cropping pattern

The new cropping pattern suggested is basically aimed at harvesting the maize in the dry season so as to avoid conditions of high aflatoxin risk. This entails planting maize in July and harvesting in November. The results of a number of studies (Kawashima et al. 1990; Kawasugi et al. 1988b; Nagler et al. 1989; Wirrawat 1984) have indicated that harvesting maize in the dry season should bring about low moisture content and produce low aflatoxin maize.

As noted earlier, there are two maize-growing seasons in Thailand. About 90% of the annual crop can be planted and harvested during the rainy season. The remaining 10% is planted during the middle and end of the rainy season and harvested at the beginning of the dry season. Thai farmers tend to plant maize as their first crop because thereafter there is more reliable rainfall for higher value cash crops such as sorghum, soybeans, or mung beans.

The likely social cost and profit of this method were

studied by Arunotong (1987), who found negative benefits to farmers even if they were able to overcome the many difficulties in modifying their cropping pattern.

Conclusion

While no overall breakthrough has yet been made with regard to aflatoxin contamination in maize in Thailand, several studies have raised awareness of the problem and have led to many improvements in farm and market practices.

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Regulatory Aspects of Mycotoxins in the European Community and USA

J. Gilbert*

Abstract

In the European Community (EC) and the USA, regulatory controls for the presence of mycotoxins are applied primarily to aflatoxins, although some countries additionally control patulin and ochratoxin A. In the EC, there are common limits for aflatoxins in animal feedstuffs, but no agreement exists on the limits to be applied to human foods. Limits in EC countries currently range from 5 μg/kg for aflatoxin B_1 alone (e.g. The Netherlands) to 50 μg/kg for total aflatoxins (e.g. Italy), with some member states considering the application of stricter limits. In contrast, a total aflatoxin limit of 20 μg/kg is applied to all food for human consumption in the USA, with no indications that this is likely to be lowered in the future. To be meaningful, regulatory limits must take account of both the difficulties of sampling and of achieving good analytical precision at low limits of detection. The effectiveness of controls and monitoring for aflatoxins in the food chain cannot be readily assessed for either the EC or the USA. However, as an example of the method of working within one EC member state, the results of enforcement action for the control of aflatoxins in bulk consignments of dried figs and pistachio nuts entering the country through ports in the United Kingdom are outlined.

ALTHOUGH mycotoxins, and primarily the aflatoxins, are regulated throughout the world (Van Egmond 1989) there is no consistent rationale for setting limits or for enforcement control (H.P. Van Egmond, these proceedings). Political and economic, as well as food safety considerations, affect the degree of seriousness with which aflatoxin contamination in human foods is viewed, these factors being inexorably linked to other circumstances, such as whether the country in question is a producer or importer of potentially contaminated materials. Thus, within the European Community, where aflatoxins are not generally a problem with locally produced foods or animal feedstuffs, limits for human foods tend to be stricter than, for example, in the USA where there would be serious economic consequences of implementing controls at very low limits of aflatoxin contamination.

EC Regulations for Mycotoxins

In the EC there are somewhat complex limits for the control of aflatoxins in animal feedstuffs. These are due to be harmonised by December 1991 by all member states. Although these limits have not yet been finally agreed, they are likely to be set at 50 μg/kg for aflatoxin B_1 in straight feedstuffs. For complete feedstuffs the limit is 50 μg/kg where the feed is not to be used for dairy cattle, calves, or lambs, 20 μg/kg for pigs and poultry, and 10 μg/kg for other animals including dairy cattle.

Likewise, there are separate limits for complementary feedstuffs set at 50, 30, and 10 μg/kg depending on the intended use. These limits may ultimately be tightened reducing the 50 μg/kg limit to 20 μg/kg and the 10 μg/kg limit for dairy feed to 5 μg/kg (Commission of the European Communities 1991). Six specified raw materials (peanuts, copra, palm kernel, cottonseed, babassu, maize, and derived products) may not exceed the limit of 50 μg/kg unless sold to a registered manufacturer who may handle ingredients contaminated at up to 200 μg/kg.

For human foods, somewhat surprisingly there are no agreed limits amongst EC countries either for aflatoxins B_1, B_2, G_1, and G_2, or for aflatoxin M_1 in dairy products. Where individual member states do have regulations for aflatoxins these are summarised in Table 1, and can be seen to range from 5 μg/kg aflatoxin B_1 for all foods (Belgium) to 50 μg/kg for peanuts (Italy). For dairy products, regulations for aflatoxin M_1 exist in only four countries, ranging from 0.01 μg/kg (Germany) for milk for infant feed to 0.2 μg/kg (Netherlands) for cheese. In the UK the absence of specific regulations for aflatoxin M_1 in milk is explained by the argument that if the animal feed regulations for aflatoxins are obeyed, then assuming about a 1% conversion from B_1, aflatoxin M_1 should not be detectable in the milk (at a limit of detection of 0.05 μg/kg).

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Table 1. Regulations for aflatoxins in EC member states

Country	Aflatoxin limits in foods (μg/kg)				
	B_1	Total	Food type	M_1	Food type
Belgium	5	–	all	0.1	milk
Denmark	–	10	peanuts	–	–
France	–	10	all	0.2	infant milk powder
	–	5	cereals, oils	–	–
	–	0.1	nut pastes	–	–
	–	5	infant	–	–
Germany	5	or 10	nuts, seeds, cereals	0.05	milk
Germany[a]	2	or 4	nuts, seeds, cereals	0.05	milk
Greece	1	or 5	nuts, seeds dried fruits, maize	–	–
Ireland	5	or 30	all	–	–
Italy	–	50	peanuts	–	–
Luxemburg	5	–	peanuts	–	–
Netherlands	5	–	all	0.05–0.2	milk/cheese
Portugal	25	–	peanuts	–	–
	5		–	infant	–
	20	–	other foods	–	–
Spain	5	10	all	–	–
United Kingdom[b]	–	10	nuts, fruit	–	–

[a] New regulations expected from May 1991
[b] Voluntary guidelines (code of practice)

The only other mycotoxins that are controlled by individual member states in the EC are patulin and ochratoxin A. Patulin, which is really a quality indicator rather than a safety issue, is controlled at a level of 50 mg/kg in fruit juices in Belgium, France, and Greece. In France there is a limit of 20 μg/kg for ochratoxin A in cereals, and in Greece a limit of 20 μg/kg in coffee beans. In Denmark, ochratoxin A is controlled by analysing all pigs kidneys that show signs of mycotoxin nephropathy and condemning the carcass if the level of toxin exceeds 25 μg/kg. If the level of ochratoxin A lies between 10 and 25 μg/kg the kidney, liver and other visceral organs are condemned (Buchmann and Hald 1985).

Regulations in the USA for Mycotoxins

In the USA, total aflatoxin levels are controlled at 20 μg/kg for all feedstuffs and feedstuff ingredients, with the exception of cottonseed (intended for feed for beef cattle, pigs, and poultry) where a limit of 300 μg/kg total aflatoxins is applied. All human foods are similarly controlled at the same limit as that for animal feeds, with a limit of 20 μg/kg for total aflatoxins. For whole milk, low fat milk, and skimmed milk there is a limit of 0.5 μg/kg for aflatoxin M_1. No other mycotoxins are regulated in the USA, except for deoxynivalenol for which there is an advisory limit ranging from 1.0 mg/kg for finished wheat and wheat products for human consumption to 4.0 mg/kg for wheat and wheat products for feed ingredients.

Problems in Drafting Effective Control Measures

Although there appears to be unequivocal agreement on the need for measures to control aflatoxin levels in foods and animal feeds, there are a number of problems in translating this need into sound and realistically enforceable regulations. Because contamination is generally not uniform but has a highly skewed distribution, an important question is what is meant by a regulatory limit. Should this limit be applied, for example, as a batch average, or should it be complied with by each-and-every unit? Does this limit allow for the uneven distribution of toxin and mean implicitly that there is acceptance that a proportion of samples (albeit a low percentage) may exceed that limit?

From the standpoint of the producer or importer of material there is a reasonable desire for any limit to be tied to a specified method of sampling. The importer can then undertake monitoring himself according to this plan to check compliance. Additionally, in the event of any enforcement action, there is a clearly agreed procedure to follow. Such sampling plans based on known distributions of aflatoxins are available, but in many instances do have the severe disadvantage of being impractical in terms of requiring labour-intensive sampling and large total weights of material. These plans are also invariably only applicable to bulk consignments of material, and nobody has properly addressed the question of sampling retail samples such as packs of nuts or jars of peanut butter.

As a general rule in sampling it is desirable to take as large a total sample weight as possible, and the larger the total number of subsamples the better. In addition, the larger the individual particle size of a commodity, the greater the need for large total weights of material. Thus, total sample weights of dried figs should, in general, be greater than total sample weights of peanuts, and where there has been some mixing, for example for fig paste or peanut butter, then the sample weight can be smaller than for the intact commodity.

One approach to sampling is to accept that it is going to be impractical to implement a statistically derived plan, but instead be pragmatic and develop an operable scheme based on the practical constraints of actually taking and subsequently manipulating the sample prior to analysis. Such an approach can be evolved from the above general considerations of the desirable features of sampling and also take account of factors such as cost of the commodity (for example, cashews and pistachios are far more expensive than figs), whether in-shell, or whether packaged in a way that makes sampling difficult.

We have recently adopted such a pragmatic approach to the sampling of figs, for which there are no statistically derived sampling plans based on the distribution of aflatoxin contamination. The pattern of contamination in figs has, however, been shown to closely resemble that of peanuts, with very high levels of aflatoxins being associated with individual fruits (Boyacioglu and Gonul 1990). If directly analogous sampling to that of peanuts were to be undertaken, because of the considerably greater mass of individual figs compared with that of peanut kernels, sample sizes required would be so large as to be impractical on cost grounds and in terms of physical handling within a laboratory.

Mathot (1989) proposed a sampling regime for figs in the Netherlands based on taking a total weight of 20 kg of whole dried figs made up of 20 subsamples, each subsample being taken from different stacks in the palletised container. The acceptance criterion for this sampling regime was 2.5 μg/kg for aflatoxin B_1, based on a legal limit of 5 μg/kg aflatoxin B_1. The difference between the legal limit and the acceptance criterion was a safety margin allowing for uncertainties in aflatoxin distribution. This plan gives a consumer risk (probability of the lot not meeting the 5 μg/kg legal limit) of 22% and a producer risk (probability of a lot just below the legal limit being rejected) of 50%.

Unfortunately, figs imported into the UK are not palletised, so although the general approach given in the above plan has been adopted, subsamples have had to be taken randomly from the most accessible boxes within the container. For fig paste where individual fruits have already been homogenised smaller sample sizes of 5 kg were taken, again being made up of 20 subsamples.

For imported containers of figs, practical constraints of time and cost of sampling at the port will inevitably always dictate the sampling plan that can be adopted. The present scheme probably represents the maximum sample size that can realistically be handled both at the port and in the laboratory.

Surveillance and Enforcement

In the UK, control of aflatoxin contamination in human foods is through the general provisions of the *1990 Food Safety Act* and the *Imported Food Regulations*, supplemented by an advisory 'action level' of 10 μg/kg for total aflatoxins. The Ministry of Agriculture, Fisheries and Food is not an enforcement body, and, in the UK, enforcement is carried out at a local level with sampling by trading standards or port health officers and analysis by designated public analysts. However, to assess the extent of possible food contamination problems and to judge whether remedial action is required the ministry does carry out extensive programmes of food surveillance (Ministry of Agriculture, Fisheries and Food 1988). Sampling for surveillance purposes is carried out at various points in the food chain although, for convenience and because they are the closest point to consumption, retail samples are frequently taken in addition to bulk consignments at the port of entry into the country.

Aflatoxin Monitoring of Bulk Consignments of Nuts and Dried Figs

The occurrence of aflatoxins in dried figs was first observed several years ago (Anon. 1974) and received public prominence in 1985 with reports of levels of tens of μg/kg contamination in retail samples imported from Turkey and mg/kg levels in individual figs (Steiner et al. 1988). Regulatory authorities subsequently increased their monitoring activities in this area, and there were published reports of aflatoxin contamination of figs from a number of importing countries. In Sweden in 1988, for example, 53 from a total of 103 fig samples tested had aflatoxin concentrations exceeding 5 μg/kg, with a maximum of 203 μg/kg being detected (Akerstrand and Moller 1989). Being similarly alerted to this problem, monitoring was initially introduced in the U.K. in 1988 when fig samples were obtained in an *ad hoc* fashion from importers. Subsequently, in 1989 and 1990, there was a rigorous systematic monitoring of all samples entering the country. In a similar fashion, EC countries were alerted in early 1990 to high levels of aflatoxin contamination occurring in pistachio nuts from Iran, and this commodity was additionally monitored from that time onwards.

The results of these surveillance exercises were intended to assess potential consumer exposure to aflatoxin contamination of figs and pistachio nuts, in particular dried figs, to gauge the effectiveness of quality control measures newly introduced by exporters in Turkey.

However, port health authorities were advised when aflatoxin levels exceeded 10 μg/kg and, where they considered it appropriate, action was taken to ensure that these contaminated consignments were not allowed to enter the U.K.

Sampling and Analysis

Samples of figs (about 5 kg in size varying from single boxes to composites from many boxes) for initial surveillance work were obtained in late 1988 directly from UK importers and retailers. Samples for subsequent work from April 1989 onwards were obtained from containers (generally 18 tonnes although occasionally smaller) at the port of entry into the U.K. Consignments were sampled randomly, taking 20 samples each of 1 kg from individual boxes of whole dried figs and 20 samples of 250 g from individual boxes of fig paste. Samples of pistachio nuts were generally about 5 kg in size made up of 20 subsamples taken from individual sacks.

Surveillance Results

The results of analyses for aflatoxins in figs obtained from the 1988 crop destined for sale over the Christmas period in the U.K. are shown in Table 2. For these initial samples, 5 of 29 (17%) of dried figs were found to contain more than 10 μg/kg of total aflatoxins with the highest concentration being 96 μg/kg, and 7 of 20 (35%) of fig pastes were found to exceed this limit, with one sample containing as much as 165 μg/kg. This preliminary work confirmed literature reports of a contamination problem with figs and clearly indicated that further action would be required in the U.K.

Table 2. Total aflatoxin levels in figs and pistachio nuts sampled at U.K. entry ports

Period of sampling	Commodity	Number of samples Total aflatoxins (μg/kg)		
		<1	1–10	>10
Dec 1988–Jan 1989	Fig paste	7	6	7
Jan 1989–Jan 1991	Fig paste	21	99	15
Dec 1988–Jan 1989	Dried figs	18	6	5
Jan 1989–Jan 1991	Dried figs	57	98	25
March 1990–Jan 1991	Pistachios	47	38	27

From 1989 onwards, rigorous screening procedures were introduced in Turkey to detect individual contaminated figs. This screening was based on observations of a correlation between blue/green/yellow fluorescence on the outside of figs and high aflatoxin levels (Steiner et al. 1988; Reichert et al. 1988). All figs were manually screened under UV light, discarding fluorescent figs, and this procedure was to be supported by analysis of each batch of figs in Turkey prior to shipment. In addition, U.K. importers of fig paste decided to undertake their own analyses of pre-shipment samples.

The results of surveillance monitoring of figs after introduction of quality control measures, from April 1989 until January 1991, are shown in Table 2. During this period a total of 135 consignments of fig paste and 180 consignments of whole dried figs were analysed for aflatoxin contamination. Some improvement was observed in the incidence and maximum levels of contamination, although 15 consignments of paste (11%) and 25 consignments of whole dried figs (14%) were nevertheless found to exceed the 10 μg/kg limit for total aflatoxins. As regards pistachio nuts, of which monitoring at ports began in March 1990, the incidence and levels of contamination with aflatoxins were higher, with 24% of consignments exceeding the 10 μg/kg limit. Port health authorities were advised of these surveillance results for both figs and pistachio nuts and they subsequently took action to refuse entry into the U.K. of these foodstuffs.

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Regulatory Aspects of Mycotoxins in Asia and Africa

H.P. Van Egmond*

Abstract

Some 60 countries have currently enacted or proposed regulations for mycotoxins in food and feedstuffs, 17 of them situated in Asia and Africa. Various factors influence the establishment of tolerances for certain mycotoxins, such as the availability of toxicological and dietary exposure data, the distribution of mycotoxins throughout commodities, legislation of other trading countries, and availability of analytical methods.

In practice, only a few countries have formally presented a rationale for regulation or selection of a particular maximum tolerance level. In Asia and Africa practically all mycotoxin regulation involves aflatoxins, and most of these regulations concern aflatoxin B_1 alone. In these countries, actual tolerance levels for aflatoxins in food for human consumption range from zero to 50 µg/kg, and in animal feed from 10–1000 mg/kg, depending on the tolerance level per product and destination.

Harmonisation of mycotoxin regulations is highly desirable, not only in Asia and Africa, but worldwide. International organisations could play a role in the rationalisation process by providing estimates of safe intake levels, based on the current scientific knowledge of the hazards of mycotoxins.

'FISH which cannot be sold, can be declared as such, and is allowed to be sold to foreigners only'. This remarkable passage of a Swiss food regulation was promulgated by the Council of Basel at the beginning of the thirteenth century. It demonstrates that municipal ordinances in the Middle Ages did not always follow the nowadays generally applied principle that food contaminated with hazardous substances is unfit for human consumption and shall therefore not be sold or offered for sale at all. Another difference with today's food laws involves the way inspections are carried out. In the past, these were relatively simple, as there were no auxiliary sciences, such as bacteriology, chemistry, and microscopy. It is only in the last century that framers of food regulations started to make use of the growing knowledge of food scientists, thus leading to more effective consumer protection, especially in the industrialised countries.

Current food laws often include specific legislation that imposes limits or tolerances on the concentrations of specific contaminants of constituents of foods. These have been set to safeguard the health and economic interests of those who ultimately consume food. The contaminants for which regulations exist may be of industrial or natural origin. The latter group includes the mycotoxins and phycotoxins. Van Egmond (1989) gives an overview of current regulations for mycotoxins. A review of regulations for phycotoxins is in preparation (Van Egmond, unpublished data). This paper first discusses the general factors influencing the establishment of mycotoxin regulations, then gives an overview of current regulations for mycotoxins in Africa and Asia. Regulations existing in the European Community and the USA are discussed by J. Gilbert elsewhere in these proceedings.

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Factors Influencing the Regulation for Mycotoxins

Various factors may influence the decisions taken by authorities to establish limits for certain mycotoxins. Among these are scientific and non-scientific factors including:

- the availability of toxicological data;
- the availability of survey data;
- the distribution of mycotoxins over commodities;
- the availability of methods of analysis;
- legislation in other countries with which trade contacts exist; and
- sufficient food supply

Further details on each of these factors follow.

Without *toxicological information* derived from animal data and, where possible, from epidemiological data, there can be no hazard assessment. Measures are most logically taken on the basis of specific toxicological effects, except when cancer is the basis for concern, as is the case with aflatoxins. The current assumption is that there is no threshold level for aflatoxins below which some effects cannot occur and that, therefore, any small dose will cause a proportionally small probability of inducing some effect. A zero tolerance would therefore be appropriate, but the problem is that aflatoxins are natural contaminants which cannot be eliminated completely without outlawing the susceptible food or feed. This makes regulatory judgments particularly difficult.

The *availability of survey results* may provide data for exposure assessment. Survey data also allow an estimate of the effects of enforcement of regulations on the availability of food, including animal products, and feed.

Hazard assessment and exposure assessment are the basic ingredients for risk assessment; that is, the analytical process by which the nature and magnitude of risks are identified. Risk, as it pertains to the health effects of mycotoxins, is the probability of disease or death of individuals exposed to these mycotoxins. (In the case of aflatoxins we are talking about death of individuals exposed to carcinogenic substances, and the risk then can be expressed numerically, for example, 1 in a million lifetimes.) The risk assessment for mycotoxins involves:

1. exposure assessment;
2. hazard assessment based on animal toxicity data and, where possible, also on epidemiological data;
3. extrapolation from animal data, obtained at relatively high exposure levels, to low exposure levels and to humans to give an estimate of safe intake; and
4. comparison of the exposure assessment to the estimate of safe intake, which indicates the degree of concern regarding a particular mycotoxin and which dictates, in part, whether the setting of tolerances or guideline levels for mycotoxins is required.

While exposure and hazard assessement are always involved in risk management, other factors may be involved to varying degrees, depending on the situation.

The *distribution of the mycotoxin(s)* in the products may pose certain very difficult problems in establishing regulatory criteria. This distribution can be very uneven, as is the case with aflatoxins in peanuts. The number of contaminated peanut kernels in a lot is usually very low, but the contamination level within individual kernels can be very high. This means that there is a good chance that the mycotoxin concentration in the lot to be inspected will be wrongly estimated, because of difficulties in representative sampling. It also means that consumption of peanuts may lead to an accidental high single dose of aflatoxins, instead of a chronic intake at relatively low level. A similar situation may occur with figs.

Accurate *methods of analysis* have to be available, because legislation calls for methods of control. If reliable methods of analysis do not exist, the fulfilment of established tolerances is not possible. In addition to reliability, simplicity is a requirement, as it will influence the amount of data that will be developed and the practicality of the ultimate measures taken. It should be borne in mind that a tolerance cannot be lower than the actual limit of detection of the method of analysis used. In the past, various tolerance levels were set at the limit of determination (and/or at the limit of confirmation of identity) of the analytical method. For these cases it may be necessary to reconsider the tolerances when the analytical methodology improves; that is, when the limits of determination go down.

The *regulations in force in other countries* with which trade contacts exist, have to be considered and, if possible, brought into harmony with the legislation under consideration. Unnecessarily strict regulative actions may create difficulties for importing countries in obtaining supplies of essential commodities such as food grains and animal feedstuffs. For exporting countries, difficulties may arise finding markets for their products. For example, because of current (and soon to be even more stringent) regulations for aflatoxin B_1 in animal feedstuffs in the European Community (Commission of the European Communities 1983, 1991), some developing countries have increasing problems with the export of feed ingredients to their European trading partners.

The regulatory philosophy should not jeopardise the availability of some basic commodities at reasonable prices. Especially in the developing countries, where *food supplies* are already limited, drastic legal measures could lead to lack of food and higher prices. It must be remembered that people living in these countries cannot exercise the option of starving to death today in order to live a better life tomorrow.

