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Mycotoxin review 3: Houses and pastures

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The two previous parts to this review have emphasised the relatively small number of mould toxins, associated with the mould genera *Penicillium*, *Aspergillus* and *Fusarium*, which may have the greatest impact in the context of human foods. There are, however, a large number of toxic mould metabolites, produced by a diverse range of other mould genera, with an extraordinary diversity of chemical structure and biological activity. The chemical structures are fascinating and worth studying because they provide clues about biosynthetic pathways and possible biological activity. The moulds producing them occur in a wide range of ecological niches from endophytes, obligate plant pathogens to saprophytes. This diversity will be explored from two points of view – damp houses and animal pastures.

Keywords: damp house illness, ergotism, toxic pastures, *Stachybotrys*, *Pithomyces*, *Rhizoctonia*, *Acremonium*

Moulds in damp houses

Although species of *Fusarium* produce a large number of trichothecene toxins there is one group of this family of compounds which is not associated with *Fusarium*. These are the macrocyclic trichothecenes, such as satratoxins, verrucarins and roridins, some of which are more toxic even than T-2 toxin. Satratoxin H (Fig 1a), low concentrations of which cause necrosis and haemorrhage in many organs, is an example of one of these molecules produced by *Stachybotrys chartarum* (= *S. atra*). This mould was originally implicated in stachybotryotoxicosis of farm animals, especially horses fed on contaminated mouldy hay, and occasionally with the people handling such hay (Forgacs & Carll, 1962). More recently *S. chartarum* has been implicated in illnesses associated with living in damp houses in which this very cellulolytic species can grow on wall paper and plaster board when they have become wet.

Professor Bruce Jarvis, of the University of Maryland, has made considerable contributions to the study of the chemistry and significance of the macrocyclic trichothecenes and his recent review of the diversity of moulds associated with damp houses emphasises the importance of *Stachybotrys* (Jarvis, 2002). There have been several well documented incidents of adults suffering a range of illnesses following exposure to the spores of *Stachybotrys* in damp buildings (Cooley, *et al*., 1998), but the more recent reports of idiopathic pulmonary haemorrhage in very young children in Cleveland, Ohio, have really put the spotlight on this group of moulds (Jarvis *et al*., 1998). *Stachybotrys chartarum* is a complex species and seems to contain at least two distinct phylogenetic species (Cruse, *et al*., 2002). There is no correlation between genetic characteristics and geographical distribution and it is still not certain whether these phylogenetic species correspond to the distinct chemotypes recognised by Andersen, Nielsen & Jarvis (2002). At least two chemotypes have been described, one characterised by the production of the cytotoxic macrocyclic trichothecenes and the second by the production of a group of diterpenoid metabolites known as atranones which induce inflammation. Both of these chemotypes, as well as a further unnamed species of *Stachybotrys*, may occur in the same water-damaged building making it difficult to determine cause and effect, a difficulty compounded by the ability of these moulds to produce several other toxic metabolites. Jarvis (2002) cautiously concludes that "more research is needed to determine the impact to health resulting from inhalation of toxigenic mould spores".

Moulds, forages and animal welfare

It is possible to demonstrate the ecological diversity of toxigenic moulds using examples implicated in the health of farm animals. This diversity includes obligate plant pathogens, obligate endophytes and saprophytes.

