# Moulds and mycotoxins

Magic mushrooms and toadstools aside, what can eating fungi do to you? A chance encounter with a mould led Fleming to a life-saving drug (albeit he didn't fully appreciate it at the time), but meeting a mould need not always be so serendipitous.

The fact is that many fungi produce chemically unusual substances — often exceedingly poisonous — called mycotoxins. Moulds are the worst offenders in this respect. They can contaminate our foodstuffs and then we inadvertently consume them.

Most people certainly won't eat obviously mouldy food, but moulds can be present without being easily visible. Alternatively, although contaminating fungi might have died or been removed by special treatments, their mycotoxins may still be intact.

Accordingly, people in the food production and preparation industries take the greatest care to prevent any fungal contamination. In the past, however, before scientists became aware of mycotoxins, matters were not so well controlled.

### Fearsome fungi

One of the most notorious mycotoxicoses — the name given to diseases that mycotoxins cause — was ergotism. The culprit, the fungus *Claviceps purpurea*, grows on some grains, especially rye. Parts of the fungus, called ergots, become fragmented and dispersed throughout the flour during the milling process.

The toxins in ergots are all derivatives of lysergic acid, which is also the precursor of the hallucinogen LSD. Consequently, victims suffer from unpleasant hallucinations as well as the other effects of the toxins, which include a constriction of the arteries that gives rise to a sensation of burning in the affected parts, and may ultimately lead to gangrene. In severe cases the victim dies.

Episodes of bizarre behaviour in villages in the Middle Ages, such as witch-burning and seeing visions of angels or devils, have been attributed to ergot poisoning. The last known case of this distressing disease in Europe occurred in a French village in 1954, when more than 200 people became ill and four died. The outbreak was well studied and was traced to gross negligence on the part of a miller.

Outbreaks of ergotism have been reported more recently in drought-affected areas of Africa and India, and may well continue to occur in these regions because of food shortages and less stringent health control measures.

People can also ingest mycotoxins second-hand, as it were. An example occurred in the 1970s in Denmark, where some pigs were found at slaughter to have kidney inflammation. The cause was eventually traced to ochratoxin A, a mycotoxin produced in this case by *Penicillium viridicatum*, which often grows on Danish barley, a constituent of the pig feed. (Barley with any hint of mould would not of course be used for human consumption.)

By law, the diseased pig kidneys were destroyed, but other parts of the carcasses were released for use. Later analyses of pork and bacon showed the presence of ochratoxin A from the feed, unchanged, in the pig fat.

A small risk therefore exists for humans who might eat this — particularly the Danish farmers who usually eat their own (uninspected) pigs. And indeed, rates of death caused by kidney failure are unusually high in some rural areas in Denmark.

### Risks in Australia?

The question naturally arises: how much at risk is the Australian population from diseases caused by mycotoxins?

We can be quite sure that we are safe from ergotism. Ergots do not occur on wheat, our staple cereal, and we have no evidence that the causative fungus is a problem on rye or triticale here. Similarly, we have no reason to suspect that ochratoxins are significant in Australia. Of course, it wouldn't do any harm to check a little further, but as usual resources and finance are limiting.

Moulds belonging to the genus *Fusarium*, growing mainly on wheat and corn, can produce a whole battery of toxins called trichothecenes. Analytical techniques to detect these are tricky, as is the identification of the different *Fusarium* species.

One of the most toxic trichothecenes, T–2, has been implicated in outbreaks of a mycotoxicosis in the U.S.S.R., occurring frequently earlier this century. In this case, the grain on which *Fusarium* grew had alternately frozen and thawed when it was left to overwinter in the fields during war-time labour shortages.

However, scientists now suspect that freezing and thawing may not always be necessary for the development of the toxin, and T2 is now known to be a cause of disease in feedlot animals in the United States. Trichothecenes cause severe internal bleeding, accompanied by muscle spasms and convulsions. Symptoms also include vomiting blood, and haemorrhages under the skin.

Fortunately, no known problems with trichothecenes occur in Australia. Serious outbreaks of *Fusarium* moulds are quite obvious when growing on a crop, and any infected wheat would automatically be downgraded and declared unfit for human consumption. (This is not always the case in poor countries.)