Weighing the various factors that play a role in the decision-making process of establishing mycotoxin tolerances is not easy. Common sense is a major factor in reaching a decision. Public health officials are confronted with a complex problem: mycotoxins, and particularly aflatoxins, should be excluded from food as far as possible. Since the substances are present in foods as natural contaminants, however, exposure of the population to some level of mycotoxins must be tolerated. Despite the dilemmas, mycotoxin regulations have been established in many countries over the past decade.

International Enquiries in the 1980s

In 1981 an attempt was made to gain an overview of worldwide mycotoxin legislation. The results were

presented by Schuller et al. (1983) to an International Symposium on Mycotoxins, held in Cairo. For that survey, the Agricultural Attaches or Counsellors of the Dutch Embassies in their various stations contacted the responsible authorities to obtain relevant information. The information collected was supplemented with data obtained through courtesy of the International Affairs Staff, U.S. Food and Drug Administration, and information published by Krogh (1977).

Since 1981, mycotoxin regulations have changed, expanded, or been introduced in various countries. The International Union of Pure and Applied Chemistry (IUPAC) and the Food and Agriculture Organization (FAO) therefore recommended that an update be made in 1987, the results of which are given by Van Egmond (1989). The updated information confirmed the conclusion that the differences between tolerated levels of mycotoxins sometimes vary widely between countries, making harmonisation of mycotoxin regulations highly desirable. Harmonisation efforts should be supported by knowledge of the rationale behind the decisions that have led to the establishment of current mycotoxin regulations throughout the world. To gather this information, IUPAC therefore requested that a new international survey be undertaken in 1989, the results of which were published by Stoloff et al. (1991). A summary of the results of this and the earlier survey by Van Egmond (1989) follows.

At the time of writing there were about 60 countries that had specific regulations or detailed proposals for regulations on mycotoxins. The fact that the law is silent on this topic in some countries does not necessarily mean that the problem does not exist or is ignored. Several countries rely on general food legislation that prohibits the introduction, delivery for introduction, or receipt in commerce of food containing substances injurious to health. Most of the existing mycotoxin regulations concern aflatoxins and, in fact, all countries with mycotoxin regulations have tolerances for aflatoxins in foods and/or animal feedstuffs. Less frequently, specific regulations also exist for patulin and ochratoxin A, and for deoxynivalenol, zearalenone, T-2 toxin, chetomin, stachybotryotoxin and phompsin.

Because of length limitations on this paper, further discussions are focused on selected aflatoxin regulations only, and details will be presented about only Asian and African countries in the next section. Readers wishing an insight into other mycotoxin regulations are referred to other publications (Van Egmond 1989; J. Gilbert, these proceedings; Stoloff et al. 1991; Van Egmond 1991). In the countries surveyed worldwide, some regulated only aflatoxins B_1, while others regulated the sum of aflatoxin B_1, B_2, G_1, and G_2, and yet others also regulated aflatoxin M_1 in dairy products. With regard to acceptable levels in foods of aflatoxin B_1 alone, a majority of the countries adopted a level of 5 µg/kg. In those countries that apply limits for the sum of the aflatoxins, such a uniformity in tolerance values does not occur. Most of these tolerances were in the range 10–30 µg/kg. In the case of aflatoxin M_1 in liquid milk, two groups of five countries each adopted levels of 0.05 and 0.5 µg/kg, respectively, with some other jurisdictions taking zero, 0.1, and 1.0 µg/kg as their respective limits. The divergence between the 0.05 µg/kg and 0.5 µg/kg levels is indeed striking, the former prevailing in several Western European countries. This low level in these European countries has in turn resulted in the fairly stringent regulation of aflatoxin B_1 in complementary feedstuffs in dairy cattle in the European Communities (EC) (Commission of the European Communities 1983). The acceptable level for aflatoxin B_1 in such feedstuffs was reduced from 20 to 10 µg/kg in 1984. Recently, the decision was taken to further tighten this acceptable level to 5 µg/kg, a measure which will be accorded legal status by the end of 1991 (Commission of the European Communities 1991).

The international enquiry of 1989 concerning the rationales for regulations on mycotoxins in human food and animal feed yielded rather meagre results (Stoloff et al. 1991). Most of the responses concerned limits for aflatoxins in food, and most of these were based on a vague, unsupported statement of the carcinogenic risk for humans. There was a general consensus that exposure to a potential human carcinogen that could not be totally avoided should be limited to the lowest practical level, the definition of 'practicality' depending on whether the country was an importer or producer of the potentially contaminated commodity. Some countries made a claim to a hazard evaluation, although specific details were rather scarce.

The Situation in Asia and Africa

Figure 1 charts those countries with mycotoxin legislation and those without, as at 1989. Obviously many Third World countries, where mycotoxin problems may be severe, had no mycotoxin regulations, a conclusion of considerable interest. In Tables 1–4 the regulations for aflatoxins in foods and animal feedstuffs that do exist in Asia and Africa are summarised.

At the time of writing, twelve Asian countries (Table 1) and five African countries (Table 2) were known to have regulations for aflatoxin(s) in food. Most of these regulations concerned solely aflatoxin B_1. It is debatable, whether a tolerance for the sum of the aflatoxins, which requires more analytical work than for aflatoxin B_1 alone, would contribute significantly to better protection of public health. From the points of view of both toxicology and occurrence, aflatoxin B_1 is the most important of the aflatoxins. It is unlikely that commodities will contain aflatoxins B_2, G_1, and G_2 and not aflatoxin B_1, whereas the concentration of the sum of the aflatoxins B_2, G_1, and G_2 is generally less than that of aflatoxin B_1 alone (Van Egmond et al. 1979).

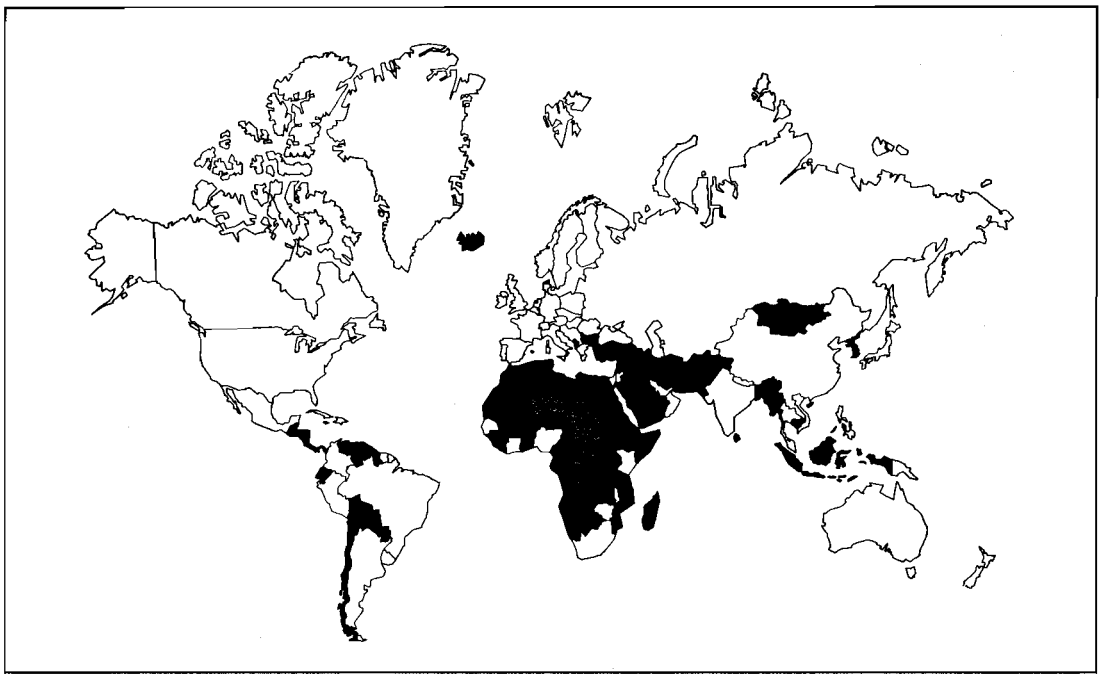

Fig. 1. Countries with (white) and without (black) mycotoxin legislation in 1989.

Table 1. Maximum tolerated levels for aflatoxins in foodstuffs in Asian countries

Country	Commodity	Tolerance (μg/kg)	
		B_1	$B_1+B_2+G_1+G_2$
People's Republic of China	Maize and maize products	20	
	Peanuts and peanut products	20	
	Peanut oil	20	
	Rice, other edible oil	10	
	Other cereals, legumes, fermented foods	5	
	Infant milk substitutes	Not detectable	
Taiwan	Cereals (rice, maize, peanuts sorghum, legumes, wheat, barley, oats etc.)	50	
Hong Kong	Peanuts and peanut products	20 (including aflatoxins M_1, M_2, P_1, and aflatoxicol)	
India	All foods	30	
Japan	All foods	10	
Jordan	Almonds, cereals, maize, peanuts, pistachio nuts, pine nuts, rice	15	30
Malaysia	All foods		35
Nepal	Cereal grains, legumes	30	
Philippines	Coconut, peanut products (export)	20	
Singapore	All foods	zero	zero
Thailand	All foods		20
Vietnam	Peanuts	zero	

Sources: Van Egmond (1989); FAO (1990); Stoloff et al. (1991)

Table 2. Maximum tolerated levels for aflatoxins in foodstuffs in African countries

Country	Commodity	Tolerance (µg/kg)	
		B_1	$B_1 B_2+G_1+G_2$
Malawi	All foods		35
Mauritius	Peanuts	5	15
	Other products	5	10 (including aflatoxins M_1 and M_2)
Nigeria	Infant foods	zero	
	All other foods	20	
South Africa	All foods	5	10
Zimbabwe	Peanuts, maize, sorghum	5	
	Peanut butter, cereals, flour and bread		20

Sources: Van Egmond (1989); Stoloff et al. (1991)

Table 3. Maximum tolerated levels for aflatoxins in animal feedstuffs in Asian countries

Country	Commodity	Tolerance (µg/kg)	
		B_1	$B_1 B_2+G_1+G_2$
Taiwan	Oilseed meals for feed (use level under 4% of mixed feed)		1000
India	Peanut meal (export)	120	
Israel	Straight feedstuffs	50	
	Complete feedstuffs, not for dairy cattle, calves, lambs	50	
	Complete feedstuffs for pigs and poultry	20	
	Other complete feedstuffs	10	
	Complementary feedstuffs for cattle, sheep and goats (except dairy animals, calves, lambs, and kids)	50	
	Complementary feedstuffs for pigs and poultry (except young animals)	30	
	Other complementary feedstuffs	10	
Japan	Peanut meal (export)	1000	
	Mixed feeds, not for dairy cattle, young cattle, young pigs, young birds and fish	20	
	Mixed feed for dairy cattle, young pigs, young birds	10	
Jordan	Animal feed	15	30
Nepal	Dairy cattle feed	50	
Oman	Complete feeds for poultry (except chicks)	20	
	Other complete feeds	10	

Sources: Van Egmond (1989); FAO (1990); Stoloff et al. (1991)

Table 4. Maximum tolerated levels for aflatoxins in animal feedstuffs in African countries

Country	Commodity	Tolerance (µg/kg)	
		B_1	$B_1+B_2+G_1+G_2$
Ivory Coast	Straight feedstuffs		100
	Complete feedstuffs for cattle, sheep andgoats (except dairy cattle, calves, and lambs)		75
	Complete feedstuffs for pigs and poultry (except young animals and ducks)		38
	Complete feedstuffs for dairy cattle		50
	Other complete feedstuffs		10
Nigeria	Feedstuffs	50	
Senegal	Peanut products (straight feedstuffs)	50	
	Peanut products (feedstuff ingredients)	300	

Source: Van Egmond (1989)

Analytically, determination of the sum of the aflatoxins requires more work and the availability of separate quantitative aflatoxin standards, a problematic situation in many of the developing countries. Monitoring agencies in those countries that apply tolerances for the sum of the aflatoxins should inspect their analytical data to see how frequently the availability of data on the sum of the aflatoxins (above that on aflatoxin B_1) has been indispensable to adequately protect the consumer.

The numbers of countries with actual legislation for aflatoxin(s) in animal feed were seven in Asia (Table 3) and three in Africa (Table 4). Again, most of these countries regulated only aflatoxin B_1. Some of the Asian limits have probably been set to comply with the EC regulations for aflatoxin B_1 in animal feedstuffs. As yet, it is uncertain what impact the new EC limit for aflatoxin B_1 in complementary feed for dairy cattle (set at 5 µg/kg as of the end of 1991) will have for the feedstuff regulations in the developing countries. No doubt the downward trend of the EC limits for aflatoxin B_1 in animal feedstuffs will lead to increasing problems for Asian and African countries, as they find themselves compelled to establish export standards meeting the requirements of their European trading partners.

For the African and Asian regions, only South Africa and India provided some background details for the regulations they had established for aflatoxins. South Africa referred to a risk determination, based on the results of an epidemiological study (Van Rensburg et al. 1974) of liver cancer rate and aflatoxin intake in a part of the region with a high cancer rate. The conclusion of a later study (Van Rensburg et al. 1985) in the same area had apparently not been considered for a redetermination of the risk. India relied on the advice of a FAO–WHO–UNICEF panel (Protein Advisory Group 1969) that considered the risk from aflatoxin in a protein supplement (peanut meal) that was being offered in the 1960s for kwashiorkor therapy, and extended the recommended limit in peanut meal to be used as a protein supplement to all foods. However, recent investigations (De Vries 1989) point to the possible causal role of aflatoxin in kwashiorkor, and the PAG-recommendation may need to be reconsidered in view of the results of these studies.

Regulations for mycotoxins other than aflatoxins hardly exist in Asia and Africa. In India a limit of 30 µg/kg has been proposed for deoxynivalenol in wheat, whereas a limit of 0.01% of ergot in pearl millet is applied. Little change is foreseen in this situation in the near future.

Conclusion

At present, information exists about regulations on mycotoxins in some 60 countries. No more than 17 of these countries are located in Asia and Africa, which means that many Third World countries do not have such regulations. Nearly all of the existing Asian and African regulations practically all refer to aflatoxins, and the tolerance levels in the various countries may differ significantly, a situation which is also apparent worldwide. The scientific basis for regulation of mycotoxins seems rather weak. It would be useful if an internationally authoritative body such as the World Health Organization were to (re-)evaluate and summarise current knowledge about the hazards of mycotoxins, so as to facilitate the establishment of harmonised limits for these natural toxins.

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Communication, Networking, and Collaboration in Mycotoxin Prevention and Control Activities

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Abstract

Various activities related to mycotoxin control have been carried out at national, regional, and international levels. Whatever the activities carried out including research, prevention at agricultural level, surveillance programs of mycotoxin contamination, etc, the need for exchange of information and experiences between the people involved has been continuously recognised and emphasised. This paper attempts to present international activities undertaken in communication and networking and the need for strengthening efforts in this field at international and regional levels in particular.

THERE is an increasing worldwide awareness of the serious consequences that undesirable levels of mycotoxins may have on food and feed supplies. The existence of contaminated food has negative effects on food consumption, nutrition, and international trade in foodstuffs.

Global concerns regarding mycotoxin contamination of food products have been discussed on numerous occasions, as has the need to control the problem through the introduction of good agricultural practices in the areas of drying, storage, and transport, through monitoring and control of mycotoxin contamination at national, regional, and international levels—including the setting of internationally agreed tolerance levels—and through the development of suitable research and education programs. If recommendations to improve the situation are to be implemented smoothly and at the same time duplication of activities is to be avoided, networking of agencies and institutions is considered to be essential.

An effective worldwide transfer of scientific and technological information has often been stressed as one of the ways to achieve development. To produce or acquire both scientific and technical information requires human and financial resources and capabilities. When these resources are limited, such as in developing countries, it is most important to have an economical way of accessing scientific and technical information and experience existing elsewhere. Thus, methods for communication and transfer of technology must be created, and networking is one such method.

Various efforts have been made at the international level to coordinate activities between countries and to exchange valid information on research programs and contamination studies, as well as on practical experiences in the prevention and control of mycotoxin contamination. Regional networking is now being developed in order to optimise use of scarce financial, human, and technical resources, to encourage closer collaboration between institutions and/or countries from the developed and the developing world, and last but not least to enhance exchange of information through established communication channels.

In this context, attention is drawn to the Technical Cooperation Among Developing Countries (TCDC) program.

The fundamental principle of TCDC is the voluntary sharing or exchange of technical resources, skills, and capabilities between two or more developing countries for their mutual benefit. TCDC is neither an end in itself nor a substitute for technical cooperation with developed countries. FAO Director General E. Saouma has stressed that TCDC is a means of building communication, of exchanging and adapting existing technology, and of collectively taking new strides. FAO is playing a catalytic and supportive role in helping member countries to identify areas of possible cooperation and then to implement TCDC activities. The TCDC potential for food, agriculture, and rural development is almost unlimited. Sharing of experiences and joint efforts to improve food quality control systems and to monitor and control food contamination have been initiated around

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the world, although financial constraints have affected activities in most countries.

Nevertheless, FAO has assisted in promoting TCDC and a regional initiative on food control is currently under way in Asia. The Training Network on Food Control in Asia is a good example of TCDC networking. This program commenced in 1986 and involves all Asian nations with five countries—China, Indonesia, India, Malaysia, and Thailand each being training centres in a particular food control subject. The project is now well known in the region and its newsletter *Network* is seen as a valuable link between food control services in the region.

Networking activities in mycotoxins could no doubt benefit from this experience and draw on its success.

Training Network on Mycotoxin Control

Following requests from, and consultations with various governments from the Asian region, an FAO/UNEP/UNEPCOM (USSR) mission was undertaken in June 1989 to identify Asian institutions that would be interested in and have suitable facilities for acting as regional reference centres for activities related to mycotoxin prevention and control. A project proposal was prepared, addressing the main concerns expressed in the region. It was designed to strengthen the capability of inspection and food laboratory personnel and extension staff in mycotoxin detection and prevention.

An FAO Regional Workshop on Control of Aflatoxins in Asia, held in Chiang Mai, Thailand in February 1990, recommended that high priority be given to establishing the network for mycotoxin control in the region, as described in the FAO/UNEP/UNEPCOM draft project.

Training facilities have been identified in India, the Philippines, and Thailand, with linkages to the USSR. The personnel who will be trained as part of project activities will later become trainers of other personnel at country level in the detection and quantification of mycotoxin contamination and the initiation of mycotoxin prevention and control programs.

The training programs are not restricted to laboratory staff. They are also targeting decision-makers, planners, and senior administrators, so as to increase awareness that mycotoxins pose serious consequences in terms of human and animal health, trade, and economic losses.

In addition, training in prevention and control measures is also to be directed to community leaders, farmers' representatives, storage managers, processors, oil millers, exporters and, above all, agricultural extension workers. The network includes mycotoxin prevention and control measures in the curricula of training programs for extension workers dealing with commodities such as peanuts and maize, which have a high potential for mycotoxin contamination.

A detailed workplan for the project has been prepared by the coordinators, and its progress will be watched with keen interest, as it could be used as a model for similar activities in other regions of the world, as well as for controlling other food contaminants. At a recent session of the Codex Coordinating Committee for Latin America and the Caribbean, representatives of Latin American countries highlighted the need for establishing similar networking activities in mycotoxin-related subjects in that part of the world.

In addition to the activities already mentioned, it is planned to issue a mycotoxin newsletter within the Asian network. This should assist in providing various types of information to concerned professionals, including details on research, prevention, and control measures undertaken in the region.

Networking Activity in Analytical Quality Assurance

The advantages of collaborative studies on mycotoxin analysis cannot be overemphasised. They include:

- validation of methodology;
- minimisation of intra and inter-laboratory error;
- improvement of the accuracy and analytical capabilities; and
- removal of variability.

An analytical quality assurance program based on the model of collaborative studies carried out under the auspices of the AOAC or IARC, mostly in developed countries, should be initiated in the developing countries and modified to suit local conditions.

A successful start in Asia was made in 1984 under the FAO Food Contamination Monitoring Project in India, Nepal, Pakistan, and Sri Lanka. Following completion of the project, the Indian Council of Medical Research extended the study through the implementation of an aflatoxin contaminants hazard evaluation project.

Based on this experience, the National Institute of Nutrition (India) has been identified to host and initiate, with FAO assistance, an Asian regional quality assurance program for aflatoxins. Collaborating laboratories have been identified in China, Thailand, Sri Lanka, Nepal, the Philippines, and Malaysia.

It is expected that this networked collaboration will assist in obtaining reliable data to evaluate existing levels of mycotoxins (aflatoxins) in foods, so as to provide a sound basis for national decisions on future food control activities concerning these contaminants. Thus, this program is to survey the quality of mycotoxin analytical data produced by food mycotoxin laboratories within the Asian region, to evaluate the findings and recommend remedial actions. Recently initiated activities involve:

- the preparation of proper food check samples with both added and natural contaminant residues;
- replicate analysis of the check samples to establish level of contamination for use in comparing later results;
- the distribution of the check samples to participating food laboratories and evaluation of the results received from them; and
- the provision of technical advice and guidance to individual laboratories whose analysis results indicate a problem.

The results of the study will be confidential and should assist in providing for follow-up assistance to correct deficiencies found.

REGNET

Since its inception in 1978, the Special Action Programme for the Prevention of Food Losses (SAP/PFL), has progressively developed action-oriented field projects in member nations that address the requirement to provide a multidisciplinary systems approach to improving national postproduction programs.

Harvested foods, particularly the durables, require some form of drying immediately after harvesting to optimise further primary processing and to ensure that storage losses are minimised, no matter what system is employed.

Previous PFL projects have aimed at drying and storage systems improvements that have the capability of not only restricting both dry matter loss and loss in quality, but also of preventing the proliferation of mycotoxin-forming moulds in harvested foods. PFL has been able to draw on the expertise of the technical services within FAO to promote mycotoxin prevention in postharvest systems, particularly for maize and cassava, through a number of national and intercountry cooperation or regional TCDC projects. It can be noted that PFL is the designated FAO coordinator for the Group for Assistance on Systems relating to Grain After-harvest (GASGA). It has therefore been able to draw upon the results of research projects implemented by individual GASGA members to more effectively target its field development program related to mycotoxin prevention.

In Asia, for example, PFL has been instrumental in providing technical assistance through a number of UNDP-funded projects, in developing a regional post-harvest network 'REGNET' in which 13 countries are currently members. The responsibility for REGNET was recently transferred to the participating designated national focal point institutions for the network. It is important to note that, during 1989, REGNET was able to implement a high level regional training program on mycotoxin prevention and control, through the Plant Pathology and Microbiology Division of the Department of Agriculture in Bangkok, Thailand, but did so in strong collaboration with the ASEAN Grains Post-Harvest Programme (AGPP), CIDA/IDRC, ACIAR, JICA, SGS, and other agencies, thus resulting in a much more effective training program.