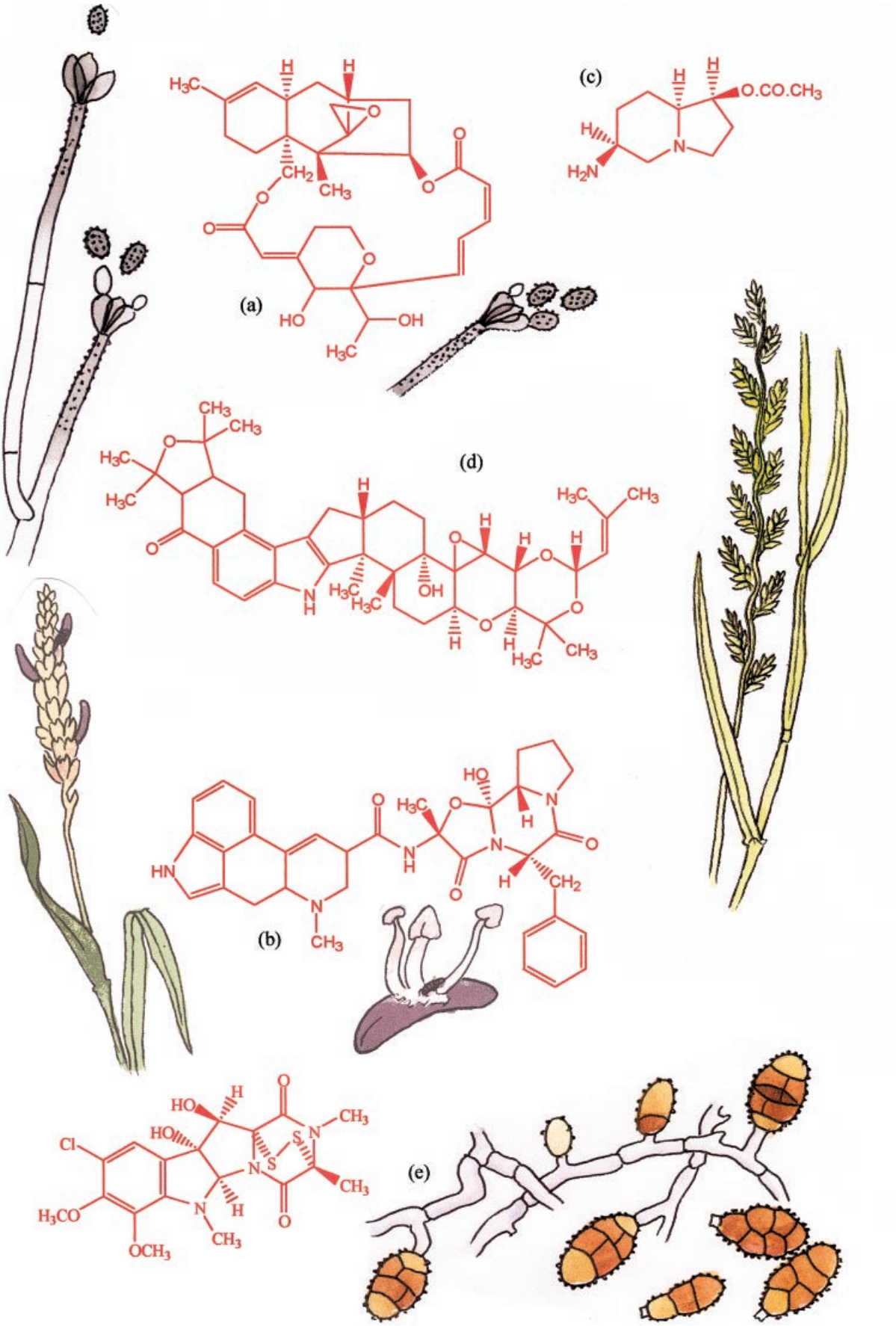

Fig 1 The sources and structure of a selection of mycotoxins. (a) *Stachybotrys chartarum* and satratoxin H. (b) *Claviceps purpurea* and ergotamine. (c) Slaframine. (d) The grass *Lolium perenne* and lolitrem B. (e) *Pithomyces chartarum* and sporidesmin.

Ergotism and Red Clover Disease

As examples of obligate plant pathogens two very different fungi are *Claviceps purpurea*, the causative agent of ergotism, and a species originally described as *Rhizoctonia leguminicola*, implicated in an illness of cattle known as red clover disease. *Claviceps purpurea* has a complex life cycle during which the infected seed of cereals such as rye become compact purplish brown masses of fungal tissue known generally as sclerotia, or in this specific case as ergots. The role of these structures in nature is to survive the winter and, without the intervention of humans, they would fall to the ground along with uninfected seed and germinate in the following spring. These structures are packed with toxic alkaloids, one example of which is ergotamine (Fig 1b), which would perhaps allow them to survive predation by insects and small mammals. However, when harvested they may be ground up with the cereals to produce toxic flour responsible for the devastating outbreaks of ergotism in Europe during the middle ages (the most recent outbreak in Europe was in France in the 1950s) and, more frequently, they will contaminate cereals and forages used as animal feed. The weather conditions in the summer of 2002 have led to an unusually high level of ergot formation in some parts of Britain leading to possible problems of ergot poisoning in farm animals. Ergot alkaloids act on various aspects of the nervous system and may cause spasms of muscle contraction, halucinations, and constriction of blood vessels, the last leading to the death of tissues in the peripheral limbs and gangrene. As is so often the case, compounds which are toxic in one situation may be beneficial in another, and ergotamine can be used to control bleeding in difficult cases of childbirth or to alleviate certain forms of migraine. Other derivatives may be of value in the treatment of hypertension, depression and Parkinson's disease. Indeed, so important are these compounds in the medical context that extensive studies have been carried out on the molecular biology of their production and the genes associated with the biosynthetic pathways have been characterized (Tudzynski, Correra & Keller, 2001).