**Harvest: the process of pulling peanuts.**

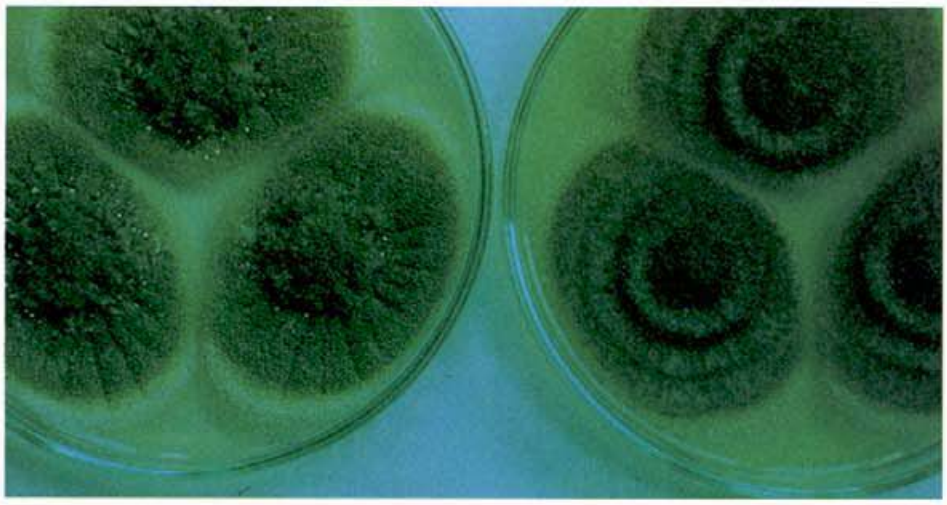

**Although some peanuts grow in northern New South Wales and the Northern Territory, large-scale production is confined to Queensland, and 85% of its crop comes from the Burnett region, with the Atherton Tableland and Rockhampton areas producing most of the rest.**

The only area where we know that we have to take particular care is the Australian peanut crop. Roast peanuts are a popular and nutritious snack, and plenty of people like their peanut butter, too — so much so, in fact, that the peanut industry is worth about $30 million a year to Australia.

However, two species of the mould genus *Aspergillus*, namely *A. flavus* and *A. parasiticus*, can grow on peanut plants and may invade the nuts themselves. Moreover, they can produce their own toxins, called aflatoxins. The name comes from '*A. flavus* toxins'.

These toxins were first detected in 1960, following the deaths in Britain of 100 000 turkeys that had been fed on peanut meal. Ironically we now know that probably the majority of *A. flavus* isolates do not produce aflatoxins, whereas virtually all *A. parasiticus* isolates do. (It requires an expert to tell the two species apart, and often moulds called *A. flavus* may include some *A. parasiticus*. For this reason we will use *A. flavus* here to denote both species unless otherwise stated.)

Aflatoxins in sufficient doses (5–10 mg) can cause acute (short-term) poisoning, which may lead to fatal liver damage. This is so exceedingly rare that the world's scientific literature contains no absolutely confirmed cases in humans. In smaller quantities aflatoxins may cause no noticeable illness at all, but, insidiously, they may be carcinogenic, acting to produce liver cancers and possibly genetic or other damage many years later.



**The mould itself, *Aspergillus flavus*, in laboratory culture.**

The fact that aflatoxins are among the most poisonous substances known need not unduly worry the Australian consumer. The peanut industry has long been aware of the problem, and Dr John Pitt, a mycologist now specialising in mycotoxins, and Ms Ailsa Hocking, both of the CSIRO Division of Food Research in Sydney, have been researching the problem in some detail.

With the help of funds from the National Peanut Council of Australia and a Commonwealth Special Research Grant, they set to work — first, to develop a culture medium that allows simple but reliable identification of *A. flavus* and *A. parasiticus*, and then, armed with this, to perform detailed surveys of soils and farms in the main peanut-growing areas.

Peanuts are particularly susceptible to *A. flavus* attack because they grow underground. The growth of the mould can occur at a number of stages: while peanuts are still maturing in the soil; after they are pulled from the ground, during drying; or finally during storage, but only if poor practice allows the moisture content of the nuts to rise above the low level that prevents mould growth.

In the laboratory, the first task was to obtain rapid identification of the mould in samples. Dr Pitt and Ms Hocking devised a special growth medium (AFPA) for the fungus. As well as all the usual compounds essential for fungal nutrition, and others to prevent bacterial contamination, this selective medium contains ferric ammonium citrate at a concentration of 0·05%. The ferric ions react with an organic compound called aspergillic acid, which the fungus produces, to give a bright and distinctive orange-yellow colour visible underneath the fungus through the Petri dish.