It is hoped that collaboration and support of this nature will continue in future network endeavours, not only in Asia but also in the Post Harvest Technology Cooperation Network that has also been established with 18 member countries in Latin America and the Caribbean.

Information Transfer

To be fully effective, all persons involved in mycotoxin prevention and control activities, including scientists, technologists, field workers, industry personnel, and policy makers, need information on mycotoxins. The benefits that will inevitably flow from access to the right information include:

- improved ability to take advantage of knowledge and experience gained elsewhere;
- rationalisation of research and development efforts according to existing information and experience on mycotoxins;
- a broader information base to assist in solving specific mycotoxin problems;
- access to methods and techniques used elsewhere, for possible adaptation to country-specific needs;
- improved effectiveness and efficiency of activities dealing with research, prevention, and control of mycotoxins; and
- improved decision-making in all sectors and at all levels of responsibility.

While worldwide exchange of scientific information is already well established through, for example, scientific and technical publications and journals, there are still some notable gaps. In particular, the holdings of libraries in the developing world can be restricted because of financial and organisational problems. Restrictions on photocopying are also an obstacle.

The International Information System for the Agricultural Sciences and Technology (AGRIS) is an FAO-sponsored international bibliographic data base covering the agricultural sciences and technology including forestry, fisheries, and nutrition. AGRIS is composed mainly of journal articles (75%), monographs (18%), and conference papers (6%). Contributions (about 120 000 items annually) come from national (143), regional (3), and international centres (16). Currently, about 1750 items are directly related to mycotoxins. Three AGRIS subnetworks have been developed as a result of regional cooperation (AGRIASIA, AGRINTER, and EURAGRIS).

CARIS (Current Agricultural Research Information System) covers information on research activities in the developing world. It is another FAO promoted network activity. CARIS enables developing countries to collect, organise, and disseminate information on their respective research institutions, workers, and projects, and to exchange this information both among themselves and with developed countries. About 44 centres participating in CARIS deal with mycotoxins.

Personal contacts between scientists and technicians in international and regional meetings also play an important role in the exchange of information and experience. It is worth noting in this context the valuable efforts made throughout the world to organise scientific meetings. In the field of mycotoxins the following recent or imminent meetings can be mentioned:

- An international symposium and workshop on food contamination, mycotoxins, and phycotoxins was held in Cairo, Egypt from 4–15 November 1990. About 197 scientists from 34 countries participated.
- A mycotoxin workshop for the SADCC states will be held in Botswana from 22–26 July 91. The workshop is inviting scientific personnel from food institutions and industries, and aims to inform personnel working on mycotoxins on current techniques for sampling, extraction, and analysis of mycotoxins, as well as discussing various methods of decontamination of feed and foodstuffs for mycotoxins.
- The Gordon Research Conference on Mycotoxins and Phycotoxins will be held from 23–28 June 1991, to discuss the latest scientific advances, their significance, and future research plans.
- An international symposium on stored grain ecosystems will be held in Canada in June next year. The purpose of the symposium is to synthesise knowledge from the different areas of grain research, and to explore directions for optimum management of stored grain in the 21st century, based on the ecosystem concept. Both scientific and practical aspects of grain storage problems (including mycotoxins and mycotoxigenic fungi in storage) will be addressed.
- And of course this international conference organised by GASGA, the Australian Centre for International Agriculture Research, and the Department of Agriculture, Thailand.

FAO has contributed to these efforts to bring together people working on mycotoxin problems. The International Conferences on Mycotoxins held in 1977 and 1987, which assisted in assessing the world mycotoxin situation and identifying strategies for actions, described in the second paper in these proceedings, can be mentioned in this regard.

At the regional level, recent activities include workshops on aflatoxin in Asia (Chiang Mai, Thailand, February 1990), Africa (Cairo, Egypt, December 1990), and Latin America (San José, Costa Rica, February 1991). These workshops reviewed the proposals made by the international conferences with a view to adapting them to the particular needs of the different regions involved.

Technical meetings on mycotoxin (aflatoxin) analysis have also been organised with FAO assistance. Training was directed to food analysts and considered mainly TLC and ELISA methods. These meetings included a training workshop in 1989 in Trinidad and Tobago for the English- speaking Caribbean countries, and a training course in Dakar, Senegal in 1990 for the French-speaking countries of Africa. Previously, other national or regional workshops also assisted in bringing together professionals involved in mycotoxin analysis in Mexico, Cuba, and various other countries.

FAO involvement in the dissemination of technical information can also be mentioned, in particular the *FAO Food and Nutrition Manuals* published and widely distributed among FAO member countries and specific institutions. A list of FAO publications available is given in Appendix 1.

A Data Base for Prevention and Control Measures for Mycotoxins

The establishment of a data base on mycotoxin prevention and control measures was requested during the 1990 Asian workshop on aflatoxin control. Documenting and disseminating information at regional level on this subject was considered by the food control agency representatives as essential to their day-to-day work.

In the past, much of the information collected on mycotoxins has been published in the research literature. Little effort has been made towards compiling a data base covering the whole spectrum of problems and activities relating to mycotoxin prevention and control. The profile for entries in such a data base could include information on existing infrastructure to control mycotoxins in each country, details of trade implications of mycotoxin contamination of commodities, and facilities for exchange of information and experience on mycotoxin prevention and control measures (see Appendix 2).

While compilation of a readily accessible data base of this type, and networking of information exchange among workers in countries of the region will be useful, obtaining the information needed may be difficult. This problem could be overcome by greater participation and involvement of the countries concerned. Coordination of the activities of different ministries and organisations, and involvement of the private trade sector, which has been generally sensitive to providing data considered as 'commercial intelligence', is essential. A focal point for mycotoxin prevention and control has to be established

in each country. Within the limits of its funds, FAO is planning such work, and the data base may be initiated as part of the activities of the Asian Training Network on Mycotoxin Control.

Conclusion

FAO is not the only organisation active in communication and networking in this field. FAO recognises the efforts of AOAC and IARC in collaborative interlaboratory studies, ACIAR involvement in prevention activities, and the *Swedish Food Laboratory Newsletters* which provide regular information on mycotoxins and are distributed free of charge to laboratories and institutions in developing countries. This is just a small sample of current activity. It is likely that many more institutions are involved in this field ... but a lack of means of communication and information dissemination means we do not know of their activities! Clearly, we need to develop information networking as an essential tool to assist in alleviating the global mycotoxin problem.

Appendix 1

List of major FAO publications on mycotoxins

- FAO Food and Nutrition Paper No. 2 — Report of the Joint FAO/ WHO/UNEP Conference on Mycotoxins, Nairobi, Kenya, 1977
- FAO Food and Nutrition Paper No. 10 — Prevention of Mycotoxins FAO, Rome 1979
- FAO Food and Nutrition Paper No. 13 — Perspective on Mycotoxins, FAO, Rome, 1979
- FAO Manuals of Food Quality Control — Microbiological Analysis, 14/4, FAO, Rome 1979
- FAO Manuals of Food Quality Control, Food Analysis: General Techniques, Additives, Contaminants and Composition, 14/7, FAO, Rome 1986
- FAO Manuals of Food Quality Control, Introduction to Food Sampling, 14/9, FAO, Rome, 1988
- FAO Food and Nutrition Paper No. 21 — Mycotoxin Surveillance a Guideline, FAO, Rome, 1982
- FAO Food and Nutrition Paper No. 29 — Post-Harvest Losses in Quality of Foodgrains, FAO, Rome, 1983
- FAO Food and Nutrition Paper No. 14/10 — Mycotoxin Analysis, FAO, Rome, 1990
- Nairobi + 10, Report of the Second Joint FAO/WHO/ UNEP International Conference on Mycotoxins, Bangkok, Thailand 1987, FAO Bangkok, 1988
- Summary 1984–85 Monitoring Data, GEMS Food — FAO/WHO –EHE/ FOS/884, WHO, Geneva, 1987
- 'Current Situations on Regulations for Mycotoxins Tolerances and Status Standards, Methods of Sampling and Analysis' and 'Current Limits and Regulations on Mycotoxins', MYC 87/9.1 and MYC 87/9.2. Working Papers for the Second Joint FAO/WHO/UNEP International Conference on Mycotoxins, H.P. Van Egmond, FAO, Rome, 1987
- Distribution of Mycotoxins, and Analysis of Worldwide Commodities Data, Including Data from the FAO/ WHO/UNEP Food Contamination Monitoring Programme, MYC 87/5, Working Paper for the Second Joint FAO/WHO/UNEP International Conference on Mycotoxins, C. Jelinek, FAO, Rome, 1987
- FAO Food and Nutrition Paper No. 47/1 — Utilization of Tropical Foods, Cereals, FAO, Rome, 1989
- FAO Food and Nutrition Paper No. 47/5 — Utilization of Tropical Foods, Tropical Oil Seeds, FAO, Rome, 1989

Appendix 2

Information needed for the establishment of mycotoxin data base and country profile

1. Summary of available infrastructure to control mycotoxins in each country

- Number of laboratories/facilities existing in terms of trained manpower, equipment, etc. for analysis of mycotoxins.
- Type of mycotoxins analysed, methods of sampling, analysis, inter- and intra-laboratory quality assurance programs.
- Training courses conducted, number of participants trained, continuous education programs offered.
- Legislations on mycotoxins, tolerance levels, administrative machinery for enforcement, ministries concerned in implementation, involvement of provincial governments, voluntary compliance procedures.
- Number of inspections carried out, frequency, methods for inspection, compliance rate, number of samples found above limits, fate of such samples.
- Prosecutions initiated, convictions, cases pending, siezures, destructions, fines, warnings.
- International harmonisation efforts, participation in Codex meetings.
- Continuing education programs on mycotoxins, information base, mechanism of dissemination.

2. Trade implications of contamination by mycotoxins

- High risk commodities for mycotoxins from trade angle.
- Total volume of export/import of high risk commodities for mycotoxins quantity, value.
- Names of countries to which these commodities are exported, from where imported.
- Official limits for mycotoxins in trade/trade limits or buyers specification and the difference between the two.
- Places of analysis of mycotoxins for trade, whether they are part of multi-national chain.
- What are the levels encountered before export/after import, total number of samples analysed, total number found above limits.
- Discrepancy of levels when analysed at port of export and the port of import.
- Rate of rejection of commodities by importing countries, their quantity, value, or amount of discount offered for or penalty imposed because of contamination, total value.
- Ways of disposing of rejected commodity.
- The aflatoxin values of commodities analysed at port of import and the mechanism to collect such data from importing countries (Japan, EEC, and East European countries such as Poland gathers information of this type).
- The sampling plan at the port of export and port of import, the variation in the procedure of sampling.
- Conditions of storage at ports, conditions for shipping, containerisation, types of vessels used, normal flag of carrier.
- List of leading exporters, importers, surveyors, analysts.
- Attempts to calculate the economic costs, methodology used, including case studies conducted on losses in trade, in terms of animal health (e.g. poultry).

3. Exchange of information and experience of mycotoxin prevention and control in various countries

- List of institutions both in the private and public sectors in each country, names and addresses of workers.
- Identification of contact points in each country, subregion.
- Mode of compilation of 'grey literature' in the form of newspaper reports, circulars, government directives, newsletters, annual reports, meetings, seminars, unpublished data.
- Details of bilateral international programs on mycotoxins, their objectives, monitoring their progress (e.g. Japan–Thailand, Australia–Indonesia, Britain–Myanmar, FAO/UNEP, ICRISAT, CIMMYT).
- Role of NGOs scientific association in promoting mycotoxin prevention.
- Interfaces between ministries of agriculture, health, commerce, food and civil supplies.

Management of the Microflora and Their Toxins — Part 2 Session Summary

Chairman: Dr J.D. Miller, Plant Research Centre, Agriculture Canada, Ottawa
Rapporteur: Dr A.D. Hocking, CSIRO Division of Food Processing, Sydney, Australia

THE session comprised five papers; two on occurrence and significance of aflatoxins in the Philippines and Thailand; two on regulatory aspects, and one on networking.

The first paper, 'Aflatoxin Studies in the Philippines', by Raquel Quitco, reviewed aflatoxin studies in the Philippines from the earliest work in 1967, which identified maize, peanuts, and copra as high risk commodities. The importance of rapid drying for peanuts and maize was stressed as a strategy for minimising aflatoxin. Some food preparation methods, particularly washing followed by a heat process, could be beneficial in reducing aflatoxin load. There is a need for regulation, monitoring and incentive schemes, backed by adequate methodology, sampling, and training to reduce aflatoxin levels in susceptible commodities.

Discussion dealt with the question of aflatoxin in cassava and the problems of interfering compounds, and methodology for testing for resistance in peanut varieties.

The second paper, 'Aflatoxin in Maize in Thailand', was presented by Ms Prisnar Siriacha. This paper outlined the complexities of the maize marketing system in Thailand, and the critical steps for aflatoxin buildup. Thai maize is almost aflatoxin free at harvest in most seasons, but because *A. flavus* is already present, aflatoxins can build up rapidly if there are delays before shelling and drying. Many methods of shelling caused kernel damage with consequent aflatoxin buildup. The National Committee to Solve Mycotoxin Problems in Agricultural Commodities, established in 1985, recommended 1–2 weeks field drying after maturity to reduce aflatoxin levels. There has been little progress in breeding of resistant varieties of maize. Studies showed that high moisture maize (36.9%) could be kept in airtight storage without mould damage or aflatoxin build up for 28 days.

Discussion covered the absence of *A. parasiticus* from Thailand, and the questions this raised regarding the use of this species for testing for resistance in cultivars. The potential problems of introducing this highly toxigenic fungus were pointed out. There was also some discussion of the agronomic practices used by Thai farmers, such as burning fields after the maize crop, which may help reduce populations of *A. flavus* in soil.

The next two papers covered regulatory aspects of aflatoxins in foods and feeds. Dr J. Gilbert's paper, 'Regulatory Aspects of Mycotoxins in the EC and USA', outlined the complex set of regulations governing acceptable limits for both total aflatoxins and aflatoxin B_1 in foods and animal feeds in the EC. The question of whether different limits should be applied to bulk and retail commodities was raised. Sampling plans should be not only clearly stipulated and soundly based, but also practical, and should provide an acceptable balance between risk to the consumer and the producer. For analysis methodology, certain steps (e.g. homogenisation and sub-sampling) need to be specified, but the actual analysis should just be that which is needed to meet certain performance criteria without being tied to a particular recommended method. Dr Gilbert then outlined U.K. surveillance systems for aflatoxins in figs, pistachio nuts, retail peanut butter, and aflatoxin M_1 in milk.

Discussion covered sampling schemes for figs, designing sampling plans to ensure balance between risks to consumer and producer, consumer reactions to aflatoxins in foods, and the problems of re entry of rejected shipments through ports with less stringent surveillance.

The second paper, entitled 'Regulatory Aspects of Mycotoxins in Asia and Africa', and presented by Dr H. van Egmond, dealt with factors influencing the establishment of regulations, outlined the current

mycotoxin (mostly aflatoxin) regulations around the world, and pointed out the discrepancies in these regulations. There is a need for rationalisation of aflatoxin tolerances worldwide, with a re evaluation of the hazards to provide a rational basis for setting limits. If it is to be effective, legislation needs to be realistic.

The discussion following this paper covered many aspects of the rationale behind setting mycotoxin limits, and the difficulties of carrying out risk-assessment studies. Canada has set mycotoxin limits based on thorough risk assessment studies, but many countries set limits on political rather than scientific bases.

The final paper in this session was 'Communication, Networking and Collaboration among Mycologists and Mycotoxicologists', by Mr R.J. Dawson. Mr Dawson outlined the concept of networking as an efficient means of providing assistance. He described the activities of various bodies, including the Network on Food Control in Asia, involving institutes in India, Thailand, Malaysia, Indonesia, and the People's Republic of China, and the ASEAN Region Network on Mycotoxin Control, created in 1987, which involves institutes in India, Thailand, and the Philippines. In these networks, an institute in each country is responsible for a particular aspect of the network, for example, training in analytical methods, sampling, or the training of extension and management personnel. Such activities are backed up by databases within the networks, and publications such as FAO manuals.

Discussion raised the bringing of newly emerging issues in mycotoxicology to the workers in developing nations. This can be accomplished in part by FAO financial support for attendance at conferences and study tours.

There was a structured discussion on 'Research and Development Needs in Storage Mycology and Mycotoxicology' following presentation of the five papers. The panel consisted of Dr R. Coker, Mr R. Dawson, Dr J.D. Miller, and Dr J.I. Pitt. The following were identified as important areas in need of more work:

- improvement and standardisation of methodology for detecting fungi in foods;
- culture collections in the ASEAN region;
- more information on *Fusarium* toxins and their significance; and
- better understanding of the fundamental mechanisms by which toxigenic fungi invade grains, either before or after harvest, and reduction of soil inocula.

Analysis of all options was needed to better direct monetary resources, and not repeat the mistakes of the past.

The worth of continued research into resistant cultivars was examined. There has been little progress in 25 years, but with powerful new genetic techniques now becoming available, this may change. The distribution of *A. parasiticus* was discussed, and grave concern expressed about its introduction (for the purpose of testing for cultivar resistance) into areas previously free of this highly toxigenic fungus.

The need for more accurate knowledge on the economic impact of mycotoxins was identified as an area for further investigation. This is now being done for food borne illness, and perhaps similar methods could be applied to mycotoxins. Better information in this area will add incentive to mycotoxin research, improve the quality of foods, particularly export commodities, and aid harmonisation of international standards.

The fate of mycotoxin-contaminated food or feed was examined. Contaminated commodities can be processed into oil, but this often produces highly contaminated presscake. It can be fed to beef cattle in some parts, as they are highly resistant to aflatoxins. There is a need for investigation of better methods, e.g. use of binding agents, detoxification.

The chairman, Dr Miller, closed the discussion by summarising it with two points: we need to provide better education and training for the people who need to manage these problems; and we need to be more proactive in elimination of the problems. Methods of attack include studying the local ecology and deciding which technologies are most applicable.

Poster Papers

Survey of Fungi and Mycotoxins Associated with Maize and other Commodities in Thailand

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FUNGI can cause two quite distinct, though not unrelated problems, in stored commodities: spoilage resulting from fungal growth, and mycotoxin formation. Mould growth in grains may cause loss of weight, loss of nutritive value, poor milling quality, or deterioration in flavour, colour, or other organoleptic properties. Losses from spoilage or down grading, though a much less spectacular problem than mycotoxicoses, may be of greater economic significance. Spoilage and down grading may be caused by the growth of fungi, such as *Fusarium* or *Alternaria*, before harvest, or may be due to inadequate drying or mishandling in storage, where fungi such as *Aspergillus* or *Penicillium* are likely invaders.

Mycotoxins may be produced by fungi when they grow in foods or feeds. They may be acute or chronic diseases, cancer, tumours or teratogenic effects. Because weather damaged grains, which are often invaded by toxigenic fungi, are commonly used for animal feeds, the risk of mycotoxins in animal feeds is high in many countries. Fungi and mycotoxins have taken a high profile in Asia in recent years following recognition of the serious problems that have resulted from their toxicity and carcinogenicity both in food and feed stuffs.

One of the major aims of ACIAR Project 8806 is to study the distribution, prevalence and importance of postharvest fungi in durable commodities in Thailand, in relation to both spoilage and mycotoxin production, over a period of three years. The project commenced in October, 1988.

Samples of Thai commodities were examined to assess their status as purchased by the grain handlers and central grain storage systems, and to assess the influence of grain handling procedures on mould development and mycotoxin production. Five climatically different regions were sampled: 1, the central area (Provinces of Saraburi, Lopburi, and Nakorn Sawan); 2, the far northern area where the climate is colder (Chiangrai); 3, the north central area (Petchaboon); 4, the drier north central area (Leoy); and 5, the dry, eastern upland region (Nakon Rajahasima).

Commodities examined included mainly maize, sorghum, soy beans and smaller numbers of samples of mung beans, wheat, paddy rice, cashews, peanuts and copra. Between October, 1988 and April 1991, a total of 367 samples from field, farm and storage has been examined mycologically. A further 233 retail samples have also been analysed. Mycotoxin testing has been carried out where mycological result indicated potential mycotoxin contamination. Over the period, an estimated 14,000 fungi have been isolated, and more than 15% of these have been subjected to full identification procedures.

Table 1 shows the species of fungi most commonly isolated from field, farm and storage samples of the major commodities surveyed. Eighty five percent of maize kernels contained some mould. The majority of species isolated were field fungi, e.g. *Alternaria*, *Lasiodiplodia*, and *Nigrospora* species, however, *F. moniliforme* was very common in many maize samples. This species is capable of forming the mycotoxins fumonisins and fusarins.

The level of infection of sorghum was not as high as the maize, with most species isolated being field fungi. Levels of storage or possible mycotoxigenic species were less than 20%. Soybeans contained very few storage fungi. Some *Aspergillus* species were isolated, but on the whole the level of contamination of the soybeans was among the lowest of any commodity examined. The major field fungi present were *Epicoccum nigrum*, *Nigrospora oryzae*, and *Cladosporium cladosporioides*.

Table 1. Fungi isolated from field samples received from Thailand, 1989-90

Commodity	Major species isolated
Maize (143 samples)	*Fusarium moniliforme, Nigrospora oryzae, Cladosporium cladosporioides, Aspergillus flavus, A. niger, Curvularia pallescens, C. lunata, Chaetomium* spp., *Penicillium citrinum, P. funiculosum*
Cashews (37 samples)	*Cladosporium cladosporioides, Nigrospora oryzae, Chaetomium* spp., *Eurotium chevalieri, E. rubrum, E. amstelodami*
Cassava (9 samples)	*Lasiodiplodia theobromae, Nigrospora oryzae, Phoma* sp.
Copra (21 samples)	*Nigrospora oryzae, Aspergillus flavus, A. tamarii, A. niger, Endomyces fibuliger*
Peanuts (39 samples)	*Aspergillus niger, A. flavus, Cladosporium cladosporioides, Lasiodiplodia theobromae, Penicillium pinophilum, Chaetomium* spp.
Rice (18 samples)	*Fusarium semitectum, Alternaria padwickii, Cladosporium cladosporioides, Nigrospora oryzae, Curvularia lunata*
Sorghum (46 samples)	*Curvularia lunata, C. pallescens, Fusarium semitectum, F. moniliforme, Nigrospora oryzae, Bipolaris maydis, Cladosporium cladosporioides, Eurotium chevalieri, E. rubrum, Aspergillus niger, A. flavus*
Soy beans (34 samples)	*Cladosporium cladosporioides, Chaetomium* spp., *Epicoccum nigrum, Fusarium semitectum, Nigrospora oryzae, Arthrinum phaeospermum, Penicillium citrinum, Eurotium rubrum, Aspergillus restrictus, A. penicillioides*

Cashew nut samples were collected from a middle man operation in Saraburi. The most commonly isolated species were *Cladosporium cladosporioides, Nigrospora oryzae*, and a number of *Chaetomium* species. Paddy rice, mung beans and soybeans were often highly contaminated with *Fusarium semitectum.* Ninety percent of peanut kernels examined were contaminated with *Aspergillus niger*; only 20% were contaminated with *Aspergillus flavus.*

Table 2 shows the dominant fungal species isolated from retail commodities from Thailand during 1990. Retail samples showed a higher rate of storage fungi than samples collected from the field and farm. Of the retail peanut samples examined, 85% contained A. flavus, with some samples being highly infected. *Eurotium* species were also common on many retail samples.

