Chapter 21
The Weaponisation of Mycotoxins

Robert Russell Monteith Paterson and Nelson Lima

21.1 Introduction

Mycotoxins as weapons is a serious issue. The word "weaponisation" in the title of this chapter is poor grammatically, although it is useful as it is generally understood. Crucially, access to accurate information is required to enable appropriate responses for potential threats. The high interest created by a recent review of fungi and toxins as weapons indicated that further publications in the field are desirable: Paterson (2006a) remained at number 1 in Science Direct's "Top 25 Hottest Articles" (*Mycological Research*) for a year. Citation numbers put it fourth since 2006: Holstege et al. (2007) is instructive as it indicates just how seriously the threat is taken in the United States of America. The authors focus on trichothecene mycotoxins and particularly T-2 toxin. Of course, the reasons for the topicality were the mass attacks on citizens which have occurred this century, and the claim that aflatoxins had the potential to be used by Iraq. The recent attacks in the USA using anthrax spores via internal post also caused a great deal of concern: massive casualties have been predicted from anthrax released into very large cites. Dohnal et al. (2007) are also concerned with T-2 toxin. Latxague et al. (2007) focuses on anticrop bioterrorism and bioweapons against the agricultural sector. They appear to be more concerned with whole organisms, rather than purified mycotoxins per se. However, it is difficult to obtain a list of the fungi with which Latxague et al. are concerned, no doubt for security reasons. Mycotoxin-producing fungi need to be on the list, as do plant pathogens. Also, countries where crops are developed almost as a monoculture are at particular risk from natural pathogens [e.g. the fungus *Ganoderma* and the oil palm crop (Paterson et al. 2009)]. Pohanka et al. (2007) considered the issue of developing bioassays to detect mycotoxins. Finally, Casadevall and

R.R.M. Paterson (✉)
Micoteca da Universidade do Minho, IBB/Centre for Biological Bioengineering, Campus de Gualtar, University of Minho 4710-057, Braga, Portugal
e-mail: russell.paterson@deb.uminho.pt

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Pirofski (2006) provide a well-argued assessment concerning why human pathogenic fungi could usefully be given greater consideration as biowarfare and bioterrorism agents.

A recent example of a biosecurity lapse in Egham, UK demonstrates why complacency cannot be permitted: poor infrastructure and inadequate funding led to accidental release of the foot and mouth disease virus. Possible causes of the breach may have involved misaligned effluent pipes and tree-root damage to the pipes, compounded by an increase in water caused by floods (HSE 2007). Obviously, those working with toxigenic fungi and fungal toxins need to exercise appropriate care. It is now absolutely necessary to consider the issue as it relates to the field of weapons.

The topic of fungal toxins as weapons is no longer taboo as it has changed to one of public interest and media attention. The remainder of this chapter discusses the toxins and not the fungi per se (i.e. the biochemical rather than the biological) although this tends to overlap when the problems with the taxonomy of the producing fungi are considered (Paterson et al. 2004, 2006). One might expect that the fungi which produce the toxins are well characterised. However, this is not the case. Conversely, this lack of knowledge may work as an advantage in terms of security as it is not always straightforward to select toxin-producing isolates on the basis of current taxonomies. It makes more difficult (a) the application of legal restrictions on the use or export of the fungi, and (b) tracing particular strains used in toxin production.

Public pressure exists on authorities to assess if occurrences of food contamination are nefarious acts (Elad 2005). The economic consequences of simply reacting to a potential attack can be huge, if what was experienced after the recent attacks in the USA is an example (Lenain et al. 2002). Furthermore, a sombre assessment of the dangers of bioweapons to the United States of America is provided by Bailey (2001). It is much more appropriate to focus on prevention, followed by readiness and response. The balance is to declassify essential information with a view to preventing aggressive acts. A compromise has to be drawn: it is self-defeating to ignore the subject given the large amount of public information already available.

Biosecurity guidelines are essential reading for those working with toxigenic fungi (Tucker 2003). They have highly significant implications which could limit research in the field, including determining which individuals do the work. However, just how representative the views are of this publication is unclear when the disclaimer states, "The views expressed in this report are those of the author alone. They do not necessarily reflect the views of the United States Institute of Peace". A public debate is required. Some well-known reports have claimed recently that aflatoxins were placed in warheads for use by Iraqis, although the effect of such a limited amount of aflatoxins dispersed in this manner would be minimal (Paterson 2006a). On the other hand, *Aspergillus flavus* may be more dangerous as a human pathogen than is generally realised (Hedayati et al. 2007).