The techniques of molecular biology have demonstrated that there is a high degree of genetic diversity within the species *Claviceps purpurea*, which also has a very wide host range (Jungehülsing & Tudzynski, 1997). Using RAPD and RFLP analysis Pažoutová & Tudzynski (1999) were also able to show that the strain ATCC 26245, previously used for biochemical and genetic studies, was not in fact *Claviceps purpurea*, but a related species *C. fusiformis*. The significance of this finding is that the latter is not able to produce the peptide alkaloids (of which ergotamine is an example) as it lacks the genes for the final stages in their biosynthesis; *C. purpurea* has the complete biosynthetic pathway for these compounds. Some plants, such as *Ipomoea argyrophylla* in the Convolvulaceae, are also able to produce ergot alkaloids (Friedmann, Dao & Gumbmann, 1989) and the application of molecular biology should make it possible to determine whether this is a property of the plant or of as yet unknown endophytic fungi.

Claviceps purpurea is an ascomycete but the form genus *Rhizoctonia* DC has been broken down into several genera correlated with teleomorphs in the basidiomycetes. The organism originally described as *Rhizoctonia leguminicola* Gough & E. S. Elliott, has been shown to fit *Botrytis* and the type of *R. leguminicola* is most probably *B. fabae* Sardina (Andersen & Stalpers, 1994). *Botrytis* P. Micheli ex Pers. is the anamorphic state of *Botryotinia* Whetzel which is, of course, also an ascomycete! This species is a pathogen of red clover in which it causes a black spot disease and produces another alkaloid, slaframine (Fig 1c), responsible for red clover disease of cattle, also known to the farmer as slobbers (Crump, *et al*., 1963). The cattle initially show symptoms which include profuse salivation, feed refusal, diarrhoea and, unless removed from the toxic forage, they eventually die from respiratory or heart failure because slaframine acts on the parasympathetic nerve system. Its biological activity in mammals requires an activation process in the liver to a molecule which, both chemically and physiologically, mimics acetylcholine (Guengerich & Aust, 1987).

Perennial ryegrass staggers

It is now known that many plants have fungal mycelium growing within their tissues in a benign relationship known as an endophyte. In at least one case an endophytic fungus, growing in the grass *Lolium perenne*, makes that grass toxic to sheep and cattle by the production of a mycotoxin. Initially it was thought that soil fungi, such as species of *Penicillium*, were involved because of their ability to produce tremorgenic metabolites such as the penitrems (Cole, 1981). However, it was demonstrated that rye-grass staggers is associated with an endophytic fungus in the leaves of *Lolium* (Fletcher & Harvey, 1982). The mould is *Acremonium loliae* and the toxins a family of compounds known as the lolitrems (Fig 1d). The lolitrems have potent tremorgenic activity and, although they may not directly cause the death of animals, they become unmanageable and when stressed may be killed by

trampling or other accidents. The endophyte has an ecologically obligate relationship with its host and is seed-borne from one generation of plant to the next. The fungus can be removed from seed by careful heat treatment, because it is significantly more heat sensitive than the plant, but the plants from such endophyte-free seed are more susceptible in the field to insect damage, such as that caused by the stem weevil *Listronotus bonariensis* (Siegel, Latch & Johnson, 1985). The lolitrems are not the anti-insect metabolites and it is possible to reinfect endophyte free seed with a strain of *Acremonium lolii* which does not produce lolitrems but still produces the insecticide.