Mycotoxins. A large number of samples was tested from the presence of a range of toxins by a thin layer chromatography screening procedure. However, in most cases, the only toxins detected were aflatoxins. During 1990, the following samples were tested for mycotoxins: maize (35), sorghum (8), soybeans (7), peanuts (3), rice (1) and cassava (3). Twenty one of the maize samples contained unacceptable levels of aflatoxins, two being in excess of 1000 µg/k. Two sorghum samples contained aflatoxins, and one was positive for ochratoxin A. The absence of G aflatoxins in the maize samples indicated the presence of A. flavus only, in agreement with mycological data.

Of 39 retail samples of peanuts from Thailand assayed for aflatoxins, 10 (25%) were positive and 8 contained more than 15 µg/kg, with >2000 µg/kg detected in one sample. The samples examined were of good appearance visually, and apparently had been hand sorted. Levels in excess of 200 µg/kg total aflatoxin in 3 samples indicates a real problem. Three samples contained G aflatoxins: in the absence of *A. parasiticus*, the source of these toxins appears to be an undescribed species closely related to *A. flavus.*

Other retail samples from Thailand were also tested for mycotoxins: black beans (5), soy beans (4), black rice (3), mung beans (1), red beans (1), rice (1) and black sesame seed (1). None of these samples tested positive for aflatoxin.

Table 2. Fungi isolated from retail commodities purchased in Thailand

Commodity	Major species isolated
Black beans	*Fusarium semitectum, Aspergillus wentii, A. flavus*
Mung Beans	*F. semitectum, A. flavus, Rhizopus oryzae*
Soy beans	*A. flavus, Curvularia lunata, Penicillium citrinum, Eurotium chevalieri, E. amstelodami, E. rubrum*
Red beans	*A. restrictus, A. wentii, A. ochraceus, E. rubrum, F. semitectum, Phoma* sp.
Black rice	*Chaetomium* sp., *E. rubrum, E. chevalieri, Alternaria padwickii*
Cashews	*Aspergillus niger, Nigrospora oryzae*
Peanuts	*A. niger, A. wentii, A. flavus, A. tamarii, P. citrinum, Papulaspora* sp.
Rice	*E. rubrum, Alternaria alternata, C. cladosporioides*
Sesame seeds	*Penicillium chrysogenum, A. flavus, Papulaspora* sp.

Problems and Perspectives of Spoilage Fungi and Mycotoxins in India

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INDIA, with its various agroclimatic regions, produces a variety of crops throughout the year. Of the 170 million tonnes of food grains produced annually, 70% are retained by the producers and stored under poor conditions for considerable periods. Extended storage in hot and humid conditions predisposes the produce to insect infestation, growth of spoilage fungi, and production of mycotoxins. In some cases, crops are prone to unexpected rains and floods, leading to damage by fungi even before harvest.

Rice is one of the most important crops grown during June–October in various parts of the country. At the time of harvest in October, the coastal regions of Tamil Nadu and Andhra Pradesh experience heavy rains which interfere with harvesting and drying of crops. In these circumstances, field fungi of the genus *Fusarium* attack the grain which has a moisture content of 18–20%. Rice straw is also infected and mould growth causes discoloration, making it unfit for animal feed.

In Assam, the rice crop is sometimes inundated by flood waters, leading to infection by fungi and sprouting of grains. Moreover, since parboiling of rice is a common practice in India, the moisture in the grain permits the growth of fungi before storage.

In Madhya Pradesh, the most important crop is soybean, which is harvested during October–November. Most of the harvested crop is exposed to rain in the threshing yard and, since soybeans are rich in protein and oil, the development of spoilage fungi and the production of mycotoxins are quite common. The damaged seeds have either very low or no germination (Table 1).

Peanuts are sown during March–April in Tamil Nadu and are ready for harvest in August. Studies on aflatoxin in peanuts at harvest revealed that *Aspergillus flavus* build-up occurs in the roots and root zone. However, harvesting in most years coincides with south west monsoon showers which interfere with proper drying of pods and haulms. Under such conditions the development of field fungi, *Fusarium* on pods and *Alternaria* on haulms, causes deterioration in quality of the produce.

In the eastern parts of India, where humidity is in the range 80–90% during June–September, aflatoxin contamination in stored rice is common. Among samples collected from various sources, such as farmers' storages, shops, and the public distribution system, unmilled rice (paddy) was relatively less infested than milled rice (Anon. 1990). Whereas just 6–9% of milled rice samples from farmers' storages were contaminated with aflatoxin to a level of 50 ppb, 29% of the samples from shops were contaminated to a level of 100 ppb (Table 2).

In the north of India, though the moisture content of wheat at harvest is only 6–8%, there is considerable increase in moisture content (12–14%) during the rainy season (June–September). This predisposes the grain to infestation by insects such as *Sitophilus oryzae* and *Rhyzopertha dominica*. The activity of insects in storage pushes up the moisture content to 16–18%, paving the way for the development of fungi, especially *Aspergillus flavus*, and leading to aflatoxin levels of 30 ppb. The presence of straw and broken grain also facilitates early development of insects and growth of fungi which later also invade the whole grain (Anon. 1990). Sinha and Sinha (1990) observed that *Aspergillus flavus* infection in insect-damaged samples was 87%, while in insect-free samples it was 25%. *A. flavus*, *Rhizopus*, *Penicillium*, *Tricothecium*, and *Fusarium* were found on stored spices (Geeta and Reddy 1990). *A. flavus* was found mainly on ginger, mustard, garlic, and pepper. The highest fungal counts (10.6×10^4/g) occurred in stored pepper.

Storage of various qualities of peanut kernels for a period of 6 months indicated that the brokens were

Table 1. Germination levels (mean %) of fungicide treated and untreated stored soybean seeds

Storage period	Treatments			
	Thiram	Bavistin	TcMTB	Control
One month	71	74	74	70
Two months	70	72	74	63
Three months	70	71	71	42
Four months	64	66	67	20
Five months	60	63	64	15

Table 2. Extent of aflatoxin contamination in paddy, milled rice, and byproducts

Commodity	No. of samples	Samples contaminated		% contamination		Aflatoxin level (ppb)
		initial	final	initial	final	
Paddy (farmers' stores)	32	2	6	6	19	20–100
Rice						
Farmers' stores	16	–	1	–	6	20–50
Shops	22	2	2	9	9	20–50
Godowns	24	6	7	25	29	20–100
Bran	14	2	4	14	29	20–100

infested at the end of 2 months by *Corcyra cephalonica* (Asaf Ali et al. 1977), the infestation causing webbing of the produce, growth of fungi, and production of aflatoxin.

Studies of the equilibrium moisture content for safe storage of copra for both absorption and desorption conditions at 18, 31, and 41°C and at various humidities, revealed that copra with a moisture content corresponding to 70% relative humidity cannot be stored safely. There was growth of *Aspergillus flavus* and *Penicillium* sp., and the level of aflatoxin reached 40–60 ppb within a storage period of 2 months. It was also observed that infestation by *Tribolium castaneum* and *Oryzaephilus surinamensis* exacerbated the growth of fungi and, in turn, the production of aflatoxin in stored copra (Anon. 1990).

In India, several agricultural commodities, including rice, sorghum, oilseeds, and pulses, often show fungal contamination and the presence of toxic metabolites such as aflatoxin (Singh et al. 1983). The aflatoxin content is highest in peanuts and its products. The toxin content of crude peanut oil varies from 20–100 mg/kg, depending on the quality of the kernels used for extraction. India has adopted the FAO/WHO maximum tolerance limit of 30 mg/kg for aflatoxin in peanut meal.

The following techniques for reducing aflatoxin levels in staple grains have been investigated.

1. Advancing the stage of harvest

It has been found that when rice crops are physiologically mature, a spray of 5% salt solution helps advance the harvest stage. As this procedure helps to reduce the moisture content of grains and straw, the possibility of infection by spoilage fungi is reduced.

2. Cleaning and separation

In most warehouses, mills, and markets, depending on the quantity of grain handled, either manual labour or air screen cleaners are generally used to remove the broken and infected kernels. The process indirectly reduces the chances of insect infestation, fungal growth, and production of mycotoxins in soybean, maize, and other crops. Steps are also being taken to introduce gravity separators at procurement centres, so as to ensure efficient separation of the good and inferior quality grain.

3. Safe storage moisture

In storage of oilseeds, the survival of *Ephestia cautella* grain moths was drastically reduced at 6% moisture content, as was, in turn, the growth of fungi. The safe moisture range for soybean storage was found to be 8–9%, for gram 8–10%, and for wheat 10–12%.

4. Solvent extraction of oil

Aflatoxins are insoluble in water, but soluble in organic solvents. It was found that the aflatoxin content of peanut meal can be reduced from 110 ppb to 8 ppb by extraction with 10% aqueous acetone at a temperature of 120°C (Pruthi 1978). Most of the modern oil extraction units in the country now use solvent extraction, which helps to reduce the mycotoxin levels in the oil produced.

5. Detoxification of aflatoxin

Studies in India on the effect of various methods of detoxification on the aflatoxin content of peanut oil cake (Table 3) revealed that, though heat treatment of oil cake at 135°C for 2 hours reduced the aflatoxin content, it also affected the nutritional quality of the cakes. Treatment with hydrochloric acid or hydrogen peroxide has thus been adopted.

Table 3 Percent reduction in aflatoxin content of peanut oil cake following various methods of detoxification

Methods	Initial ppb	Final ppb	Percent reduction
Heat treatment at 135°C for 2 hours	8 × 103	1 × 103	88
Hexane + ethanol (79:21) for 1 hour	10 × 103	2 × 103	80
Hexane + ethanol + water (72:15:13) for 1 hour	12 ×103	3 × 103	75
1% calcium chloride for 1 hour	11 × 103	4 × 103	64
1% sodium bicarbonate for 1 hour	9 × 103	5 × 103	44
Hydrogen peroxide (6%) for 30 minutes at 80°C	12 × 103	2 × 103	83
2% sodium hydroxide for 24 hours at normal temperature	11 × 103	2 × 103	82
5% hydrochloric acid for 1 hour	13 × 103	2 × 103	85
2% sodium chloride for 1 hour at 80°C	12 × 103	5 × 103	58

Infection by spoilage fungi causes deterioration in seed quality, especially in soybeans. After oil extraction, soybean meal can be exported to other countries to earn foreign exchange. However, if fungal infection in seeds affects the quality of meals also, serious economic losses result. The same applies to stored spices, because India is already exporting large quantities of these commodities to other countries.

Physical and chemical methods of aflatoxin detoxification are being used. Steps may be taken to identify chemicals of plant origin that may reduce the aflatoxin levels in food and feedstuffs at lower cost and with greater convenience.

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Mycotoxin Contamination of Peanuts after Harvest in Sukabami, West Java, Indonesia

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A study was undertaken to assess the extent of mycotoxin contamination in peanuts after harvest. Twenty samples of peanuts collected from local farmers and 80 from markets were analysed for aflatoxins, cyclopiazonic acid, and ochratoxin A.

All samples contained aflatoxin B_1, B_2, G_1, and G_2, but none contained cyclopiazonic acid. Traces of ochratoxin may have been present in some samples. The lowest total aflatoxin levels detected were 126 ppb in farmer samples and 81 ppb in market samples, while the highest were 1603 and 14565 ppb, respectively. Levels of aflatoxin contamination were higher in market than in local farmer samples.

Microbiological Quality of Paddy during In-store Drying

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METHODS developed for in-store drying in temperate regions have been technically successful. However, the microbiological status of the grain in the system needs to be assessed, as commodities which carry high microbial loads can deteriorate more rapidly, particularly in humid tropical climates. In-store drying has been carried out on partially dried long grain paddy of $17 \pm 1\%$ moisture content in both dry and wet seasons under humid tropical conditions in Malaysia. It has been shown that in-store drying can maintain the microbiological quality of paddy in bulk storage in both seasons. No significant increase was observed in the total number of viable mesophilic bacteria, moulds or yeasts, and *Aspergillus flavus*. However, if loading was delayed, the microbiological quality of the paddy was affected even in the dry season. A 100 times increase in *Aspergillus flavus* was detected in the dry season when it took 7 days to fill a 750-tonne storage bin.

Control of Storage Fungi through Heat Treatment of Wet Paddy Grains

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HARVESTING times for multi-seasonal paddy (rough rice) crops fall during the wet season, except for one crop which is harvested in summer. Paddy is generally harvested at moisture contents (m.c.) of 18–20%. However, during adverse weather conditions, moisture contents above 28% may prevail. If paddy grains above 18% m.c. are not dried immediately, deterioration of grain quality occurs as a consequence of biological activity of the grain itself (i.e. respiration), and of microorganisms that infest it (Shukla and Stickney 1985). The basic cause of deterioration of wet paddy is growth of fungi. Several methods have therefore been suggested for reducing fungal deterioration of paddy.

Sun drying of crops is traditionally practiced in all Asian rice-growing countries. However, during adverse weather sun drying is unreliable. Application of fungicides has been suggested by Calverley (1968), Bandong et al. (1982), and Kuthubutheen (1984). Dewatering of wet paddy in a 4–5% dry salt solution has been suggested by Shivanna and Kudarathulla (1971), and use of bleaching powder has been recommended by Subrahmanyan (1972) for reduction of fungi in wet paddy grains. Application of potassium chromate has been suggested by Desikachar et al. (1976)and salt solution treatment, combined with other chemical treatment, has also been suggested by Anthoni Raj (1980) to prevent deterioration in wet paddy grains. None of these methods has yielded a permanent solution to the problem. There is thus scope for investigation of heat treatment as a possible method of controlling storage fungi.

It has been reported by Joarder and Majumdar (1981) and Rukundin and Man (1982) that biological activity in wet paddy may be reduced by brief heat treatment. At a moderate temperature (60°C and above) it was found that most fungi and other organisms are either inactivated or killed. Shukla and Stickney (1985) also reported that heating the paddy grains (24% initial m.c.) for 5 minutes at 110–125°C on a hot plate markedly reduced the growth of fungi during storage. Similar results were obtained on heating the wet paddy in an air oven for 5 minutes at 100°C. However, practical application of these methods is not feasible under field conditions in Asia. A more convenient way of handling the wet paddy after threshing was then developed. The threshed paddy was treated with hot water then held for a period of 120 hours. This was found to be as effective in controlling fungi as the methods used in the earlier experiments by Shukla and Stickney (1985).

The method was found to be feasible at times of adverse weather during harvesting. Grain harvested in rainy weather already carries moisture contents above 35% and rewetting during hot water treatment adds little more water. Moreover, the method is not expensive and is within the reach of ordinary farmers in India. Large-scale trials were therefore carried out.

A furnace for heating the water in a barrel was developed. It is fired using wastes — brokens and dust particles or rice straw, or any other agricultural waste — as fuel. The barrel, which contains about 400–500 litres water, is readily available in local markets. The amount of water in the barrel was kept at a level such that a 50 kg jute bag of paddy can be easily immersed. The water in the barrel was heated to a temperature of about 98°C. The paddy was loaded into the bag to 90% capacity. With the help of two hooks, the bag was then immersed in hot water and left for 5 minutes.

There was enough space in the bag (since it was loaded to only 90% capacity) for the whole mass of paddy grains to attain a suspended state and thus for almost every grain to be in contact with hot water during the treatment period. A drop of 2–3°C in water temperature was observed after the immersion of the bag of

paddy. This drop in temperature was insignificant as the fire under the barrel was maintained during the full course of the experiment. Thus, the paddy in bag was treated at about 95°C for five minutes. At the end of the treatment, the paddy bag was removed from the barrel and kept in a godown on a wooden rack for 4–5 days. Bags of freshly harvested paddy were also kept in the same store on similar racks for the same period of time. Samples from the top, middle, and bottom of the treated and untreated bags were taken during the storage period and analysed for fungal growth. The standard blotter technique of the International Seed Testing Association was followed for fungal analysis of the samples. Results are shown in Table 1.

Table 1. Presence of pathogens in fresh and hot-water treated paddy before and after storage for 120 days

Pathogens	Fresh paddy before storage	Treated paddy before storage	Fresh paddy after storage for 120 days	Treated paddy after storage for 120 days
Alternaria longissima	20	1	17	1
Anguillospora spp.	2	–	4	–
Aspergillus spp.	1	–	1	–
Cephalosporium	3	–	4	–
Cercospora oryzae	10	2	18	2
Cardana spp.	1	1	2	2
Corynespora spp.	50	1	59	1
Curvularia spp.	48	1	57	1
Drechslera oryzae	3	–	4	–
Drechslera rostrata	5	–	8	–
Drechslera sorokiniana	1	–	1	–
Drechslera spp.	–	–	–	–
Fusarium equiseti	4	–	10	–
Fusarium moniliforme	45	1	65	1
Fusarium semitectum	–	–	40	3
Gerlachie oryzae	–	–	3	–
Neurospora oryzae	1	–	5	1
Nigrospora oryzae	10	1	10	1
Penicillium spp.	–	–	1	–
Periconia spp.	12	2	16	3
Phoma spp.	6	–	2	–
Phyllosticta glumarum	–	–	7	–
Pethomyus spp.	–	–	1	–
Pyricularia oryzae	–	–	30	1
Sarocladium oryzae	15	2	20	2
Trichonicella padwickii	26	1	28	–

Fungi were reduced to near zero levels after the heat treatment, and these levels were maintained during the storage period (Table 1). However, in the control sample the incidence of fungi increased. Only a few fungi such as *Fusarium semitectum* were detected in freshly harvested paddy, but were found in treated paddy only after the storage period. The reason for their growth after hot water treatment is not understood.

The freshly harvested paddy had a moisture content of 35%, and there was almost no reduction in moisture at the end of the storage period. During the storage period the temperature and humidity were 32–35°C and 85–90%. No discoloration of kernels was observed at the end of storage period in treated paddy whereas some grains in the untreated paddy were blackened.

It is concluded that this heat treatment method has potential for protecting wet paddy from spoilage. The method is cheap enough to be within the reach of farmers. There is a need to develop equipment for application of this method suited to the resources of farmers.

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Comparative Toxicity of *Fusarium* Species

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FUSARIUM species are known to produce highly toxic mycotoxins which are associated with a number of animal diseases. These are most commonly oestrogenic and feed refusal syndromes (Marasas et al. 1984). In this communication, the toxicities of Fusaria in section Gibbosum (*F. acuminatum, F. compactum, F. equiseti, F. longipes,* and *F. scirpi*) were assessed. *F. equiseti* is known to produce a metabolite, fusarochromanone, which can induce tibial dyschondroplasia (T.D.) in chickens (Walser 1987) and *F. compactum* has been implicated in deaths of large numbers of sandhill cranes feeding on naturally contaminated peanuts (Cole et al. 1988). There is little information on the toxicities of the other species in this section.

Cultures of these species were obtained from soil samples collected in northern and eastern Australia. A total of 206 isolates was selected for toxicity testing in a chick bioassay. Cultures were grown on 85 g of crushed malted whole wheat biscuits, moistened with 50 mL of water and grown at 25°C in the dark for 2 weeks. Mycotoxin extraction and bioassay procedures followed those of Kirksey and Cole (1974). Four chickens were dosed per culture and housed in cages situated in an air-conditioned (32°C), continuously illuminated room. A commercial chick feed and water were provided *ad libitum* for the 4 days of the bioassay. Mortality was assessed daily. Results are tabulated below.

Species	No. of isolates	% toxicity
F. compactum	34	95
F. acuminatum	42	85
F. scirpi	6	58
F. equiseti	114	11
F. longipes	10	5

F. compactum and *F. acuminatum* were highly toxic, whereas *F. equiseti* and *F. longipes* showed the least toxicity. Preliminary chemical analysis has indicated the presence of trichothecenes in toxic extracts of *F. acuminatum*. Isoneosolaniol and other unidentified tricothecenes have been identified previously in toxic extracts of *F. compactum* (Cole et al. 1988). The extraction procedure used in this study would not have assessed the possible production of polar compounds, such as those causing T.D., by these *Fusarium* species.

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Toxicity of Feed Grain Moulded with *Alternaria*

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MOULDS of the genus *Alternaria* are ubiquitous in nature, with many being important plant pathogens that cause spoilage of commercially important commodities (Watson 1984). *Alternaria* produces an array of toxic secondary metabolites including alternariol and its monomethyl ether, tenuazonic acid, altenuene, and altertoxins (Watson 1984; Blaney 1991). There are also reports that feed grain may from time to time contain *Alternaria* toxins which, if ingested, could give rise to a toxicosis and reduced productivity (Bryden and Burgess 1985; Andrews and Lukas 1988).

The genus *Alternaria* is the most common fungal contaminant of Australian cereal grains (Klein 1987; Ali et al. 1991) but there is little information on the toxigenicity of Australian *Alternaria* isolates. In these studies, grain (barley, rice, sorghum, and wheat) samples collected in New South Wales were surface sterilised and *Alternaria* isolated (200 isolates) in pure culture. The isolates were incubated for 3 weeks at 24°C on wheat and then extracted with 80% acetone. The extract was intubated into the crop of day-old chickens. After dosing, the chicks were observed for 14 days. Bioassay results showed that 13% of the isolates were highly toxic to chicks, causing deaths within 3 days of dosing. The majority of isolates, however, were chronically toxic and caused a reduction in growth (63%) and feed conversion efficiency (67%). Preliminary analysis of the acetone extracts of a number of isolates that caused mortality (toxic isolates) and others that had no apparent effect (non-toxic isolates) showed that the toxic isolates produce *Alternaria* toxins in much higher concentrations (Bakau 1990).