There is nothing to be gained from being coy about discussing this issue. There is a great deal of information in scientific papers, published journals, newspapers, and the World Wide Web. The most comprehensive, and well considered source

of information/best practices is the Australia Group (http://www.australiagroup.net). This is a body which meets every year and represents numerous nations whose aim is, inter alia, to control the spread of chemical and biological weapons. For example, it is used by the UK Department of Trade and Industry for export control of a wide range of potentially and obviously dangerous material with possible utility as weapons. The web site is essential reading for anyone involved in the field. The mycotoxins considered are diacetoxyscirpenol, T-2 toxin, HT-2 toxin, and aflatoxin B_1.

Information is becoming increasingly available on these bioweapons (Bennet and Klich 2003; Miller et al. 2005; Paterson and Lima 2005; Stark 2005; Paterson 2006a); in addition to the dubious mixture of the informative (Locasto et al. 2004) and illegitimate material on the World Wide Web. A great deal is known about botulinin from *Clostridium botulinum* which is the most toxic compound in the world (human lethal dose 0.2–2.0 μg kg^{-1}), and so there is little point in not discussing fungal toxins as weapons which are, after all, less toxic. To obtain some level of calibration at the extremes, mycotoxins are (a) not as dangerous as nuclear weapons and (b) more dangerous than teargas. Ease of conversion to a weapon is a crucial factor (i.e. "weaponization"). It is crucial that rational discussion appears in reputable journals, books and media.

This chapter does not concern primarily fungi that cause disease per se. Obvious growth of fungi on animals is called mycosis and they are primary pathogens e.g. *Histoplasma capsulatum* (Bennet and Klich 2003). An excellent overview of fungi as weapons is provided in Casadevall and Pirofski (2006). Dietary, respiratory, dermal and other exposures to mycotoxins are called mycotoxicosis, and this area is more relevant to the creation of weapons. However, Hedayati et al. (2007) reported the aflatoxin-producing fungus *A. flavus* as the second most serious *Aspergillus* for causing human and animal infections (*Aspergillus fumigatus* is the first). The importance of this fungus increases in regions with a dry and hot climate. Consequently, the potential as a bioweapon may have been underestimated.

Furthermore, the use of fungi in technologies (e.g. biocontrol) requires revision because of the current heightened security awareness. There is an apparent similarity between fungal biocontrol agents (FBCA) and weapons, in that toxin-producing fungi are mass-produced and, for example, sprayed onto crops. (This also has ramifications for the health and safety of those who use these organisms for mass production and/or in non-sterile conditions.) Which raises the question, what are the natural levels of fungi (Gonçalves et al. 2006) and toxins (Paterson 2007a) in the environment? Interestingly, Bucheli et al. (2008) describe the occurrence of deoxynivalenol and zearalenone in river water which is of relevance to this topic. Pharmaceuticals from fungi are also relevant to the discussion. The difference between a compound being a toxin or a drug may be a shift in a decimal point of concentration and/or a change in a simple moiety. How these compounds are classified depends to some extent on the prevailing "climate". For example, mycophenolic acid, ergot alkaloids, penicillin and perhaps patulin, can be either.

21.2 Fungal Toxins and Mycotoxins as Weapons

There has always been great concern about toxins from the macro fungi (e.g. mushrooms), often from accidental consumption of the fruiting body. However, scientific endeavour began in mycotoxins per se with the discovery of aflatoxins in the 1960s. It is an extremely complex field due to its multidisciplinary nature. General discussions on mycotoxins are dealt with elsewhere in the current book, and Venâncio and Paterson (2007) can be consulted.

HACCP protocols have been developed to prevent unintentional contamination of food with mycotoxins and fungi. However, further control and analytical steps may be required for intentional contamination. The universality of applying HACCP has been questioned in any case (Sperber 2005). Mycotoxins are (a) below microbiological, some phytotoxins and phycotoxins and (b) above anthropogenic contaminants, pesticide residues, and food additives in terms of acute health risks. Significantly, they are the highest chronic risk factor in the diet (Kuiper-Goodman 2004). However, chronic effects are of little interest to weapon manufacturers. Furthermore, there may be fungal metabolites which are more toxic than mycotoxins (e.g. aflatoxins) although they are not normally detected in the environment (see Cole and Schweikert 2003a, b; Cole et al. 2003). These may be revealed through natural product screenings for drugs where toxic compounds are removed from screens at early stages in the process. Organisations such as culture collections (i.e. biological resource centres) that work with numerous fungi need to be more aware of this fact and improve biosecurity measures accordingly.