The method is reliable (Dr Pitt established that false negatives occur in only 2·9% of cases and false positives in only 1·4%), and gives results within 42 hours when incubated at the correct temperature, instead of the 4 days generally required with the older identification medium.

## In the field

To get a general picture of the problem, Dr Pitt and his staff examined hundreds of soil samples from land in which peanuts had recently grown, from under pastures, and from virgin forest in nearby areas.

Nearly 90% of samples from peanut farm land contained *A. flavus* spores, but the levels varied widely — from 100 spores per gram of soil to 50 000 per gram. High levels of *A. flavus* in soils are not necessarily associated with high levels of invasion of the peanut plants, because other factors are also important. However, farms with low numbers of *A. flavus* spores in the soil are less likely to have the mould in their peanuts at pulling.

By contrast, Dr Pitt found that *A. flavus* is largely absent from virgin soils and

**The distinctive orange colour produced by *A. flavus* when it grows on Dr Pitt's specially designed identification medium.**

**Conventional drying of peanuts in the field after harvest. A CSIRO worker is sampling for *A. flavus*.**

pasture land. Of about 30 virgin soil (that is, undisturbed forest soil) samples, only three contained detectable levels of the fungus, and one of these had come from a drainage path of a peanut field.

By analysing spore numbers Dr Pitt also showed that any substantial rainfall caused a quick and dramatic (ten- to a hundred-fold) decrease in numbers of spores in the soil. At first glance, this seems rather strange for a mould, which one might expect to like damp climates, but Dr Pitt has shown that the rain causes population increases in many other soil micro-organisms, some of which prey on *A. flavus*. His research has also demonstrated that low moisture levels favour the invasion of the plants by *A. flavus*, as well as its survival in the soil, because moisture stress reduces the plant's vigour and renders it more susceptible to infection.

Dr Pitt and his colleagues monitored the levels of the mould growing in the flowers, pegs, and developing nuts of the peanut plants. Then they checked the nuts, after harvest, for *A. flavus* and for moisture content, and for the quantity, if any, of actual aflatoxins.

Like most agricultural pests, *Aspergillus* spp. cause more trouble in some years than others, related to climate and seasonal factors. Dr Pitt wanted to know whether they invaded peanuts after pulling and, if so, what would prevent this? He also wanted to know whether it was possible to predict a bad year with heavy *A. flavus* infestation in advance; if so, then farmers could be forewarned and suitable precautionary measures, which he hoped to identify, could be taken.

After continuous sampling from 1980 to 1984, he established that peanuts are not invaded by *A. flavus* after pulling. The nut itself is relatively resistant to extensive fungal growth.

However, peanuts colonised by the mould while still underground suffer a marked build-up of aflatoxins after pulling. Rapid drying, to levels of humidity in the peanut of less than 12%, minimised the build-up, because not even this relatively drought-tolerant mould can survive at such a low water level.

Normal drying, taking from 6 to 10 days, involves leaving the pulled peanuts outside without cover. Indoor mechanical ventilation makes drying more rapid and efficient, but is expensive. Dr Pitt's work shows that only those peanut crops extensively invaded before pulling need the costly mechanical drying to prevent aflatoxin build-up.

With regard to possible prediction, unfortunately the work concluded that invasion of the flower or peg by *Aspergillus flavus* bore little relation to levels of the mould in the nuts at harvest. However, a good warning to the industry can come from the state of the nuts during the last week before pulling.

If, in any year, samples taken then from several farms show heavy fungal contamination (quite simple to confirm with Dr Pitt's selective growth medium), not only will the more rapid drying method be recommended, but also the peanut-shellers can be warned to be especially alert for aflatoxins. Conversely, in a good year, much of the expensive drying on the farm, and some of the exhaustive testing by the shellers, could be reduced.

But conclusions in this area cannot be clear-cut. Remember that many *Aspergillus flavus* isolates do not produce the toxin. And the moulds of *A. parasiticus*, although they appear capable of it, do not produce the toxin all the time. Toxins are generally only produced at one stage in the life cycle, and then probably only if the food source is adequate. These factors complicate any predictions of aflatoxin production.

## Precautions

If aflatoxins are present, what happens then?

First, they must be detected. Fortunately, they have the property of fluorescing with a blue-green colour under ultraviolet light. The sample of peanuts to be tested is homogenised, and then thin-layer chromatography is used to separate aflatoxins from the many other compounds present in squashed peanuts.