In subsequent studies, extracts of naturally moulded white and red sorghum and clean white sorghum were tested for acute toxicity in a chick bioassay as described above. The sorghums were also fed to male broiler chickens and weaner pigs. Neither the chicks intubated with extracts of naturally moulded sorghum nor the pigs fed mouldy sorghum showed any signs of intoxication. However, the chickens fed mouldy white sorghum gained less weight ($P < 0.05$) and consumed less feed ($P < 0.05$) than chickens fed clean sorghum. Feed conversion efficiency was the same between the sorghum diets and an increase ($(P < 0.05)$ in relative liver weight of chickens fed the mouldy white sorghum was the only significant macroscopic effect noted at necropsy. Moulding of sorghum had no effect on the amino acid profile of the grain but did cause a reduction in the metabolisable energy content of the white sorghum.

Alternaria is a common contaminant of cereal grain and most isolates are toxigenic. Animals ingesting *Alternaria*-moulded feed grains may suffer production drops.

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Determination of Ochratoxin A in Cereal Grains in Thailand

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CEREAL grains from various parts of Thailand were investigated for ochratoxin A after harvest. A modification of published analytical methods was used. This involved extraction of samples in organic solvent mixture, partial purification by partition in basic and acidic solutions, and final clean-up by celite column. Using this method, the samples were cleaned sufficiently to permit unambiguous interpretation of ochratoxin contamination. It was not necessary to confirm the presence of ochratoxin by derivatisation.

Analysis of data provided a basis for determining the initial magnitude of ochratoxin contamination. The method will be used for monitoring commodity ochratoxin levels.

Detection of Aflatoxin B_1 by Enzyme-Linked Immunosorbent Assay in Thailand

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A specific microtest plate enzyme immunoassay has been developed in Thailand for the rapid detection of aflatoxin B_1 at concentrations as low as 0.1 ppb per assay. Two methods for immunisation were tested for the production of antiserum. Multiple-site injection of rabbit backs with an aflatoxin B_1 carboxymethyloxine-bovine serum albumen conjugate, followed by monthly booster injection in tight muscle, gave an antiserum titre up to 1:15 000. Intramuscular injection with an aflatoxin B_1–CMO–BSA conjugate, followed by intraveinal booster injection, gave similar titres.

Dilution 1:5000 of purified antibody was used for microplate coating in the direct competitive enzyme-linked immunosorbent assay (ELISA) for aflatoxin B_1 detection. The sample extract was then incubated with aflatoxin B_1–carboxymethyloxine–horseradish peroxidase conjugate for 20 minutes. The amount of enzyme bound to antibody was determined by the change in absorbance at 490 nm after the addition of O–phenylenediamine substrate. The reaction was stopped with 0.1 N HCl. Aflatoxin B_1 concentration in test samples was calculated by comparison with the standard curve of aflatoxin B_1.

The ELISA technique shows good potential for aflatoxin B_1 detection in agricultural commodities in Thailand.

Monoclonal Antibodies for the Detection of Fungi in Stored Products

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CONVENTIONAL methods for detecting fungi causing postharvest spoilage are generally time-consuming, require considerable expertise to interpret results, and depend on isolation of these organisms from infected material. Information obtained by these methods cannot give a clear picture of the extent of mycelial growth of fungi. Quick, user friendly assays are now needed to detect and quantify fungi involved in postharvest spoilage.

Immunoassays have usually been applied to the detection of pathogenic organisms. This paper describes the application of simple immunodiagnostic assays (Dip-stick and ELISA) for the detection and quantification of fungi in cereal grains.

The immunoassays use monoclonal antibodies (mAbs) raised to two fungi associated with postharvest spoilage of rice: *Penicillium islandicum*, a potentially toxigenic fungus, and *Humicola lanuginosa*, a thermophilic fungus causing heating in damp harvested paddy.

Immunoassays

Mice were immunised by injecting directly into the peritoneum cell-free surface washings of agar slant cultures of *P. islandicum* and *H. lanuginosa*. Hybridoma supernatants were screened by ELISA. Most mAbs raised cross-reacted with other storage fungi and/or uninfected rice grains but three of the *P. islandicum* mAbs were species specific. One IgM antibody (EC6) to *H. lanuginosa* did not recognise antigens from rice grains and cross-reacted significantly with only one other fungus — *P. variabile*.

Immunofluorescence studies showed that the mAb PI01, which is an IgG1 antibody raised against *P. islandicum*, binds to fungal antigens present in the hyphal wall region. The IgM mAb EC6 raised against *H. lanuginosa* also binds to fungal antigens present in hyphal walls and immature aleuriospores.

Immunodiagnostic assays (ELISA and Dip-stick assay) have been developed using these mAbs (PI01 and EC6) to detect *P. islandicum* and *H. lanuginosa* in rice grains.

Comparison of Conventional Detection Methods with Immunoassays

Sterilised rough rice grains (300 g, 24% moisture content) were inoculated with 4×10^5 spores of *H. lanuginosa*. Growth of *H. lanuginosa* at 45°C was monitored over 7 days using:

(a) direct plating of untreated and surface sterilised grains on Yeast Starch agar (Fig. 1).;

(b) ergosterol content by the HPLC method of Seitz (Fig. 2); and

(c) Dip-stick and ELISA immunoassays (Figs 1 and 2).

Ergosterol, a sterol found in fungi and not in higher plants, can be used to measure fungal biomass. ELISA and ergosterol content can both be used to quantify fungal biomass (Fig. 2), while the Dip-stick immunoassay and direct plating can be used to measure % infection of individual grains by fungi (Fig. 1).

Figures 1 and 2 show that:

(i) growth of *H. lanuginosa* was detected after 1 day by ELISA, Dip-stick, and direct plating of untreated grains;

(ii) by day 2 there was a significant rise in ergosterol content, ELISA response and direct plating of surface sterilised grains indicating penetration of the grain by fungal mycelium;

(iii) by day 5, ELISA, ergosterol, and direct plating results had approached their maxima; and

(iv) ELISA and Dip-stick assay results for pre and post-inoculation showed no response, while direct plating indicated 40% grains infected.

Results indicate that ELISA and Dip-stick assays can be successfully used to detect growth of *H. lanuginosa* at an earlier stage than would be possible by either monitoring ergosterol content or by direct plating of surface-sterilised grains. The immunoassays have additional advantages in rapidity and specificity with results obtained within 24 hours.

The Dip-stick assay shows potential as a simple field test kit requiring no laboratory facilities other than reagents and a coolbox. The ELISA is a more sensitive assay allowing quantification of fungal mycelium rather than presence or absence, as detected by the Dip-stick assay.

The binding of the two mAbs to mycelia but not to mature spores means that assays employing these mAbs can be used to detect active growth of the fungi and differentiate it from the presence of spores.

Such assays may improve interpretation of mycological data on spoilage problems and provide an early warning system for detection of deterioration due to fungi.

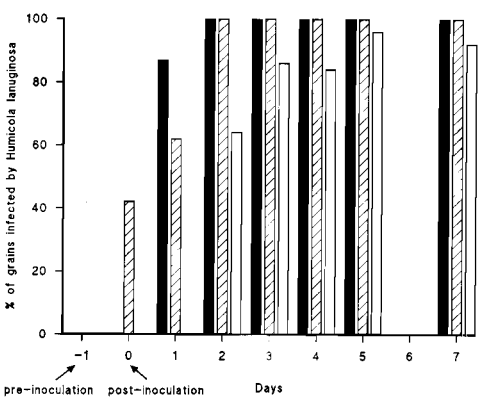

Fig. 1. Growth of *Humicola lanuginosa* monitored by direct plating and Dip-stick immunoassay.

Dip-stick Direct plating NSS

Direct plating SS

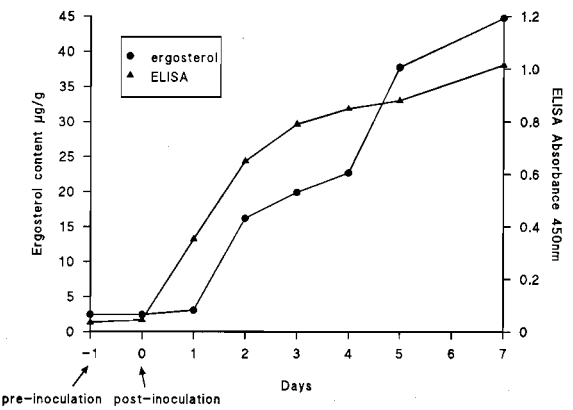

Fig. 2. Growth of *Humicola lanuginosa* monitored by ELISA and ergosterol content.

Effects of Aflatoxin B_1 in Ducklings: Effect on Hepatic Microsomal Drug Metabolising Enzymes

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STUDIES on the toxicity of aflatoxins have largely focused on aflatoxin B_1 because it is the most abundant and toxic metabolite. It is highly hepatotoxic, hepatocarcinogenic, and teratogenic to laboratory animals, and highly mutagenic to microbial assays systems (Hsieh and Wong 1982). There is strong evidence that aflatoxin B_1 requires metabolic activation for its toxic, mutagenic, and carcinogenic effects (Ames et al. 1973). Aflatoxins are primarily metabolised by the microsomal mixed function oxidase system, a complex organisation of cytochrome coupled, O_2-and NADPH-dependent enzymes located mainly on the endoplasmic reticulum of liver cells (Dalezios and Wogan 1972). There are few data on the hepatic microsomal drug metabolising enzymes in liver microsomes of ducklings, most reports dealing with rat, mouse, monkey, and guinea pig enzymes (Kamdem et al. 1983; Toskulkao et al. 1986; Obidoa and Achike 1986; Dalezios and Wogan 1972; Pier et al. 1989). The present study therefore examined the activity of cytochrome b_5, NADPH cytochrome *c* reductase, aniline hydroxylase, and NADH ferricyanide reductase in liver microsomes of ducklings injected with a single dose of aflatoxin B_1.

Materials and Methods

Animals: Eighteen 1-day-old Cherry Barley ducklings (Hamada shoten, Saitama prefecture, Satte city, Japan) were fed conventional feeds (National Institute of Animal Health, Japan, standard diet) for 15 days. Ducklings were then divided into 3 groups. The first group served as a control. The second and the third groups were intramuscularly injected with a single dose of 0.25 and 0.5 mg aflatoxin B_1/kg body weight, respectively, using olive oil as a diluent. Ducklings were housed in isolators held at identical environmental conditions. At 3, 14, and 21 days after administration, livers were collected for determination of hepatic microsomal drug metabolising enzymes.

Preparation of microsomes: Ducklings were anaesthetised by injection of 25 mg/kg body weight sodium pentobarbital (Abbott Lab., U.S.A.), following which 20–50 mL of blood were collected from carotid arteries for biochemical determination. Livers were washed via the portal vein with 50–100 mL of 0.9% normal saline.They were then removed and, under cold conditions (0–4°C), cut into small pieces. One gram of liver was homogenised with three volumes of 0.25 M sucrose, 5 mM EDTA and 5 mM tris–HCl at pH 7.0, then centrifuged twice at 10 500 rpm for 10 minutes. Sediment was discarded and the supernatant centrifuged twice at 33 700 rpm for 90 minutes. Sediments of microsomes were washed twice in 6 mL of 0.25 M sucrose, then mixed with 3 mL of deionised water and decontaminated with nitrogen aeration. After calculation for protein concentration of microsomes (Bio-RAD protein assay, USA), they were held at –20°C for enzyme determination.

Enzyme determination: Cytochrome b_5 was determined according to the method of Strittmatter and Velick (1956); aniline hydroxylase was measured using the method of Kato and Gillette (1965); and the method

of Phillips and Langdon (1962), was used to measure NADPH cytochrome *c* reductase and NADH ferricyanide reductase activity.

Results and Discussion

The effect of aflatoxin B_1 on cytochrome b_5 in ducklings is summarised in Figure 1. Three days after administration, levels of the enzymes in the 0.25 and 0.5 mg groups were 0.088 and 0.094 nM/mg protein, respectively, both of which were higher than in the control group (0.029 nM/mg protein). At 14 days, the enzyme levels had fallen slightly in both groups, to 0.051 and 0.069 nM/mg protein, respectively, but they marginally increased again in the 0.25 mg group (0.061 n mol/mg protein) at 21 days after administration.

Figures 2 and 3 showed the effects of aflatoxin B_1 on NADPH cytochrome *c* reductase and aniline hydroxylase activities. The pattern of effects on the enzymes is similar to cytochrome b_5. Figure 4 indicates that, at 3 days after administration, NADH ferricyanide reductase levels in the 0.25 and 0.5 mg groups were 9.18 and 7.64 unit/mg protein/min, respectively, lower than in the control group (10.68 unit/mg protein/min). The levels of enzyme in the 0.25 mg group decreased to 6.14 and 5.21 unit/mg protein/min at 14 and 21 days after administration, respectively. In the 0.5 mg group, the enzyme activity increased to 10.38 unit/mg protein/min but then fell markedly to 4.57 unit/mg protein/min at 21 days post-administration.

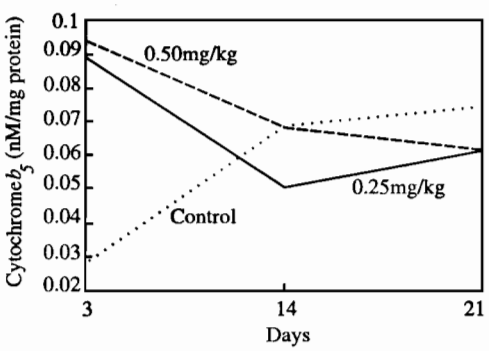

Fig. 1. Effect of aflatoxin B_1 on cytochrome b_5 in ducklings

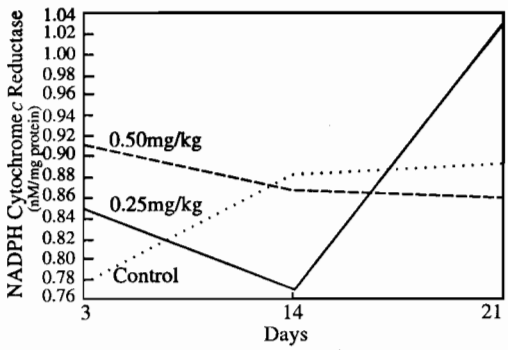

Fig. 2. Effect of aflatoxin B_1 on NADPH cytochrome *c* reductase in ducklings

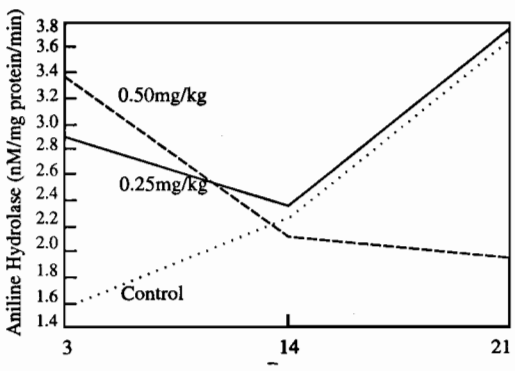

Fig. 3. Effect of aflatoxin B_1 on aniline hydroxylase in ducklings

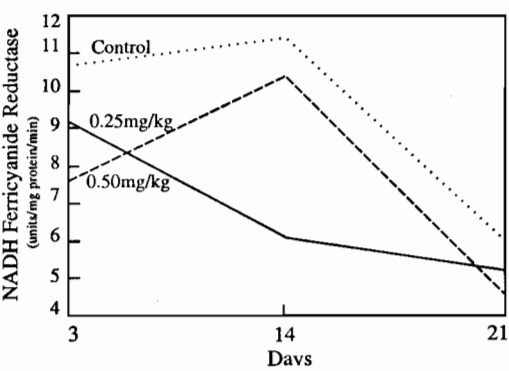

Fig. 4. Effect of aflatoxin B_1 on NADH ferricyanide reductase in ducklings

Effects of aflatoxin B_1 on aniline hydroxylase and NADPH cytochrome *c* reductase in this study were similar to those reported by Yoshizawa and Ueno (1980), Yoshizawa et al. (1979, 1980), and Obidoa and Achike (1986). However, Toskulkao et al. (1986) reported that while potentiation of aflatoxin B_1-induced hepatotoxicity in male rats, with ethanol pretreatment did not cause any significant change in the activity of NADPH cytochrome *c* reductase, it did increase aniline hydroxylase activity and decrease cytochrome b_5. However, in this study ducklings were intramuscularly injected with a single dose of 0.25 and 0.5 mg aflatoxin B_1/kg body weight but most other studies relate to intraperitoneal injection of a single dose of 0.3–0.6 or 0.5–2.0 mg/kg body weight in rats (Kamdem et al. 1983; Toskulkao et al. 1986; Obidoa and Achike 1986). The acute oral LD_{50} of aflatoxin B_1 in one-day-old ducklings is 0.335 mg/kg body weight (Butler 1974; Busby and Wogan 1979, 1981; Wogan and Busby 1980). The results of this study suggest that chronic changes induced in ducklings fed contaminated aflatoxin B_1 feeds may be shown more clearly.

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Effects of Aflatoxin B_1 in Ducklings: Effect on Liver Lesions

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AFLATOXINS have been shown to have carcinogenic activity in many species of animals including birds (Wogan 1973). Wogan et al. 1971 reported the toxicity series in day-old ducklings as aflatoxin B_1 > aflatoxin G_1 > aflatoxin B_2 > aflatoxin G_2. In ducklings, which are very sensitive to aflatoxin B_1 (Wogan and Busby 1980), liver is the primary target of aflatoxin action: it becomes infiltrated with fatty deposits when sufficiently high levels of aflatoxin are administered (Newberne and Butler 1969). Moreover, aflatoxins have been shown to induce hepatocellular carcinomas(Wogan 1973; Wogan and Newberne 1967; Newberne and Wogan 1968). In the study reported here, the appearance of liver lesions was expected to indicate evidence of toxicity of aflatoxin B_1, when an adequate dose of the toxin was intramuscularly administered to ducklings.

Materials and Methods

The animals used for histopathological examination were those described in the previous paper (Anong et al. 1991). At the time of sampling each liver for enzyme analysis, a small part was excised and fixed in 10% buffered formalin for histopathology. Conventional methods of paraffin embedding, sectioning, and haematoxylin and eosin staining were used.

Results and Discussion

Histopathological changes in livers of ducklings given a single dose of 0, 0.25, or 0.5 mg aflatoxin B_1/kg body weight at 3, 14, and 21 days after administration are summarised in Table 1. At 3 days after administration, slight bile duct hyperplasia was found in the ducklings administered 0.25 mg aflatoxin B_1/kg body weight.

Table 1. Histopathological changes in liver of ducklings given a single dose of 0, 0.25, or 0.5 mg aflatoxin B_1/kg body weight at 3, 14, and 21 days after administration.

Liver lesions[a]	3 days			14 days			21 days		
	0	0.25	0.50	0	0.25	0.50	0.0	0.25	0.50
1. Vacuolation of hepatic cells	0	1.0	3.0	0	0	1.0	0.5	0.5	0
2. Diffuse necrosis	0	0.2	2.0	0	0	1.0	0	0	0
3. Individual hepatic cell necrosis	0	0.2	1.0	0	0	0	0	0	0
4. Heterophilic infiltration	0	0	2.0	0	0	0	0	0	0
5. Lymphocytic.infiltration	0	0.2	0.5	0	0	0	0	0	0.5
6. Bile duct hyperplasia	0	1.0	4.0	0	1.0	1.0	0	0.5	1.0
7. Mitosis of proliferating bile duct epithelia	0	0	2.0	0	0	0	0	0	0

[a]Liver lesions were scored on a scale from 0 (no lesion) to 5 (very severe lesion)

In ducklings administered 0.5 mg aflatoxin B_1/kg body weight, marked bile duct hyperplasia was found, accompanied by mitosis of bile duct epithelia (Figs 1 and 3). Diffuse necrosis of the liver, individual hepatic cell necrosis, and hepatic cell vacuolation were also found, accompanied by heterophil infiltration (Figs 1–3). At 14 days after administration, slight bile duct hyperplasia was found in one case only (dosage 0.25 mg aflatoxin B_1/kg body weight) and slight bile duct hyperplasia accompanied by slight diffuse necrosis in one other (dosage 0.5 mg aflatoxin B_1/kg body weight). After 21 days, one of two cases dosed at 0.25 mg aflatoxin B_1/kg body weight showed slight hyperplasia of the bile duct, as did one case dosed at 0.5 mg aflatoxin B_1/kg body weight. No significant histopathological changes were found in the livers of control animals (Fig. 4).

Bryden (1985), reporting on the response of one day old Pekin–Aylesbury ducklings fed 0.1, 0.2, 0.4, and 0.8 mg aflatoxin B_1/kg diet at 7, 14, and 21 days of age, found the most severe bile duct proliferation in 14-day old animals administered 0.2–0.4 mg toxin. Bile duct proliferation was extensive in all treated groups. Clearly, duckling bioassay should be considered as only semiquantitative or qualitative (Butler 1974).

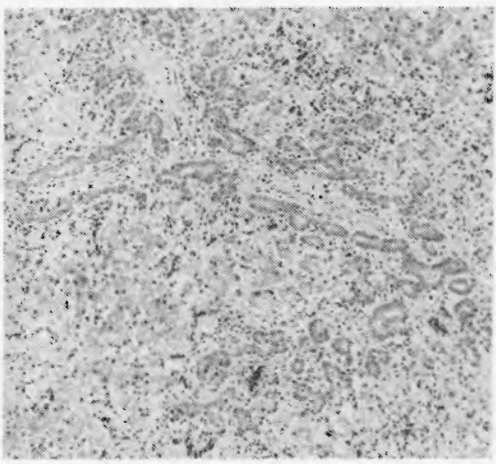

Fig. 1. Duckling at 3 days after administration of 0.5mg aflatoxin B_1/kg body weight, 18 days of age. Marked bile duct hyperplasia is evident in the periportal area. (Haematoxylin and eosin staining; ×110.)

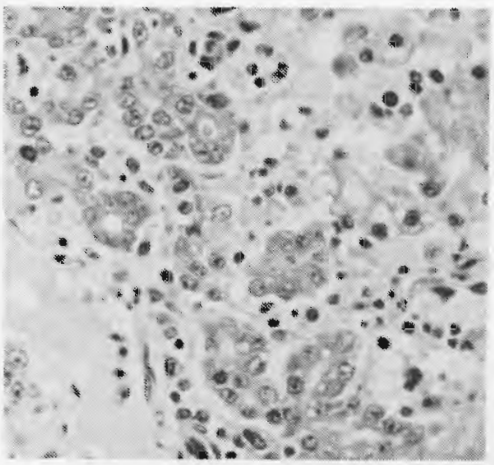

Fig. 2. A section of Figure 1 at higher magnification (×440). Hepatic cell vacuolation and necrosis are evident. (Haematoxylin and eosin staining.)