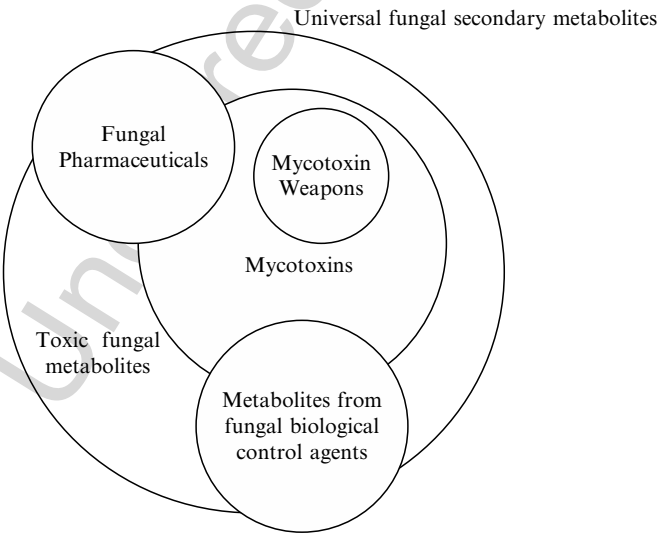

Fig. 21.1 Venn diagram of the relationship between fungal metabolites in terms of toxicity. The sizes of the circles are in proportion to the actual number of compounds only in a general manner

Table 21.1 Approximate classification of some toxins of fungi t1.1

	Weapon	Mycotoxin	Pharmaceutical	FBCA toxin	Other entomopathogen toxin	t1.2
Aflatoxins	+	+[a]			+	t1.3
Ochratoxin A		+[a]			+	t1.4
Cytochalasins		+		+		t1.5
Beauvericin		+		+		t1.6
Eniantins		+		+		t1.7
Destruxins				+		t1.8
Oosporein				+		t1.9
Moniliformin		+		+		t1.10
Efrapeptins				+		t1.11
Beauveriolides				+		t1.12
Amanita phalloides toxins	+	+[b]				t1.13
Patulin		+[a]	+[c]			t1.14
Mycophenolic acid		+	+			t1.15
Penicillin		+	+			t1.16
T-2 toxin	+	+[a]				t1.17
Ergot alkaloids		+[a]	+			t1.18

FBCA, fungal biological control agent t1.19
[a]Regulated in food
[b]Arguably mycotoxins as the whole fungus in ingested
[c]As originally investigated

The overlapping relationships between mycotoxins, pharmaceuticals, FBCA toxins, and fungal biochemical weapons are represented diagrammatically in Fig. 21.1. Even the same compound can be represented in different fields. This is a reason why a sound knowledge of which fungi produce which toxins (i.e. fungal chemotaxonomy) is crucial. Presently there are only a few metabolites considered to be mycotoxins from potentially thousands. There are probably more toxic metabolites from FBCA than there are mycotoxins using this narrow definition as only a few (i.e. three) of them (Table 21.1) can be considered as mycotoxins. However, a minute percentage of toxin fungal metabolites have been considered seriously as weapons: two (aflatoxins and T-2 toxin) are obviously mycotoxins whereas one is a toxin from a macroscopic fungus, i.e. α-amanitin. Holstege et al. (2007) mentions a rather wide range of compounds from fungi including trichothecenes in general, although in practice only T-2 toxin is considered in depth. Paterson and Lima (2005) list the ergot alkaloids (see CAST 2003) as other possibilities. 140 141 142 143 144 145 146 147 148 149 150 151 152 153

21.2.1 Fungal Toxins 154

Many fungi are not pathogens although they do produce toxins (Cole and Schweikert 2003a, b; Cole et al. 2003) and fungal toxins as weapons or "expressions of 155 156

discontent" are taken seriously (Paterson 2006a). Here we will consider the fungal toxins intrinsically as weapons which could be employed by governments and/or small groups of individuals. An individual could use them in a revenge attack on an employer for example. The supposed governmental deployments have ranged from the thin evidence of the use of T-2 toxin by the Soviet Union, to the development of aflatoxins by Iraq, the efficacy of which is minimal (Bennet and Klich 2003). It is worth pointing out that T-2 toxin does appear to be a valid weapon. It is axiomatic to state that any government would be interested in developing such weapons if they had desirable characteristics as defined by that government.

The factors which are fundamental for producing a serviceable biochemical weapon are (a) efficient manufacture, (b) ease of conversion to a weapon ("weaponization"), (c) longevity of the organism or toxin in storage, (d) efficient dispersal, and (e) stability when exposed to the environment. Other factors are concealment and ability to obtain the toxin or organism.

In the early twentieth century, biotoxins were investigated militarily, and were rejected because of the difficulty of conversion to weapons. For example, it has been stated that the US has no current offensive biological weapons capability. Interest has been stimulated by developments in biotechnology (e.g. "black biology" (Casadevall and Pirofski 2006). They are less expensive than nuclear and conventional chemical weapons and may appeal to countries or terrorist organizations where cost is an important issue (Locasto et al. 2004).