Currently, these techniques can detect aflatoxins in quantities as small as 1 $\mu$g per kg of peanuts, which is one part in a thousand million. The legal limit, as recommended by the National Health and Medical Research Council, is 15 $\mu$g per kg. While it takes 1 mg (1000 $\mu$g) to cause acute sickness in humans, lower doses could induce cancer and we do not have precise figures, for obvious reasons.

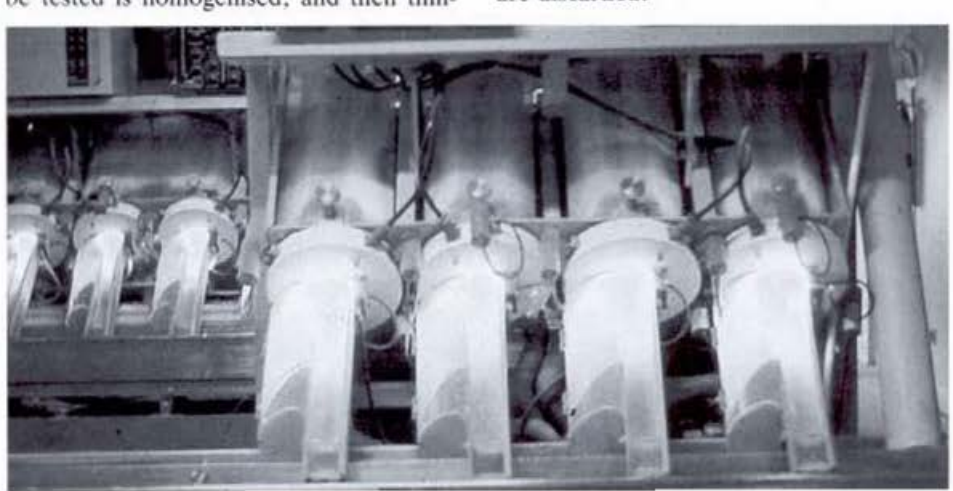
Every 10-tonne batch of peanuts is routinely sampled for aflatoxins, but this is just the first step. The problem is that just one extremely heavily infected individual peanut could contain as much as 0·1 g (100 mg) — the lethal limit. Therefore, every individual peanut must be checked in some way.

It's clearly not possible to analyse each and every one, as the process involves the destruction of the nut; but, fortunately for us, the kernels of mouldy nuts almost always have a darker colour than their uninfected brethren. Based on this, the Peanut Marketing Board, independent peanut-shellers, and peanut-processors all use electronic sorting machines that scan each individual nut at least once, and often three times or more. If a discoloured nut is detected as the nuts stream past the electronic eye, it is knocked out by a blast of air. Human sorters are on hand to check this process.

Mouldy nuts cannot be sold, and are used to make peanut oil. The refining process adds an alkali that removes the toxins. However, peanut oil fetches a lower price than the nuts themselves, and so a heavily contaminated crop represents a severe economic loss.

Alkali treatment cannot be applied to whole nuts, but can we remove or destroy aflatoxins in any other way? Short of destroying the nut, the only possibility may be heating. Unfortunately, aflatoxins are

**Banks of colour sorters at the Peanut Marketing Board, Kingaroy. Rigorous sorting ensures that any mouldy peanuts are discarded.**



## Mycotoxins on the farm

As well as affecting us, mycotoxicoses can be a problem for our livestock.

Facial eczema in sheep is caused by eating perennial ryegrass infected with the fungus *Pithomyces chartarum*, which produces a toxin. The disease occurs sporadically in Western Australia and Victoria, and average annual losses are small.

But the most serious known mycotoxic disease of stock in Australia is lupinosis, which causes annual losses amounting to perhaps $16 million. The bulk of this represents the production lost through leaving lupin stubbles ungrazed.

As the name lupinosis implies, the animals become ill and may die after eating post-harvest lupin stalks and roughage. The lupin is infected by a mould grandly called *Phomopsis leptostromiformis*. The mould is present on the living plant, but flourishes on the dead leaves after harvest — specially if the humidity is high.

The disease is more of a problem in Western Australia, where 500 000 ha were sown with lupins in 1984, as against 70 000 ha in the eastern States. Each year farmers report, on average, more than 1000 sheep killed by the disease, and losses also occur through the decrease in production caused by sickness in the animals that survive.

Lupinosis is not a new condition. Indeed, in the 1870s scientists in Germany suggested the possibility of fungal involvement in the disease. In the 1950s it appeared in Western Australia and South Africa, and the role of the fungus was confirmed by scientists in both countries in the early 1970s.