A disorder of farm animals feeding on tall fescue (*Festuca arundinacea*) in the USA is known as fescue summer syndrome and also involves an endophytic species of *Acremonium*. This was initially considered to be the anamorph of *Epichloë typhina* but has been described as a distinct species *Acremonium coenophialum* (Morgan-Jones & Gams, 1982). Endophytic members of the Clavicipitaceae, such as *Epichloë* and *Balansia* with their anamorphs *Neotyphodium* and *Ephelis*, are also able to produce ergot alkaloids and efforts are being made to produce alkaloid non-producing mutants because these endophytes also increase the fitness of their hosts.

Sheep facial eczema

Fungi play an important role in the biosphere in the degradation of organic material of both animal and plant origin and the maintenance of the carbon and nitrogen cycles, but a number of these saprophytic moulds may also produce mycotoxins. Facial eczema in sheep, which is especially important in New Zealand, is caused by sporidesmin (Fig 1e) produced by *Pithomyces chartarum*. This species grows on dead grass material close to the soil, especially after frost damage during the winter. If there is a cold wet spring, leading to slow growth of forage grasses, sheep may graze too close to the soil and pick up spores of this mould containing sporidesmin (named after an earlier binomial for this species, *Sporidesmium bakeri*). The spores are readily detached from their original substrate and dispersed by rain and wind to attach to the new growth of grasses which then becomes toxic without being overtly mouldy. Sporidesmin causes irreversible liver damage of the animals eventually leading to their death. In the early stages this liver damage gives rise to increased concentrations of the breakdown products of chlorophyll in the blood. These compounds are photoactive and cause sunburn, or ezcema, around the muzzle of the sheep. *Pithomyces chartarum* is world wide in its distribution and sheep facial eczema does occasionally occur in different parts of the world but it seems to be a particular problem in New Zealand and it is not easy to understand why. It may be a combination of climatic factors with the intensity of sheep farming and the fact that most forage grasses are not indigenous but have been introduced. Although first recorded in New Zealand at the beginning of the twentieth century the aetiology was difficult to study and it was not until the middle of the century that the disease was associated with *Pithomyces chartarum* (Thornton & Percival, 1959) and subsequently with pure sporidesmin (Mortimer & Taylor, 1962). Although it is increasingly possible to predict the probability of outbreaks of facial eczema, it is still important to monitor the occurrence and increase in the number of spores of *Pithomyces chartarum* in order to judge what preventive action may be necessary. They are relatively easy to recognise (Fig 1e) and can be monitored by sampling and microscopy. However, an immunoassay has been evaluated which is sensitive over the range 0.4 - 40 ng/ml and shows good correlation with the numbers of spores in samples (Collin, Briggs & Towers, 1995). If an outbreak has been forecast the farmer may respond by dosing sheep with zinc, or moving them to safer pastures, although there is some progress in the development of breeds of sheep with intrinsic resistance to facial eczema (Morris, *et al.*, 1995).

Conclusion

This remarkable diversity of metabolites, and the diversity of biosynthetic pathways leading to their production, begs the question, what is their role in the biology of the producing organisms? The observation that secondary metabolism, as a process, has a role in an environment of nutrient imbalance and the products are essentially a matter of chance, is no longer satisfactory. A metabolite such as aflatoxin B_1 (Moss, 2002) is produced at the end of a long, complex biosynthetic pathway involving many enzymes (and associated genes, all of which have been characterized) and its production must have some relevance to the survival of those few species producing it (*Aspergillus flavus*, *A. parasiticus*, *A. nomius*, *A. ochraceoroseus*, *A. pseudotamarii* and *A. bombycis* at the last count!). We see a comparable complexity in biosynthetic pathway and genetics in the biosynthesis of ergotamine and related alkaloids (Tudzynski, *et al.*, 2001). The antibiotic activity of many mycotoxins, and their toxicity to insects and small mammals, may play a role in their success in colonising plant products in competition with all these other living organisms. The mycelium and other

structures of moulds are themselves nutritious and potential food for a wide range of fungivorous animals, especially amongst the invertebrates, and these toxic metabolites, some of which have antifeedant properties, may play a role in the protection of moulds from such activity. Again this is speculation and a resolution of this interesting problem requires some subtle tools of thought and experiment which may not yet be available!

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