Biotoxins need to be produced from precursors. For example, a growth medium containing carbon and nitrogen as the predominant precursors must be inoculated with a live fungus in a suitable vessel (e.g. a bioreactor). The toxin is produced as a consequence of the metabolism of the fungus and is then purified to some degree. This can be undertaken in large bioreactors, hence producing a high yields of toxin. So the toxin is a chemical and has no living component. This implies that these compounds need to be classified as chemical weapons. For example, they will not spread from person to person beyond the locality of attack as would infectious diseases. So there is surely a need to reclassify these compounds. A dangerous scenario can be imagined where authorities respond to a biotoxin as a biological attack (perhaps involving isolating large numbers of people to prevent disease spread) whereas a much more localised concentration of effort may be more appropriate. Furthermore, in a British Broadcasting Cooperation web site it is mentioned that 30,000 kg of fungus was produced as a bioweapon to spray on crops (http://news.bbc.co.uk/1/hi/world/americas/1618420.stm). Presumably a similar quantity could be achieved if required to produce a large amount of fungal toxin. Finally, one simply has to consider how much penicillin has been produced from initially minute yields to realise that a massive amount of toxin as a weapon could be produced.

The threat of fungal toxins and mycotoxins were made apparent in Garber et al. (2005), Miller et al. (2005), and Stark (2005). The current terrorist tactics have shifted attention to protection of food supplies and consequently it is a world-wide concern for the twenty-first century. The impact on society could be anything from low to "catastrophic". A huge range of actions and programmes are being developed and implemented to prevent, deter, and respond to potential attacks.

For example, (a) enhanced laboratory capability, (b) advanced tracking, (c) increased examinations, (d) better surveillance, (e) more training, (f) recovery plans, and (g) new medical treatments.

Mycological/chemical sampling and detection methodologies need to be improved to meet the new concerns. Inactivation of mycotoxins and decontamination of food plants requires urgent consideration. Also, foods have to be ranked in terms of vulnerability to attack, as do the risks to people when foods are intentionally contaminated. The development of algorithms to differentiate natural from unnatural food contamination (Paterson and Lima 2005) is required: international and national collaborations are essential. Finally, biomarkers in humans and animals need to be further developed with particular relevance to metabolomics.

21.2.1.1 α-Aminitin

α-Aminitin from *Amanita phalloides*, amongst others, is a major concern as it is extremely toxic, water-soluble, and heat-stabile (Garber et al. 2005). However, mass production would presumably be limited to solid substrate bioreactors if basidiocarps were used, or conventional bioreactors if mycelium can produce the compound. The background counts of α-amanitin and T-2 toxin were useful as a demonstration of what is required to be done to distinguish abnormal from normal concentrations of biotoxins. In some cases the background was higher than the spiked samples, which is interesting. In some ways Stark (2005) is simultaneously alarming and reassuring in what is possibly an overly dramatic paper, at least in the introduction. The author mentions that large-scale tactical weapons are impractical but that the sabotage arena is suitable for mycotoxins; this is slightly reassuring, in that antidotes do already exist for some mycotoxins.

21.2.1.2 Aflatoxins

The concept of liver cancer from aflatoxin as a battlefield weapon beggars belief, and the reports of acute toxicity are uncertain. However, the threat of the use of the compound may have a psychological effect on a potential enemy. It is not widely known that *A. flavus* is the second most serious *Aspergillus* disease (see above) and cannot be discounted as a potential weapon, which was not emphasised in Paterson (2006a).

21.2.1.3 T-2 Toxin

Exposure to T-2 toxin of a few milligrams is potentially lethal. The concept of mass-production of the compound may be impractical simply from the point of view of obtaining sufficient growth medium to grow the fungus (Stark 2005). Whether another cheaper and plentiful medium could be used is a possibility,

although what this might be is uncertain (although large amounts of biomass of fungi have been produced (see above).

21.3 An Example

A revenge attack by a disgruntled employee is a possibility, for example by contaminating a water distribution system (Mays 2004) and so it remains worthwhile to illustrate the problem further:

Paterson et al. (1997) first reported the natural occurrence of any fungal secondary metabolite in water after attempting to produce aflatoxin in water in a bioreactor. The control water, which contained aflatoxins B_2, G_2 was from a water tank used to serve the laboratory where the work took place. An *A. flavus* strain was isolated from the control water, indicating how the aflatoxins may have arisen. However, it is not known if this was from the natural growth of a fungus or from intentional addition from a disgruntled employee. The water was used for a variety of purposes such as washing benches, cleaning floors, and supplying showers. So the levels of aflatoxins would have tended to accumulate over the years and be inhaled. Only a survey of similar water tanks would indicate if this contamination was normal or deliberate. It may be worth mentioning here that zearalenone and other metabolites from *Fusarium graminearum* were demonstrated to be capable of being produced in water (Paterson 2007b). Also, similar concentrations of zearalenone and deoxynivalenol have been detected in river water (Bucheli et al. 2008). In addition, T-2 toxin can be used as a food- or water-borne (Paterson and Lima 2005) poison. Currently, T-2 toxin is the only biologically active toxin effective through (a) dermal exposure, (b) respiratory and (c) gastrointestinal (GI) portals. Tissues involved in high cellular turnover (e.g. GI and respiratory epithelium, bone marrow cellular elements) are the most susceptible (Locasto et al. 2004).