Phomopsin, the toxin produced by the fungus, damages the liver, eventually causing cirrhosis. The animal stops feeding, and death may occur within a few days of the appearance of the disease.

Strangely, sheep seem particularly susceptible to the toxin. Just 1 mg per kg of body weight, given by mouth, is a fatal dose for them. Rats and some other mammals can tolerate higher doses.

A group of scientists led by Dr Claude Culvenor, of CSIRO's Division of Animal Health in Melbourne, has for some years studied lupinosis, as well as other stock diseases caused by toxins. The researchers have isolated phomopsin, the toxin, and, no easy feat, determined its structure. It is a peptide with six modified amino acids and an unusual large ring.

We also know that phomopsin prevents cell division, and it does this by attaching to tubulin, an important protein within cells. Tubulin forms the spindle — the delicate apparatus that pulls the chromosomes apart during cell division. Phomopsin is, therefore, starting to be seen as an interesting tool for molecular and cell biologists probing the mechanism of cell division.

Sheep and their owners are no doubt less enthused by it.

We have no 'cure' for lupinosis. The only answer is not to use lupins if rain has fallen after harvest. Some good news is that, as far as we know, the fungus in the field grows only on lupins.

The structure of Phomopsin A, a mycotoxin produced by *Phomopsis leptostromiformis* (Kühn) Bubak ex Lind. J.A. Edgar, C.C.J. Culvenor, J.L. Frahn, A.J. Jones, C.P. Gorst-Allman, W.F.O. Marasas, P.S. Steyn, R. Vleggaar, and P.L. Wessels. In 'Tricothecenes and Other Toxins', ed. J. Lacey. (John Wiley and Sons Ltd: Chichester 1985.)

heat-stable. Temperatures of 100°C will only destroy from 30 to 50% of the aflatoxins, and this is clearly not sufficient.

### Other crops

*Aspergillus flavus* can grow on many other plants, but will seldom produce toxin unless a sufficiently concentrated nutrient source is available. For example, it grows on coffee plants without apparently producing toxin. A danger is probably only present with oily nuts and seeds, which in Australia could include pistachio nuts, sunflower seeds, and corn. Dr Pitt is not aware of any aflatoxin problem with these products, but he is unable to check this because of lack of resources at present.

Around the world, mycotoxins are starting to be implicated in various known diseases. They have also, sadly, been strongly implicated in warfare; many scientists consider that the notorious 'yellow rain' in South-East Asia contained trichothecenes.

The aflatoxin story itself is not yet fully unravelled. Aflatoxins exist in more than one form. Dr Pitt suspects that most of them are not, in fact, carcinogenic by themselves. Evidence is accumulating to suggest that they are only carcinogenic in 'collaboration', as it were, with the virus that causes hepatitis B. This may help explain some observations from overseas that communities exposed to relatively high

**What the consumer will not meet; mouldy, discoloured peanuts rejected by the electronic eye.**

levels of aflatoxins in some areas do not develop the expected levels of liver cancer.

All of this shows that apparently 'pure' scientific studies such as those relating to moulds may reveal important facts that require urgent action. In the case of aflatoxins, we do not have a magic 'cure' to prevent the growth of the mould on the peanut plants. What is possible, however, is to be sufficiently aware of the problem, and its status from year to year, so that we can control the public health aspects of it.

### Why does the mould do it?

One final question you may be asking yourself: why does this little mould produce such lethal compounds? Dr Pitt speculates that the answer is mites. Apparently, these tiny arachnids are voracious predators on many moulds; but when they hit our aflatoxic friends, they curl up and die, leaving the mould in peace. But Dr Pitt is wary of proving this by introducing any experimental mites because they constitute frightening agents of contamination in any mycology laboratory. As they chew their way from one harmless mould to another, they carry spores and cause chaos in the ordered cultures of our mycologists — a 'mitey' important problem!

*Roger Beckmann*

### More about the topic


The significance of potentially toxigenic fungi in foods. J.I. Pitt. *Food Technology in Australia*, 1984, **36**, 218–19.

Mycotoxins in human health. J.I. Pitt. *CSIRO Food Research Quarterly*, 1981, **41**, 31–7.

An improved medium for the detection of *Aspergillus flavus* and *A. parasiticus*. J.I. Pitt, A.D. Hocking, and D.R. Glenn. *Journal of Applied Bacteriology*, 1983, **54**, 109–14.

Mycotoxins in food products. J.I. Pitt. *Culture*, 1985, **6**(2).