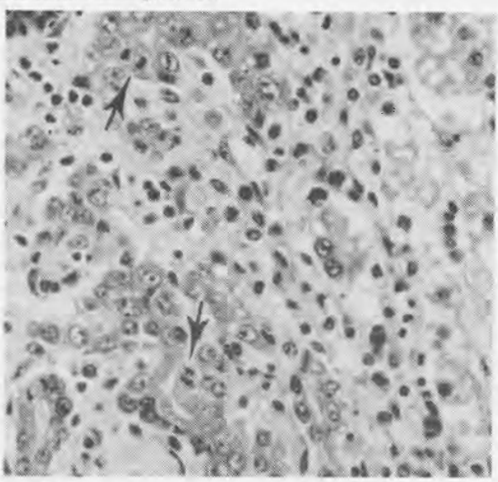

Fig. 3. A section from the same duckling as Figure 1, showing bile duct hyperplasia and epithelial mitosis (arrow). (Haematoxylin and eosin staining, × 440.

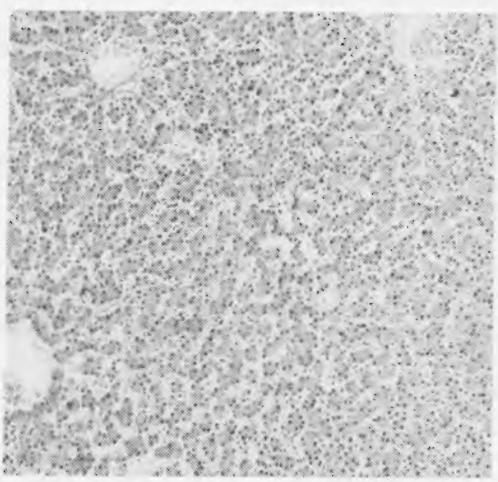

Fig. 4. Control duckling, 18 days of age. No significant changes are evident. (Haematoxylin and eosin staining, ×110.)

Studies of the effects of aflatoxin B_1 on liver histopathology in animals report swollen hepatocytes, fatty degeneration, bile duct hyperplasia, hepatic necrosis, hepatocellular necrosis, biliary cell proliferation of the periportal areas, liver cirrhosis, vacuolation in hepatocytes, interlobular fibrosis, periportal lipidosis, periportal lymphocytic infiltration in chicks, goslings, ducklings, turkey poults, guinea pigs, and barrows (Chaltopadhyay et al. 1985; Dalvi 1986; Uchida et al. 1988; Pier et al. 1989; Harvey et al. 1988). After withdrawal of the food containing aflatoxins, all apparent gross lesions of aflatoxicosis disappeared, with no evidence of any lesions 8 days after removal of the contaminated diets (Chen et al. 1985).

The numbers of animals in each group in this study were small, so it is difficult to draw general conclusions. However, it is clear that the most severe bile duct hyperplasia, accompanied by acute degenerative changes, occurred three days after intramuscular administration of aflatoxin B_1, especially at the higher dosage used (0.5 mg aflatoxin B_1/kg body weight). At 14 and 21 days postadministration, recovery from bile duct hyperplasia and degenerative changes was evident.

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Effects and Fate of Cyclopiazonic Acid in Poultry and Sheep

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CYCLOPIAZONIC acid (CPA) is a mycotoxin produced by some species of the fungal genera *Aspergillus* and *Penicillium* (Cole 1984). Chemically, CPA is an indole tetramic acid which has been shown to be toxic to all animals and birds tested. CPA produces tissue degeneration in a wide range of organs, especially the liver, kidney, and gastrointestinal tract. It appears to act primarily as an entero-nephrotoxin in chickens and pigs, and primarily as a hepatotoxin in rats (Cole 1984). It is of concern because it may be produced under the same circumstances as aflatoxin and because residues of CPA have been demonstrated in the muscle tissue of rats and chickens (Norred et al. 1985, 1988).

Several experiments were conducted to study the effects of oral doses of CPA in poultry and the lactating ewe. In both species, CPA reduced feed consumption and decreased body weight. Chickens developed ulcerations in the digestive tract and focal necrosis of the liver when given doses of 5 mg CPA/kg body weight (Suksupath et al. 1989, 1990b). At this dose, sheep ceased eating but recovered if no further doses of toxin were administered (Cole et al. 1988). CPA also impaired the utilisation of dietary calcium, protein, amino acids, and apparent metabolisable energy in broiler chickens (Suksupath et al. 1990b). In mature male chickens, CPA reduced reproductive performance by decreasing semen volume and sperm concentration, and increasing the number of abnormal spermatozoa (Suksupath et al. 1990a). Further studies showed that CPA caused lower egg production and poor egg-shell quality in laying hens (Cole et al. 1988; Suksupath et al. 1989). Analysis of egg whites and yolks from these hens showed much higher concentrations of CPA in the whites. The toxin was also detected in the milk of lactating ewes (Dorner et al. 1990).

The studies indicate that CPA contamination of poultry and ruminant diets will result in reduced production, leading to economic loss. It should be noted that these studies were conducted with purified toxin, and any naturally occurring toxicosis involving CPA would quite possibly be exacerbated by the presence of other toxins, depending on the fungi present in the contaminated feed. Moreover, the presence of the toxin in meat, milk, or eggs could also pose a health risk to the consumer.

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Protective Effect of Smoke on Aflatoxin Accumulation in Coconuts

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PREVENTION of aflatoxin formation in agricultural commodities is one of the best means of avoiding the threat of toxic and carcinogenic hazards to humans and animals arising from consumption of aflatoxin-contaminated foods. Many antifungal agents and chemicals have been examined for their ability to control fungal growth and aflatoxin accumulation in foods (Ray and Bullermann 1982). However, they are of limited acceptability due to possible toxic effects of the chemicals themselves. Smoke curing of foods appears to be more acceptable in view of its long usage in preservation of meat and fish in many countries in the world. Drying of coconut kernels using coconut shell smoke is practised widely in Sri Lanka.

Although fresh coconut is highly susceptible to aflatoxin production (Arseculeratne et al. 1969), copra produced industrially in Sri Lanka by smoke curing was found to be resistant to aflatoxin accumulation. However, fungi were observed in cracks or on newly exposed surfaces produced during bad handling after curing. In this study, the effects of smoke on fungal growth on grated coconuts and 'smoke broth' were examined.

Materials and Methods

Solid media. Freshly grated coconuts were dried to constant weight in electric ovens, by sun drying, or in a chamber using hot smoke produced by burning coconut shells in a limited supply of air (Arseculeratne et al. 1976). Replicate samples of dried coconuts (10 g) were re-moistened to different levels and inoculated with a suspension of *Aspergillus parasiticus* NRRL 2999 in 0.1% Tween 80. Flasks were incubated in the dark at 25°C and shaken twice daily. The flasks were withdrawn every two days and analysed for aflatoxins (Samarajeewa and Arseculeratne 1975).

Liquid media. Smoke obtained by burning coconut shells was aspirated into potato dextrose broth. Different concentrations of 'smoke broth' were prepared by diluting it with sterile water. Broth aliquots held in 500 mL conical flasks were inoculated with toxigenic fungi and incubated as above. Samples from the replicates were withdrawn at 24 hour intervals. The mycelia were separated and dried to constant weight. The broth was analysed for aflatoxins.

Results and Discussion

In time course studies using grated smoke-dried coconuts and sun dried coconuts, highest aflatoxin levels and durations noted were as shown in Table 1.

Table 1. Comparison of maximum aflatoxin levels (μg/g) found in smoke- and sun-dried coconuts

Treatment	Aflatoxin B_1	Aflatoxin G_1
Sun dried	88 (5 days) 45 (19 days)	88 (5 days) 48 (22 days)
Smoke dried	0.06 (19 days)	0.33 (22 days)

In sun dried coconuts, two maxima of aflatoxin production were observed, whereas smoke dried coconuts showed only one maximum which corresponded in time to the second maximum of the sun dried coconuts. In smoke dried coconuts, the concentration of aflatoxin detected was lower, clearly indicating suppression of aflatoxin production by constituents in smoke.

Aflatoxin B_1 was detected in electrically dried coconuts at and above 15% moisture content but smoke dried coconut did not support aflatoxin production below 30% m.c.(Fig. 1), indicating the protective effect of smoke even at relatively high moisture levels. However, at 50% m.c. the electrically dried and smoke dried re-moistened coconut produced equal concentrations of aflatoxins.

In 'smoke broth' containing graded levels of smoke, the smoke constituents suppressed aflatoxin accumulation to a greater extent than mycelial growth. Suppression of mycelial growth required higher concentrations of smoke in the broth (Fig. 2), indicating that the smoke constituents have high potential for controlling aflatoxin production in coconuts.

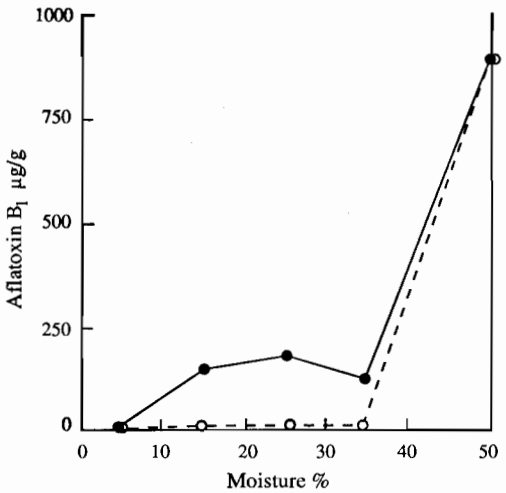

Fig. 1. The effect of coconut shell smoke on aflatoxin production in grated coconut in relation to % moisture content; unsterile substrate, smoke for 4 hours, incubated for 7 days at 25°C with manual agitation once daily.
o—o smoked; ● -● unsmoked

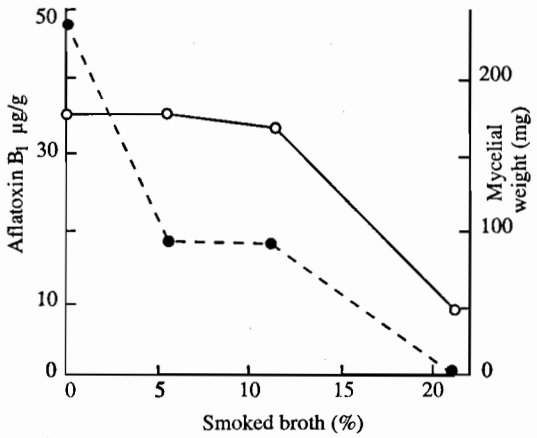

Fig. 2. Mycelial dry weight of *Aspergillus parasiticus* NRRL 2999 and aflatoxin B_1 production in graded 'smoke broth'. o—o mycelial weight; ●—● aflatoxin B_1

In industry, smoke curing of coconuts appears to provide greater protection of the kernels during storage than does sun drying or oven drying. The smoke curing of coconut kernels on a commercial scale may be one of the main reasons for the low aflatoxin levels reported in copra produced in Sri Lanka (Samarajeewa and Arseculeratne 1983) compared with other countries in the region which do not practice smoke drying. The same effect appears to occur in other foods. Skrinjar and Skenderevic (1987) observed no aflatoxins in any of the market samples of meat products, although Aspergilli were detected.

In the industrial application of smoke drying, the 'standard Ceylon copra kiln' designed for production of clean, soot free, smoke-dried copra may be useful for drying other agricultural commodities. Spraying atomised liquid smoke, already practiced in the meat industry, may be an alternate method for controlling aflatoxin contamination.

As smoke may itself contain carcinogenic compounds, screening of smoke constituents for non-carcinogenicity on the one hand and inhibition of aflatoxin production on the other is suggested before smoke is used to control aflatoxin contamination of foods during storage.

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Use of a Medicinal Plant (*Illicium verum* Hook L.) to Control Seed-Borne Fungi of Soybean

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SOYBEAN (*Glycine max* L.) var. Chiengmai 60 was used for isolation of seed-borne fungi. Seed samples were placed on potato dextrose agar containing 40% sodium chloride after surface disinfection with 10% sodium hypochlorite. Seed-borne fungi were periodically examined under a stereo microscope and isolated into pure cultures. Fungal cultures were maintained in PDA tube slants and kept in a refrigerator for further use.

The following seed-borne fungi were isolated and identified: *Alternaria alternata, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Curvularia lunata, Fusarium solani,* and *Penicillum terrestre.* These species were tested for inhibition of growth using star anise (*Illicium verum* L.) incorporated in an inhibitory culture medium or applied to seeds at various concentrations.

Star anise incorporated in culture media inhibited fungal growth.

For application to seeds, crude ethyl alcohol (95%) or hot water extracts of star anise were used at concentrations of 0, 10 000, 20 000, and 30 000 ppm to drench soybean seeds. Results showed that all concentrations used significantly inhibited growth of all fungal species tested. However, the ethyl alcohol extracts were more effective in inhibiting spore production.

It is concluded that the use of *Illicium verum* to control the seed-borne fungi on soybean depends on the fungal species, and the *Illicium* extraction method and concentration level.

Potential Plant Materials for the Control of *Aspergillus* Infection in Maize

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PLANT species reported to have medicinal value and fungicidal properties were collected and extracted using water. Crude water extracts of 6 of 127 plant species screened *in vitro* for fungicidal properties showed inhibitory activity against *Aspergillus flavus* and *A. parasiticus*.

Crude garlic (*Allium sativum*) extract was found to be most effective against aflatoxin-producing fungi. Also effective against *A. flavus* were, in decreasing order, the following plant extracts: kalachuchi (*Plumiera acuminata*) > bulak manok (*Ageratum conyzoides*) > sampa sampalukan (*Phyllantus nirure* L.); and, against *A. parasiticus*, talisay (*Terminalia catappa* L.) > kakawati (*Gliricidia sepium* Jacq.). Aflatoxin was not detected on potato dextrose broth incorporating the above-mentioned crude extracts and inoculated with aflatoxin-forming organisms.

Since garlic was found to yield the most effective antifungal extract in *in vitro* tests, its antifungal activity in stored maize was investigated. Maize seeds of 16.7% moisture content were inoculated with agar discs containing spores of *Aspergillus flavus* and treated with different amounts of chopped garlic. To test the possible protective and eradicative effects of garlic, experimental samples were treated with chopped garlic 2 days before or after inoculation, and stored for 3, 6, or 9 weeks.

No amount of chopped garlic applied significantly inhibited kernel infection and aflatoxin production by *Aspergillus* for any of the storage periods. Clearly, further study of the active antifungal principles of garlic is required, as well as of appropriate methods of practical application to grain.

Production of Deoxynivalenol in Maize by *Fusarium proliferatum*

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FUSARIUM proliferatum is a common isolate from various food and feedstuffs collected throughout Peninsular Malaysia. While there have been some reports of toxigenicity, the toxic components have never been characterised. In a recent survey of animal feeds, several species of *Fusarium* were isolated from mixed feeds and feedstuffs. An isolate was observed to be highly toxic towards brine shrimp larvae. The isolate, initially identified as *F. moniliforme*, was later confirmed to be *F. profileratum*. The isolate displayed culture characteristics typical of species in the section Liseola. Microconidia were produced in long chains (> 30), rendering the culture powdery on PSA. Macroconidia were rare. It was differentiated from *F. moniliforme* on the basis that polyphialides were produced on lateral conidiophores only after prolonged incubation.

Cultures grown on moistened, autoclaved, crushed maize, and incubated at room temperature in the dark, were extracted weekly with acetonitril:water (3:1) and defatted with n-hexan. After evaporation of solvent and reconstitution in brine shrimp medium, the crude extract indicated that the isolate produced highest toxic activities towards brine shrimp after a 4-week incubation period. Its toxic activity was estimated to be 5000 unit activity (UA)/g of inoculated maize. Thin-layer chromatography revealed the presence of deoxynivalenol (DON). Quantitation with gas chromatography showed that DON was produced at a level of 184 µg/g inoculated maize. This is the first report of DON production by *Fusarium* isolates in Malaysia.

The Effects of Phosphine on Some Biological Aspects of *Aspergillus flavus*

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THE tropical climate of Indonesia provides conditions favourable to growth of storage fungi; that is, temperatures of 5°–35°C and relative humidities between 70–90% (Christensen 1978). *Aspergillus* and *Penicillium* species are the most common fungi found infecting stored products (Pitt and Hocking 1985). They can cause loss in weight, discoloration of seeds, heating and mustiness, and production of mycotoxins, the most important of which are aflatoxins, produced by *Aspergillus flavus* and *A. parasiticus*.

According to Dharmaputra et al. (1991), treatment with phosphine, a fumigant in widespread use for control of storage insects, at a concentration of 2 g/tonne for 5 days reduced the percentage of grains of maize infected by the fungal species *Eurotium chevalieri* and *E. repens*, and also reduced the population of *Aspergillus wentii*.

The objective of this study was to investigate the effect of different concentrations of phosphine on mycelial growth, sporulation, spore germination, and aflatoxin production of *A. flavus*.

Materials and Methods

Isolates of *A. flavus*: *A. flavus* BIO-17 and BIO-18 isolated from maize (*Zea mays*) were used, because they produce more than 20 ppb of aflatoxin B_1 on Coconut Extract Medium (CEM) 10% at 28°C, after 10 days of incubation in dark conditions (Lin and Dianese 1976).

Preparation of fungal cultures: Test cultures were grown on Aspergillus Differential Medium (ADM) (Bothast and Fennell 1974) at 28°C for 7 days. Mycelial plugs (5 mm in diameter) from the ADM plate served as inocula for Petri dishes containing Potato Dextrose Agar, to study the effect of phosphine om mycelial growth, sporulation, and spore germination. Gall beakers (volume 100 mL) containing 50 mL CEM 10% were similarly inoculated with mycelial plugs to study the effects of phosphine on aflatoxin production. Cultures were incubated for 2 days before exposure to phosphine.

Phosphine treatment: The experiment was conducted in sterile 3.3 L jars (gastight) at ambient temperature (27° ± 1°C) and a relative humidity of 75 ± 5%. The Petri dishes or glass beakers containing the fungal culture (2 days old) were covered with sterile muslin and placed inside the experimental jar. Phosphine was generated from aluminium phosphide tablets, which release phosphine gas on contact with ambient air (FAO 1969; reproduced in AFHB/ACIAR 1989). The fungus was treated with air (as control) or phosphine at 0.5, 1.5, 2.5, and 3.5 mg/L for 5 × 24 hours. Nine replicates were used for each treatment.

The effect of phosphine on mycelial growth, sporulation, spore germination, and aflatoxin production: Mycelial growth were assessed by measuring the diameter of the colony of each isolate before and after fumigation. The data were then transformed into colony size (Πr^2) and the change in colony size before and after fumigation was calculated. Sporulation was determined by visual observation of the density of spores after treatment.

Spore germination was observed by spreading 1 mL of spore suspension (concentration 10^5 spores/mL) of each isolate on a Petri dish of PDA. The spore suspension was derived from the fungal cultures which were fumigated with different concentrations of phosphine for 5 x 24 hours, and the dishes were then incubated at room conditions for 6 hours. The percentage of spore germination was calculated from a total of 100 spores observed. Nine replicates were used for each treatment.

Aflatoxin production: Fungal cultures were extracted , then analysed for aflatoxin B_1 content by thin layer chromatography (TLC) (Blaney et al. 1984).

The data were analysed using a factorial completely randomised design.

Results and Discussion

Effect of phosphine on mycelial growth and sporulation: Based on the analyses of variance there were significant differences between the two isolates of *A. flavus*, phosphine concentrations, and interactions between the isolates and phosphine concentrations (Table 1). The changes in colony size of isolates BIO-17 and BIO-18 before and after fumigation with phosphine were 974 mm^2 and 2419 mm^2, respectively. Isolate BIO-17 was more sensitive than isolate BIO-18 (Table 2).

Table 1. Analyses of variance on the effect of *A. flavus* isolates, phosphine concentrations, and their interactions on mycelial growth

Source of var.	df	SS	MS	F-value
A	1	47004986.17	47004986.17	391.28**
B	4	73377830.74	18344457.68	152.70**
A x B	4	3832504.04	958120.01	7.98**
Error	80	9610529.4	120131.6	

A = Isolates of A. *flavus*
B = Phosphine concentrations
A x B = Interaction between isolates of A. *flavus* and phosphine concentrations

* = Significantly different at 95% confidence level
** = Significantly different at 99% confidence level

The change in colony size decreased with increasing phosphine concentrations. In the control, the changes in colony size of isolate BIO-17 and BIO-18 were 2372 mm^2 and 3885 mm^2, respectively, while at 3.5 mg/L phosphine concentration, the changes in colony size were 55 mm^2 and 752 mm^2, respectively (Table 2). Mycelial growth inhibition commenced at concentration at 0.5 mg phosphine /L, and for isolate BIO-17 was almost totally inhibited at 3.5 mg phosphine/L.

When incubated at normal conditions after treatment the fungi were still able to grow, indicating that the mycelia were inhibited rather than killed by phosphine treatment.

According to Hocking and Banks (1990) storage of moist paddy rice in phosphine (100 ppm) for 14 and 28 days at 28°C slowed the growth rate of most storage fungi, but did not prevent growth .

Based on visual observation, inhibition of sporulation of isolates BIO-18 and BIO-17 began at 0.5 mg/L and 1.5 mg/L phosphine, respectively. At 3.5 mg/L phosphine, no sporulation occurred in isolate BIO-17, but inhibition of sporulation of isolate BIO-18 commenced(Table 3).

Effect of phosphine on spore germination: Analyses of variance showed that there were significant differences between the two isolates, phosphine concentrations, and the interactions of those two factors on spore germination (Table 4).

Percentage spore germination of each isolate treated with different concentrations of phosphine is shown in Table 5. Germination began to decrease at 0.5 mg phosphine/L and decreased with increase in phosphine concentration.