21.4 Water as a Vector

Drinking or non-drinking water may be effective media for mycotoxin dispersal as a weapon and worthy of a separate section. The threat from contaminated drinking water is obvious. In the case of non-drinking water, the toxin could be spread by water from a shower and then inhaled. Work places where high volumes of water are employed, such as farms or car washes, could be susceptible. As an example, stored water which was demonstrated to contain aflatoxins was used to clean laboratories, and contaminated dust could be spread amongst the workers (see above). Furthermore, drinking water for animals may be at a considerably higher level of risk than that for human consumption. Toxins in water are possible naturally, unnaturally and from inoculation of fungi (Paterson and Lima 2005; Paterson 2006a, 2007a, b; Bucheli et al. 2008). However, it is interesting

that high concentrations of the mycotoxin ochratoxin A (OTA) were degraded in a model waste water treatment system and that the activated sludge/microbial consortium remained effective (Nogueira et al. 2007), indicating that water treatment plants could cope with a threat from biotoxins to some extent.

21.5 Fungal Biological Control Agents

There are some interesting parallels between fungal weapons and FBCA. FBCA technology needs to be reassessed in terms of safe use (Skrobek et al. 2005), which is relevant especially in the current high-security climate. For example, the procedures involve applying natural fungal pathogens to crops in the field or in storage, to control insect pests or disease. They have been advocated by some as an effective and, more particularly, an environmentally sound means for controlling pests, disease and undesirable organisms. An immediate concern about these preparations is how safe they are in terms of toxin production (Strasser et al. 2000; Skrobek et al. 2005). After all, these could affect the general population and workers producing FBCA. They have been introduced or tested disproportionately in developing countries where, ironically, the mycotoxin problem is more acute. In a large study on risk assessment Strasser et al. (2000) did not consider compounds produced by FBCA which are already known mycotoxins. For example, cytochalasins are not discussed, although they are produced by the FBCA, *Metarhizium anisopliae*, which is considered by the authors. Cytochalasin D in particular is very toxic. If it is satisfactory to use the fungi mentioned because the levels in the environment would be low, is it equally safe to use *A. flavus* which produces aflatoxins, or *Aspergillus ochraceus* which produces OTA (Paterson 2006a)? Futhermore, there are reports where the FBCA forms a symbiosis with the plant to be protected (Wagner and Lewis 2000). In biocontrol terms this is considered to be desirable as it may provide long-term protection. In terms of persistence in the environment it is worrying.

An example of an FBCA is the use of *Fusarium oxysporum* (i.e. *F. oxysporum* f. sp. *exythoroxilum*) to kill coca plants in some Latin American countries (Connick et al. 1998; de Vries 2000). Interestingly, the idea is to introduce, rather than cure, a disease. The ultimate objective is to stop the manufacture of cocaine. Table 21.2 indicates the toxins associated with the fungus and it appears that the effect of these had not been considered sufficiently. The concepts of what constitutes a species in this taxon are complex, and toxin production from individual special forms has simply not been clarified satisfactorily. The technology has a great deal of similarity to what would be required to apply a bioweapon. After all, mycotoxins have been ranked as the most important chronic risk factor in the diet above pesticide residues, synthetic contaminants, plant toxins and food additives (Bennet and Klich 2003). They are also considered to be more acutely toxic than pesticides. Those interested in applying this technology need to collaborate more fully with those who know

t2.1 **Table 21.2** Some toxins or secondary metabolites of potential biocontrol fungi

t2.2 Entomopathogens	Secondary metabolites/toxins
t2.3 *Aspergillus flavus*[a]	Aflatoxins
t2.4 *Aspergillus ochraceus*[b]	Ochratoxin A
t2.5 *Metarhizium anisopliae*	Cytochalasin C, D, helvolic acid, destruxins
Beauvaria bassiana t2.6	Beauvericin, dipicolinic acid, oosporein, isoleucylisoleucyl anhydride, cyclo-(L-isoleucyl-L-valine, cyclo-(L-alanyl-L-proline), bassianolide
t2.7 *Beauvaria brongniartii*	Beauverolide L, La
t2.8 *Verticillium lecanii*	Helvolic acid, bassianolide
t2.9 *Paecilomyces fumosoroseus*	Beauverolide L, La
t2.10 *Colletotrichum gloeosporioides*	Gloeosporone
Trichoderma harzianum t2.11	Koninginin A,C, peptaibols, harzianum, cyclonerodiol, trichorzianines A,B
t2.12 *Penicillium oxalicum*	Oxalic acid, oxaline, secalonic acid
Fusarium oxysporum t2.13	Moniliformin, hydroxylated fumonisin C1, fumonisin C4, fusaric acid, benzoic acid, enniatins, fusaric acid, fusarin C, ipomeamarones, sambutoxin.A

t2.14 [a]Not used
[b]Not used. Pathogen of *Ceratitis capitata* (Castillo et al. 2000) amongst other insects. Well-known producer of ochratoxins