In the controls (air), the percentages of spore germination of isolates BIO-17 and BIO-18 were 59.1%

Table 2. The effect of *A. flavus* isolates, phosphine concentrations and their interactions on mycelial growth

Effect	Mean change in colony size before and after fumigation (mm^2)*
A. flavus isolates	
BIO-17	973.59 b
BIO-18	2418.96 a
Phosphine concentrations	
Control	3128.90 a
0.5 mg/L	2114.70 b
1.5 mg/L	1524.00 c
2.5 mg/L	1309.90 c
3.5 mg/L	403.90 d
Interaction between *A. flavus* isolates and phosphine concentrations	
A. flavus BIO-17	
Control	2372.20 a
0.5 mg/L	1177.02 b
1.5 mg/L	644.30 c
2.5 mg/L	619.20 c
3.5 mg/L	55.22 d
A. flavus BIO-18	
Control	3885.60 a
0.5 mg/L	3052.33 b
1.5 mg/L	2403.70 c
2.5 mg/L	2000.53 d
3.5 mg/L	752.62 e

* Numbers followed by the same letter do not differ significantly according to LSD test at 95% confidence level

Table 3. The effect of phosphine concentrations on sporulation of *A. flavus* BIO-17 and BIO-18

Treatment	Sporulation	
	BIO-17	BIO-18
Control	+++	+++
0.5 mg/L	+++	++
1.5 mg/L	++	++
2.5 mg/L	++	++
3.5 mg/L	+	++

+++ Good sporulation
++ Sporulation was started to be inhibited
+ No sporulation

Table 4. Analyses of variance on the effect of *A. flavus* isolates, phosphine concentrations, and their interactions on spore germination

Source of var.	df	SS	MS	F–value
A	1	4528.10027	4528.10027	131.21**
B	4	32828.82228	8207.20557	237.81**
A¥B	4	4955.79642	1238.94910	35.9**
Error	80	2780.87369	34.51092	

A = Isolates of *A. flavus*
B = Phosphine concentrations
A¥B = Interaction between isolates of *A. flavus* and phosphine concentrations
* = Significantly different at 95% confidence level
** = Significantly different at 99% confidence level

and 52.5%, respectively, while at 3.5 mg phosphine/L the germination rates were 2.4% and 2.0%, respectively.

Spore germination was only inhibited by phosphine, as the spores still had the capacity to germinate when incubated under normal conditions after treatment.

Effect of phosphine on aflatoxin production: Based on the analyses of variance there were no significant differences in aflatoxin production between isolates, but there were very significant differences among phosphine concentrations and the interactions between isolates and phosphine concentrations on aflatoxin production (Table 6).

Aflatoxin production levels of isolates BIO-17 and BIO-18, 152.3 ppb and 146.6 ppb, respectively (Table 7), were not significantly different. Nevertheless, aflatoxin production of isolate BIO-18 was inhibited more than that of isolate BIO-17.

Aflatoxin production decreased with increase in phosphine concentration, from 241.2 ppb (air control) down to 65.3 ppb (3.5 mg phosphine/L) (Table 7). Aflatoxin production of each isolate treated with different concentrations of phosphine is also shown in Table 7.

The two isolates were still able to produce aflatoxin after treatment with 3.5 mg/L phosphine concentration for 5 × 24 hours, but the amounts were low. A phosphine concentration higher than 3.5 mg/L would be necessary to prevent aflatoxin production.

Table 5. The effect of *A. flavus* isolates, phosphine concentrations, and their interactions on spore germination

Effect	Mean spore germination (%)
A. flavus isolates	
BIO-17	31.5 a
BIO-18	17.4 b
Phosphine concentrations	
Control	55.8 a
0.5 mg/L	34.0 b
1.5 mg/L	21.8 c
2.5 mg/L	8.7 d
3.5 mg/L	2.2 e
Interaction between *A. flavus* isolates and phosphine concentrations	
A. flavus BIO-17	
Control	59.1 a
0.5 mg/L	53.7 a
1.5 mg/L	32.1 b
2.5 mg/L	9.8 c
3.5 mg/L	2.4 d
A. flavus BIO-18	
Control	52.5 a
0.5 mg/L	14.8 b
1.5 mg/L	10.6 b
2.5 mg/L	7.6 bc
3.5 mg/L	2.0 d

* Numbers followed by the same letter do not differ significantly according to LSD test at 95% confidence level

Table 7. The effeet of *A. flavus* isolates, phosphine concentrations, and their interactions on aflatoxin production

Effect	Mean of aflatoxin B_1 production (ppb)
A. flavus isolates	
BIO-17	152.3 a
BIO-18	146.6 a
Phosphine concentrations	
Control	241.2 a
0.5 mg/L	174.6 b
1.5 mg/L	150.1 c
2.5 mg/L	116.0 d
3.5 mg/L	65.3 e
Interaction between A. *flavus* isolates and phosphine concentrations	
A. *flavus* BIO-17	
Control	231.1 a
0.5 mg/L	187.3 b
1.5 mg/L	160.4 c
2.5 mg/L	120.3 d
3.5 mg/L	62.3 e
A. *flavus* BIO-18	
Control	251.3 a
0.5 mg/L	162.0 b
1.5 mg/L	139.9 c
2.5 mg/L	111.7 d
3.5 mg/L	68.2 e

* Numbers followed by the same letter do not differ significantly according to LSD test at 95% confidence level

Table 6. Analyses of variance on the effect of *A. flavus,* phosphine concentrations, and their interactions on aflatoxin production

Source of var.	df	SS	MS	F–value
A	1	723.1802	723.1802	1.62
B	4	310603.4769	77650.8692	173.72**
A x B	4	6360.8855	1590.2214	3.56*
Error	80	35759.0277	446.9878	

A = Isolates of *A. flavus*
B = Phosphine concentrations
A ¥ B = Interaction between isolates of *A. flavus* and phosphine concentrations
* = Significantly different at 95% confidence level

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Degradation of Aflatoxin B_1 and Loss of Mutagenicity in Peanuts and Copra Meal on Chlorine Gas Treatment

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WHILE prevention of fungal growth and aflatoxin production in foods is the best means of controlling contamination, it is sometimes not possible to do so. Thus, detoxification methods become important as a second line of defence in recovering food commodities already contaminated. Among many chemicals that have been examined for their ability to destroy aflatoxins, chlorine has shown promise against the pure toxin (Sen et al. 1988). However, the method has not been tested with contaminated agricultural commodities.

Chlorine gas is more acceptable than other chemicals because it is already used in the food industry to bleach flour, purify water, and clean food-processing equipment.

In the study reported here, concentrations of chlorine gas needed to destroy aflatoxins in peanuts and copra meal were estimated and the mutagenicities of extracts from the treated commodities assessed.

Materials and Methods

Samples: Copra meal samples collected from a commercial oil mill in Sri Lanka were spiked with pure aflatoxin B_1 to concentrations of 10 mg/g. Sunrunner peanuts were moistened and inoculated with *Aspergillus parasiticus* NRRL 2999 in 500 mL conical flasks. They were incubated at 25°C in the dark for varying periods to produce required levels of aflatoxins. The infected peanuts were dried at 70°C and ground, then blended with ground, uncontaminated peanuts or peanut flour before chlorination.

Chlorination: Chlorine gas generated as described by Sen et al. (1988) was used. Samples (10 g) were placed in 290 mL flasks which were then evacuated and predetermined amounts of chlorine gas injected using a syringe. Flasks were sealed by closing the taps and kept in the dark for required durations, with or without stirring (Samarajeewa et al. 1991).

Quantification of aflatoxins: Peanuts were extracted using AOAC method 26.035 (AOAC 1984) and the copra meal by a modified 70% aqueous acetone method (Samarajeewa and Arseculeratne 1975). Aflatoxins were estimated by thin-layer chromatography using a chloroform:acetone:isopropanol (85:15:2.5) solvent system. For quantification, the aflatoxin B_1 spots were scanned with a Kratos model SD 3000 spectrodensitometer at a wavelength of 362 nm.

Mutagenicity: Mutagenicities of the chlorinated and control samples were tested in dimethylsulphoxide by plate incorporation using rat liver S-9 mix (Ames et al. 1975).

Results and Discussion

Degradation of aflatoxin B_1: More than 75% degradation of aflatoxin B_1 occurred in peanuts and copra meal on treatment with 35 and 16 mg chlorine/g of substrate, respectively. Instantaneous generation of heat occurred in the flasks on admission of chlorine gas, with deposition of moisture on the walls. Optimum destruction of aflatoxin in peanuts was observed after 24 hours, whereas in copra meal the destruction was complete in 2.5 hours. The percent degradation of aflatoxin B_1 in copra meal did not increase on exposure for longer than 24 hours or on stirring the samples continuously for 24 hours.

Interactions of peanut constituents: When the possible interactions of constituents of peanuts, namely oil and protein, were examined by chlorinating blends of infected peanuts with uninfected peanuts (high oil content) or peanut flour (low oil, high protein) at ratios of 1:9, 3:7, and 5:5, no significant differences in percent degradation of aflatoxins were observed. All treatments showed percent degradations of $87 \pm 5\%$ (Samarajeewa et al. 1991). The composition of peanuts did not appear to be a factor influencing levels of aflatoxin B_1 degradation by chlorine gas treatment.

Mutagenicity: The decrease in mutagenicity of peanuts and copra meal on chlorine gas treatment, as indicated by the Ames test, showed the following linear relationships with percent degradation of aflatoxin B_1 estimated chemically:

for peanuts $y = 0.75\,x + 19.5;\ r^2 = 0.88;\ P = 0.001$

for copra meal $y = 1.2\,x - 24.4;\ r^2 = 0.88;\ P = 0.01.$

The linear relationship between the destruction of aflatoxin and mutagenicity confirmed both the loss in mutagenicity of aflatoxin B_1 associated with chlorine gas treatment and the absence of new mutagenic products resulting from interactions between chlorine and peanut or copra meal constituents.

While further tests are needed, present evidence indicates that chlorine gas treatment has potential for detoxifying aflatoxins in peanuts and copra meal.

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Potential of Ammonium Benzoate for Aflatoxin Control

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AMMONIUM benzoate at 0.4 and 0.8 g/100 g in potato dextrose broth showed complete inhibition of toxigenic *Aspergillus flavus* and *A. parasiticus*, respectively. When using an antifungal agent, 10 spores/g of both *A. flavus* and *A. parasiticus* were completely inhibited by ammonium benzoate at 0.64 and 0.32 g/100 g, respectively, in pre-treated peanuts and maize. Meanwhile, no aflatoxins could be detected by thin-layer chromatography from any of the treatments. Aqueous extracts from treated peanuts and maize showing complete inhibition of mould growth, injected into 48-hour fertilized hens eggs, had no observable effect on the development of chick embryos. *Escherichia coli* strain PQ 37 in SOS Chromotest was used as a model to investigate the mutagenicity of aqueous extracts from treated peanuts and maize stored for 7, 14, 21, and 28 days. No mutagenic effects were detected.

Chemical Detoxification of Aflatoxin B_1

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THE aim of this study was to find a simple and economic method to detoxify aflatoxin-contaminated stored products. The biological safety of treated products was confirmed by modified Ames' test.

Methods

Peanut and maize samples were obtained from the local markets in Chiang Mai province and treated with the following chemicals: ammonium carbonate, ammonium bicarbonate, calcium hydroxide, hydrogen peroxide, ammonium hydroxide, sodium bicarbonate, and calcium hypochlorite. The treatment conditions — concentration, time, moisture content, and temperature — were varied. The combined effects of two chemicals, and of ammonium bicarbonate with gamma-rays were studied. Aflatoxin B_1 in the treated samples was quantitatively determined by thin-layer chromatography (TLC) and spectrofluorometry. Finally, the mutagenicity in extracts of the treated samples was tested by *Salmonella*/microsomal assay (McCann et al. 1975; Yahagi et al. 1977).

Results

Effect of chemical concentration: Several concentrations of ammonium carbonate varying from 0.5 to 5% could reduce aflatoxin B_1 by from 22% up to 69%. The most effective concentration was 3% or higher. With ammonium bicarbonate, the optimal concentration for detoxification was also 3%, which reduced aflatoxin B_1 by 80% (Fig. 1). For calcium hydroxide, 5% gave optimal reduction of the toxin (58%). Ammonium hydroxide, sodium bicarbonate, and calcium hypochlorite at concentrations of 25, 5, and 5%, were found to decrease the toxin by 60, 55, and 57%, respectively. Hydrogen peroxide (30%) detoxified only 25% of the toxin.

Effect of time: The optimal treatment period was 3 hours for all the chemicals which were mixed with samples at room temperature (25°C) and 20% moisture content.

Effect of moisture content: The optimal commodity moisture content was found to be 20% for all chemicals tested. Addition of water could enhance the detoxifying effect of the chemicals.

Effect of temperature: Heat alone (100°C for 1 hour) could destroy aflatoxin B_1 by 10% in peanuts. It appeared that the effects of heat and chemical treatment were additive.

Effect of combined two chemicals: The combination of 3% ammonium carbonate and 30% hydrogen peroxide (5 mL) could reduce the toxin by 76%. Hydrogen peroxide alone yielded 25% detoxification and ammonium carbonate 69%. If the treated samples were further incubated at 100°C for 3 and 24 hours, the reduction of toxin was slightly higher at 77 and 81.5%, respectively.

Effect of gamma irradiation: The samples were irradiated with gamma-rays at doses of 0.1 to 5 M-rad using Cobalt-60 as the source. It was found that aflatoxin reduction depended on the intensity of irradiation and was constant around 28–30% at higher doses (1–5 M-rad).

Effect of combined ammonium bicarbonate and gamma irradiation: By increasing the dosage of gamma-rays from 0.1 up to 1 M-rad, the toxin reduction was between 79 and 82%. The combined detoxification did not significantly increase by using gamma-rays at the higher dose (5 M-rad) (Fig. 2).

Mutagenicity test: After treatment of aflatoxin-contaminated samples with ammonium bicarbonate for from 3 hours up to 7 days, their mutagenicities decreased proportionally. The reduction of mutagenicity was much higher after the combined treatment with ammonium bicarbonate and gamma irradiation.

Discussion

In this study, it was demonstrated that the most effective and suitable chemical for aflatoxin detoxification was ammonium bicarbonate. Certain treatment conditions which give additive effects should also be considered for practical use. In this context, gamma irradiation was found to be not feasible in this study because it did not significantly increase the detoxifying effect of ammonium bicarbonate treatment. The mechanism of detoxification by ammonia released from the ammonium salts may involve the destruction of lactone ring of aflatoxin B_1 into the nontoxic aflatoxin D_1 (Park et al. 1988). Some toxicity and mutagenicity remained in the treated samples. The treatment conditions or factors could be further improved to yield higher detoxifications.

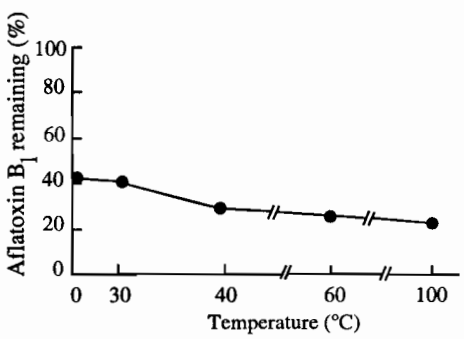

Fig. 1. Effect of 3% ammonium bicarbonate on aflatoxin B_1 at room temperature for 3 hours then heating at different temperatures for 1 hour.

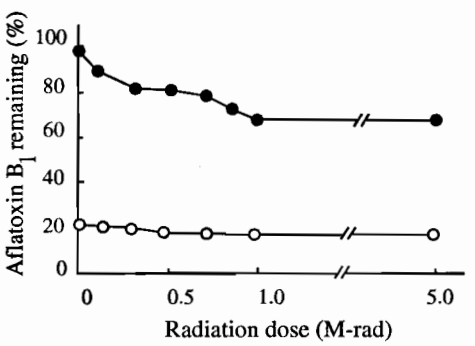

Fig. 2. Effect of gamma irradiation with and without 3% ammonium bicarbonate on aflatoxin B_1. Without NH_4HCO_3 treatment; ●–● With NH_4HCO_3 treatment; O–O

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Chitinase Activity in Maize Seeds and Their Fungal Resistance

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CHITINASE catalyses the hydrolysis of chitin into N-acetylglucosamine. In the study reported here, chitinase activity in extracts from local maize seeds and their hybrids was assessed by a biochemical method. The *in vitro* susceptibility to infection of *Aspergillus flavus* inoculated on those seeds was also determined. The specific activities, units per mg protein and units per g seed, were compared with the percentage fungal infection. The correlation coefficient, – 0.352, indicated that there was a negative relationship between chitinase activity and fungal infection in maize seeds. Endogenous chitinase in fungal-resistant seeds may act on chitin in cell walls of fungi and inhibit their further invasion.

Screening for Resistance to Aflatoxins Contamination in Peanuts in China

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AFLATOXINS contamination in peanuts is a serious problem in southern China. Research on utilisation of genetic resistance to seed invasion by *Aspergillus flavus* and/or aflatoxins commenced in 1987 at the Institute of Oil Crops. Some 1529 genotypes have been tested in the laboratory for resistance to *A. flavus*. Levels of seed infection ranged from 8 to 100%, with 54 genotypes < 15%, 145 in the range 16–30%, 231 in the range 31–50%, and 1009 > 50%. By selection of resistant seed, the average level of seed infection of 39 genotypes was decreased from 46% in 1989 to 26% in 1990. Preliminary tests were conducted on some genotypes for their ability to support low aflatoxin production (SLAP–ability). A new screening method for SLAP-ability was developed at the Institute of Oil Crops.

Field and Laboratory Techniques for Evaluating Resistance of Maize to *Aspergillus flavus*

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PLANT disease control by using plant resistance is an ideal, simple, and practical method, and is less costly to farmers than other approaches. In order to identify resistant cultivars, reliable, practical screening techniques are needed. This research developed simple, reliable, and practical field and laboratory screening techniques for resistance to *Aspergillus flavus* infection and aflatoxin contamination in maize.

For field inoculation: the most effective and practical technique for large-scale screening is injection of an *A. flavus* suspension inoculum of 10^7 conidia/mL concentration onto the silk channel of maize plants at 3 weeks after 100% silking.

For laboratory evaluation: kernel inoculation by dipping in a suspension of 10^7 conidia/mL, then incubation in a sterile petri dish with two layers of filter paper on top of three layers of straw paper, is preferred. Only the second layer of straw paper is moistened by soaking in water. This is a modification of the blotter method.

These inoculation techniques have been used routinely at Kasetsart University during research on *A. flavus* infection of maize, and specifically for resistance to *A. flavus* infection and aflatoxin contamination in maize.

Strategies for the Prevention and Control of Fungi and Mycotoxins in Central and South America

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The Problem

THE occurrence of mycotoxins in foodstuffs depends on their formation by specific strains of fungi and is influenced by environmental factors such as humidity and temperature. This mycotoxin contamination of foodstuffs may vary with geographical conditions, production and storage methods, and also with the type of food.

Most toxigenic fungi are ubiquitous and the potential for contamination of foodstuffs and animal feeds is widespread. Toxin-producing fungi can infect growing crops as a consequence of damage by insects or other agencies, and may produce toxins before harvest, or during harvesting and storage.

The moisture content and temperature of the substrate are the most important factors regulating fungal growth and mycotoxin formation.

The need for great care in handling and processing grains under local conditions in warm, humid climates has been overlooked.

In Latin America and the Caribbean where such conditions are common, surprisingly, very few official efforts are being made to:

- assess the situation;
- control and monitor mycotoxin levels in food and feedstuffs;
- prepare and train professionals in methods of mycotoxin prevention and control;
- establish workable local programs aimed at arresting the problem and involving community members in rural and urban areas; and
- design and implement education and extension programs for schoolchildren in rural areas and for other target groups such as mothers' clubs, rural promoters, etc.

The Situation

The occurrence of aflatoxins in grain is an important contamination problem in many regions. Some important fungi associated with this problem—*Aspergillus flavus* and *Penicillium* spp.—are increasingly creating worries for farmers, traders, animal feed producers, and the research and extension sectors. The extent of *A. flavus* contamination in maize at the postharvest period was put by one study at more than 50% of samples examined, although at low levels. *A. flavus* has been found to be present even before harvest and to be generally associated with insect damaged grains.

In general, it is observed that total grain contamination by fungi is of the order of 80%. The relative composition of field and storage fungi varies as the grain moves through the postharvest system. Field fungal populations decrease from around 50% contamination at the time of harvest to 12% during silo storage. Storage fungi start from 22% at the time of harvest and reach 78% during the initial phases of silo or warehouse storage. All this happens in a period of about 4–5 months

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Among field fungi, *Fusarium* spp. invade more than 50% of the grains. *Penicillium*, a known storage fungus, is widely present in fresh harvested grain at levels of 15–37%. This indicates the importance of these two genera in maize.

In Costa Rica, in the Central American subregion, high levels of aflatoxin contamination, mainly in maize from the southern region, have been reported recently. During the postharvest period it is reported that 70% of samples had levels of 5–20 μg/kg of aflatoxins, while 50 % had levels between 16–35 μg/kg. After harvest, during handling and storage periods, 23% of samples of unshelled maize had aflatoxin levels between 16 and 100 μg/kg. Forty percent of husked unshelled maize samples had aflatoxin levels of 16– 80 μg/kg.

A similar pattern could be expected in other countries in the region.

In Latin America and the Caribbean Region, some 19 countries recently submitted updated information on their mycotoxin-related problems and activities. Activities in the prevention, control, monitoring, and training and extension fields were assessed and recorded. The results, shown in Table 1, generally indicate only low levels of activity.

Table 1. Summary of Latin American and the Caribbean activities for the prevention and control of fungi and mycotoxins

Country	Prevention	Control	Monitoring	Training/extension
Argentina	N	Y	P	P
Antigua and Barbuda	N	N	N	N
Barbados	N	N	N	N
Belize	N	N	N	N
Bolivia	N	N	N	N
Colombia	P	P	P	P
Costa Rica	P	P	P	P
Cuba	P	Y	Y	P
Chile	P	P	P	Y
Dominican Republic	N	N	N	N
Ecuador	N	N	N	N
Guatemala	P	N	P	P
Honduras	N	N	P	P
Mexico	P	Y	Y	Y
Nicaragua	N	N	N	N
Panama	N	N	N	N
El Salvador	N	N	N	N
Uruguay	N	P	N	N
Venezuela	N	P	N	N

N = No activity
Y = Active program
P = Partial activity

In Mexico in a period of almost 50 years from 1932 to 1979 there were 40 published papers on aflatoxins and related topics. Except for two authors, no mention was made of prevention and control aspects. However, from 1979 onwards, through a collaborative effort among university research centres and government institutions, attention is now being focused on these aspects.

In Chile, a National Mycotoxins Council has recently been created by research, university, and private and official sectors to help remedy, prevent, and control the problem of fungi and mycotoxins. Two FAO and UNDP postharvest projects have promoted, guided, organised, and implemented the initial coordinating activities and infrastructures to make possible this effort.

In Bolivia, the Regional Council for Food and Nutrition (CRAN) plans to launch cooperative programs for the prevention and control of fungi and mycotoxins. Due to the multidisciplinary and complex nature of these problems, the coordinated participation of government entities, the private sector, nongovernment organisations (NGOs), university and research institutions, and community members is believed to be of paramount importance.

Studies must be associated with nutritional surveys and programs, so as to identify dietary components and intake levels, particularly those suspected of being contaminated, and to promote nutritional education and awareness of the mycotoxin problem, and facilitate the adoption of appropriate technologies.

Constraints to Solving the Mycotoxin Problem

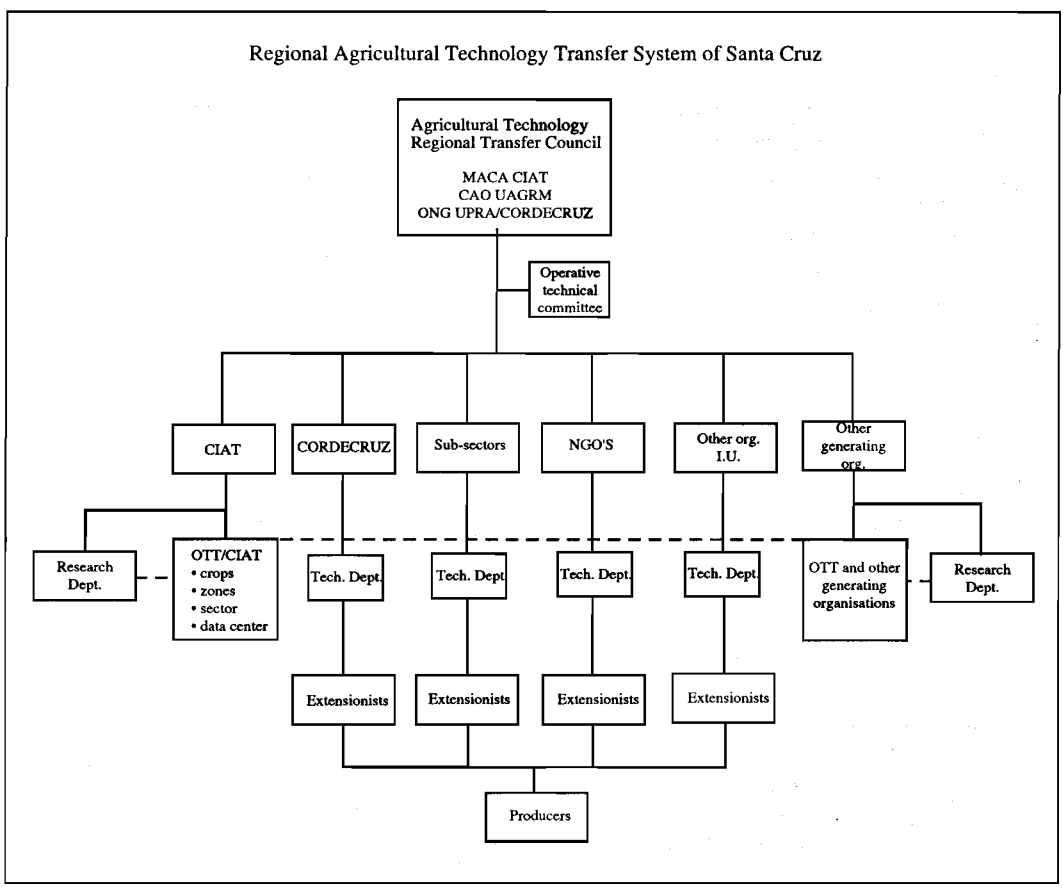
- Lack of trained personnel in official institutions.
- Lack of trained and experienced scientists in universities and research institutes
- Lack of research programs at government, university, and other research centres
- Lack of funds
- Lack of liaison between government, the private sector, scientists, and extension agents.
- Too much emphasis on analytical, toxicological, and regulatory aspects, to the detriment of prevention strategies and methodologies.
- Controls are more strictly applied to imported foodstuffs and animal feeds than to local production
- Lack of data on the mechanisms and extent of mycotoxin contamination
- Non-existent or poor flow of technical assistance to small farmers to prevent the problem
- Lack of proper identification of community leaders to support extension programs
- Lack of correspondence between the objectives and resources and the problems of fungi and mycotoxins at the farm level
- Failure to see the problem from a socioeconomic perspective
- Failure to tackle the problem using a comprehensive approach applying postharvest systems technologies

Needs and Strategies for Solving the Mycotoxin Problem

1. Socioeconomic appraisal of farmers' status, organisation, and authority and leadership flows.
2. Technical diagnosis of crops qualities and problems at both pre- and postharvest levels.
3. Existence and levels of extension and technology transfer efforts in the project area.
4. Type and degree of education and training at all levels of the farmer–extension worker complex.
5. Type and degree of understanding and utilisation of postharvest technologies by the farmers.
6. Identification, coordination, and implementation of cooperative activities with NGOs and other institutions working at the local level
7. Other physiographic and environmental studies.
8. Identification and quantification of specific problems along the postharvest chain.
9. Promotion and coordination of participation by farmers/traders and other groups in the problem identification process and in the search for solutions (cooperative networks).
10. Identification, selection, adaptation, and or adoption of specific locally acceptable technologies for the prevention and control all problems in the postharvest system.
11. Making clear to farmers project objectives and procedures of procuring and supplying technology to them.
12. Planning, designing, organising, and implementing thorough and comprehensive programs of motivation, education, training, and technology transfer for each agroecological and socioeconomic condition.
13. Monitoring, testing, and reorienting prevention and control of field and institutional activities at all levels.

Postharvest project and programs require:

- community training and other measures to promote and assist in the continuous monitoring of the postharvest system by 'local people' to identify, report, and take action;
- design and implementation of training and extension activities based on practical and locally acceptable technologies to prevent and control problems caused by moulds and mycotoxins; and
- through motivation, education, extension, and production and distribution of training materials, collaboration with local farmers in extending to 'other' farmers and community members information on those technologies already tested and proved effective. Credibility is important!



Poster Papers
Session Summary

Chairman: Dr J.I. Pitt, CSIRO Division of Food Processing, Sydney, Australia
Rapporteur: Dr R.A. Samson, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands

SURVEYS of fungi and mycotoxins in Asian commodities remain important. Surveys indicated a wide range of fungi occur in commodities in the region, though the introduction of more modern methods has helped to define the problem fungi more effectively. Surveys of mycotoxins continue to indicate excessive aflatoxin levels in maize, peanuts, copra and, sometimes, rice.

Control of aflatoxins in commodities also featured largely in the poster papers, which indicated that a wide variety of techniques has been studied, ranging from chlorine gas, various chemicals and preservatives, natural compounds, fumigants, and even plant chitinases. The use of smoke in copra drying showed promise. Several papers dealt with methodology for detection of aflatoxins or important spoilage fungi using monoclonal antibodies, with some encouraging results.

Toxicity studies were reported on some *Fusarium* and *Alternaria* species grown on grain, and with pure aflatoxin B_1.

Conference Summary
(including recommendations made by the meeting)

DURING this final session, summaries of each of the conference sessions were presented by their respective chairmen. These summaries are given at the end of the papers for each session in these Proceedings.

Also presented were the recommendations of two conference working parties, one convened by Dr J.I. Pitt to consider mycological aspects, the other led by Dr H.L. Trenholm examining mycotoxicological aspects of the storage fungi and mycotoxin problem. These recommendations and a summary of the discussions following their presentation are given below.

Dr R.V Bhat, supported by Dr Marietta Adriano, then led a discussion on the need for studies of the economic impact of fungi and mycotoxins.

The meeting next discussed a proposal for an ongoing working party to ensure contact was maintained among participants after the conference and that the recommendations made were implemented to the fullest extent possible.

Finally, Dr B.R. Champ tabled an overall summary of the conference.

General Issues Raised during the Conference Summary Session

Mycotoxin research in many developing countries has focused primarily on the aflatoxins. The reasons given for this include the lack both of trained personnel and of the highly sophisticated instruments, such as HPLC and GC–MS, needed for work on the other mycotoxins.

Personnel involved in research, instruction, and regulation of mycotoxins must be trained, generally at high (MSc or PhD) levels in chemistry and mycology if they are to gain a solid understanding of the basis for the development of analytical methodologies, of the formation of mycotoxins, and of the ecology of mycotoxin-producing fungi. There is a particular need to develop methodologies for both mycotoxin and fungal analysis that are fast, need minimal resources, and are suitable for screening, monitoring, and surveillance activities.

Existing facilities in the region must be upgraded. In the Philippines, for example, several agencies have ceased mycotoxin work simply because they lack safety equipment as basic as fumehoods. Others have been restricted to aflatoxin work because they do not have the apparatus needed for analysis of other mycotoxins. This is unfortunate because several species of *Fusarium*, *Penicillium*, and other important fungi have been isolated from major agricultural commodities

Sampling of commodities is another area that needs more intensive study. The distribution of mycotoxins has yet to be established and, consequently, a sampling protocol has to be formulated. As regards the latter, difficulties are encountered in obtaining representative samples, particularly from bulk commodities.

Summary of Needs

1. Personnel development

(a) Training of all personnel involved in research, teaching, and regulation of mycotoxins, in analytical methodologies, particularly those other than for aflatoxins and aflatoxin-producing fungi.

(b) For mycologists and chemists, graduate courses on the ecology of mycotoxin-producing fungi, and on analytical methodologies for both fungi and mycotoxins.

2. Facilities

Existing facilities for work on fungi and mycotoxins need upgrading to improve their effectiveness and safety.

Facilities needed include appropriate analytical equipment, cold storage for samples and standard solutions of mycotoxins, and sample preparation rooms and equipment.

3. Techniques and supporting manuals
Sampling plans suited to local conditions in the region have to be devised, including methodologies for identification of fungi and mycotoxins. Identification and procedural manuals and handbooks are needed relating specifically to fungi and mycotoxin problems in the Southeast Asian region.

Recommendations of the Working Party on Mycology

1. This conference clearly identified the need for improvement in the methodology for identifying and quantifying food spoilage and mycotoxigenic fungi in stored food and feedstuffs.

Therefore it is recommended that the International Commission on Food Mycology be approached, with a view to forming a working group and eventually a Subcommission in the Southeast Asian region. (Recommendation 1)

The aims of this group would be:
(a) to conduct a survey to obtain information on methods being used for enumeration and isolation of fungi from food and feedstuffs in Southeast Asia;
(b) to promote collaborative studies of modern mycological methods, so as to select and develop systems suitable for use in tropical areas; and
(c) in collaboration with local organisations and granting agencies, to organise one or more workshops to facilitate standardisation and dissemination of standard food mycology methods in Southeast Asia.

and

It is recommended that one or more fungal culture collections be established in the region as soon as possible. (Recommendation 2)

These collections would:
(a) maintain accurately identified cultures of major spoilage and mycotoxigenic fungi obtained from within the region and elsewhere;
(b) provide such cultures to regional mycologists so as to facilitate accurate identification of and effective research on food-borne fungi; and
(c) collaborate with each other and with existing collections elsewhere in the world.

2. The conference identified a pressing need for improvements in knowledge of the ecology and etiology of the major mycotoxin-producing fungi in the region.

Therefore, it is recommended that studies on the ecology and etiology of aflatoxin-producing fungi, and of major Fusarium *and* Alternaria *species that occur on the most important crops and commodities, be implemented by competent mycologists as soon as possible.* (Recommendation 3)

3. With the recent recognition that toxigenic *Fusarium* species are widely distributed in tropical regions, especially in maize, a need was identified for greatly improved knowledge of the susceptibility of maize cultivars to *Fusarium* infection. Increased introduction of new cultivars in other crops, such as sorghum, may also lead to an unacceptable increase in susceptibility to mycotoxigenic fungi, especially *Alternaria* species.

Therefore, it is recommended that studies on the susceptibility of maize cultivars to Fusarium moniliforme *and other toxigenic* Fusarium *species, and of sorghum cultivars to* Alternaria *species, be undertaken as a matter of urgency.* (Recommendation 4)

Discussion on Mycology Recommendations

There was unanimous support for the aforementioned recommendations with discussion emphasising the need for a better understanding of the factors leading to mycotoxin contamination (Recommendation 1) and on the establishment of accessible culture collections (Recommendation 2). As regards the latter, it was suggested that a realistic starting point would be provision of extra support for existing collections.

Recommendations of the Working Party on Mycotoxins

1. It was recognised that there are limited laboratory facilities in developing countries, including Southeast Asia, for carrying out mycotoxin analysis, researching the nature, extent, and economic impact of mycotoxins, and responding with advice and information when mycotoxin problems emerge. This was exacerbated by the lack of adequate training programs for local analytical laboratories, and unavailability of inexpensive mycotoxin reference standards, check samples, and analytical methodology optimised for local laboratory conditions. As a consequence, local data defining the problem were sparse and there was an urgent need for quantitative surveys of mycotoxin incidence to supplement qualitative data on the nature and extent of contamination.

Therefore, it was recommended that funding agencies aim to strengthen programs of core research laboratories involved in mycotoxins research and development. (Recommendation 5)

This would enable them to:

(a) research and develop practical methods for sample preparation, mycotoxin analysis, and screening techniques;

(b) define the nature, extent, and costs associated with mycotoxin contamination; and

(c) act as clearing houses for information on mycotoxins, support training and network communication, and provide reference standards and check samples.

2. The conference highlighted the lack of established communication both between local laboratories and between these laboratories and those in developed countries. There was no up-to-date list of laboratories working in the area and there was a need to certify methods suitable for developing countries, including screening methods for mixtures of mycotoxins.

Therefore, it was recommended that international agencies, through multilateral or linked bilateral activities, support networking and provision of other services aimed at standardising and improving laboratory and sampling methodologies, communication, and training in the core laboratories. (Recommendation 6)

This would be achieved by:

(a) facilitating communication between laboratories in developing and developed countries;

(b) preparing and disseminating audiovisual aids (videos, manuals, etc.) for training;

(c) training of personnel involved in research and analytical services, including graduate studies for mycologists and chemists to gain a broader understanding of the extent and nature of mycotoxin problems;

(d) providing ongoing advice for regional analytical services;

(e) organising collaborative studies on analytical methods;

(f) establishing standard methodology, standard laboratory safety services, and regional laboratory certification;

(g) maintaining an inventory of available analytical services;

(h) advising new laboratories on appropriate analytical methods; and

(i) promoting development of new, rapid screening methods.

Discussion of Mycotoxin Recommendations

Again there was unanimous support for the proposed conference recommendations, discussion focusing on the characteristics of the central research laboratories promoted. It was suggested that they would house multidisciplinary teams including not only mycological and mycotoxicological specialists, but also socioeconomists, veterinarians, etc. There was general agreement that the most realistic approach to fulfilling objectives in mycotoxins research would lie in strengthening existing laboratories rather than establishing new ones.

Economic Impact of Fungal and Mycotoxin Contamination

The conference then focused its attention on economic issues that had been highlighted on many occasions during the meeting. This related to the general problem of identifying the direct and indirect losses occasioned by spoilage fungi and mycotoxins, and the need to quantify these and the benefits that would accrue by preventing them. This had become mandatory in soliciting support for both research and measures to overcome the perceived threat to food supplies and human and animal health. The problem is how to structure economic impact assessments so that they provide firm data that will convince policy makers of the need for action.

The economic consequences of fungal and mycotoxin contamination are generally better recognised than health implications. Nevertheless, an intensive and extensive review of the existing information on economic implications of mycotoxins needs to be undertaken. The methodology needed for economic studies has to be evolved from real data on the costs to national exchequers of economic losses in agricultural and livestock productivity, in foreign exchange, in terms of quality of plant and animal products, and so forth. Some of these data must be collected in countries of the region.

Therefore, it is recommended that research effort on spoilage fungi and mycotoxins be extended to include studies on the economic implications of their contamination of food and feedstuffs and the development of appropriate methodology for these economic assessments be a matter of high priority. (Recommendation 7)

Working Party on Fungi and Mycotoxins in Asian Food and Feedstuffs

The Chairman indicated that in discussions during the conference a recurrent theme was the desirability and need for regular interaction between the various workers associated with the mycotoxin problem. The conference had demonstrably been instrumental in providing for many of the participants a valuable updating of the situation in the region and the state of the art of mycology and mycotoxicology. In view of the seriousness of the problem and the prognosis of possibly much more serious problems emerging in the near future, it appeared highly desirable to ensure an opportunity for interested participants to meet regularly to exchange information and to ensure that recommendations coming from the conference were promoted and implemented to the fullest extent possible. The Chairman, responding to numerous requests from participants, proposed that a Working Party be established to achieve these aims. He indicated that, in his opinion, it would be appropriate if GASGA, a cosponsor of the conference and a truly international consortium of postharvest grain technology expertise and resources, were to provide an umbrella for such an activity. The individual member organisations of GASGA could then support participation of their own representatives.

Therefore, it is recommended that GASGA be approached to convene a Working Party on Fungi and Mycotoxins in Asian Food and Feedstuffs to enable regular exchange of information and to ensure recommendations from the conference were implemented to the fullest extent. (Recommendation 8)

Chairman's Summary

In my summary of the conference, I do not propose to reiterate what the chairmen of the various sessions of the meeting have so capably said already. Our rapporteur for this closing session has their reports, as well as your comments and recommendations. The recommendations will be collated and circulated to discussion leaders for comment before they are published in the Proceedings of the Conference.

Three main messages arise from the presentations and discussion at this conference:

- aflatoxins in food and feedstuffs are a major problem, particularly in developing countries;
- there are, as well, other problems, such as those arising from the *Fusarium* toxins, with potentially serious consequences for human and animal health; and
- the deleterious effects of mycotoxins are not restricted to nutrition and carcinogenicity: the discovery of immunosuppressive activity in these compounds adds a further serious dimension to the mycotoxin problem.

Some common threads have run through discussions over the past four days.

Firstly, the need for proper identification of food-spoilage fungi — particularly the mycotoxigenic species — has often been mentioned, as well as the need for correct association between species and their toxins, and elucidation of the conditions favouring production of the latter.

A second issue which has emerged is the need to gain an economic perspective of mycotoxin problems. We must be able to put figures on the various types of losses that accrue because of the presence or the possibility of presence of mycotoxins in food and feedstuffs. We must also try to apply some measures to the effects on the health of humans and livestock. Last but not least, we must be able to measure the benefits from mycotoxin reduction programs, because only by doing so will mycotoxin work attract the funding needed for further progress.

Also articulated on a number of occasions was the need for harmonisation of approaches to the mycotoxin problem, of the methodology for dealing with it, and of national and international regulations affecting trade in food and feedstuffs.

How do we achieve this? The following is a distillation of points — not in any order of priority — all of which need attention if we are to overcome, or at least reduce the importance of the problem.

1. Standardised fungal identification methodology, including handbooks on identification
2. Regional culture collections
   - Location and affiliation
   - Scope/support
   - Terms of availability of material
3. Ecology of mycoflora
4. Standardised mycotoxin sampling procedures and analytical techniques, including handbooks
5. Collaborative mycotoxin analytical programs including performance monitoring schemes
   - Convening group
   - Reference analytical grade materials
   - Samples containing certified amounts of mycotoxins
   - Listing of participating laboratories
6. Definition of field aspects of fungal infection, including ecology and etiology
7. Selection for resistance to mycotoxigenic fungi
   - Character identification
   - Guidelines
8. Economic evaluation and benefit/cost studies
   - Establishing standard methodology for economic assessment of the mycotoxin problem and measuring potential benefits from proposals to alleviate the problem

9. A regional working group, organised under the umbrella of GASGA, which meets regularly to ensure recommendations from the Conference are followed through.

10. Consolidation of Mycotoxin Newsletter

- Listing of regional laboratories and workers
- Research news and summaries

Eight recommendations have been formulated during the conference. If these are implemented they will go a long way towards solving the spoilage and fungi and mycotoxin problem. The recommendations are:

1. That the International Commission on Food Mycology be approached, with a view to forming a working group and eventually a Subcommission in the Southeast Asian region.

2. That one or more fungal culture collections be established in the region as soon as possible.

3. That studies on the ecology and etiology of aflatoxin-producing fungi, and of major *Fusarium* and *Alternaria* species that occur on the most important crops and commodities, be implemented by competent mycologists as soon as possible.

4. That studies of the susceptibility of maize cultivars to *Fusarium moniliforme* and other toxigenic *Fusarium* species, and of sorghum cultivars to *Alternaria* species, be undertaken as a matter of urgency.

5. That funding agencies aim to strengthen programs of core research laboratories involved in mycotoxins research and development.

6. That international agencies, through multilateral or linked bilateral activities, support networking and provision of other services aimed at standardising and improving laboratory and sampling methodologies, communication, and training in the core laboratories.

7. That research effort on spoilage fungi and mycotoxins be extended to include studies on the economic implications of their contamination of food and feedstuffs and the development of appropriate methodology for these economic assessments be a matter of high priority.

8. That GASGA be approached to convene a Working Party on Fungi and Mycotoxins in Asian Food and Feedstuffs to enable regular exchange of information and to ensure recommendations from the conference were implemented to the fullest extent.

I commend these to you, and ask you to take them away and do all that you can to expedite their implementation.

Lastly there is the matter of training at all levels, the need for which we must certainly endorse. How such training might be provided is probably beyond the ambit of the cosponsors of this meeting and more the role of FAO and bodies with a similar mandate. What we *can* do is to reinforce at every possible opportunity the need for support for such training.

I must now finalise this conference summary and, since it will be my last opportunity to do so, I wish to make some acknowledgements. Firstly, thanks are due to Mrs Dara Buangsuwon and her staff from the Plant Pathology and Microbiology Division, Department of Agriculture, and particularly Mrs Prapaisri, for the superb local organisation of the conference. No one could fail to have noticed how smoothly proceedings have gone this week. I must also convey our thanks to Ms Irene Villapando of the ASEAN Food Handling Bureau, who has so capably handled much of the international liaison associated with this conference.

Finally, I must thank all the presenters of papers, chairmen, rapporteurs, and participants who have combined and never flagged in their enthusiasm to make this meeting so productive and stimulating.

Dr B.R. Champ
ACIAR

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