315 how to control the secondary metabolism that produces the toxins, although control
316 of production in the field will be difficult in a predictable manner.

317 21.6 Taxonomy

318 The first thing to mention is that fungal taxonomy is in an immature state (Burnett
319 2003; Santos et al. 2009). Fungi have not received as high a level of attention as
320 other kingdoms (plants, bacteria, animals, etc.). Species concepts are confused
321 largely from the use of inconsistent characters. Many of the asexual isolates have
322 been given species status when this may be inappropriate (see Paterson et al.
323 2006). There is the issue of stating that mycotoxins are not produced from species
324 when what is meant is that they were not detected (Paterson et al. 2004). The
325 fungus may produce small, difficult-to-detect amounts, or large amounts under
326 other specific circumstances. Paterson et al. (2004) suggested identifying fungi to
327 an easily recognised morphological character (e.g. a conidiophore) and undertak-
328 ing a biochemical analysis for the toxin of interest. In the case of OTA-producing
329 penicillia, an identification of *Penicillium* OTA " + " may be obtained. Interest-
330 ingly, a somewhat similar scheme has been reported previously for *A. flavus*
331 (Cotty 1989). Strains that produced only detectable aflatoxin Bs were designated
332 "B" whereas those that produced detectable aflatoxins B and G were designated
333 "BG". An approach as described in Paterson et al. (2004) may be useful as there
334 are many very similar taxa within the *A. flavus* group (Hedayati et al. 2007). The
335 situation is similar for trichothecene-producing fusaria where various taxa can
336 produce biotoxins for possible use as weapons (Glenn 2007).

The history and current state of the taxonomy of mycotoxin-producing fungi is of relevance to the weapons issue. For example, (a) authorities may need to trace particular cultures used for research or in the mass production of mycotoxigenic fungi, and (b) if fungi were used to contaminate commodities it would be necessary to determine if the fungi produced particular toxins. There is often a great deal of confusion concerning species concepts and what is meant when a fungal species is described as "producing a particular mycotoxin": it is not as simple as "only this species produces this toxin".

Furthermore, PCR methods are used increasingly for identification and other taxonomically related purposes for fungi. The limitations of this technique may not have been considered adequately (Paterson 2006b) and certainly the lack of internal amplification controls in most previous studies requires attention (Paterson 2007c, 2008). Also, the method by which the fungi are grown has not been considered where mutagenic compounds may be produced in culture and affect detrimentally the DNA of the fungi of interest (Paterson et al. 2008; Paterson and Lima 2009). Too often the technique has been used by those whose primary skill is not in biochemistry or molecular biology. They appear to have simply followed published methods although adapted superficially to their particular situation, without fully considering the ramifications of so doing, and alternative methods would be useful (see Santos et al. 2009).

21.7 Genetically Modified Fungi (GMO)

It is possible to speculate about altered strains which could be more virulent and/or produce higher yield of toxins than the wild type. The use of "black biology" was discussed above. Countries with large resources could perhaps develop such strains. It may be something of a worst-case scenario. However, considering the developments in genetics it is probably only a matter of time before such an organism exists, if it does not already. In developed countries, transfer of cultures from culture collections to second parties involves the completion of a "material transfer agreement" which forbids genetic modification of the received cultures unless containment is increased to accommodate the GMO. However, it is not clear what GMOs mean. Does it specifically require that genetic information is transferred from one taxon to another? A useful example is how the titre of penicillin has been vastly increased from the original meagre amounts through strain improvement techniques, and this could conceivably be undertaken for toxin production.

21.8 Security of Laboratories and Obtaining Pure Mycotoxins

These issues are discussed in depth in Tucker (2003) although some controversial points are raised, such as which individuals and laboratories could be allowed to

work in the field, and whether the degree of control possible in the USA can be applied to other countries. It needs to be determined what degree of risk pertains to each situation. Put crudely, does working with certain pathogenic bacteria equate in safety terms with working with fungi (Casadevall and Pirofski 2006)? Is the risk from the fungus (i.e. biological) or the toxin produced (i.e. chemical)? If it is the toxin then would not chemical security procedures be more appropriate and which have been established longer? Some relevant concerns are raised in WHO (2003). Recent global events and more local events such as those in Egham, UK (see earlier in this chapter), have underlined the need to design laboratories and the materials they contain in a way that will protect people, livestock, the environment and agriculture. However, there are distinctions between laboratory biosecurity and biosafety. Biosecurity measures prevent intentional release, loss, misuse, theft and diversion of pathogens and toxins. In contrast, biosafety measures are containment procedures implemented to prevent unintentional exposure of pathogens/toxins or accidental release. Indeed, security precautions need to become routine laboratory practice, according to WHO (2003).

In the OECD (2007) report on biological resource centres (BCRs) (i.e. culture collections), it is stated that many are entrusted with the maintenance and exchange of hazardous biological resources. The report makes some self-evident statements such as, "staff should have relevant qualifications, training and competence to carry out their duties". Also, it contains such tautological sentiments as "To achieve quality assurance in BRCs, best practices for quality are clearly needed". Biosecurity could be enhanced at BRCs if they limited activities to the maintenance of organisms and did not become involved in, for example, research and development where the organisms have to be grown and extracted in large scale. However, this may not find favour with most BRCs. The menace of bioterrorism has changed the geo-political landscape and BRCs need to make special efforts to prevent loss or theft. Facilities have to be protected and to promote a sense of security. Often a risk assessment of fungi and toxins is involved, and the assigning of work activities occurs at an appropriate level of security suitable to the risk involved. This implies that the workers are aware of the toxins produced by the various fungi they hold, which at least implies awareness of current literature on the subject. It needs to be recognised that novel toxins can be discovered, and that compounds referred to as mycotoxins are not necessarily the most dangerous. An apparently safe, or unknown, fungus in term of toxin production may produce high yield of a novel toxin. Therefore, the undertaking of so-called bioprospecting projects (i.e. screening unknown fungi for bioactivity) (see the Iwokrama project in Paterson 2008) needs to be carefully considered from safety perspectives. A form of pre-toxicity screening is surely required, perhaps by a biological assay method. Grading security risks from high to low is dependent on the knowledge or motivation of the assessor or manager involved. The potential of fungi to produce dangerous chemical compounds needs to be assessed. Some further procedures may include methods for regular decontamination of work places. Before working with fungi, researchers should undertake a literature search on secondary metabolites known to be produced from the fungi.

However, the high costs estimated for a UK culture collection in the area of technical compliance of 100,000 to 340,000 Euros (OECD 2007) did not take account of even some of these points.

In addition, workers attempting to purchase pure mycotoxins from the chemical companies will realise that it has become more difficult. Often legitimate proof of use is required. Presumably this is because of security and not from an increased level of concern for workers' health per se. There is more paper work and security surrounding sending toxigenic cultures *inter*-laboratories. Concern with respect to health and safety is mostly related to the mass production of fungi and especially dried conidia which can be so easily inhaled. Similarly, a great deal of care is required when handling the purified and dried toxin preparations.

21.9 Mycotoxicosis Treatment

Supportive therapies for mycotoxicosis consist of improved diet and hydration of patients (Locasto et al. 2004), which are fairly obvious. Taking super activated charcoal orally may be effective if toxins are swallowed (e.g. T-2 toxin), and indeed the route of entry and dose indicate the clinical course for T-2. From a detailed study of OTA toxicity and activation metabolism of aflatoxin B1, it was discovered that the sweetener aspartame is very protective against OTA intoxication, and that Oltipraz effectively protects against AFB1 acute toxicity and carcinogenicity (Stark 2005). Oltipraz has been tested in China on populations exposed to aflatoxins (Bennet and Klich 2003). Some strains of *Lactobacillus* effectively bind dietary mycotoxins and may be an effective treatment. However, it is noted that management of fungal-related weapons is not discussed in Shannon (2004).

21.10 Mycotoxin Decontamination

Biotoxins from fungi would be difficult to remove from food and water (Paterson and Lima 2005). The methods devised by Castegnaro et al. (1991) at least would be effective for the mycotoxin-contamination of environments such as rooms. The most common procedure for decontamination is washing with bleach which effectively oxidises most aflatoxin and some other mycotoxins (Stark 2005). Potassium permanganate under alkaline conditions appears to be effective for a wider range of mycotoxins and for more situations than bleach, points which have been overlooked. The use of an enzyme to degrade the toxin might be feasible technically but is probably not yet applicable as a routine or emergency procedure. Finally, Sharpira (2004) provides extensive details on decontamination of foods.

21.11 Some Priorities

It needs to be recognised that it is the low molecular weight toxins from fungi that present the biggest threat. It is not the fungus that is the direct threat, apart from the remote possibility of a genetically engineered one causing unconstrained damage (although it is true that fungi have been underestimated as weapons). The use of fungal plant pathogens to devastate crops is a serious threat. An understanding is needed of what are normal levels of fungi and toxins in the environment (Strasser et al. 2000; Gonçlaves et al. 2006, Paterson 2006a, 2007a; Bucheli et al. 2008). The acute (e.g. T-2 toxin) or chronic (e.g. aflatoxins) nature of each mycotoxin needs to be established. Better methods for analysing the toxins are required. Fortunately, methods for multimycotoxin analysis based on chromatography exist (Paterson and Lima 2005). The single method procedures for hundreds of compounds are of particular value and standardised protocols could be based on these. PCR methods for the fungi can be employed (Paterson 2006b, c) although these methods have been compromised by the lack of suitable controls (Paterson 2007c) and optimal protocols for growth of the fungi for testing (Paterson and Lima 2009). There are vast amounts of data on the levels of the more well-known mycotoxins in a variety of foods. CAST (2003) and Venâncio and Paterson (2007) are excellent source materials. However, there are more data from other surveys. It is worthwhile listing those compounds which are water-soluble, as this will be a crucial factor in water and food contamination. Determining which foods would be expected to be contaminated with particular mycotoxins and which would normally not be expected is essential information.

Furthermore, it is crucial to appreciate the uncertainties in mycotoxin analysis (CAST 2003; Whitaker and Johansson 2005). For example, samples of corn contaminated with aflatoxin at (a) 10 ng g^{-1} and (b) 10,000 ng g^{-1} are estimated to vary in a repeated subsequent analysis by (a) 0 to 33.9 ng g^{-1} and (b) 8,992–11,008 ng g^{-1} respectively. One can therefore immediately understand the problem of deciding if a sample was intentionally contaminated. It should go without saying that practical classifications of the fungi that produce the toxins are required. There is a strict requirement to be able to unequivocally identify those isolates from commodities that produce toxins of relevance. A novel scheme is discussed in Paterson et al. (2004, 2006) where it is required to identify consistent morphological characters and then analyse for the toxin of relevance.

21.12 Future Trends

The authors of this chapter predict that there will be more compounds considered as mycotoxins within 10 years. Mycotoxins will become acceptable only at ever-decreasing concentrations tending towards background levels, and increasing numbers will be shown to be toxic and present in different foods. So the mycotoxin

circle will become bigger in terms of Fig. 21.1. We predict that the number of FBCA will decrease. Also, compounds from these may begin to be considered more seriously as mycotoxins (e.g. destruxins) and consequently result in fewer FBCA. The trend for weapons is difficult to predict. It may be that they will begin to be considered as "not effective". Alternatively, they could expand into the "non-mycotoxin toxins" (e.g. Cole and Schweikert 2003a, b; Cole et al. 2003). This type of activity is reported in the literature but the compounds are not usually found, or investigated, in food.

21.13 Conclusions

A previous review which considered fungi and toxins from fungi as potential weapons created high interest. The demand for such work was the recent aggressive attacks on innocent citizens and concomitant increased security by governments. We need to be able to prevent rather than react to such events. The Australia Group deliberations are essential reading for anyone working in the field (http://www.australiagroup.net). Bioweapons, mycotoxins, FBCA and even pharmaceuticals need to be considered in the context of the new paradigm. None of the toxins are as (a) toxic as botulinin toxin from *C. botulinum*, and (b) dangerous as nuclear weapons. However, they are more dangerous than, for example, teargas. A toxin may be considered as a pharmaceutical and vice versa simply by a small change in concentration or a moiety. Fungal toxins of use as weapons may be defined as any toxic compound from fungi which could be "weaponised". The current list of fungal toxins as biochemical weapons is small although awareness is growing of the threats they may pose. T-2 toxin is perhaps the biggest concern. A clear distinction is required between the biological (fungus) and chemical (toxin) aspects of the issue. Various factors need to be considered and not simply overall toxicity or notoriety. Ease of "weaponisation" is important. The classification of toxins as potential biological weapons appears anomalous as they are chemicals and it is suggested here that they be considered as chemical weapons. There is an obvious requirement to be able to trace the fungi and compounds which are produced in the environment and to know when concentrations are abnormal. Many FBCA produce toxins and so the use of these preparations requires additional consideration.

The chemotaxonomy and identification of the producing fungi needs to be reconsidered. There is a great need to be able to link consistently toxin production with particular fungi isolated from commodities. On the positive side, it is possible to treat mycotoxicosis and to decontaminate mycotoxins. There is considerable confusion and inconsistency surrounding the topic of bioweapons which requires to be assessed in an impartial and scientific manner. It is fundamental to be able to differentiate between abnormal and normal concentrations of toxins or fungi in the food/water supply.

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